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## **China-Bulgaria Rural Revitalization Development Cooperation Forum**

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УНИВЕРСИТЕТ ЗА НАЦИОНАЛНО  
И СВЕТОВНО СТОПАНСТВО



ФОНД  
НАУЧНИ  
ИЗСЛЕДВАНИЯ  
МИНИСТЕРСТВО НА ОБРАЗОВАНИЕТО И НАУКАТА

# PROCEEDINGS

## China-Bulgaria Rural Revitalization Development Cooperation Forum

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Agricultural University-Plovdiv, University of National and World Economy-  
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Shengquan Che, Hrabrin Bachev, Bozhidar Ivanov, Dimitar Terziev, Lyubka Koleva

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## CONTENT

		Page
1.	<b>Bioremediation of secondary salinization soil by a novel isolated strain <i>Bacillus megaterium</i> NCT-2 and its nitrate assimilation related enzymes</b> <i>DanZhang, ShaohuaChu, WeiweiShi, PeiZhou</i>	4
2.	<b>Tourism factors and rural development in Bulgaria</b> <i>Bozhidar Ivanov, Hrabrin Bachev, Dilyana Mitova, Daniela Dimitrova and Vasil Stoychev</i>	16
3.	<b>Grape Breeding in China</b> <i>Jiang Lu1, Yu Gao1</i>	29
4.	<b>Development of the Viticulture and Wine Industry in Bulgaria - Problems and Perspectives</b> <i>LyubkaKoleva, AdelinaHarizanova, DesislavaToteva, SvetlaYancheva, HristinaYancheva</i>	49
5.	<b>Effects of inhaling ylang-ylang essential oil on anxiety, social interaction and sleep behaviors in mice</b> <i>Nan Zhang, Lei Yaoa, Linyin Feng</i>	63
6.	<b>Development of the production of aromatic oil crops in Bulgaria</b> <i>Mariana Petkova1, Nurettin Tahsin, Svetla Yancheva, Hristina Yancheva</i>	71
7.	<b>Evaluation of rural landscape recreation quality in metropolis: Case study in Shanghai</b> <i>Shengquan CHE, Zhijie YANG, Anze LIANG</i>	87
8.	<b>The Sustainability of farming enterprises in Bulgaria</b> <i>Hrabrin Bachev</i>	109
9.	<b>Research on the Landscape of Shanghai Rural Settlements</b> <i>YANG Zhi-jie, CHE Sheng-quan, DU Yu-yu</i>	128
10.	<b>Economic Viability: Sustainability and Safety through Human Values and Economic Modes</b> <i>Dimitar Terziev, Donka Radeva</i>	141
11.	<b>The straw organic fertilizer workshop in the container: Reearch and application of integrated technology for distributed straw cleaning and fertilizer fermentation</b> <i>Dan Zhang, Haiwei Feng, Jie Feng, Minglei Yu, Pei Zhou</i>	149
12.	<b>Study on the Spatial Structure of Shanghai Urban Agriculture Tourism</b> <i>WANG Ling, CHE Shengquan, LIANG Anze, LIU Xiao</i>	157
13.	<b>History, Challenge and Opportunity of Grape Wine in China</b> <i>Yu Gao1#, Jiang lu1</i>	169

# Bioremediation secondary salinization soil by a novel isolated strain *Bacillus megaterium* NCT-2 and its nitrate assimilation related enzymes

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## Abstract

Large accumulation of nitrate in soil has resulted in “salt stress” and soil secondary salinization. *Bacillus megaterium* NCT-2 which was isolated from secondary salinization soil showed high capability of nitrate reduction. The resting cells could remove nitrate over 200mg/L within 48h. In order to further investigate the nitrate assimilation function of the NCT-2, genome sequencing was performed and submitted to the NCBI GenBank (AHTF00000000). The genes encoding assimilatory nitrate and nitrite reductase from NCT-2 were indentified, cloned and over-expressed in *Escherichia coli*. The putative 3D protein structures of these genes were modeled by SWISS MODEL, and shown to be highly similar to the nitrate assimilation related genes in the PDB database. The molecular mass of nitrate reductase was 87.3 kDa and 80.5 kDa for electron transfer and catalytic subunit, respectively. The large and small subunit of nitrite reductase was 88 kDa and 11.7 kDa, respectively. The purified recombinant enzymes showed broad activity range of temperature and pH. The effects of metal ions and electron donors on enzyme activities were analyzed. Moreover, the kinetic parameters of enzymes were measured. These results revealed adaption of NCT-2 to secondary salinization condition and shed light on the role played by the nitrate assimilatory pathway in NCT-2.

**Keywords:** *Bacillus megaterium* NCT-2; secondary salinization soil; nitrate reductase; nitrite reductase

## 1. Introduction

Since nitrogen fertilizers increase agricultural productivity [1], nitrate has been used more than is actually required for crop and vegetable cultivations [2, 3]. Large accumulation of nitrate in soil not only contributes to air pollution and climate change [4], but also affects the plant growth negatively, and this is known as “salt stress” [5, 6]. Furthermore, high concentration of soil nitrate has resulted in soil secondary salinization [7], which is considered to be one of the major factors that limits sustainable

development of agricultural production in the protected farmland of China [8]. Therefore, excess nitrate should be removed from agricultural soils for the cultivation and production of safe products [2]. Bioremediations using microbes have been successful for the removal of various pollutants from diverse environments [2, 9, 10], so that similar strategies may be applicable to the nitrate removal from secondary salinization soil. Nitrate is a significant nitrogen source for microorganisms [11]. It can be converted to the biologically useful form during assimilation that provide for nitrogen incorporation into the carbon backbone of organic compounds [12]. The nitrate assimilatory pathway is mediated by two enzymes, nitrate reductase and nitrite reductase, which catalyse the stepwise reduction of nitrate to nitrite and nitrite to ammonia, respectively [13].

Nitrate reductase is the first enzyme involved in the chain of nitrate reduction. Three classes of nitrate reductases can be identified in prokaryotes according to their cellular location and function: membrane-bound respiratory (Nar), periplasmic dissimilatory (Nap), and cytoplasmic assimilatory (Nas) nitrate reductases [12, 14]. In comparison with Nar and Nap in both heterotrophic and phototrophic bacteria, Nas have been scarcely studied, especially in heterotrophic bacteria [15]. Nas in the anabolic pathway of nitrate assimilation catalyses the reduction of nitrate to nitrite and has been considered the rate-limiting step for the conversion of nitrate to ammonium [16]. Two classes of Nas are have been found in bacteria: ferredoxin (flavodoxin)-dependent Nas and NADH-dependent Nas [12]. The ferredoxin-dependent Nas consists of one catalytic subunit (70-85 kDa) [17]. The NADH-dependent Nas is a heterodimer containing a catalytic subunit (90-105 kDa) and a subunit that is responsible for the electron transport (45-50 kDa) [18]. The researches on nitrite reductase are focused on dissimilatory pathway, especially the denitrification process. Assimilatory nitrite reductase contains sirohaem and iron-sulfur cluster [11]. There are also two types of this enzyme: the ferredoxin-dependent enzyme typical of photosynthetic organisms and the NAD(P)H-dependent enzyme of most heterotrophic organisms [14, 19]. In comparison with assimilatory nitrate reduction in fungi, algae and plants documented for a long time, data on nitrate assimilation in bacteria are relatively scarce [20]. The genes coding for assimilatory nitrate reduction systems which are normally clustered have been sequenced in some bacteria, however, biochemical studies on bacterial assimilatory nitrate reductases have not kept pace with genetic characterization, and this situation needs to be redressed in future studies [14].

Most nitrate and nitrite reductases were generally isolated from organisms inhabiting “normal environments, only a few papers were available on this two enzymes from microorganisms living in stressful environments [16, 21-25]. However, this information is extremely important both for revealing general mechanisms of adaption to stress and for applied

investigations with due regard to their roles in nitrogen turnover in nature [12]. To our knowledge, there are few studies related to their properties of bacteria isolated from secondary salinization soil. A novel bacterium *B. megaterium* NCT-2 which was isolated and identified from secondary salinization soil from the greenhouses in China, can utilize nitrate as its only nitrogen source for growth [26]. Thus, *B. megaterium* NCT-2 would be applied as biological components to eliminate excessive amounts of nitrate in soil. In the present study, the morphological features and nitrate uptake of NCT-2 were studied. The NCT-2 genome sequencing was performed and submitted to the NCBI GenBank. The sequences of the nitrate assimilation related genes were identified. NasBC (the nitrate reductase electron transfer subunit NasB and the catalytic subunit NasC) and NasDE (the nitrite reductase large subunit NasD and the small subunit NasE) NCT-2 were prepared as recombinant proteins, and their catalytic properties were analyzed.

## **2. Materials and methods**

### ***2.1 Morphology and nitrate uptake of B. megaterium NCT-2***

The morphology was observed by electron microscope (Sirion 200, USA). Uptake of nitrate by the resting cell of strain NCT-2 was carried at 25°C and 150 rpm, when the concentration of nitrate was from 100 to 1,000 mg/L. And, the concentration of resting cells was about 20 mg/L in this experiment. The concentration of nitrate was measured continuous flow analysis (SEAL AutoAnalyzer3, USA).

### ***2.2 Genome sequencing and gene annotation***

*B. megaterium* NCT-2 genome was sequenced on the Next Generation Sequencing Solexa platform by Major Bio BioPharm Biotechnology Co., Ltd. The contigs were generated with optimized Khmer parameter by the Velvet assembler. The obtained contigs were combined and predicted by three gene prediction softwares as follows:

Glimmer3 (<http://www.cbcb.umd.edu/software/glimmer/>)

Genemark (<http://exon.biology.gatech.edu/>)

Gismo (<http://www.cebitec.uni-bielefeld.de/groups/brf/software/gismo/>)

The same predicted genes, from at least both two softwares, were compared with the non-redundant (Nr) protein sequences database by using the NCBI BLASTP program. The fine map of the NCT-2 was submitted to the NCBI GenBank (AHTF00000000).

### ***2.3 3D structure modeling and prediction***

Gene 3D structures were predicted by two methods. (1) The SWISS-MODEL online modeling system, automated mode. The amino acids of the protein of interest were used for searching against the SwissModel Template Library (ExpDB) with its own rules [27-29]. The resulted amino acids sequences with the most similarity of the protein were used for generating models based on the comparing templates from the ExpDB. (2) The NCBI standard protein BLAST, BLASTP. The amino acids sequences of protein of

interest were compared with the Protein Data Bank (PDB) protein database. The alignments from the protein searching results that had the most similarity were used for model generation. All 3D structures were generated by using the software Cn3D (version 4.3, NCBI structure).

#### **2.4 DNA amplification and construction of co-expression plasmids**

Genomic DNA was extracted from *B. megaterium* NCT-2 using a Bacteria Genomic DNA Extraction Kit (TIANGEN Biotech, Beijing, China). The sequences of *nasB*, *nasC*, *nasD* and *nasE* were amplified using genome of *B. megaterium* NCT-2 as the template with primers listed in Table 1. The PCR products were purified and then added to A-tailing reactions with DNA A-Tailing Kit (Takara Biotechnology, China). The fragments encoding subunits *nasB*, *nasC* and *nasE* were cloned into the pMD18-T vectors, and the fragment encoding subunit *nasD* was cloned into the pUCm-T vector. Then all fragments were transformed into *E. coli* DH5 $\alpha$  competent cells. Transformants containing the pUCm-T vector harbouring *nasD* gene and the pMD18-T vectors harbouring *nasB*, *nasC* and *nasE* were selected. The plasmids (pMD18-T-*nasB*, pMD18-T-*nasC*, pUCm-T-*nasD* and pMD18-T-*nasE*) were isolated from the transformants and sequenced.

The expression plasmids pETDuet-*nasB* was constructed by ligation of gene *nasB*, digested by *Bam*HI and *Sac*I from pMD18-T-*nasB*, into the corresponding restriction sites of the pETDuet plasmid and transformed into *E. coli* BL21 (DE3). Likewise, gene *nasC* was digested by *Eco*RV and *Kpn*I from pMD18-T-*nasC*, and ligated into the corresponding restriction sites of pETDuet plasmid. The expression plasmid pETDuet-*nasC* was then transformed into *E. coli* BL21 (DE3). To over-produce nitrate reductase in *E. coli*, a co-expression plasmid pETDuet-*nasBC* was constructed by ligation of pETDuet-*nasC* and pMD18-T-*nasB*, both digested by *Bam*HI and *Sac*I. *E. coli* BL21 (DE3) and Transetta (DE3) cells were transformed with the co-expression vector.

The expression plasmids pETDuet-*nasD* was constructed by ligation of gene *nasD*, digested by *Bam*HI and *Eco*RI from pUCm-T-*nasD*, into the corresponding restriction sites of the pETDuet plasmid and transformed into *E. coli* BL21 (DE3). Likewise, the gene *nasE* was digested by *Nde*I and *Kpn*I from pMD18-T-*nasE*, and ligated into the corresponding restriction sites of pETDuet plasmid expression plasmid. Then pETDuet-*nasE* expression plasmid was transformed into *E. coli* BL21 (DE3). To over-produce nitrite reductase in *E. coli*, a co-expression plasmid pETDuet-*nasDE* was constructed by ligation of pETDuet-*nasD* and pMD18-T-*nasE*, both digested by *Nde*I and *Kpn*I. *E. coli* BL21 (DE3) and Transetta (DE3) cells were transformed with the co-expression vector. All plasmids were sequenced through the entire encoding sequence to verify the absence of undesired mutations introduced by polymerase chain reaction (PCR).

## ***2.5 Over-expression and purification of recombinant nitrate and nitrite reductase***

*E. coli* expression strains were grown at 32°C and 200 rpm in 50 mL LB medium containing ampicillin (25 µg/mL) until the OD<sub>600</sub> reached 0.8. Expression conditions were optimized with regard to expression strain BL21 (DE3) and Transetta (DE3), isopropyl-β-D-thiogalactoside (IPTG) concentration (0.05 mM, 0.08 mM, 0.1 mM, 0.4 mM and 0.7 mM), and duration (6, 8, 10 and 22 h) at 20°C and 150 rpm.

After expression, cells were harvested by centrifugation at 12000 rpm and 4 °C for 10 min, and then washed with 50 mM Tris-HCl buffer (pH 8.0). Cells were collected by centrifugation and lysed with an AH-1500 high pressure homogenizer (ATS engineering limited, AH-1500, Shanghai, China) at 900 bar for three cycles. The temperature of the entire homogenization process was maintained between 3 and 5°C with a recirculating cooling system. The lysates were centrifuged at 12000 rpm and 4 °C for 10 min to remove the cell debris, and the supernatant was filtered through a 0.22 µm filter. The filtrate was loaded on a pre-equilibrated gravity flow column equilibrated with equilibration buffer (500 mM sodium chloride, 50 mM potassium phosphate, pH 7.5). The column was then washed with the same buffer containing 25 mM imidazole, and the buffer containing 300 mM imidazole was applied to elute the recombinant protein. The purified proteins were then concentrated, and imidazole and sodium chloride were removed.

Samples including cell pellets and purifications were boiled for 5 min for denaturation, and then estimated by Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE).

## ***2.6 Analytical methods***

Nitrate concentration was measured with hydrazine sulfate colorimetric method using a high-resolution digital colorimeter (AA3, SEAL company, Germany). Protein concentrations were determined according to Bradford [30], using bovine serum albumin as a standard protein.

Nitrate and nitrite reductase activities were assayed by the method of MacGregor [31] and Martínez-Espinosa [18], respectively.

For the effect of temperature, the activities of purified nitrate and nitrite reductase were determined at different temperatures ranging from 25°C to 80°C, at pH 7.1. The optimum pH was determined by incubating the purified enzymes at 30 °C in phosphate buffer (pH 5.9-7.4) and Tris-HCl buffer (pH 7.4-9.0). Enzyme activities were expressed in percent form and the highest values were taken as 100%.

The effect of metal ions on enzyme activity was investigated at 30 °C and pH 7.1. The purified enzyme was incubated with the addition of 1 mM solution of the following metal salts: MgCl<sub>2</sub>, MnCl<sub>2</sub>, ZnCl<sub>2</sub>, CaCl<sub>2</sub>, CuCl<sub>2</sub>, BaCl<sub>2</sub>, FeCl<sub>3</sub>, AlCl<sub>3</sub> and EDTA for 10 min to determine residual activity.

Results were expressed as percentages and values of the native enzyme without metal ions addition were set as 100%.

The optimum electron donors for nitrate and nitrite reductase were studied by incubating the purified enzymes in phosphate buffer (pH 7.1) at 30 °C in the presence of 20 mM NADH, MV + Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>, MV + Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> + DTT, and MV + Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> + EDTA, respectively. Values of purified enzyme activity incubated in standard assay conditions without electron donor were taken as control. Enzyme activities were expressed in percent form and the highest values were taken as 100%.

Kinetic parameters of nitrate and nitrite reductase were determined under standard assay conditions with different concentrations of KNO<sub>3</sub> and KNO<sub>2</sub> (0.5 mM, 1 mM, 2 mM, 3 mM, 5 mM and 10 mM), respectively. Values of Michaelis constant ( $K_m$ ) and maximum velocity ( $V_{max}$ ) were obtained using a Lineweaver-Burk plot.

### 3. Results and discussion

#### 3.1 Morphological features of *B. megaterium* NCT-2

A novel nitrate-uptake bacterium, strain NCT-2, was isolated from greenhouse soil. And, it was registered in China General Microbiological Culture Collection Center (CGMCC No. 4698). It could use nitrate as sole sources of nitrogen. It formed grey, convex, smooth with entire margins and homogeneous colonies in inorganic salts medium. Strain NCT-2 was gram positive, long rhabditiform, and measured 1.2-1.5 μm in width and 3.5-5 μm in length (Fig.1).

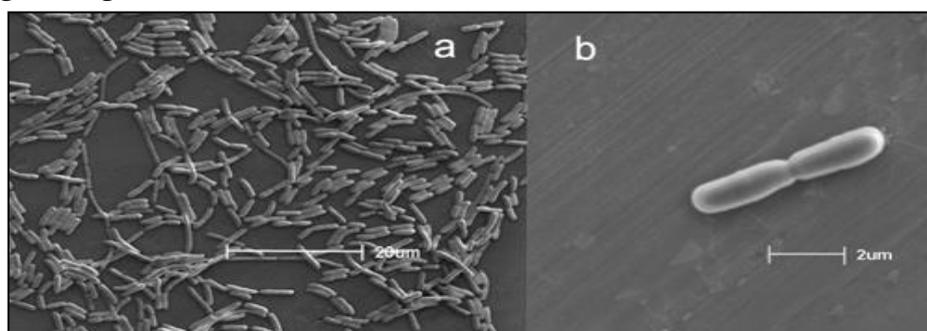


Fig. 1 Scanning electron micrographs of isolate *B. megaterium* NCT-2. Masses of cells (a) and single cell (b).

#### 3.2 Nitrate uptake by the resting cells of *B. megaterium* NCT-2

In this study, the resting cells of strain NCT-2 was able to transform nitrate-N efficiently at 25°C and 150 rpm. The uptake of nitrate-N at various initial concentrations was investigated and results were shown in Fig. 2. The resting cells could utilize 100mg/L nitrate-N within 24 h. And 300 mg/L nitrate-N was taken up nearly completely within 48h. However, when the initial concentrations of nitrate-N were more than 300 mg/L, the nitrate-N was taken up incompletely. When NCT-2 was incubated in the media containing 400, 600 and 800mg/L nitrate-N, nitrate-N removals were 78.3±0.6, 64.5±0.5, 44.9±0.2%, respectively within 60h. When the initial concentration of nitrate-

N was 1000 mg/L, over 500 mg/L nitrate-N still remained in the culture. And, little Nitrate-N could be taken up when the inoculation was more than 48h.

The resting cells of the *B. megaterium* NCT-2 used in the present study were found to be not only tolerant to very high concentrations of Nitrate-N but also effective in Nitrate-N removal. Though with Nitrate-N concentration increasing nitrate removal ratio decreased, nitrate removal amount increased. Little nitrate was removed after 60h probably due to the depletion of nutrients in the media. This study demonstrated that NCT-2 has nitrate removal activity.

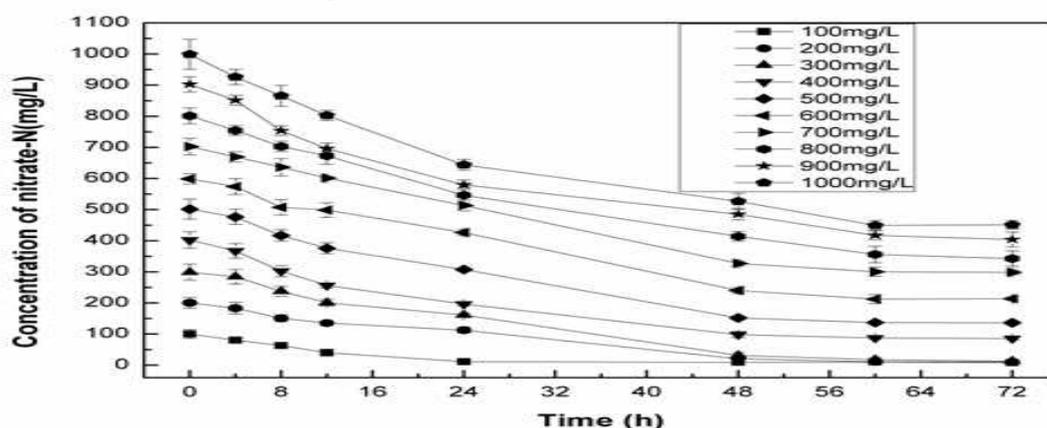


Fig. 2. Removal of nitrate-N by resting cell of *B. megaterium* NCT-2. Cultivations were carried out at 25°C and 150 rpm in inorganic salts medium containing different amounts of nitrate-N.

### 3.3 Genome sequencing and nitrate assimilation related genes sequence identification of *B. megaterium* NCT-2

The genome of *B. megaterium* NCT-2 was sequenced on the Next Generation Sequencing Solexa platform by Major Bio BioPharm Biotechnology Co., Ltd. A total of 6,736,868 reads pair were generated, with an average read length of 81 bp, yielding a total sequence of 1,091,372,616 bp. This represents 192× coverage of the estimated 5.68 Mb genome. The results were then assembled with optimized Khmer parameter by the Velvet assembler. The fine map of the NCT-2 was submitted to the NCBI GenBank (AHTF00000000).

After obtaining the genome assemblies, genes were predicted by three software and then annotated by performing BLASTP against the Nr protein sequences database from NCBI. After which, a search was performed in the annotated genes pool to find out the nitrate assimilation related genes. The genes found were the nitrate reductase electron transfer subunit nasB (781 aa) and the nitrate reductase catalytic subunit nasC (716 aa), the nitrite reductase [NAD(P)H] large subunit nasD (804 aa) and the nitrite reductase [NAD(P)H] small subunit nasE (108 aa).

### 3.4 3D structure prediction of nitrate and nitrite reductase

The putative 3D protein structures of the nitrate assimilation related genes were predicted by SWISS MODEL according to the templates on its own database. Also the structures of the known proteins from the PDB

database with the most similarity to the nitrate assimilation related genes were screened out by NCBI BLASTP and displayed. By comparing of the two modeling results, a brief idea of how the protein 3D structure of these genes products may look like will be suggested. The nitrate reductase has two components as follows: the nitrate reductase electron transfer subunit (*nasB*) and the nitrate reductase catalytic subunit (*nasC*) (Fig. 3). The nitrite reductase has two components as follows: the large subunit (*nasD*) and the small subunit (*nasE*) (Fig. 4)

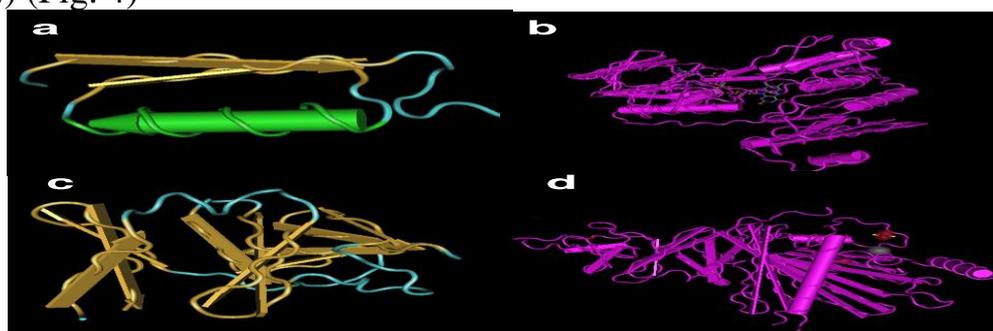


Fig. 3 3D structure modeling of nitrate reductase. a *nasB* predicted by SWISS MODEL, b *nasB* most similar protein predicted by NCBI BLASTP, c *nasC* predicted by SWISS MODEL, and d *nasC* most similar protein predicted by NCBI BLASTP

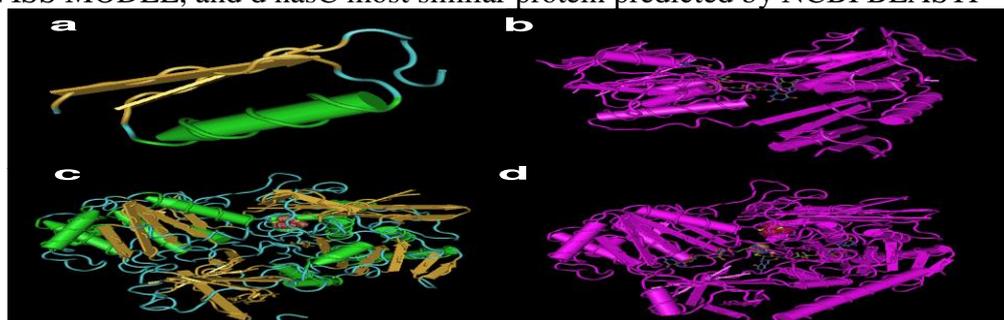


Fig. 4 3D structure modeling of nitrite reductase. a *nasD* predicted by SWISS MODEL, b *nasD* most similar protein predicted by NCBI blastp, c *nasE* predicted by SWISS MODEL, d *nasE* most similar protein predicted by NCBI blastp

Due to its relatively low homology to the modeling template, the nitrate reductase electron transfer subunit *nasB* predicted by SWISS MODEL was only partially modeled. Thus, it only displayed a partially 3D structure compared with its most similar known protein from the PDB database by NCBI BLASTP. While the 3D structure of nitrate reductase catalytic subunit *nasC* predicted by SWISS MODEL was highly identical to that of the protein model from the PDB database by NCBI BLASTP.

### 3.5 Heterologous expression of nitrate and nitrite reductase

In order to express the nitrate and nitrite reductase, gene *nasB*, *nasC*, *nasD* and *nasE* were cloned into pETDuet, and all of them were over-expressed in *E. coli* BL21 (DE3) (Fig. 5a and b; Fig. 6a and b). When *NasB* and *NasC* were co-expressed using one vector in soluble form in *E. coli* BL21 (DE3), the His6-tagged *NasB* protein appeared to be accompanied by *NasC* protein (Fig. 5c). Similarly, *NasD* and *NasE* were also successfully co-

expressed (Fig. 6c). It is possible that the co-elution is a result of specific non-covalent interactions.

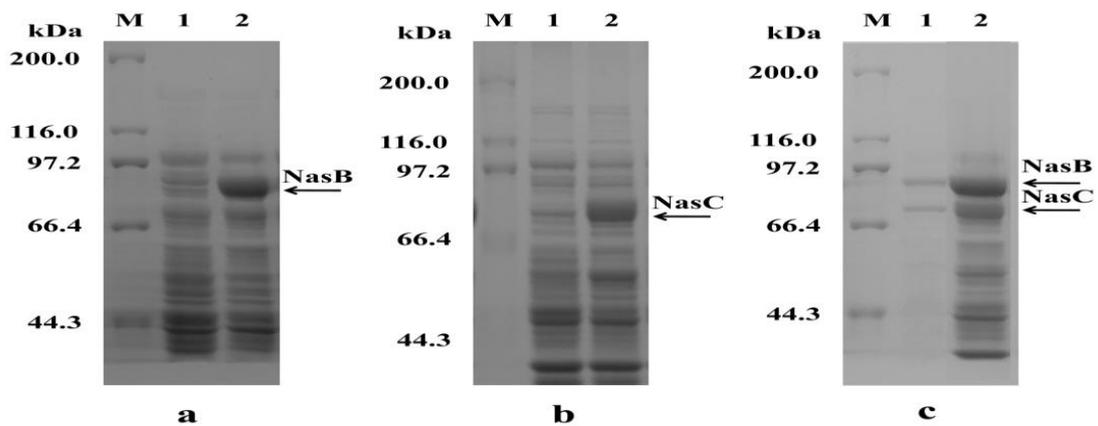


Fig. 5 SDS-PAGE analysis of nitrate reductase expressed in recombinant *E. coli* BL21 (DE3). (a) Expression of subunit NasB in the transformant *E. coli* BL21 (DE3). Lane M, protein marker; lane 1, IPTG-induced total fraction of *E. coli* BL21 (DE3); lane 2, IPTG-induced total fraction of *E. coli* BL21 (DE3) harboring pETDuet-nasB. (b) Expression of subunit NasC in the transformant *E. coli* BL21 (DE3). Lane M, protein marker; lane 1, IPTG-induced total fraction of *E. coli* BL21 (DE3); lane 2, IPTG-induced total fraction of *E. coli* BL21 (DE3) harboring pETDuet-nasC. (c) Co-expression of subunit NasB and NasC in the transformant *E. coli* BL21 (DE3). Lane M, protein marker; lane 1, purification of cell extraction in *E. coli* BL21 (DE3) harboring pETDuet-nasBC; lane 2, IPTG-induced total fraction of *E. coli* BL21 (DE3) harboring pETDuet-nasBC.

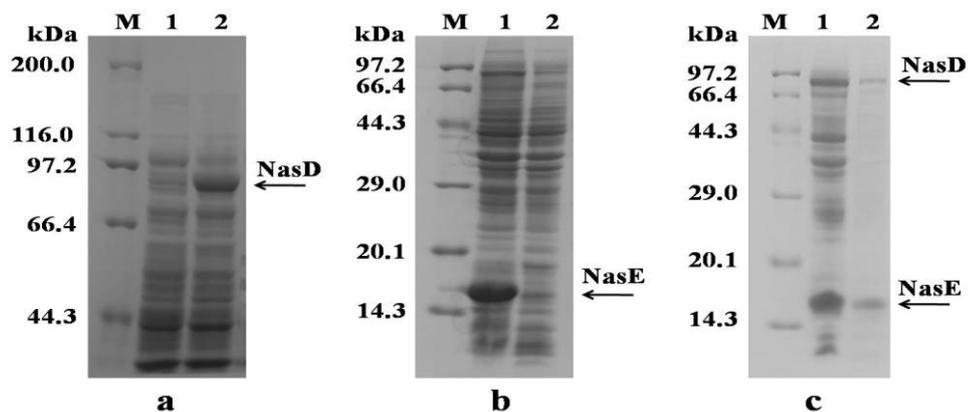


Fig. 6 SDS-PAGE analysis of nitrite reductase expressed in recombinant *E. coli* BL21 (DE3). (a) Expression of subunit NasD in the transformant *E. coli* BL21 (DE3). Lane M, protein marker; lane 1, IPTG-induced total fraction of *E. coli* BL21 (DE3); lane 2, IPTG-induced total fraction of *E. coli* BL21 (DE3) harboring pETDuet-nasD. (b) Expression of subunit NasE in the transformant *E. coli* BL21 (DE3). Lane M, protein marker; lane 1, IPTG-induced total fraction of *E. coli* BL21 (DE3) harboring pETDuet-nasE; lane 2, IPTG-induced total fraction of *E. coli* BL21 (DE3). (c) Co-expression of subunit NasD and NasE in the transformant *E. coli* BL21 (DE3). Lane M, protein marker; lane 1, IPTG-induced total fraction of *E. coli* BL21 (DE3) harboring pETDuet-nasDE; lane 2, purification of cell extraction in *E. coli* BL21 (DE3) harboring pETDuet-nasDE.

The optimum co-expression condition of nitrate and nitrite reductase was obtained with *E. coli* BL21 (DE3) and 0.1 mM IPTG for 10 h when expression was carried out at 20 °C and 120 rpm in LB medium. Specific activities of purified nitrate and nitrite reductase were 243 and 22 U/mg, respectively. SDS-PAGE analysis of the extracts of *E. coli* BL21 (DE3) harbouring pETDuet-*nasBC* induced by IPTG, revealed the presence of electron transfer subunit protein around 87 kDa and the catalytic subunit protein around 80 kDa (Fig. 5c), which was in accordance with the predicted molecular mass based on the amino acid sequence. The presence of large subunit protein of NasDE was around 88 kDa (Fig. 6c), which was in accordance with the predicted molecular mass. However, the small subunit appeared on the gel was larger than the calculated value of 11.7 kDa, suggesting its unusual running behavior in SDS-PAGE.

### **3.6 Biochemical characterization of nitrate and nitrite reductase**

The purified recombinant enzymes showed broad activity range of temperature and pH. The maximum activities were obtained at 35 °C and 30 °C, pH 6.2 and 6.5, which was similar to the condition of greenhouse soils. Maximum stimulation of the enzymes occurred with addition of Fe<sup>3+</sup>, while Cu<sup>2+</sup> caused the maximum inhibition. The optimum electron donor was MV + Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> + EDTA and MV + Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>, respectively. Kinetic parameters of  $K_m$  and  $V_{max}$  were determined to be 670 μM and 58 U/mg for nitrate reductase, and 3100 μM and 5.2 U/mg for nitrite reductase.

## **4. Conclusion**

In summary, *B. megaterium* NCT-2 was isolated from secondary salinization soil and showed high capability of nitrate reduction. We have obtained the fine map of the NCT-2 genome, which was submitted to the NCBI GenBank (AHTF00000000). The assimilatory nitrate and nitrite reductase of NCT-2 were cloned, expressed, purified and characterized. Expression of recombinant enzymes was optimized in *E. coli* BL21 (DE3) to obtain active and soluble protein. The purified productions allowed us to characterize the enzymes showing broad activity range of temperature and pH. In addition, the stimulative and inhibitive metal ions, optimum electron donors,

and kinetic parameters were identified. This work provided information on a novel assimilatory nitrate reductase from *B. megaterium* NCT-2, suggested its superiority in secondary salinization soil remediation of greenhouse cultivation, and explained part of the reason why this strain can survive in such condition.

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# Tourism factors and rural development in Bulgaria

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## Abstract

The object of the study are two typical rural areas in Bulgaria that are characterised as relatively peripheral, significantly rural burdened by the contemporary challenges reported in the development of rural areas, namely: demographic crisis, high unemployment, lack of diverse opportunities for job, domination by the agricultural sector, regression in their socio-economic vitality, etc. They itself possess critical cultural (tangible and intangible) values to alleviate these weaknesses and to improve their status. The study is done as observation in a time span of few years, trying to make comparison for the rural tourism within different conditions. It is found out that in many peripheral rural regions the multifunctional character of rural tourism proved to be faint, as well as the contribution of rural tourism activities to the more general multifunctionality, diversification and development of local communities. Some of the objectives in the study are to identify good examples that contribute for the bigger role of the cultural values for the overall development in rural areas and appraise the resilience of such places to persist the crisis situations and to take advantages upon the presence of such potential. The study works with the data collected by the secondary, official statistical sources along with deep interviews conducted as with the stakeholders from the rural areas (entrepreneurs, persons in charge of the cultural values management and community leaders, etc) as well as with the guests and tourists.

## Introduction

The decline of traditional rural industries such as in agriculture, mining, and forestry over the past decades has required many rural communities to explore alternative means to strengthen their economic base (Allen, Hafer, Long, & Perdue 1993; Gilbert 1989; Long, Perdue, & Allen 1990). Liu (2006) indicates that tourism is an option to enhance rural lifestyles and to induce positive changes in the distribution of income in underprivileged regions

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given the fact that tourism has comparative advantage effects in income and employment generation. Of course, a successful tourism development must be planned and managed responsibly (De Oliveira, 2003; Inskeep, 1991; Southgate & Sharpley, 2002). This means putting efforts in combining and complementing all possible elements of tourism, of which cultural values play an important role.

The cultural values are considered to bear merit and to subserve for creating a tourism product in the rural areas, which to reach the consumers, to satisfy their expectations and attitudes, to tie them closer to the rural environment, working for a reverse process of inward migration back to the rural areas in some cases and retaining the immigration of the local citizens. For the majority of the contemporary tourists, the key motivation represents the desire to have something new and different leading to psychological and physical relaxation (Georgiev, 2010). This creates a need of direct contact with the nature and evokes feelings of enjoyment and love for peaceful and natural living in rural ambience, which should be used to vitalize the rural areas, to halt their further social and economic degradation and regress and to facilitate for cohering the living standards between urban and counterpart rural areas. The rural tourism through incorporating the cultural values should be explored to create a better condition for doing business, having in mind the uniqueness and the diversity of the cultural heritages and the opportunities to procreate a immanent cluster pivoted on the tourism and branched with food and entertainment sectors, agriculture, some manufacturing and other services. Thus, the main issue is to find a way and to devise a system, which to propitiate the creation of a vibrant tourism product in rural areas, based on the endogenous resources and bringing added value and multiplication effects, delivering a better and wealthy rural environment.

The purpose of this paper is to identify the role and potential of the cultural values to incite the development of the rural tourism, reckoned as a mighty factor for overall rural development, which to stir up their vitality and to have turning around the negative demographic and migration movements, stopping their further degradation and cessation of processes leading to disappearances of accumulated cultural and historical heritage in rural areas. Cultural tourism is understood as such a tourism product in which the motivation of the tourist (providing the supply side) is getting acquainted with new cultures, participate in cultural events and visiting cultural attractions and the demand side's core element is the peculiar, unique culture of the visited destination (J. Csapó (2012)).

The objectives of the study are: (1) to analyze the effects and benefits from the offered rural tourism in two different areas in Bulgaria and to investigate the role of cultural values and (2) to identify factors influencing the development of the rural tourism and the contribution of the cultural values and to evaluate the feasible effects from those factors. An additional

objective in the study is to illustrate good examples that contribute for the bigger role of the cultural values for the overall development in rural areas and appraise the resilience of such places to prevent the crisis situations and to take advantages upon the presence of such potential.

The object of the study are two typical rural areas in Bulgaria that are characterised as relatively peripheral, significantly burdened by the contemporary challenges reported in the development of rural areas, namely: demographic crisis, high unemployment, lack of diverse opportunities for job, domination by the agricultural sector, regression in their socio-economic vitality, etc. They itself possess critical cultural (tangible and intangible) values to alleviate these weaknesses and to improve their status.

The study is done as an observation on a time span, using previously collected data and the survey run during the Bilateral Bulgarian – Chinese project implementation. The study works with the data collected by the secondary, official statistical sources along with deep interviews conducted as with the stakeholders from the rural areas (entrepreneurs, persons in charge of the cultural values management and community leaders, etc) as well as with the guests and tourists. The analysis is carried out by statistical methods – descriptive statistics, regression and correlation, as none-secondary data is collected by interviews with different related stakeholders from both areas. The encompassed group of interviewees are tourists, visiting the areas and spending time there as well as people working for different services engaged in the tourism – accommodation places, tourist centres, recreation institutions, food sites. The total number of the interviews from both areas is 45, as 29 are customers and stakeholders from Strelcha area, whereas 16 are from the same constellation connected to Zasele-Zimevitsa (municipality of Svoge) rural domain. The interviewees are inquired for their behaviour and perception changes and statement to the researched situation, occurred effects and influential factors for the period 2012 and 2017.

### **Tourism and area specificities and background**

The territory of Strelcha municipality and Zasele-Zimevitsa areas are gifted with natural endowments and artifacts, possessing cultural value suitable for development and creation of a thorough tourism product. Although, the potential and the scale of the cultural values in the selected areas are pretty diverse, both areas have feasibilities to attract tourists and to develop tourism activities. Strelcha, as an area with mineral water sources is favored to station on such priority, whereas Zasele-Zimevitsa domain relies primarily on the natural values and ambience rather than the cultural values conducting mostly as a backdrop. The mineral springs and balneological clinic along with cultural-historical heritages render possibility to immerse into the atmosphere of Strelch region in Bulgaria. The community and the municipal authority fit in their vision and back up the notion for valorization of the natural capital of the territory and driving the tourism as an engine of the

socio-economic development, vesting this vision as a goal and main priority. It is also recognized and shared by the community that the traditional modes of tourism and partial oriented strategies are no longer a successful form for organization of tourism business. It is important the territory of Strelcha to supply a holistic tourism product, combining the possibilities of mineral and hot springs with the historical heritage and natural beauties.

As for Zasele-Zimevitsa domain, the advantages of the region is the mountainous landscape, fresh air and beautiful ambience, supposing good conditions for relax and natural sightseeing, as the cultural values are linked predominantly to intangible capital – local folklore, traditional food and bright festivals. The tourism in Zasele-Zimevitsa domain is a relatively new initiation started by several enthusiasts based mostly on the rural tourism principals, generating energy since the beginning of the new millennium coinciding with the contemporary development of the rural tourism in Bulgaria. It is assumed that the mineral water in Strelcha might transform in a focal point, which complemented and stuck by other tourism alternatives may contribute for a better and prosperous development of the territory. Zasele-Zimevitsa relies on the natural favourites to create a tourism niche and to provide jobs and incomes of the people engaged in these activities, as cultural values into different extent are thought to complement the creation of a tourism product.

Regarding the cultural heritages in Strelcha, they are several and have different importance and role for the formation of the tourism image of the area:

- The Thracian cult complex "Frog Mound" composed of tomb-mausoleum-shrine and temple. The complex dates back to the V-IV century BC. The tomb was unearthed in the mid 70-ies of XX century and stands for the largest and most impressive Thracian tombs in Bulgaria and in the area of Strelcha, where there are about 300 Thracian tombs. The tomb is surrounded by a moat of 30 meters wide and 3-4 meters deep.

- The Strelcha fortress is located 2,5 km away from Strelcha. Its impressive 8-meters high protective walls clearly illustrate the character of this age and have been preserved to this day. The fortress was also the centre of a medieval town named "Shooter".

- Dyulevsko Kale has the shape of an ellipse and covers an area of about 1 ha. Perhaps, it has served to guard the mountain gorge or it was part of a feudal castle.

- Mryanskoto Kale fortress (ruins) is situated about 11 kilometers north of Strelcha. Originally it was built as a fortified residence of a Thracian king from the Odrisi tribe who lived in the IV century BC. It is believed that later it used to be a Roman fortress (II-III c. AD), which patrolled the road through Sredna Gora mountain.

- In the town, the Strelcha museum runs, founded in the 40s of XX century. Initially the museum was housed in the local library, but in 1958 was moved to a specially re-constructed for this purpose building -the House of Angel Nedelev - participant in the April uprising. At the beginning of 90 - years the House of Chorbadji Nesho Grozev was also restored which now houses the museum's history.

#### Factors for tourism development

As it was mentioned the development of the tourism in both areas is quite different and their background and conditions for promoting the tourism are dissimilar. However, by different success at both areas, the tourism is assumed to address the income leakages, volatility, declining multiplier, low pay, imported labor and the conservatism of investors (Butler and Clark, 1992). The least favoured circumstance in which to promote tourism is when the rural economy is already weak, since tourism will create highly unbalanced income and employment distributions. It is better supplement for a thriving and diverse economy than as a mainstream of rural development. It is somehow shared that in many peripheral rural regions the multifunctional character of rural tourism proved to be faint, as well as the contribution of rural tourism activities to the more general multifunctionality, diversification and development of local communities (ESRS, 2013).

For the sake to identify the factors determining the development of the tourism in both areas was conducted the survey with the tourists. They were asked to report and range the influence of the factors, which propel their decision to visit one of two areas. They were requested to estimate from 0 to 2 (lack of significance to quite important), each of the external and internal factors as well as to point out additional factors that drives their decision. The results from the survey are posted in Fig.1. The characteristics of both areas regarding the tourism potential and their endowments with tourism objects were corroborated. The Strelcha area is associated mostly by the tourists with the mineral springs followed by the cultural material heritage and the ancient monuments. The tourism image of the area is constituted by these core elements, which accounts for an estimation between 0,76 (ancient monuments) to 1 (mineral springs).

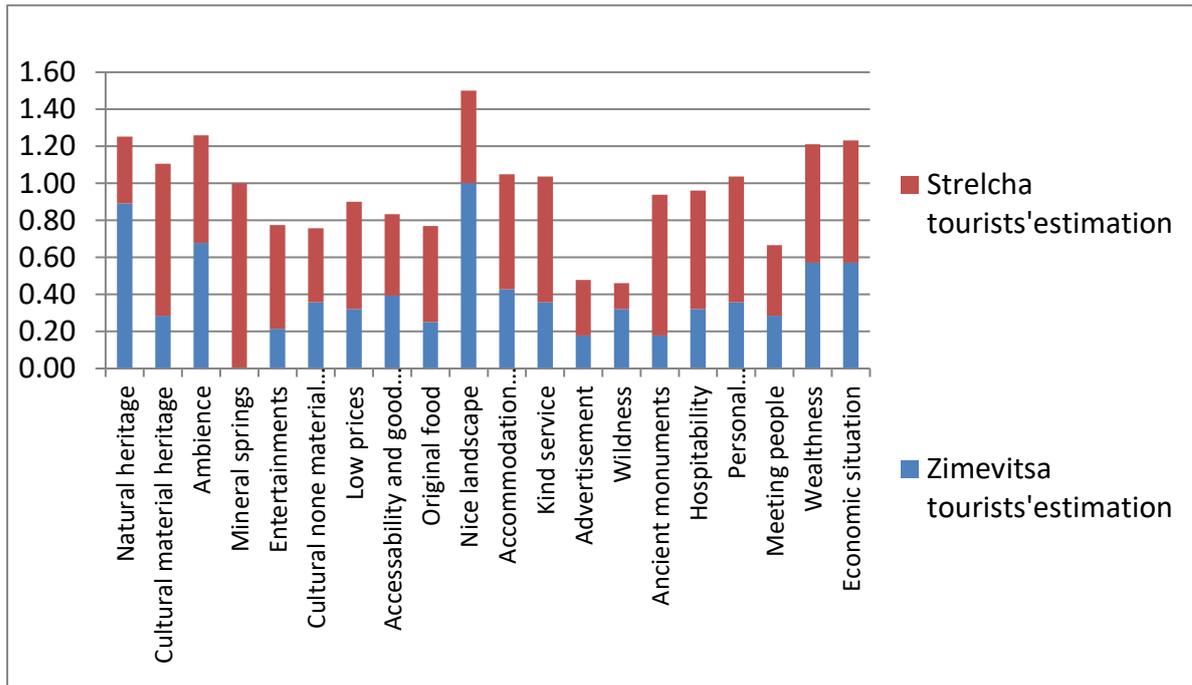


Fig. 1 Tourists estimation of the factors driving the tourism in investigated areas  
 Source: Field survey

As regards the Zasele-Zimevitsa domain, the discerned strong in the tourism term sides as natural heritages and nice landscape were elicited by the respondents as the most important with scores 1 for landscape and 0,89 for natural heritage. The common things for both groups were the similar relation to the factors economic situation and wealth of the tourist. The tourists admit that economic situation is not the crucial factor determining their intention to travel and spend time in the rural area even though it must be taken into account.

The mineral springs in Strelcha are the main driver for the development of the tourism in Strelcha, as all interviewees admit the role of the mineral water for their stay and interest to this site. However, the level of the advertisement and information about the opportunities to go and use the services connected to this natural resource are very stunted and the contribution of the advertisement in inciting these people to visit Strelcha is hardly 0,3. Taking into account that the spa tourism, which is considered as the main factor for tourism development in Strelcha assumes a longer stay of the customers and a good relations to them by the hosts, the level of the services in Strelcha is estimated as satisfactory 0,68. Contrarily, the level of the services in Zasele-Zimevitsa domain is viewed by the visitors as not very high-0,36. This is prescribed to less traditions of the tourism business in Zasele-Zimevitsa domain to work with tourists and to establish a system and manner, which to conform to the tourists and to predispose for their satisfactory experience.

At the same time, the perception of the accommodation in both areas is estimated by the tourists as different too. Strelcha accommodation availability

is scored with up to 0,62, while Zasele-Zimevitsa domain is estimated up to 0,43. The prices that tourists pay for the service they get are relatively high in Zasele-Zimevitsa domain, where 0,32 is the rate of the prices as a factor driving their decision to visit the place. As regards Strelcha, the prices of the services the tourists obtain are viewed as acceptable with a rate of 0,58. This spells for a well-balanced marketing in Strelcha in terms of the services delivered to the tourists and the prices bidden to them, which is not a point in Zasele-Zimevitsa domain. In addition, the significance of the entertainment in both areas in relation to the tourism development is quite low in Zasele-Zimevitsa (0,21) and not enough high in Strelcha (0,56) to stick tourists longer, amusing and spending nicely the leisure.

**Table 1 Experts' estimation of the tourists' visits and overnight stays**

Experts estimation of the tourists' visits in surveyed areas	Visits of tourists by 40 years age	Visits of tourists over 40 years old	Annual overnights	Visits of tourists by 40 years age	Visits of tourists over 40 years old	Annual overnights
Zasele-Zimevitsa	650	150	300	900	250	600
Strelcha	8000	8000	63000	7000	7000	60000

*Source:* Field survey

The different potential of the areas in terms of the tourism is revealed by the number of the visitors coming to these places annually and the number of the overnight stays. These figures can be seen in Table 1 and they stand for approximate estimations done by the tourism business and other experts interviewed. It should be noticed that the number of the tourists yearly in Strelcha is about 20 times higher than in Zasele-Zimevitsa domain but this is due mainly to the role of rehabilitation and healthy centres operating at the area. About 65% of the guests are coming under the treatment programs run by the National Security Institute and the internal programs of the Ministry of Interior Affairs. These two bases of the Ministry of Interior Affairs and Balneo-sanatorium "Roza" after reconstruction and modernization in 2001/2002, "Roza" has been transformed in a modern rehabilitation center for physiotherapy and water cure and works all the year, under a Program of the National Insurance Institute. The hotel part has 130 beds in double and triple rooms, included 2 apartments; restaurant with 200 places, conference room and café-bar. The balneo-sanatorium offers wide set of healing and diagnostic procedures. Besides the balneology centers, on the territory are founded and operates 4 hotels and rest-houses, which entice the predominant number of the short visits that are coming mostly during the weekends and spend averagely up to 2 overnights.

#### **Estimated effects and consequences from tourism development**

As it might be supposed the tourism development brings about different positive effects expected by the local stakeholders and tourist operators. By the survey with the local stakeholders was found out that there is robust correlation of the tourism development with entrepreneurs' return, the remuneration of the people engaged and employment level and a slighter relationship with the infrastructure development and the socio-economic vitality. The defined effects related to the tourism development are estimated and envisaged by the local stakeholders in three scenarios – status quo, recessive and growing. The results from the estimations are indicated in Table 2, as the figures are aggregated for both areas and for all three scenarios.

**Table 2 Relationship of the tourism development in both areas and the estimated effects**

The relationship of the tourism development scenarios in both areas with concrete effects	Employment	Staff incomes	Entrepreneurs' return	Socioeconomic vitality	Infrastructure
<b>Multiple R</b>	0,91	0,92	0,96	0,82	0,85
<b>R Square</b>	0,84	0,85	0,93	0,68	0,73
<b>Standard Error</b>	0,34	0,48	0,31	0,41	0,49
<b>Coefficients</b>	0,54	0,80	0,83	0,42	0,56

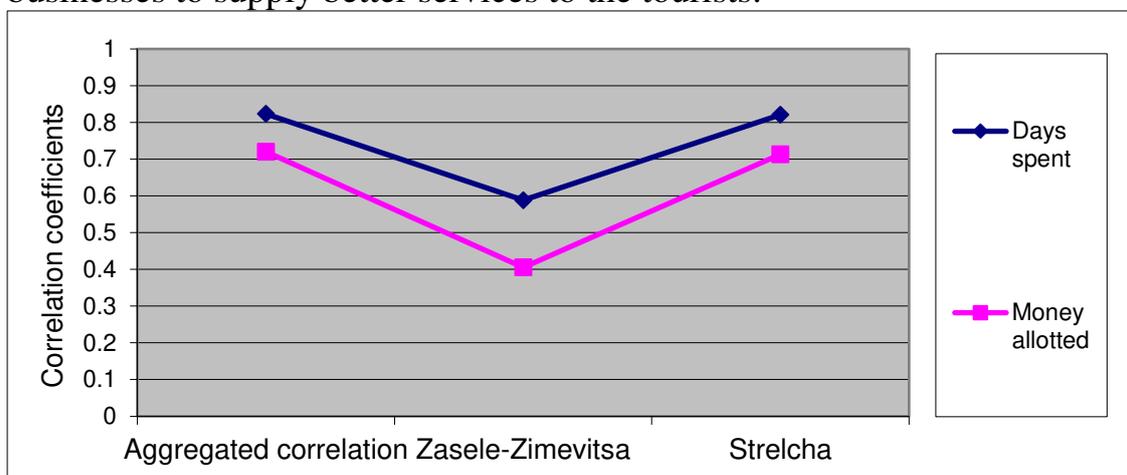
*Source:* Field survey.

By the estimations of the local stakeholders the correlation R<sup>2</sup> of the level of rural tourism in areas is highest linked to the entrepreneurs' return. They assume that a growing and ascending development of the rural tourism in their area will inevitably lead to an increase of their economic earning and vice versa the deflating station of the tourism activity will curb their profitability and their stimulus. This posit alleged by the stakeholders shows a relatively low standard error, tallying up to 0,31, which is a moderate dispersion among the estimations. At the same time, the regression coefficient of the entrepreneurs' return to the independent variable is 0,83, which is quite high and spells for a tall determination of the situation in the tourism to the economic return. On the opposite pole is the correlation of the tourism development to the overall socio-economic vitality. The coefficient R<sup>2</sup> is 0,68, while the standard error is also significant, indicating for presence of distraction among the estimations.

The regression coefficient of 0,42 testifies for a stunted change of the expected socio-economic situation as a result of any improvement of the tourism conditions. This is quite relevant because the tourism in both areas and especially at Zasele-Zimevitsa domain might not be seen as a puissant generator capable to animate the overall development of the territories. In less extent, the same stance is assumed for Strelcha where the growing development of the tourism may propel the socio-economic activities because

the potential of the mineral thermal sources is significant. However, the experience and results found out in other places, where the tourism is expanded, the socio-economic situation depends mostly on the model of the tourism pattern. The tourism development based on the external drivers and concentration of the tourism business and providers to large centres and enterprises may contribute less to the local welfare. The effects when the tourism is run by the local tourism operators and there is a tourism product composed in a completion and wholeness complemented by the element of the historical and other cultural values available on the territory is the way to improve the socio-economic conditions and to create prepositions for synergic advantages.

As for the infrastructure, it is really very important for the successful development of the tourism and for the prospective features to create new employment opportunities, increased community visibility leading to other economic development opportunities and new induced job positions and increased tax base. For planning and management purposes, transportation infrastructure in the region can be seen as comprising transports means: land transport systems and routes, water supply, sewage, telecommunications, roads, power generation. Meanwhile, the improvement of the infrastructure is also linked to the economic vitality and economic possibilities delivering funding for utilities and facilities ameliorating and assisting the tourism businesses to supply better services to the tourists.



Source: Field survey.

The infrastructure for the tourism in both areas is not on the wished level, especially in terms of the accession and road network, transport connections, sewerage and other assisting facilities. For example, the utilization of the thermal water by the tourism operators in Strelcha offering balneology and spa treatments is quite complicated and each of them is impelled to set up own techniques at the aquifer when starts to use thermal water thereof the access to the thermal aquifers is subject to some restrictions.

The results in Fig. 2 try to discern and check out the relationship between the tourism development estimated and ranged by the local stakeholders and presented in 3 scenarios (shown in Table 2 and discussed there) and the attitudes and behaviour of the tourists. Entire pool of 39 tourists are encompassed with this part of the survey and they are asked for the real days and money spent during their stay in Zasele-Zimevitsa and Strelcha areas (status-quo scenario) and for their intended behaviour in case of recession and growing modes. The results are demonstrated as separately for each of the areas as aggregately likewise the response of the tourists to the assumed changes in the scenarios.

The calculated correlation spells for a robust relationship between the tourism development and the planned stay of the guests, as  $R$  is 0,82 in terms of the common case. However, there is significant difference within the areas, as the calculated correlation of the tourism development and days spent by the tourist is quite less in Zasele-Zimevitsa domain compared to Strelcha area. Whereas, in Strelcha the improvement of the tourism and raising the quality of the tourism product will bring about a symmetrical increase of the intended stay by the tourists ( $R$  is 0,82) and vice versa, concerning Zasele-Zimevitsa domain, this connection is much slighter ( $R$  is 0,58). It means that the feasibility of the scenario with a growth of the tourism in Strelcha to lead to the reported effects shown in Table 2 is quite more plausible, while concerning Zasele-Zimevitsa domain, the estimations of the stakeholders for the sequential effects are not confirmed by tourism intentions.

According to Georgiev et al (2005)“the positive impressions about the effects on economy are result of rise in employment, stated by more interviewed, together with overall positive effects due to the specific financial investments in the local areas thereof the tourist flow has been increased”. The effects on tourism are perceived as being positive because the respondents think that the activities resulted in sustainable development of tourism infrastructures (26.3%), positive effects in general (21%), rise in employment (15.8%) and improvement of areas’ promotion (15.8%). Other denoted reasons are money investments, increase in tourist flow, business effects and others (Georgiev et al, 2005).

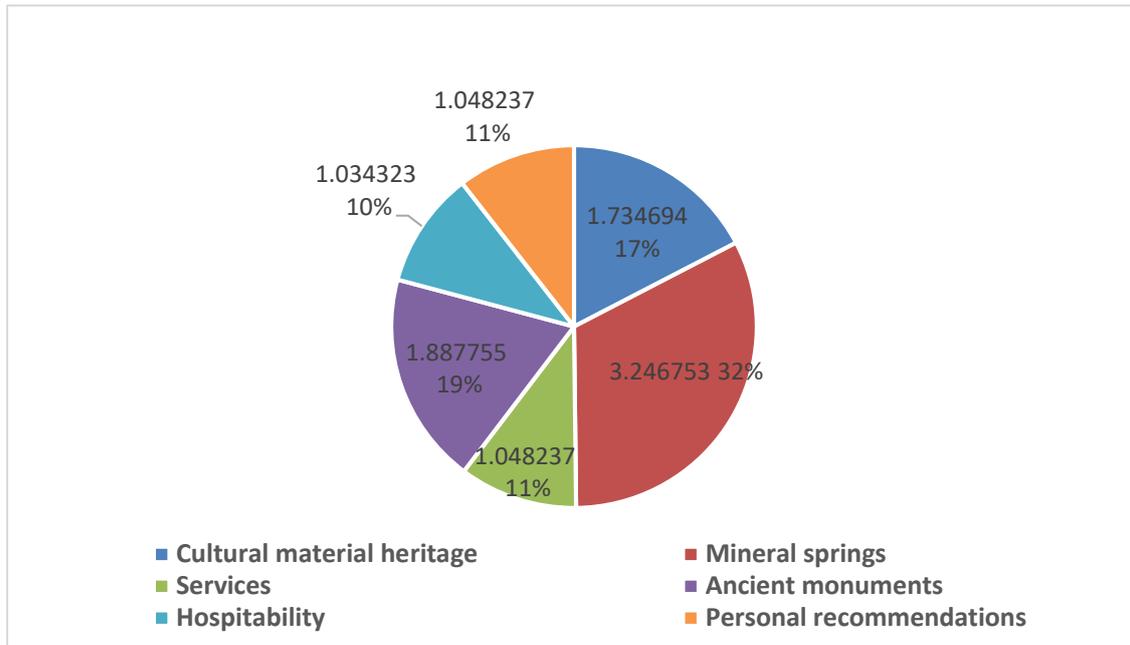


Fig.3 Difference in the amount of money spent from a tourist per day in both areas, total= 10 Euro

Source: Field survey

Regarding the correlation between the tourism development and the estimation of the tourists for the budget of their stay, it is lower than the relationship with the tourism staybut still quite high. The aggregated correlation summarised the estimations of the respondents from both areas is 0,72, as for Strelcha area the coefficient R is quite similar 0,71. In Zasele-Zimevitsa domain, the reciprocal correlation coefficient is 0,40, which rather implies for a slim relationship between the level of the tourism and the tourists' attitudes to determine their spending to it. This indicates that the relationship between tourism development and the tourism costs planed by the tourists is not proportional and depends on different factors. The investigation shows that the areas, which possess higher tourism potential and their tourism product is estimated and preferred mostly keep stronger possibility to agitate for higher spending by the tourists compared with places, where the tourism product doesn't have some specifics and distinctions. The role of different factors for the development of tourism is shown in Fig.3 and 4.

As shown in Figures 3 and 4, six factors are mostly determining for the development of tourism in the two investigated areas, while at the same time they are of different weight and importance. These factors are the cultural material heritage, mineral springs, services, ancient monuments, hospitality and personal recommendations. Based on the survey, it is found that while comparing both regions the difference in the amount of money spent per person per day is EUR 10 and the difference in the duration of the stay is 2.75 days.

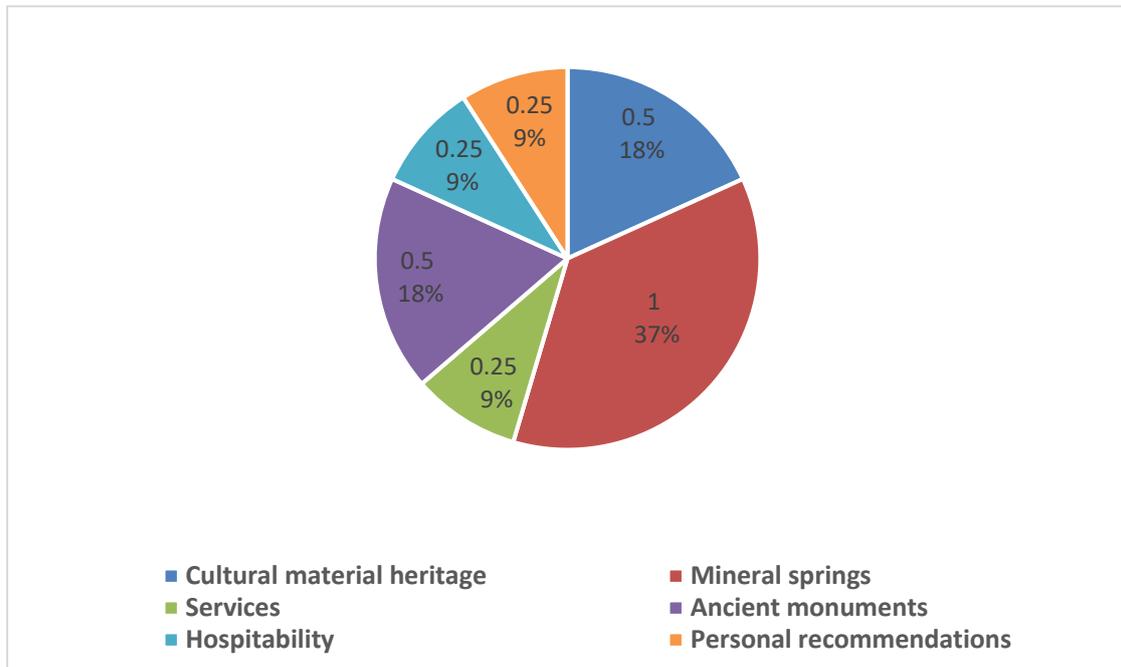


Fig.4 Difference in the length of stay of a tourist in both areas, total = 2.75 days  
Source: Field survey

The leading factor that determines to the greatest extent the choice of tourists to visit a region, to spend time and certain amount of money at the place is the availability of mineral springs there and the accompanying infrastructure such as health and rehabilitation services, water entertainment etc. (i. e. the possibility of the place to allow rest and health restoration of the tourists) – respectively 32% for the money spent and 37% for the length of stay. The second place of influence in determining the choice of tourists to visit a place and spend there some money is shared by the factors cultural material heritage and ancient monuments (18-19%) followed by the factors personal recommendations, services and hospitability – 9-11% (fig.3 and 4). In this way, it is possible to determine the most important things that influence the development of tourism in an area, respectively to outline the contribution and the results of tourism. Against this background, stakeholders and institutions can plan and outline effective actions to make the areas more attractive to tourists and thus contribute to the revitalization and prosperity of rural areas.

### Conclusions

The purpose of this paper is to identify the role and potential of the cultural values to incite the development of the rural tourism, reckoned as a mighty factor for overall rural development, which to stir up their vitality and to have turning around the negative demographic and migration movements, stopping their further degradation and cessation of processes leading to disappearances of accumulated cultural and historical heritage in rural areas.

Generally, it might be asserted that the relationship between the tourism level and the effects linked to them as increase of the tourists and their stay, enhancement of the people engaged in the tourism services and raise of the incomes is quite probable and relevant. Tourism creates jobs, both through direct employment within the tourism industry and indirectly in sectors such as retail and transportation. When these people spend their wages on goods and services, it leads to what is known as the "multiplier effect," creating more jobs. The tourism industry also provides opportunities for small-scale business enterprises, which is especially important in rural communities, and generates extra tax revenues.

From the analysis might be concluded that tourism in both studied areas is estimated to contribute to different positive effects mentioned above but it strongly depends on the potential of the tourism capital. In Fig. 1 clearly is underlined the difference not only in the available tourism factors in both areas but also in their intensity and importance. Whereas, Strelcha area possesses a relevant base for deploying tourism product built on the mineral springs and complemented harmonically by the cultural heritage, Zasele-Zimevitsa domain is much more limited and may rely predominantly on the natural resources and landscape, as the cultural values may play only an auxiliary role. These findings are the reason to state that tourism product created in both areas will be different and will lead to different effects, as the feasibility for more visitors, money spent and days realized are more relevant for Strelcha area compared to Zasele-Zimevitsa domain.

The positive impact of the tourism for rural development is accompanied by a strong contribution of the cultural values unto overall tourism and rural prospective. The cultural values are directly related to the development of the tourism and as substantiated and presented are they as growing can be the prospects of tourism development stirring the whole rural vitality up. It is also identified that for both areas, the regression coefficients determining the changes of tourism and rural development level by the changes of the material cultural value estimations are higher than those parameters for none material cultural values. This posts for a stronger determination of the tourism and rural development propelled by the material cultural values collated by none material cultural values. Altogether, the cultural tourism products will only be able to survive and attract more and more tourists – taking into consideration the basic principles of sustainable tourism – by applying an up-to-date and competitive cultural tourism product development approach which, according to a recent cultural and heritage tourism product research paper created in Ontario in 2009, is mainly based on quality, distinctiveness, economic benefit and creativity.

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## Grape Breeding in China

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### Abstract

China has a long history of planting and breeding grapevine. The vast areas, diverse climate types of China resulted in many wild *Vitis* species after natural selection. These wild grapevines distribute throughout the mainland and are valuable genetic resources (e.g. disease resistance) for breeding works. In last century, the large scale survey and collection of wild *vitis* species have been done and the considerable achievements of studying and utilizing these resources have been made. The review summarizes the major works concerning the production and breeding works by using these wild grape species, and the trend in future grape breeding has also been discussed.

### Keywords

Grapevine; wild vitis species; breeding; germplasm resources; genetic improvement

### **General Introduction**

China is one of the original centers of *Vitis* species. It has been reported that the grapes were used for Chinese as food from earliest times (Sun 1979). The domestic grapevine, *Vitis vinifera* L., has been grown in China for more than 2000 years. The acreage of grapevine, following apple, citrus, banana, pear, jujube and peach, ranks 6th among fruit cultivation in China. Moreover, grape production comprises 5% of the total fruits in China. In recent years, China has achieved great success in the establishment of many large production zones, and new grape planting technology has been developed especially since 1980's. There are at least more than 13 national and local grape research institutes involving in programs of breeding and genetic improvement of grapevines. More than 20 universities and colleges are engaging in education and research concerning grape breeding. While most grape breeding programs are carried out by public institutions sponsored by government, more and more private ones have been established in recent years. More than 50 table grape cultivars were released from 1960 -1999, in about 40 years of the breeding effort before the new century, and releases of new grape cultivars have been accelerated since 2000 as a total of 38 table grape cultivars, including 12 seedless ones, have been released in the 21 century.

### **History and Major Viticultural Areas in China**

Grape growing in China dates back to 2300-2500 years ago. The Chinese government paid great attention to the development of viticulture and grape breeding after 1949 when the People's Republic of China was established. The acreage and production of grape increased steadily since then. The major viticulture areas are located in the Northern China. For quite a long time, the five leading provinces and autonomous regions are Xinjiang, Hebei, Shandong, Liaoning, and Henan, which were accounted for more than two thirds of total grape production in China. The absolute minimal temperature of -17°C is generally considered the limit for vineyards without winter protection. Unfortunately the main viticulture areas in China are distributed in winter temperatures often lower than -17°C which means a winter protection is necessary for the grapevine to survive. The map shows the main grape growing regions is shown as Fig.1



A



B



C



D



E

**Figure 1. Chinese grape growing regions (specified zones in red). A: Northeast China; B: Northern China; C: Northwestern China; D: South Central China; E: East China.** Source: The map was adopted from <http://en.wikipedia.org/wiki/>.

### ***1.1 Main grape production areas with protection in winter***

**North East:** The wine grapes growing successfully in the North East are cultivars selected from *V. amurensis*, a wild species native to China, and the hybrids derived from crosses between *V. amurensis* and *V. vinifera*.

**North China and Bohai Ocean Bay:** This region is the oldest and the most important grape-producing zone in China, including production areas in Tianjin, Beijing, Hebei Province (Langfang, Zhangjiakou, Qinhuangdao), and Shanxi Province (Qingxu).

**Ningxia:** Grape production in this region started in the 1980s. The wine industry has been expanding rapidly since the 90s of last century. Many

vineyards have been established East of the Helan Mountain, a semiarid to arid area where there is usually ample water for irrigation.

**The Hexi Corridor:** The Hexi Corridor is along the ancient silk route in Gansu Province. Grape production in this region is largely concentrated in Wuwei, Jiuquan, and Zhangye. This is one of the premier wine grape production areas in China.

**Xinjiang:** Chinese grape culture began in Xinjiang Uygur Autonomous Region. Its acreage and annual production of grape ranks first in China. With annual precipitation ranging from 16 to 200 mm. Xinjiang has less disease issues than other viticulture regions in China.

### ***2.2. Main viticulture areas without winter protection***

**Shandong Peninsula:** This region locates in south of the Bohai Ocean Bay. It is the most important wine grape-producing region in China. The Changyu Winemaking Company, the first and the largest (thus far) Chinese winery was established in 1892 in this region.

**The Loess plateau:** This region includes the west part of Henan and Shanxi provinces, and most of Shaanxi province. Scattered vineyards are found in this region.

**The old course of the Yellow River:** This region is the alluvial plain of the Yellow River along the Longhai Railway. Several wineries were established in 1950s and more in recent years. However, wet and hot summer weather conditions in this region are not favourable for producing good quality wine grapes.

**Southwest Plateau:** This region includes some high altitude areas of Yunnan and Sichuan Provinces. ‘Rose Honey’, an old cultivar introduced by missionaries 100 years ago, is a major grape cultivar in this area. Some *V. vinifera* and hybrid cultivars had been planted in recent years.

## **3. Wild Vitis Germplasm and utilization in China**

### ***3.1. Distribution of Chinese Vitis Species***

Survey and collection of wild *Vitis* has been carried out throughout China in large scale by most research institutes since mid-1950’s (Kong 2004, Liao 1988; Lin 1988; Qiu 1990, 1992; Shen 1989; Shi 1995; Wang 1978; Wei 1991; Wen 1989; Yu 1994; Zhou et al 1995). The most comprehensive collection and evaluation among the Chinese grape species is *V. amurensis* Rupr. Since the 1970’s, more collection trips were carried out, especially in Xinjiang and Tibet (Zhou et al 1986). This effort resulted in discovery of a new species, *V. piasezkii* in Xinjiang Region (Lin 1998). According to The Chinese Flora (Vol.48 (2), Vitaceae) (Li 1998), there are 37 species, one subspecies and 10 variation species of wild grape in China, and nearly 30 species were investigated and named in last 20 years. Of course, whether all these are true species is still under debate.

Wild *Vitis* species are distributed throughout China (Table 1). Of which, 80% are found in central China and 20% of them are in restricted and

scattered areas. Zuo et al (1981) classified 31 species and cultivars according to geographical distribution, and found that Hunan, Hubei, Guangxi, and Jiangxi provinces were most abundant in Vitis species. Regions further away from these provinces have fewer grape species. For example, only two species are found in the Northeast Region, three in Tibet, three in Hainan Island and four in Taiwan. Of the 42 species / subspecies known before 1986, Kong (1986) found that 33 were distributed in Henan, Hunan, Hubei, Jiangxi and Guangxi provinces.

**Table 1. Provincial distribution of East Asiatic species in China.**

Species	Gd	Gx	Jx	Fj	Zj	Hn	Hb	Ah	Js	Yn	Gz	Sc	Sx	Sd	Hn	Shx	Hb	Gs
V.adenoclada		*	*			*												
V.adstricta	*	*	*	*	*	*	*	*	*	*		*	*			*	*	
V.amurensis					*			*						*		*	*	
V.amurensis var. baihuashanensis																	*	
V.balanseana	*	*																
V.balanseana var. ficifolioides		*																
V.balanseana var. tomentosa		*																
V.bashanica													*					
V.bellula							*					*						
V.bellula var. pubigera	*																	
V.bellula var. pubigera	*	*				*												
V.betulifolia						*	*			*		*	*		*			
V.bryoniaefolia var. ternate					*													
V.chunganensis	*	*	*	*	*	*		*										
V.chungii	*	*	*	*														
V.dividii	*	*	*		*	*	*	*	*	*	*	*	*					*
V.davidii																		
var.ferruginea	*		*	*	*													
V.davidii var.cyanocarpa							*	*		*								
V.erythrophylla			*		*													
V.fengqingensis										*								
V.flexuosa	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*
V.hekouensis										*								
V.heyneana						*												
V.heyneana	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*		*
V.heyneana subsp.Ficifolia									*				*	*	*	*	*	*
V.hHancockii			*	*	*			*										

V.hui(Lushan))			*		*														
V.jinggangensis			*			*													
V.lanceolatifoilosa	*		*			*													
V.longquanensis			*	*	*														
V.luochengensis		*																	
V.menghaiensis											*								
V.mengziensis											*								
V.piasezkii						*						*	*	*		*			
V.piasezkii Var.pagnucii													*		*	*	*	*	*
V.piloso-nerva	*		*	*															
V.pseudoreticulata	*	*	*	*	*	*	*	*	*						*				
V.retordii	*	*										*							
V.romaneti							*	*	*			*	*		*				*
V.ruyuanensis	*																		
V.shenxiensis													*						
V.silvestrii							*						*						
V.sinocinerea			*	*	*	*	*		*	*									
V.tsoii	*	*		*															
V.wenchouensis					*														
V.wilsonae				*	*	*	*	*	*	*	*	*	*		*				*
V.wuhanensis			*				*								*				
V.yunnanensis										*									
V.zhejiang-adstricta					*														

Note: 1. Materials come from The Chinese Flora (Vol.48 (2), Vitaceae) (Li *et al.* 1998) and The Guangxi Vitaceae (Wang *et al.* 1988). In table, “\*” denotes distribution of species in this province. Some species or variations of species that are most limited in scope are not listed in this table.

2. Gd: Guangdong; Gx: Guangxi; Jx: Jiangxi; Fj: Fujian; Zj: Zhejiang; Hn: Hunan; Hb: Hubei; Ah: Anhui; Js: Jiangsu; Yn: Yunnan; Gz: Guizhou; Sc: Sichuan; Sx: Shanxi; Sd: Shandong; Hn: Henan; Xhx: Shanxi; Hb: Hebei; Gs: Gansu.

The wild *Vitis* species are formed through a long-time natural selection. While fruits from the wild species are short of expectation for human consumption, explorers always selected better ones for domestic cultivation, and further breeding for new grapes with better qualities.

### 3.2 Grape Germplasm Preservation in China

While a good number of regional / provincial research institutes and universities maintain various *Vitis* germplasm throughout the country, Zhengzhou Grape Germplasm Repository (Henan), Taigu Grape Germplasm Repository (Shanxi) and Zuojia *V. amurensis* Grape Repository (Jilin) are three National *Vitis* Germplasm Repositories collecting and preserving grape materials designed by the Chinese Agricultural Ministry.

### 3.3. Utilization of Chinese Wild Grapes

### *3.3.1 Use for Table Grape*

Among the Chinese wild *Vitis* species, *V. davidii* has relatively large berry size (average 1.6 cm in diameter). It is also disease resistant, and tolerant to hot climates. Clonal selections of *V. davidii* have been used as table grape cultivars (Hu 1956; Liao et al. 1988). The people who live in Fujian, Jiangxi, Jiangsu, Hunan, and Guizhou provinces usually cultivate this wild *Vitis* species as a table grape. *V. davidii* ‘Tangwei’ and ‘Xuefeng’ are perfect flower cultivars with good viticulture characteristics. The former was originally found in Yushan County of Jiangxi province (Wang 1980; Zhang 1985), while the latter was found in Xupu County of Hunan province, respectively (Yu 1994). Residents living in the mountain area of Anhui, Henan and Shaanxi provinces also plant this wild species in their courtyards as a table grape (Wang 1978, 1980). In 2008, the *V. davidii* grape cultivation reached 6800 ha in Hunan Province, and produced 2.55 million tons of fruit. About 99.8% of the fruit was used for table consumption while only 0.2% was used for making wine and juice.

### *3.3.2. Cultivar Development from Clonal Selections among the Wild Chinese Vitis Species*

Since 1950’s, there have been many achievements on utilizing the wild grape germplasm for new cultivar development. *Vitis amurensis* is the most commonly known and used for grape varietal development. Selection of superior clones from natural variation, followed by cross pollination, is the common approach used by many breeding programs in China. Since the 1950s, Institute of Botany, CAS, Institute of Pomology of CAAS and Northeast Institute of Agricultural and Forestry Sciences started comprehensive grape breeding programs using cold hardy *V. amurensis* germplasm from Northeast China. Clonal selection of *V. amurensis* such as ‘Shuangqing’, ‘Shuangyou’ and ‘Changbai No.4’ were among a dozen *V. amurensis* cultivars selected directly from the wild clones. Crosses between *V. amurensis* and *V. vinifera* were also made and several new F1 hybrids such as Beichun, Gongniang No.1 and Gongniang No.2 were developed as new grape cultivars.

#### **Cultivars developed from *Vitis amurensis***

*Vitis amurensis* is the peculiarly valuable resource of the Changbai Mountain in the northeast, where the grape can sustain temperature as low as -40°C. Fruits have been used for making wine for many years. *Vitis amurensis* is characterized by small clusters and berries, low yields and soluble solids content (Brix), and high titratable acidity (TA) and tannins. *Vitis amurensis* is dioecious, and due to its inherently low yields and difficulty in rooting, domestic cultivation is limited. One important objective of genetic improvement is to select high productive clones with high Brix, low TA, large clusters and large berries (Hao 1982). In 1957, Professor Shen-Jun, the former president of the Society of Chinese Horticultural Science, led the effort to make clonal selections from wild *V. amurensis* in the Changbai Mountain area.

Subsequently, Tonghua Winery and Changbai Winery in Jilin province, and the Special Animals and Plants Research Institute of Chinese Academy of Agricultural Sciences, joined the same effort as well in 1961 (Lin et al. 1982, 1993). A series of new selections with large clusters and berries, such as Tonghua No.1, Tonghua No.2, Tonghua No.3, Changbai No.6, Changbai No.9, Zuoshan No.1 and Zuoshan No.2, were selected and released for commercial production (Lin et al. 1991) (Table 2). Among them, the most valuable one was a hermaphroditic clone Changbai No.11 which was originally discovered by Changbai Winery in Jiaohe County of Jinlin Province in 1963. The hermaphroditic character is stable and its clusters are small (mean 43.2 g). In 1975, the selection was renamed as ‘Shuangqing’ in 1975 (Lin 1982). “Shuang –” means both in Chinese and many perfect flowered selection are named with a prefix “shuang – “

**Table 2. Some superior clonal selections of *V. amurensis***

Selections	Sex of Flower	Units	Year of selection	Year of naming
Changbai No.6	♀	Special Plant and Animal Research Institution of CAAS Changbaishan Winery	1963	
Changbai No.9	♀		1963	
Changbai No.11 (Shuangqing)	♂+♀perfect		1963	1975
Zuoshan No.1	♀	Special Plant and Animal Research Institution of CAAS	1973	1984
Zuoshan No.2	♀		1974	1989
Tonghua No.3	♀	Tonghua Winery	1977	1991
Tonghua No.7	♀		1977	1991
Tonghua No.10	♀		1977	1991

The selection of superior clones of *V. amurensis* promoted the commercial planting in large scale. Since the discovery of superior hermaphroditic *V. amurensis*, higher yields and more stable production were achieved. By using perfect flowered parents, breeding hermaphroditic *V. amurensis* hybrids / new cultivars became possible.

In 1975, Shen Yu-Jie in the Institute of Special Animal and Plant Sciences of CAAS found an individual with abnormal growth from the intraspecific hybridization of *V. amurensis* (Tonghua No.3 X Shuangqing). It has large and thick leaves and more clusters, and the mean berry weight is 1.1 g. This line was proved as tetraploid by chromosome counting ( $2n = 4X = 76$ ). This is the first tetraploid *V. amurensis* grape in the world.

In 1995, Mudanjiang Fruit Research Institute of Heilongjiang Province selected Mushan#1 from the natural seedlings of *V. amurensis*. The cluster is conical with a mean weight of 195 g, the berry is black with green fresh, 16.0 Brix, and 60.0% juice extraction rate, of which the flesh and the peel are easy to separate. It ripens in early September and in the middle and southern parts

of Heilongjiang Province, and it does not need burying in soil for winter protection (Shan 2011).

### **Cultivars developed from *V. quinquangularis***

Since the 1980s, the wineries in Luocheng, Duan and Yongfu counties of Guangxi Province made wines with *V. quinquangularis* Rehd. In the 1990s, the Horticultural Research Institute of Guangxi Academy of Agricultural Sciences made a systematic selection of *V. quinquangularis* in Guangxi, and 15 superior individuals were selected with high Brix and yield. After evaluation for many years, two superior individuals, GSH-2 and ZHJ-5, were selected as wine grape cultivars.

Since the 1995, Science Committee of Duan County of Guangxi Province and Horticulture Institute of Guizhou Provincial Academy of Agricultural Sciences selected two well-adapted cultivars, Zhonggu No.2 and Zhongjiu No.5 from *V. quinquangularis* (Huang et al. 2003). Based on the abundant germplasm resources of *V. quinquangularis* in Guangxi, Biotechnology Institute of Guangxi Academy of Agricultural Sciences selected Yeniang No.1 as a new wine grape cultivar, but it has shortcomings of low yield and susceptibility to diseases. Then they selected a hermaphrodite strain Yeniang No.2 in 2011. The drought and disease tolerance was strong, and it was suitable for hot and humid environments. It had been planted widely in mountainous regions in Guangxi. The combination of Luocheng Administration of Fruit Production, Guangxi and Guangxi Fruit Production Technical Guidance Station selected pistillate flower cultivars, Shuiyuan No.1 and Shuiyuan No.11, from *V. quinquangularis*. In 2012, they passed Guangxi cultivar registration, and they bloomed in the beginning of July and ripened in the end of September. The fruit of Shuiyuan No.1 is reported to have light strawberry flavor.

### **Clonal Selections of *Vitis davidii***

The origins of *V. davidii* are Hunan, Jiangxi, Fujian and Zhejiang Province in the south of Yangtze River. In 1985, Professor Zhang of Jiangxi Agricultural University found that 80% of the grapes in Yushan County of Jiangxi Province were clonal progeny from the domestication and cultivation of *V. davidii*. The centralized growth was in Tangwei Village of Yushan County, and thus he named it as ‘Tangwei’ grape, which was the first reported hermaphroditic *V. davidii* in the world (Zhang et al. 1985). Its mean cluster weight is 905 g, mean berry weight is 2.9 g., mean Brix is 15.1, TA is 6.2 g/L, and juice rate is 64.7%. It is a good grape used for both table consumption and wine making. Another hermaphrodite cultivar ‘Xuefeng’ was selected from *V. davidii* in Xupu County of Hunan Province (Zhang et al. 1989). Later on, *V. davidii* ‘Gaoshan No.1’, ‘Ziqiu’, and ‘Jinzhi Ciputao’ were selected. (Xiong et al., 2006; Shi et al. 2008). *Vitis davidii* is another good example of successful domestic cultivation of Chinese wild grape species. Its popularity and acreage is just behind *V. amurensis*.

#### 4. Table Grape Breeding Programs in China

Table grape breeding in China began in the 1950's. Red berry skin, muscat flavour, early maturity and large berry size were the main breeding objectives for table grape breeding in the 1950s and 1960s. 'Muscat Hamburg and' and 'Pearl of Csaba' were main parents used in many table grape breeding programs at that time. For example, in 1955, the Chinese Pomology Institute of CAAS in Liaoning Province selected an early maturing seedling with strong muscat flavour from an open pollinated population of 'Muscat Hamburg' and named it as 'Zaotian Meiguixiang'. Unfortunately it did not become a main grape cultivar because of its low yield. In 1958, Special Plant and Animal Institute of Hubei Academy of Agricultural Sciences developed 'Zijixin' from the cross between 'Baijixin' and 'Pearl of Csaba'. Since then, many early maturing cultivars such as 'Zhengzhou Zaohong', 'Zaomeigui', 'Zaojinxiang', 'Jingzaojing', 'Jingkejing', 'Zaohong', 'Zaohuang', 'Honglianzi', and 'Hongxiangjiao' were subsequently developed by Zhengzhou Pomology Institute of CAAS, Northwest Agricultural University, Beijing Botanical Garden of CAS, and Shandong Grape Research Institute. Different grape breeding programs based on functions will be introduced in the rest of the Chapter.

##### 4.1 Northeast China

Northeast China includes Heilongjiang, Jilin and Liaoning Provinces, and part of Eastern Inner Mongolia. The climate in Northeast China is cold and dry in the winter, warm and humid in the summer since rainfall appears mainly in the summer months. The precipitation changes a lot from year to year.

Dalian is a major region and seaport in the south of Liaoning province. It is the southernmost city of Northeast China. Dalian Agricultural Science Institute started a table grape breeding program in the 1970's, and selected 'Fenghuang No.12' [Muscat of Alexandria × (Flame Tokay x Pobeda)], and 'Fenghuang No.51' (Muscat of Alexandria × Cardinal) (Wu *et al.* 1989). 'Fenghuang No.51' is an early maturing cultivar, which has large berries, purple-red in colour, thin skin, thick pulp, high quality and high yield. From the 1980's to 1990's, 'Fenghuang No.51' had been cultivated as the main early ripening cultivar in most table grape growing region throughout China. In 2002, two *vinifera* x *labrusca* hybrid cultivars were released: 'Jumeigui' and 'Mihong Putao', which were derived from crosses between *V. vinifera* 'Shenyang Meigui' and American hybrids 'Kyoho' and 'Black Olympia' (Wang *et al.* 2003; Zong *et al.* 2009a). Other cultivars released by the same institution include 'Heiguixiang', 'Jumeigui', 'Mihong Putao' and 'Heimeixiang'. These mid-season varieties have muscat flavour, high yield, and are disease resistant. Among them, 'Jumeigui' has been expanded rapidly and has become one of the main table grape cultivars growing in China today.

Liaoning Provincial Saline-Alkali Land Research Institute made crosses in 1960, and selected 'Zifeng' from 'Black Hamburg' × 'Niagara' (Li et al. 1985). 'Zhuosexiang' was selected from 'Delaware' × 'Royal Rose' in 2009, which was early maturing, with strawberry flavor, salt tolerant, cold hardy and disease resistant (Yang et al. 2012).

From the 1980's to 1990's, the Horticultural Institute of Liaoning Academy of Agricultural Sciences had selected hybrid cultivar 'Zizhenxiang' by crossing a 'Muscat Hamburg' Mutant (7601) × 'Zixiangshui' Mutant (8001) (Xu et al. 1992). In the mean time, hybrid cultivars 'Zuijinxiang', 'Xiyanghong', and 'Guixiangyi' were released from selections in a cross of 'Muscat Hamburg' Mutant 7601 × Kyoho (Xu et al. 1994; Wang et al. 1999; Wang 2000). Among those hybrid cultivars, 'Zuijinxiang' has good quality and good appearance, and can easily become a seedless cultivar by treating with plant growth regulators. Now, 'Zuijinxiang' is accepted by many grape growers in south China. In 2005, Xiangyue, a mid-season ripening, large berry, good quality, high yielding, disease-resistant cultivar was bred by crossing Shenyangmeigui and 8001 (Xu et al. 2003). Zhuangyuanhong (Kyoho × Guixiangyi), a midseason cultivar, with muscat flavour, was selected in 2006 (Jin et al. 2007).

The Pomology Institute of Jilin Academy of Agricultural Sciences selected an elite hybrid cultivar from natural seedlings of Kyoho, and named it Tianfeng in 1988 (He et al. 1989). Jilin Agricultural College bred Bixiang Wuhe from a cross between *V. vinifera* 18-5-1 × Pearl of Csaba. Bixiang Wuhe is green, seedless, early ripening, with muscat flavour (Li et al. 2008).

#### **4.2 North China**

Northern China includes Beijing Municipality, Tianjin Municipality, Hebei Province, Shanxi Province, and Inner Mongolia Autonomous Region. The North China climate is cold and dry in the winter, warm in the summer, with a substantial diurnal temperature fluctuation. Rain appears mainly in the summer but the precipitation pattern changes a lot from year to year.

Beijing Botanical Garden of CAS began a table grape breeding programme in 1960. To 1994, a total of 10 cultivars were released by Institute of Botany of CAS. Four of them are seedless grapes: 'Jingzaojing' (Queen of the Vineyard × Thompson Seedless) (Fan et al. 2004), 'Jingkejing' (Blue French × Hongwuzilu), 'Jingzijing' (Queen of the Vineyard × Hongwuzilu), and 'Jingdajing' (Queen of the Vineyard × Hongwuzilu). Four are early ripening with large berries, which are named as 'Jingxiu' [Pannoniavinesa × 60-33 (Muscat Hamburg × Hongwuzilu)] (Yang et al. 2003), 'Jingyu' (Italia × Queen of the Vineyard), 'Jingyou' (Black Olympia seedlings (Fan et al. 2004) and 'Jingya' (Black Olympia seedlings) (Yang 1990). Two are early to midseason cultivars: 'Jingfeng' (Queen of the Vineyard × Hongwuzilu) and 'Jingchao' (Kyoho natural seedlings). 'Jingxiu' is an elite early ripening and good quality cultivar, and has been grown in China fairly extensively. The

fruit ripening time of ‘Jingzaojing’ is 20 days earlier than ‘Thompson Seedless’. ‘Jingzaojing’ has been widely grown in Gansu, Inner Mongolia, Jilin, and Beijing. In the new century, ‘Jingmi’ (Fan et al. 2008), ‘Jiangxiangyu’ (Fan et al. 2008), ‘Jingcui’ (Fan et al. 2008), ‘Jingyan’ (Fan et al. 2012) were selected from a cross of ‘Jingxiu’ and ‘Xiangfei’.

In 1973, the Forestry and Pomology Institute of Beijing Academy of Agricultural Sciences started to breed table grape cultivars. The objective was to produce early-maturing or seedless cultivars. They successively bred a series early-maturing cultivars with good quality, such as ‘Zizhenzhu’ (Muscat Hamburg × Pearl of Csaba), ‘Zaomeiguixiang’ (Muscat Hamburg × Pearl of Csaba), ‘Aishenmeigui’ (Muscat Hamburg × Jingzaojing), ‘Yanhong’ (Muscat Hamburg × Jingzaojing), ‘Zaomanao’ (Muscat Hamburg × Jingzaojing), ‘Cuiyu’ (Muscat Hamburg × Jingzaojing) (Li et al.1987; Tang et al. 1992), ‘Xiangfei’ [Cardinal×73-7-6 (Muscat Hamburg × Pearl of Csaba)] (Xu et al. 2001), ‘Ruiducuixia’ (Jingxiu×Xiangfei)(Xu et al. 2008). A series of mid- and late-maturing cultivars with large berry size, muscat flavour, seedless, good for storage and transportation, were also released. These include ‘Ruiduxiangxu’ (Jingxiu × Xiangfei) (Xu et al. 2009), ‘Ruiduwuheyi’ (Xiangfei × Ruby Seedless) (Xu et al. 2001). ‘Fenghou’ (a seedling of Kyoho) is a late ripening cultivar with large berry size, good appearance and storage ability, with good eating quality (Xu et al. 2000).

Changli Pomology Institute of Hebei Academy of Agricultural Sciences (in Changli County of Hebei Province) started a table grape breeding program in 1979. They selected early ripening cultivars, ‘Chaokangmei’, ‘Chaokangfeng’, ‘Chaokangzao’ from an open-pollination seedling population of ‘Campbell Early’ (Kong, 2004). ‘Wuhezaohong’ (8611) and ‘Hongbiao Seedless’ are triploid seedless cultivars derived from ‘Zhengzhou Zaohong’ (a diploid seeded cultivar) × ‘Kyoho’ (a tetraploid seeded cultivar) in the 1990’s (Zhao et al. 2000, 2003). They ripen very early, 30 days earlier than ‘Kyoho’, with large berries, black purple in colour, high in quality, productive, disease-resistant, and have been planted widely in most grape regions. In recent years, this institute has released ‘Yueguang Wuhe’ (Muscat Hamburg × Kyoho) and ‘Xiaguang’ (Muscat Hamburg × Jingya), which are early ripening, with large berry size, high yields, and disease-resistant (Tao et al. 2012).

In the past 5 years, Hebei Normal University of Science & Technology (in Changli County of Hebei Province) released several table grape cultivars. ‘Jintian 0608’ is a new late-ripening cultivar. Its mean cluster weight is 905 g. Its berry looks like a chicken heart in shape, and is purple-black in colour. The mean berry weight is 8.1 g, Brix is 22.0, and the flesh tastes sweet with a light aroma. The overall quality is excellent. The fruit matures in late September in the east of Hebei (Xiang et al. 2008). ‘Jintian Meigui’ is derived from Muscat Hamburg × Red Globe. Its mean cluster weight is 608 g. The berry, average weight about 7.2 g, is round, purple-red to dark purple-red in colour, with 20.5

brix. The flesh is juicy and tastes sweet. The fruit mature in the late August in the east of Hebei (Xiang et al. 2008). ‘Jintianmi’ (9603×9411) mean cluster weight is 616 g. Its berry is round, and green-yellow in colour, mean berry weight is 7.2 g, and the Brix 14.5%. It tastes sweet with aroma. The quality is excellent (Xiang et al. 2008). ‘Jintian Feicui’ is a late-ripening cultivar derived from ‘Fenghuang No.51’ (maternal plant) x ‘Victoria’ (paternal plant). Its mean cluster weight is 920 g. Its berry is near orbicular. The mean berry weight is 10.6 g, and Brix is 17.5. The flesh is white colour with fragrance, the pulp is crisp and succulent, and the quality is excellent. Harvest is early September (Wang et al. 2012). ‘Jintian Meizhi’ is a late-ripening cultivar derived from ‘Niunai’ (maternal plant) x ‘Minicure Finger’ (paternal plant). Its mean cluster weight is 802 g, its berry is a long ellipsoid in shape, bright red in colour, mean berry weight is 10.5 g, and Brix is 19.0. The quality is excellent. Harvest is from late September to early October in east Hebei Province (Wang et al. 2012).

The Pomology Institute of Shanxi Academy of Agricultural Sciences started a table grape breeding program in the 1970’s. They first released ‘Guibao’ (Ispissar × Vera Rose) in 1988 (Ouyang et al. 1989). Since 2000, more cultivars were released. They are very early ripening ‘Zaoheibao’ (Guibao × Zaomeigui) which has big berry size, fine quality and high yield (Chen et al. 2001). ‘Qiuheibao’ is a mid-late season table grape cultivar derived from a cross between Guibao × Taifi Meigui. It is very vigorous and productive with good adaptability to environmental adversities and has moderate disease resistance. Clusters can hang on the vine for a very long period of time, with good shipping and storage characteristics. The clusters are large (508 g), conical, well filled. The berries are uniform, large, short oblong, with a mean weight of 7.1 g, reddish purple in colour. The flesh is firm, crisp and sweet, with a pleasant lychee-like flavour. Its fruits, with 21.8 Brix, TA 2.5 g/L, and the sugar:acid ratio is 8:7 (Chen et al. 2007). ‘Zaokangbao’ is another early season seedless grape cultivar derived from the cross between ‘Guibao’ x ‘Centennial Seedless’ made in 1998. The clusters are conical with a mean weight of 216 g, but can attain 417 g. The berries are obovate, uniform in size, with a mean weight of 3.1 g and can attain 5.8 g. The berry skin colour is purple-red. Flesh is firm, crisp, juicy, and seedless or with one to two aborted seeds; the flavour is sweet with a Muscat aroma, and has high eating quality. It matures in early August. The vines have good adaptability but its disease resistance is medium (Chen et al. 2009). ‘Qiuheibao’ is a new mid-maturing tetraploid grape cultivar which was selected from the cross of ‘Guibao’ x ‘Christmas Rose’ and then doubling the chromosome number by colchicine treatment. Its cluster is large and conical with a mean weight of 437 g. The berries are oblong with a mean weight of 7.13 g and are black purple in colour. The flesh is soft, sweet with a rose flavour. Its fruit quality is rather good with one to two big seeds, Brix is 23.4, and TA 4.0 g/L. The sugar:acid

ratio is 4.9:1. The maturity of fruit is in mid to late August. Its vines are normal, fruitful, with good adaptability to environment and highly disease-resistant (Ma et al. 2010). ‘Lihongbao’ is a new grape cultivar which is bred by crossing ‘Guibao’ x ‘Centennial Seedless’. Its clusters are large and conical with a mean weight of 300 g. The berries are chicken-heart shaped, with a mean weight of 3.9 g, having a purple-red skin colour. The flesh is crisp with rose aroma and seedless. It has Brix of 19.40, and TA of 4.7 g/L. The sugar:acid ratio is 3.55:1. It matures in mid-late August in Jinzhong of Shanxi Province (Chen et al. 2011). ‘Wuhe Cuibao’ is a new early-ripening seedless grape cultivar which was bred by crossing ‘Guibao’ x ‘Centennial Seedless’. Its fruit clusters are large and conical with a mean weight of 345 g. The berries are ovoid shape, with a mean weight of 3.6 g, with a yellowish skin colour. The flesh is crisp with rose aroma and seedless or with one to two vestigial seeds. It has Brix of 17.20, and TA of 3.9 g/L. The sugar:acid ratio is 4.0:1. The vine is vigorous, with strong resistance and adaptability (Tang et al. 2012).

#### ***4.3 Northwest China***

Northwest China includes Gansu Province, Qinghai Province, Shaanxi Province, Ningxia Autonomous Region and Xinjiang Autonomous Region. The northwestern part of China has very different climate conditions from the east China. The terrain is arid and dry. The historic Silk Road snaked from its eastern terminus at Xi'an across the mountains and deserts to Central Asia. There is very little rainfall here in summer months and day-time temperatures can get above 100F (37°C). The night temperatures drop radically after sunset so that evenings are cool. Winters are very cold with snow at times.

Xinjiang Center of Grape and Melon selected ‘Xinpu No.1’ from an open pollination seedlings of Rose de Italia from 1984 to 1990, named and registered it in 1996. ‘Xinpu No.1’ has moderate vigour, is fruitful with stable yield, large clusters, good quality, and late maturity (Luo et al. 1997). ‘Xinyu’ is a hybrid cultivar derived from E42-6 (selected from ‘Red Globe’) × ‘Rizamat’, ripening in mid September in Shanshan City area. The berry is egg-like, purple-red in colour. The mean berry weight is 11.6 g. The fruit cluster is conical, weighting > 800 g, Brix 16-19, and TA 3.3-3.9 g/L, with excellent characteristics such as strong vigour, high yield and good quality (Luo et al. 2007).

Shihezi Grape Institute in Xijiang Region released ‘Shuijing Seedless’ (Xinpu No.2) and ‘Kunxiang Seedless’ (Xinpu No.3), which were selected from the hybrid population of ‘Queen of the Vineyard’ × ‘Kang Nairuo’. The former is early-ripening, large berry size, high quality, fertile and adaptable. The latter is mid-season, with muscat flavour, strong resistance, wide adaptability, high yield, good quality, with easy cultivation. ‘Zixiang Wuhe’ (Xinpu No.4; ‘Muscat Hamburg’ × ‘Black Monukka’) was bred in 2004. It is

an early to mid season cultivar, with disease resistant, good for storage (Chen et al. 2001; Rong et al. 2004).

‘Zuirenxiang’ is a mid-late ripening table grape cultivar which was selected by Pomology Institute of Gansu Academy of Agricultural Sciences from the offspring of ‘Kyoho’ × Чарас Мускатный. The cross was made in 1985. Its cluster is medium in size, weighing 700 g. The mean berry weight is 9 - 11 g. The Brix is 18, with very good and strong flavours of strawberry and muscat. This cultivar ripens in early September in Lanzhou. The vine is vigorous, productive and disease resistant (Gao et al. 2001; Hao et al. 2001).

#### ***4.4 South Central China***

South Central China includes the provinces of Guangdong, Hainan, Henan, Hubei, Hunan, and the Guangxi Zhuang Autonomous Region. The Central China climate is affected by the monsoons and as the season changes, the temperature changes quickly. In the summer, the monsoon from the sea takes rainfall to Central China, and in the winter, the monsoons from the continent bring the cold air to this area. The Central China climate changes with the different monsoon in the different time and climate creates the four seasons of the Central China.

Hubei Special Animal and Plant Research Institute (Wuhan) made crosses in 1958, and released ‘Zijixin’ (‘BaiJixin’ × ‘Muscat Hamburg’) in 1973 (Kong 2004).

Zhengzhou Fruit Research Institute of Chinese Academy of Agricultural Sciences started grape breeding in the early 1960s, with the goals of developing new cultivars with early ripening, large berry size and better fruit quality. ‘Zhengzhouzaoyu’ (trial #18-5-1) (Liu et al. 2003), ‘Zhengzhouzaohong’ (‘Muscat Hamburg’ × ‘Pearl of Csaba’) were released in the 90’s. In 2009, an early maturing cultivar, ‘Xiazhihong’ (‘Cardinal’ × ‘Muscat Hamburg’), was released (Liu et al. 2011). Its shape of cluster is conical, the mean weight is 750 g, the berry shape is round and the mean berry weight is 8.5 g, with fruit colour red to mauve, Brix 16.0-17.4, and TA 2.5-2.8 g/L. This grape cultivar has good yield, attractive appearance, high adaptability and good storage. It can be used in greenhouse cultivation as an early season cultivar.

#### ***4.5 East China***

This region include the provinces of Anhui, Fujian, Jiangsu, Jiangxi, Shandong, and Zhejiang, as well as the municipality of Shanghai. Since the Chinese government claims Taiwan and the few outlying islands of Fujian governed by the Republic of China (Taiwanese government) as its territory, China's pseudo-province “Taiwan Province, People's Republic of China” is also classified in this region.

Shandong Grape and Wine Institute started grape breeding in 1963, and released several early ripening grape cultivars in the 1970’s, included ‘Zaohong’, ‘Zaohuang’, ‘Honglianzi’, and ‘Quanlongzhu’ which are all

hybrids of ‘Muscat Hamburg × ‘Queen of the Vineyard’, and ‘Cuihong’ and ‘Hongxiangjiao’ (both are selected from seedlings of ‘Muscat Hamburg’ × ‘Golden Muscat’) (Kong 2004). In 1990, the institute also released mid-late maturing grape cultivars ‘Hongshuangwei’ and ‘Fengbao’ (both ‘Queen of the Vineyard’ × ‘Hongxiangjiao’), and ‘Heixiangjiao’, ‘Hongyuni’, ‘Guifeimeigui’, and ‘Feicuimeigui’ (all ‘Hongxiangjiao’ × ‘Queen of the Vineyard’) (Kong 2004).

Shanghai Academy of Agricultural Science started a grape breeding program in the 1980’s, and has released five new table grape cultivars thus far. ‘Shenxiu’ and ‘Shenbao’, developed from ‘Kyoho’ family, are early maturing with excellent quality, productive and stable yield (Jin et al. 1996; Jiang et al. 2009). Triploid cultivar ‘Hupei No.1’ (‘Himrod’ × ‘Kyoho’) is mid-early maturing, strong aroma, disease resistant, good for storage (Jiang et al. 2007). Another triploid cultivar, ‘Hupei No.2’ (‘Youngeer’ × ‘Zizhenxiang’), is an early maturing, bright skin colour, and seedless (Jiang et al. 2008). Late maturing ‘Shenfeng’ (‘Jingya’ × ‘Zizhenxiang’) has high yields, and strawberry flavour (Jiang et al. 2007).

Hong Shan Horticulture Farm in Pingdu County, Shandong Province, made crosses in 1956, and released ‘Zexiang’ and ‘Zeyu’ (both from cross of ‘Muscat Hamburg’ × ‘Longyan’) (Kong 2004). Jiangxi Agricultural University started making crosses in 1963, and released ‘Zaomeikang’, ‘Baimeikang’, and ‘Zimeikang’ (all ‘Muscat Hamburg’ × ‘Campbell Early’) and ‘Meiyehei’ (Muscat Hamburg × *V. flexuosa*) (Fan et al. 1985).

## **5. Conclusion**

From 1950's to the present, Chinese breeders have selected and released > 200 grape cultivars and advanced lines (those have never been named as cultivars). More than 120 of which have been registered (protected with plant new cultivar right), and 82% of them are table grapes, and 16% are wine grape cultivars (Tao et al. 2012). Overall, the objectives of table grape breeding programs focus on large berry, muscat flavour, firm texture, novel colors and shapes, early maturation, and seedlessness. The objectives for wine grape breeding are cold hardiness and disease-resistance by incorporating characteristics from the Chinese wild grapes.

Clonal selection, mutation breeding, open-pollination seedling selection, designed hybridization and embryo-rescue methods have all been used in various grape breeding programs. Among them, cross pollination, the conventional approach of grape breeding, is the most widely used and most Chinese cultivars are released by this approach. ‘Hupei No.1’ and ‘Hupei No.2’ were developed by using embryo rescue technology (Jiang et al. 2007; Jiang et al. 2008).

The most commonly-used parents among the Chinese grape breeding programs in last century are ‘Muscat Hamburg’, ‘Queen of the Vineyard’, ‘Pearl of Csaba’, ‘Kyoho’, ‘Thompson Seedless’ and ‘Black Monukka’. In the

mean time, Chinese wild grape species were also used in some programs for improving the tolerance to biotic and abiotic stress. In general, the germplasm used in the Chinese grape breeding programs for decades are quite narrow, and not enough attentions have been paid for using the Chinese wild grape species, especially for improving biotic and abiotic stress tolerances of current varieties. The future grape breeding in China should take advantage of these germplasm resources and expand the parentage base available around the world.

Conventional breeding approach that selects hybrids based mainly upon phenotypes is time-consuming, expensive, low efficient. Using marker assistant selection strategy can accelerate the breeding cycle and improve the selection efficiency. Chain Agricultural University and Northwest A&F University are now using the molecular breeding strategy to integrate the good fruit quality from *V. vinifera* grapes and the stress tolerance from the Chinese *Vitis* species.

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# Development of the Viticulture and Wine Industry in Bulgaria - Problems and Perspectives

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## Abstract

Vine-growing in Bulgaria is a traditional branch of its agriculture. Undoubtedly, its companion is the wine production, which gives the fine image of this industry. Since ancient times people in our lands have grown grapevines and have prepared wine. Even the ancient Thracians have known that the grape quality determines the wine quality more than any other factor. On its part, the grape quality depends to a large extent on variety, as well as climatic conditions during the growing season, soil fertility, growing technology, etc. Contemporary scientific knowledge has tools and provides the opportunity to regulate and control these factors in order to improve the production quality. The present review aims at observing the viticulture from a biological point of view. We will pay attention to some of the main problems it faces and the possibilities for their solution. The review does not claim a comprehensiveness of the topic, it only brings to light some key points and problematic issues for research.

## Introduction

The unique soil composition and climatic conditions necessary for the vine growing and development, as well as the Antiquity and Middle Age experience passed down from generation to generation, have been an important prerequisite for the viticulture development in our lands. The Eurasian vine (*Vitis vinifera* L.) is one of the ancient crops closely related to the human history. The plant fruit has been used for thousands of years. At present, vine is the world's most widely grown fruit crop. Because of its high nutritional value and unique chemical composition, the fruits are used both, fresh and processed - for wine production. The healing properties of wine have been known since antiquity. The identification of the chemical compounds in it and their impact on human health is of great interest for modern medicine and pharmacy.

It is believed that modern Eurasian vine varieties (*V. vinifera* ssp. *sativa*) originate from wild precursors (*V. vinifera* ssp. *silvestris*). When and where exactly this happened, it is still unclear. The location of Bulgaria between

Asia and Europe near the West Caucasus is essential for varietal diversity as it is the area where primitive varieties from the east enter. The Bulgarian vines of *Vitis vinifera* ssp. *sylvestris* and their genetic relationship with the local Bulgarian varieties have not yet been fully studied. Their detailed study can help solve the problems facing modern viticulture and wine production (Dzhambazova et al., 2009).

***Status of viticulture and wine-making in Bulgaria – historical overview***

Wine has been produced for more than 5000 years in the Bulgarian region. The first vines have been transported to our lands from the Middle East. Since the Thracian times, on our territory wine has been part of the everyday life and pagan rituals. There are evidence all around the country - findings from the archaeological excavations such as: vessels in the shape of jugs, rhytons, amphorae, etc. known to have been used for wine serving.

Homer describes the Ancient Thrace as a land of marvelous wine. Evidence of well-developed viticulture and wine-making was also found in the works of other ancient authors such as: Plato, Xenophon, Athenaeus, Polyen, etc. It was no chance that in Ancient Thrace people worshipped especially Dionysus (Zagreus), the God of fertility, wine and joy, proved by numerous archaeological monuments: Belentash, Perperikon (Fig.1).



Fig. 1. Perperikon – a Thracian sacred temple of Dionysus (Zagreus), God of wine (Own collection).

Thracian people considered the grape harvest a feast related to many rituals in honour of Dionysus (Zagreus). Images of grape harvesting and wine production were found on ceramic vessels, frescoes and mosaics in residential and public buildings, and tombs.

Long time ago, in Thracian times, people picked the grapes in baskets, poured it into baskets or barrels, and then carried it to special places for squeezing the grape juice. The latter were facilities made of wood or stone. The oldest technique for separating the juice from the grapes was crushing it with feet in large rock pools - the so-called "sharapans" (Figure 2, Figure 3).



Fig.2. Thracian sharapans (Perperikon)  
(Own collection).



Fig. 3. Thracian sharapans  
(the village of Nenkovovo).

[www.facebook.com/nepoznatiterodopi/photos](http://www.facebook.com/nepoznatiterodopi/photos)

After the formation of Bulgaria as a state, over the centuries, people on these lands have continued the wine strong traditions inherited by the Thracian tribes. At the end of the 19th and the beginning of the 20th century the largest vine-growing cooperatives were founded, which turned the country's wine-making from a small private business into a future wine industry. At the beginning of the 20th century several strong wine centers were formed around the country. Nowadays these are Lovech, Melnik, Plovdiv-Pazardjik, Pleven Suhindol, Sliven and Chirpan. Meanwhile, world-famous varieties such as: Cabernet, Merlot, Muscat Ottonel, Sauvignon Blanc, Chardonnay, Traminer, are being planted. Rkatziteli Georgian vine variety is also widespread. In the 1960s it occupied nearly 40% of the white wine vineyards.

In the 1960s and the 1970s the Bulgarian wine industry was oriented towards the huge Soviet Union market and the former Eastern Bloc market. Large wine factories were built. In the 1980s Bulgaria searched for new wine markets and achieved some successes in Great Britain, Germany, Japan, etc. Nevertheless, about 90% of exports went to Russia.

After the fall of the Berlin Wall, wine-making has been one of the most affected industries in Bulgaria. While in the 1980s Bulgarian wines (mostly Cabernet and Merlot varieties) were well accepted for the price-quality ratio, in the mid-1990s sales began to decrease. On one hand, there was a strong competition in this cheaper market niche of New World wines. On the other hand, it was related to the internal processes in the industry. A serious problem was the control of the purchased grapes quality. The shortage of raw material every autumn unnaturally raised its price. A regular practice was grapes harvest before reaching technological maturity, which significantly decreased the wine quality.

Since 2000 the situation in the Bulgarian wine-growing industry has changed. Due to the pre-accession to agricultural funds and programs, such as SAPARD and PHARE, many Bulgarian wine-growers and foreign investors have managed to implement their projects for modern wineries and vineyards. This gradually began to change the look of the whole wine industry. For a few

years small, medium and large excellently designed and well-equipped new wineries have appeared. Examples include: Bessa Valley in the region of Pazardzhik, Zagrey in Parvomay, Edoardo Miroglio in Nova Zagora, Katarzyna near Svilengrad, on the southeastern border with Greece and Turkey, Terra Tangra, Castra Rubra in Harmanli, Borovitsa and Chateau de Val in northwestern Bulgaria, etc. Along with other private wine investments, the image of Bulgarian wine has changed dramatically for only a decade. ([http://www.haskovo-bulgaria.com/en/page\\_41\\_96.html](http://www.haskovo-bulgaria.com/en/page_41_96.html)) ([www.daflorn.com/history.html](http://www.daflorn.com/history.html))

### ***Bulgaria as a viticulture area (regions and grape varieties)***

The territory of modern Bulgaria has a unique location in the northeastern part of the Balkan Peninsula. It is surrounded by mountains and cut by many rivers. Geological processes formed a huge variety of soil types. The climate in Bulgaria is temperate, with a warm summer and a relatively cool winter. The average temperature sum, required for the vine, is between 3500 and 3700 °C. The annual rainfall is between 470 and 953 l/m<sup>2</sup>. There are various types of soil - 14 soil types and 42 subtypes, such as cinnamon and gray forest soils, humus-carbonate, leached chernozems and alluvial meadow, including deep and shallow sandy soils, etc. They are extremely favorable for the vine cultivation, for good grapes ripening, and for the production of quality white and red wines. (<http://nationalsoils.com/maps/aez/>)

According to State Gazette no. 67 / 16.08.2005, Bulgaria is divided into two wine production regions: the Danube Plain and the Thracian Plain. According to an old decree of the Council of Ministers No 162 / 13.07.1960, Bulgaria is divided into five viticultural regions - <http://nationalsoils.com/maps/aez/> (Figure 4).

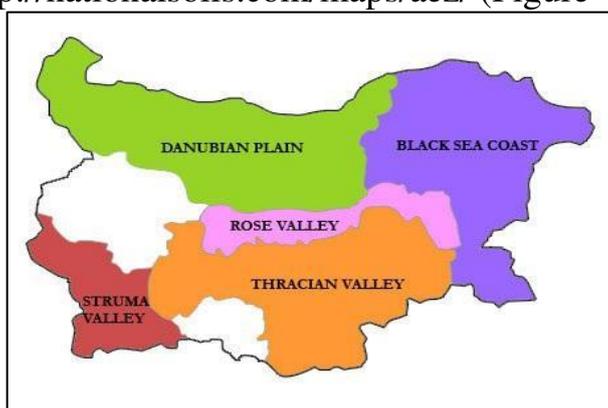


Fig.4. The wine regions of Bulgaria. ([www.nationalsoils.com/maps/aez/](http://www.nationalsoils.com/maps/aez/))

**North Region (Danubian Plain).** The climate in this region is continental - cold winter and hot summer. The sum of the active temperature is from 3600 to 3980 °C for its various parts. Annual rainfall is between 532 and 850 mm. The soils are deep and of good nutritional quality. They are represented by carbonate and typical chernozems to eroded chernozems located near the Danube. There are also gray and dark gray forest soils in the

region. The main varieties grown in this region are Muscat Ottonel, Cabernet Sauvignon, Merlot, Chardonnay, Aligoté, Pamid and the local Gamza variety.

**East Region (Black Sea).** It is characterized by a warm and prolonged autumn, which is important for the accumulation of sugars and the production of fine semi-dry wines. Very low winter temperatures (average from -12° to -16 °C) are not typical for this region. The sum of the active temperature varies from 3500 to 4000 °C. The annual rainfall is between 470 (Pomorie) and 630 mm (Shumen). High atmospheric humidity in the coastal zone largely compensates the deficiency of soil moisture. Soils are deep and rich in composition. All subtypes of black soil and eroded cinnamon forest soils are presented. There are also gray forest soils mixed with sea sands. The region is suitable for growing dessert and wine varieties. Specific varieties of the region are: Dimiat, Ugni Blanc and Muscat red. Muscat Ottonel, Chardonnay, Traminer, Sauvignon Blanc, etc. are also cultivated. Here are some of the best dry and semi-dry wines in our country.

**South Region (The Thracian Lowland).** The climate is moderate continental. It includes the central parts of the Thracian Valley, as well as parts of the Sakar mountain. The sum of the active temperature is from 3500 to 4000 °C. The average annual absolute minimum temperature is from -14 to -18 °C, which is a risk of grapes freezing. Annual rainfall ranges from 560 to 650 mm. The valley drained by the Maritsa River has a Mediterranean climate, with mild rainy winters and warm dry summers. There are various soil types: cinnamon forest, humus-carbonate, leached resins, alluvial meadows, etc. In the upper parts of the Sredna Gora mountain and the Rhodope Mountains the soil layer is shallow. The area concentrates most of the red grape varieties: Mavrud, Merlot, Cabernet Sauvignon, Red Misket, Pamid, etc. The region specific varieties are: Mavrud, Muscat red and Pamid.

**The Sub-Balkan Valleys (The Valley of Roses)** - the region borders the Balkan Mountains to the south. The dominant varieties grown here are: Muscatel, Riesling, Rkatziteli, Cabernet Sauvignon and Merlot. Predominantly dry and semi-dry white wine, and less red wine are produced in the area.

**South-western Region (The Struma Valley).** It is a small region having different climatic characteristics due to the strong Mediterranean influence from the south. The sum of the active temperature is above 4000 °C. Low temperatures below -16 °C occur rarely and for a short time. Annual rainfall is small (up to 550 mm), with a peak in the autumn-winter period. The region terrain is hilly and the vineyards are planted exclusively on slopes. The soils are mainly cinnamon-forest, medium lean, shallow at many places. The region is suitable for cultivation of dessert and wine varieties, for the production of quality red dry and table wines: Shiroka melnishka (local variety), Cabernet Sauvignon and Merlot. (<https://bulgariandrinks.com>; Popov et al., 2017)

*Variety of vines in Bulgaria*

Natural conditions in Bulgaria are suitable for growing various dessert and wine (red and white) grape varieties, which have different maturing time and different quality characteristics. There are various kinds, both local and imported. Red wine varieties occupy about 58% of the area, and white wine varieties - 42%. The biggest share of reds has Pamid - 16% (local variety), followed by Cabernet Sauvignon - 10%, Merlot -11%, Gamza - 4% (local variety), Shiroka Melnishka - 3% (local variety), Mavrud - 2 % (local variety), and many more, including Syrah - 2%, Cabernet Franc, Pinot Noir, etc. of less industrial importance. Among the whites, the biggest share has Rkatziteli - 14%, followed by Dimiat - 7% (local variety), Muscat red - 6% (local variety), Muscat Ottonel - 5%, Chardonnay - 3%, Sauvignon Blanc – 2%, Ugni Blanc, Aligote, Riesling, Traminer - 1% and many more of less industrial importance.

There has been a full control over the planting of wine grape varieties in Bulgaria since 2002. Changes in the national legislation have created conditions for restructuring and renewal of vineyards. The preservation and expansion traditional Bulgarian wine varieties (Table 1) have been encouraged (Dimitrova et al., 2013).

**Table1. Some of the grapevine cultivars in Bulgaria (Dzhambazova et al., 2009)**

Local native cultivars	Local cross-bred cultivars	International cultivars
Bolgar	Afrodita	Cabernet Sauvignon
Dimiat	Aheloj	Chardonnay
Gamza	ArmiraB	King Ruby
Mavrud	Buket	Merlot cl. ENTAV 181
Misket red	Brestovitsa	Michele Palieri
Misket Vrachanski	Cherna Perla	Pinot Noir cl. ENTAV 115
Pamid	Chernomorski Brilyant	
Shiroka Melnishka	Chernomorski Eleksir	
Tamyanka	Diana	
Zarchin	Dunav	
	Druzhiba	
	Evmolpiya	
	Hebros	
	Orfej	
	Plovdivska Malaga	

### ***Cultivation technologies***

The variety, the applied agro-technical sources, and the quality of used planting material, are important for obtaining quality grapes. Vine can be propagated in two ways: generative (sexual reproduction) - by seeds, and vegetative - by cuttings, layering, grafting and using tissue cultures. Reproduction by seeds is used only in the selection practice to obtain new varieties by hybridization. Vegetative reproduction of vines is used as a main reproduction type in viticulture around the world. Unlike seed reproduction, the vegetative one preserves the variety qualities; the obtained plants are better

and faster in growth and have earlier fruit production. Propagation by layering is used to replenish empty plots, to change plant density, to replace old or damaged vines, to reproduce hard-rooted varieties. Reproduction by cuttings has been widely used before the appearance of phylloxera. Today, it is used to create rootstocks for rootstock cuttings in areas with soils consisting over 70% sand, unfavorable to the development of phylloxera. Modern viticulture in Bulgaria relies to a great extent on the production of grafted rooted vines - more than 80% of the industrial vineyards are located in areas infested with phylloxera. For now, the only rational, though indirect, way to fight this enemy is to graft the unsustainable phylloxera varieties from the European-Asian vine group having valuable economic qualities on rootstocks of resistant American varieties and their hybrids (Braykov et al., 2005).

Tissue culture propagation is a modern and promising method that ensures preservation of valuable varieties and their characteristics. It creates virus-free propagation material and ensures high reproduction efficiency in varieties and rootstock production. The application of adapted micropropagation methods and specially created culture media, containing only auxins combination, leads to economical pricing production of healthy, viable and strong plants. It limits the possibility of somaclonal variations and ensures the authenticity of the propagation material produced (Figure 5) (Yancheva et al., 2018).



Fig. 5. Propagation of vines through tissue cultures (*in vitro* and adaptation)(Yancheva et al., 2018).

In Bulgaria there is a strict regulation control on the production and marketing of vine planting material.

In our country two technologies are applied for vine cultivation - low earth and stem (medium and high stem). The first option is used only in areas where absolute minimum temperatures have fallen below -18 °C for at least 10 years. These technologies are applied in small areas - in private farms and collections. In stem technology, the stem height varies between about 100 and over 100 cm with one-sided or double-sided branches. The planting distances depend on the biological variety characteristics, terrain and soil. Distance between rows is most often from 2 to 2.50 meters, and inter-row distance – from 1 to 1.20 meters for wine varieties and 1.20 to 1.50 meters for dessert

varieties. The supporting structures are made of wooden, metal or iron-concrete stakes (Figure 6, Figure 7) (Popov et al., 2017).



Fig. 6. Vineyards with metal constructions. (Agricultural University-Plovdiv own collection)



Fig. 7. Vineyards with iron-concrete constructions (Agricultural University-Plovdiv own collection)

There is also a growing interest to the biological viticulture, although there are a number of restrictive conditions. Plant protection measures and crop protection remain the main problem. The use of biological control, as well as authorized bio-stimulators and effective microorganisms, increase the resistance of crop plants, improve soil fertility and have a beneficial effect on the quality and quantity of the obtained grapes. Unfortunately, these modern technologies are still not widespread (Dimitrova et al., 2013; Tsvetkov et al., 2014).

#### **Areas occupied by vines and yields**

In recent years there has been a steady decrease in vineyard areas (Table 2). There are many reasons for this. In 2016 the vineyard areas in farms were 50 892 ha; 36 551 ha of them were harvested. The produced grapes decreased by 19% compared to the previous year (2015). In the south-east region there was a production of 37% of the grapes, and in the south-central region - 34%. Red wine varieties retain their dominant role in vineyard structure. About 3% of farm holdings are young, non-fertile vineyards. More than 2,680 hectares of productive vineyards are not harvested in result of the poor weather conditions.

**Table 2. Areas in Bulgaria occupied by vineyards for the period 2008-2017**

Year	Areas of vines on holdings (ha)	Vines outside agricultural holdings (ha)	Total area (ha)
2008	88 570	22 246	110 816
2009	74 018	27 416	101 434
2010	56 968	25 707	82 675
2011	52 567	25 901	78 468
2012	62 701	14 640	77 341
2013	58 236	4 900	63 136
2014	52 587	10 298	62 885

2015	50 705	12 086	62 791
2016	50 892	12 024	62 916
2017	n.a.	n.a.	60 583

*Source:* Ministry of Agriculture and Forestry, Agrostatics Department -

*Monitoring the production of grapes and wine*

Over 10,000 ha are not ploughed because of economic and social reasons. Unsupported vineyards, as well as those of undersized and fragmented plots, out of farms, are 12 024 hectares. The total size of vine areas in Bulgaria in 2016 were 62 916 ha.

In 2017 the newly planted vines, wine varieties, were 990.7 ha, of which 585.4 ha white wines and 405.3 ha red wines. The most planted varieties are traditional for Bulgaria - Chardonnay, Muscat Ottonel, Sauvignon blanc and Riesling, as well as the modern-day Pinot Griffin and Violet. Taking into account the red wine varieties, the established ones are: Cabernet Sauvignon, Merlot and Cabernet Franc. There is a constant tendency for planting Syrah, Malbec and Pinot Noir. There is also an interest in varieties, such as Mourveder, Caladoc, Petti Verde and Marcellan. The most planted Bulgarian varieties are: Misket Vrachanski, Rubin, Mavrud, Melnik 55 and Shiroka Melnishka.

Grape yields are determined by the used vine varieties, the growing technologies and the climatic conditions. Grape yields have varied in recent years, with a tendency to increase slightly (Figure 8). Yields in 2017 were strongly influenced by climatic conditions. In March, temperatures and rainfall in some areas slowed the beginning of juice-move phase. The low morning temperatures in April in some places led to frosting of young shoots. The rainfall and high air humidity during flowering at the end of April led to mold contamination with mildew. This led to a yield decrease of some varieties, mainly the Merlot variety in Haskovo region, where the average yields sharply decreased. The warm summer encouraged the accumulation of sugars in grapes. Fallen hailstorms in the districts of Rousse and Haskovo caused harvest damage up to 100%. High temperatures and dry weather in August and September helped to speed up the accumulation of sugars and mature grapes. The grape harvest campaign started in the mid-August and finished at the end of October. As a result of the above-mentioned nature-climatic factors, the grape harvest quantity in 2017 was about 5% less than the quantity in the previous 2016, but not worse in quality (Table 3).

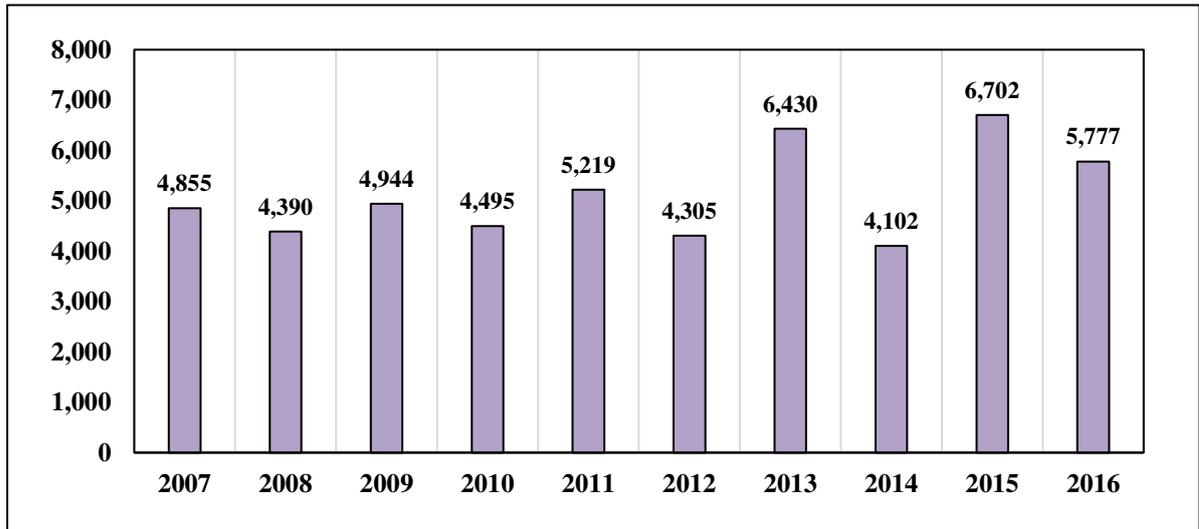


Fig. 8. Average yields, winegrapes, kg/ha.  
Source: MAF, Agrostatistics Department

**Table 3. Average yields from main varieties grown in Bulgaria for 2017**

For the production of white wines		For the production of red wines	
Variety	Average yield kg/da	Variety	Average yield kg/da
Vionie	4740	Cabernet Sauvignon	6183
Misketred	5736	Cabernet Franc	5817
Muskat Ottonel	6622	Merlot	6210
Chardonnay	6386	Mavrud	7740
Traminer	6871	Syra	7459
Souvignon blanc	5950	Pamid	4705
Tamyanka	4500	Pinot Noir	6324
Dimiat	6010	Kot	3550
Riesling Rheinsky	6290	Gamza	5233
Ugniblan	5800		

Source: EAVW data for 2017

There is also a changeable trend in country's wine production, with ups and downs over the years. (Figure 9) In 2016, 173 503 t of grapes were used for wine production in industrial conditions, and 26 765 t - in non-industrial conditions. The total quantity of wine produced in industrial conditions is 1 207 784 hectoliters: wines produced with PDO - 9 510 hectoliters, PGI wines - 360 984 hectoliters, and other wines - 837 290 hectoliters. Wine produced in non-industrial conditions is 126,196 hectoliters.

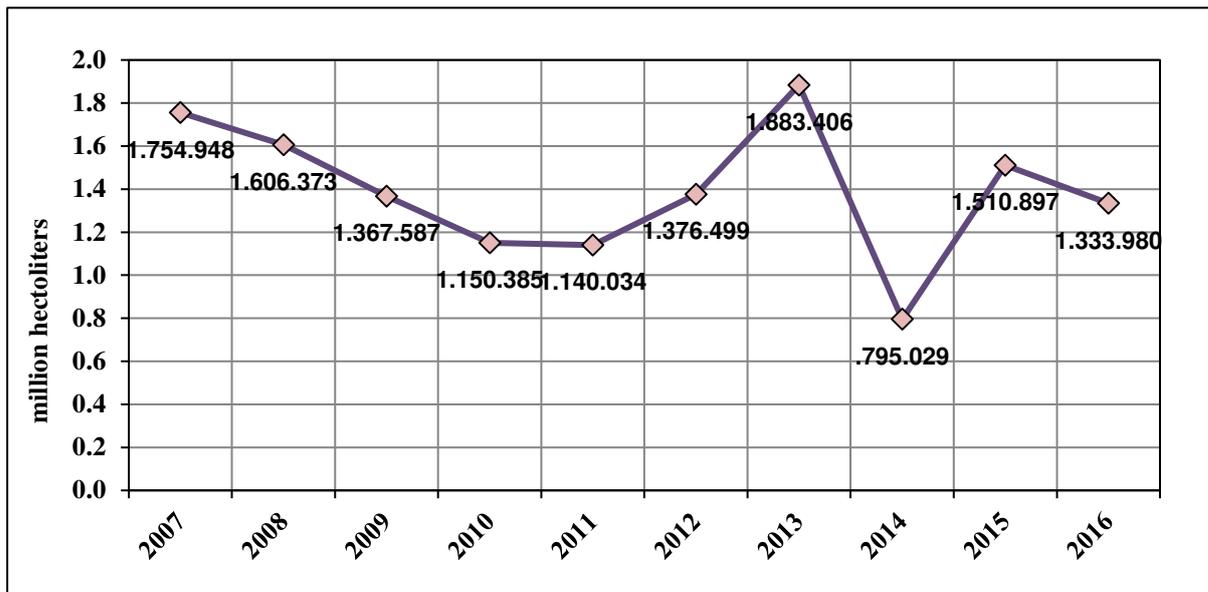


Fig. 9. Wine production in the country, 2007-2016, million hectolitres. Source: MAF, Agrostistics, Eurostat and own calculations.

### Main problems in viticulture

Today, there are many problems faced by the sector, the solution of which requires the implementation of modern economic solutions and the use of instruments for a market-oriented approach of management, determined by the specifics of the sector. Vine-growing requires large capital injections, which are paid over an extended period of time. Some of the main problems in the industry are:

1. Significant reduction in areas under vines. The reasons for the reduction of the vineyards are numerous - from the lengthy land reform and the fragmentation of the agricultural lands in the country to the unfavorable age and variety of the plantations, the lack of funding for proper agro-technical measures and some EU policy decisions for the sector. In 2007, the European Commission announced a decision to reduce vineyards "on a voluntary basis" by around 200 thousand hectares across the EU. Only in Bulgaria, and only in the period 2008-2014, the area of these massifs has been reduced by 47 thousand hectares to 53 521 hectares. ([www.expert.bg](http://www.expert.bg))

2. Unsatisfactory average yields of grape varieties. This can be due to many reasons, one of which is inappropriate agrotechnical and plant protection measures. The widespread use of chemical pesticides to suppress plant pathogens and synthetic fertilizers to stimulate plant growth causes a reduction and damage to the beneficial microflora in the soil, which generally reduces the quality of production. While the tolerance of different pathogens to pesticides is increasing, soil impoverishment determines the reduction in yield and quality.

3. The variety structure, although concentrated in 4 red and 5 white grape varieties, meets the modern requirements of wine making. There is an increased interest in more demanding and modern varieties.

4. The age structure is unfavorable - over 70% of the vineyards registered in the vineyard register were created more than 30 years ago. The share of newly created and young plantations is small.

5. Low level of application of new technologies and plant protection measures, accompanied by the presence of large-scale plantations with large inter-distances and a small number of plants per hectare.

6. Following the accession of Bulgaria to the EU, there is a stronger fluctuation in the quantities of wine produced. A negative trend is the decrease in the quantities of wine produced with PDO (protected designation of origin). It reduces the competitiveness and share of Bulgarian wines on the international market.

7. Lack of modern and adequate marketing strategy for realization of Bulgarian wine. Weak strains are based on unique, local varieties - Mavrud, Gamza, Shiroka Melnishka, Vrachanski Misket, Tamyanka etc., which over the years have been proven and accepted well on the Bulgarian and international markets.

8. A positive feature is the increase, albeit minimal, of the average value of exported wine, as well as the increase in market share in some third countries - China, Japan, the United States. However, this export is still low in quantitative terms. There is a loss of market positions on the traditional Russian market.

### **Possible solutions and perspectives**

In order to change the negative tendency of reduction of areas and the production of wine grapes, and in order to eliminate the problems in the future development of viticulture and wine production in Bulgaria, it is necessary to take a number of measures:

1. to create conditions for the consolidation of the vine plantations for easier cultivation, mechanization and application of plant protection measures. It is necessary to implement new innovative solutions in the wine grape production. In the world of science and practice, there is a sufficient number of examples and good practices to conduct agro-technology and other technologies leading to the achievement of high-efficient and productive vineyards and environmentally friendly solutions.

2. to put an end to the continuing reduction in the area, it is necessary to create more quickly new vines varieties oriented to the market.

3. to use vine seedlings of good quality. Our country has enough potential and traditions to produce grafted rooted vines from basic and certified vines. This would lead to an increase in employment in rural areas, where traditions are kept.

4. to change the agrarian policy regarding abandoned and unsupervised vineyards.

5. to increase the level of association in the sector, in order to ensure the possibility of using common and expensive wine-making equipment, in order to maintain adequate and fair prices for grape harvest. The new RDP 2014-2020 provides for the measure continuation of establishing and support of producer organizations.

6. to construction or restore the hydromelioration system in the vineyards. The main measure for the new programming period - restructuring and conversion allows activities for the establishment of drip irrigation systems and other meliorative activities, allowing the impact of environmental conditions on the development of wine-growing to be reduced.

7. In wine-making and plant cultivation, there must be a focus on producing quality wines with Protected Geographical Indication and Protected Designation of Origin (<http://foodspice.eu/index.php/en/ustoichivo-razvitie/15-zashtiteni-geografski-oznachenia>), which are valued by consumers and wine markets all over the world, and are in a higher price niche. Special attention needs to be paid to the wine production from local and unique varieties, that our country boast on the world market.

8. One of the factors for increasing the competitiveness of Bulgarian wine grapes and wine is the introduction of the GlobalGAP and ISO standards. Observing good manufacturing practices in grape and wine production and the quality requirements that producers would meet would undoubtedly lead to a better reception of Bulgarian wine on international markets.

9. In order to improve the image of the wine sector, it is necessary to comply with the rules of fair competition. The indications of grapes products and the indications of table and quality wines origin produced in certain regions must be protected. In this connection, it is necessary to put funds to use related to investments in the processing industry.

Disclaimer: Information in this report is collected from publicly available sources such as specialized and daily printed and electronic Bulgarian media, books, published surveys of consulting companies, web pages, etc.

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# Effects of inhaling ylang-ylang essential oil on anxiety, social interaction and sleep behaviors in mice

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## Abstract

Essential oil from *Cananga odorata* (ylang-ylang, YYO) is usually used in reducing blood pressure and improving cognitive function in aromatherapy in human. Few reports showed its effects on anxiety, sleep and social behaviors. To investigate these effects of YYO, ICR mice were subjected to three behavior models including elevated plus maze test, pentobarbital sleeping time experiment and three-chambered social approach task after acute YYO exposure. The results showed that YYO exposure reduced the anxiety behaviors and improved the social behaviors of the mice. It significantly increased the time of the mice spent on open arms in the EPM test and the social interaction time with novel mice in the test phase 2 in the three-chambered social approach task. In the pentobarbital sleeping time experiment, YYO did not prolong the sleep time of the mice. The above results suggested that inhaling YYO could be helpful to relieve anxiety and improve social behaviors and the anxiolytic effect of YYO exposure was not work through sedative effect.

**Key words:** *Cananga odorata* essential oil; Anxiolytic effect; Sedative effect; Social behavior.

## 1. Introduction

Anxiety disorder is a main cause of depression and it is easily to relapse. Traditional clinical drugs, no matter the benzodiazepines, or other drugs developed later such as buspirone, SSRIs, MAOIs, are more or less leading some side-effects involving with cardiovascular, stomach and liver. A side-effect and tolerance free substitution therapy is expected to be used in clinic. Various aromas from nature plants were assumed to cause both physiological and psychological changes in human. Aromas of essential oil, such as oils

from lavender (Shaw et al., 2007), sandalwood (Satou et al., 2014) and sweet orange (Faturi et al., 2010) were usually used to help people relax mind and calm down.

Ylang-ylang essential oil is obtained from fresh and mature flowers of tropical tree *Cananga odorata* (Lam.) Hook.f. & Thomson. It is one of the most popular fragrance raw materials around the world. Several experiments in human showed that ylang-ylang essential oil inhalation could reduce blood pressure (Hongratanaworakit et al., 2006) and affected the cognition and mood on healthy participants (Moss et al., 2008). However, there still have no studies focus on its effect on mental symptoms. In this study, the effects of ylang-ylang essential oil exposure on anxiety, sleep and social behaviors in mice were assessed for evaluating its application value in aromatherapy.

## **2. Materials and methods**

### ***2.1 Animals***

ICR mice were purchased from the Shanghai Experimental Animal Center of Chinese Academy of Sciences (Shanghai, China). The using of animals and experimental procedures was performed following the rules of the Association for Assessment and Accreditation of Laboratory Animal Care International and approved by the Institutional Animal Care and Use Committee of Shanghai Institute of Material Medica, Chinese Academy of Sciences. Mice were housed under a 12 h/12 h light/dark cycle and controlled temperature ( $21\pm 2$  °C) with free food and water. At the beginning of experiment, the weight of male mice were 25-30 g and female mice were 22-25 g.

### ***2.2 Chemical and treatment***

Ylang-ylang essential oil (YYO) obtained from flowers of plant *Cananga odorata* (Lam.) Hook.f. & Thomson was purchased from LiYing Trading Company Limited (Guangzhou, China). The constituents of the YYO used in this study were analyzed by GC/MS in our previous study (Zhang et al., 2016). Seven compounds of related content (peak area %) higher than 5% were identified as: benzyl benzoate (20.25%), linalool (11.00%), benzyl salicylate (9.53%), benzyl alcohol (9.10%), benzyl acetate (7.48%), geraniol (6.79%) and methyl benzoate (6.08%). Different volumes of essential oils were added into 10 ml 1% Tween 80 water solution to make different concentration of emulsion. In the odor exposure procedure, oil/water emulsion was heated to  $70\pm 3$ °C. For the control group, the same volume of water was used instead of the oil. Diazepam (DZP) injection (Shanghai Xudong Haipu Pharmaceutical Co., LTD., China) diluted with saline was intragastric administrated to mice due to the concentration of 1mg/kg as a positive control drug. Behavioral tests were carried out 30 min after the DZP administration.

### ***2.3 Inhalation apparatus***

The square inhalation apparatus (65 × 65 × 45 cm) was made of stainless steel with a closely fit cover. Eight walls with holes were used to divide the apparatus into 4 small chambers (25 × 25 × 30 cm) and made a cross shaped area in the middle where a controllable heater was settled to heat the oil emulsion. During the odor exposure, mice were placed individually into the apparatus for 10 min and then submitted to behavioral tests.

#### ***2.4 Behavioral test***

##### *Elevated plus maze test*

The elevated plus maze (EPM) (MED Associates, USA) consisted of four arms (open arms: 35 × 6 cm; closed arms: 35 × 20 × 6 cm). There was a square platform of 6 × 6 cm in the middle. Mice were individually placed into the platform by head toward open arms, and allow them to free explore for 5 min. The total amount of time of the mice spent in the open arms and closed arms were recorded during the 5 min test period. The time spent in open arms was calculated with the following formula: the time spent in open arms / the total time spent in open and closed arms × 100.

##### *The open field test*

In the open field (OF) test, mice were individually placed in the center of a black rectangular area (45 × 45 cm) with apparent walls (40 cm) around (TSE Systems, Germany). The apparatus was illuminated with a 500-lx light at floor level. Mice were allowed to explore the area for 5 min. The total move distance of the mice was recorded.

##### *Three-chambered social approach task*

The three-chambered social approach task was mainly used to test the normal social behavior of mice and their interest in novel social interactions. The experimental procedure was based on the study of Kaidanovich-Beilin et al. (2011). At the beginning of the experiment, mice were placed in the intermediate area for 5 min. In the test phase 1, stranger mouse 1 was placed in the restraining cage 1, the test mice were free to explore the box for 10 min. In the test phase 2, stranger mouse 2 was placed in the restraining cage 2 and the test mice were free to explore the box for 10 min. Male C57 mice (25-30 g) were used as the strangers. The time the mice sniffing the restraining cage 1 and cage 2 were recorded. After the test, feces and urine were removed. The restraining cages were thoroughly cleaned with 10% alcohol.

##### *Pentobarbital sleeping time test*

Pentobarbital sodium (provided by Shanghai Institute of Materia Medica) was diluted with 0.9% physiological saline and intragastric administrated to mice at 10 ml/kg due to the concentration of 50 mg/kg to induce sleep. The mouse was then placed individually in cage. The behavior of the mice was recorded by a camera. The sleep latency was defined as the time between the administration of pentobarbital and disappearance of the righting reflex. Sleeping time was recorded from the disappearance of the righting reflex to

when the mouse spontaneously turns over. DZP was administered at 1mg/kg 30 min prior to administration of pentobarbital as the positive drug.

### 2.5 Experimental procedures

After 1 h habituation in the test room, three concentrations (0.1%, 1% and 10%, v/v) of YYO were given to the mice separately for 10 min in the inhalation apparatus individually. Their behaviors in the EPM test, three-chambered social approach task and pentobarbital sleeping time test were examined immediately after the odor exposure. Mice were intragastric administered by a dose of 1mg/kg DZP 30min before behavioral test. All experimental procedures occurred between 10:00 am and 5:00 pm.

### 2.6 Statistical analyses

Animals' behaviors were analyzed using the software Noldus EthoVision XT10.0. One-way ANOVA followed by a post hoc Duncan test was used to analysis the effect of YYO. All statistical analyses were conducted using the SPSS 17.0 software.

## 3. Results

### 3.1 The anxiolytic effect of YYO after acute odor exposure

In the OF test, only DZP significantly increased the total explore distance of male mice compared to the control group ( $F(4, 60)=3.64$ ,  $p<0.05$ )(Fig. 1, a). Acute YYO exposure did not affect the locomotor behaviors of the mice. In the EPM test, YYO showed anxiolytic effect by a dose-dependent manner ( $F(4, 60)=4.08$ ,  $p<0.05$ ). The mid (1%, v/v) and high (10%, v/v) concentration treated groups significantly increased the percentage of time spent on open arms compared to the control group ( $p<0.05$ , Fig. 1, b).

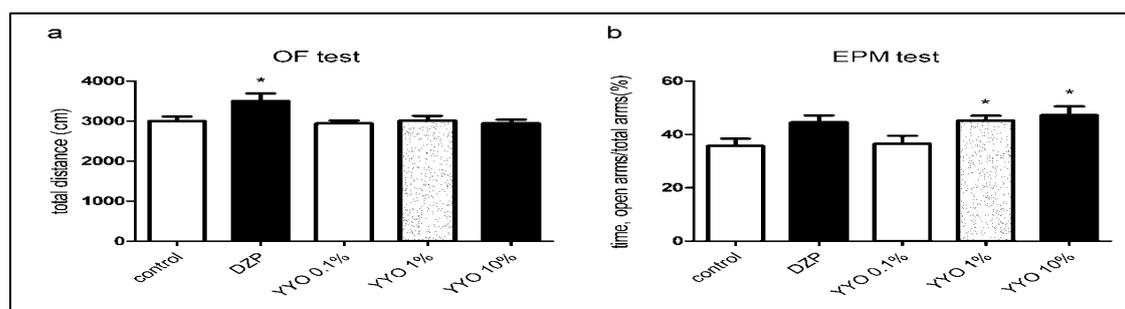


Fig. 1. The anxiolytic effect of acute YYO exposure in the OF test (a) and EPM test (b) after acute YYO exposure with three concentrations (0.1%, 1%, 10%, v/v). Values represent the mean  $\pm$  SE ( $n=12\sim15$ ), \*  $p<0.05$ . One-way ANOVA followed by a post hoc Duncan test was used.

### 3.2 Effects of acute YYO exposure in the pentobarbital sleeping time test

Drug administration could affect the sleep latency ( $F(3, 22)=5.51$ ,  $p<0.05$ ) and sleep duration ( $F(3, 22)=6.29$ ,  $p<0.05$ ) of the mice in the pentobarbital sleeping time test (Fig 3). Compared with the control group, DZP and 10% YYO treatment could significantly shorten the sleep latency of mice ( $p<0.05$ ). The 1% YYO treatment showed no significant effect on the

sleep latency of mice ( $p>0.05$ ). Compared with the control group, DZP significantly prolonged the sleep time ( $p<0.05$ ) while 1% and 10% YYO treatments had no significant effect on the sleep time of mice ( $p>0.05$ ).

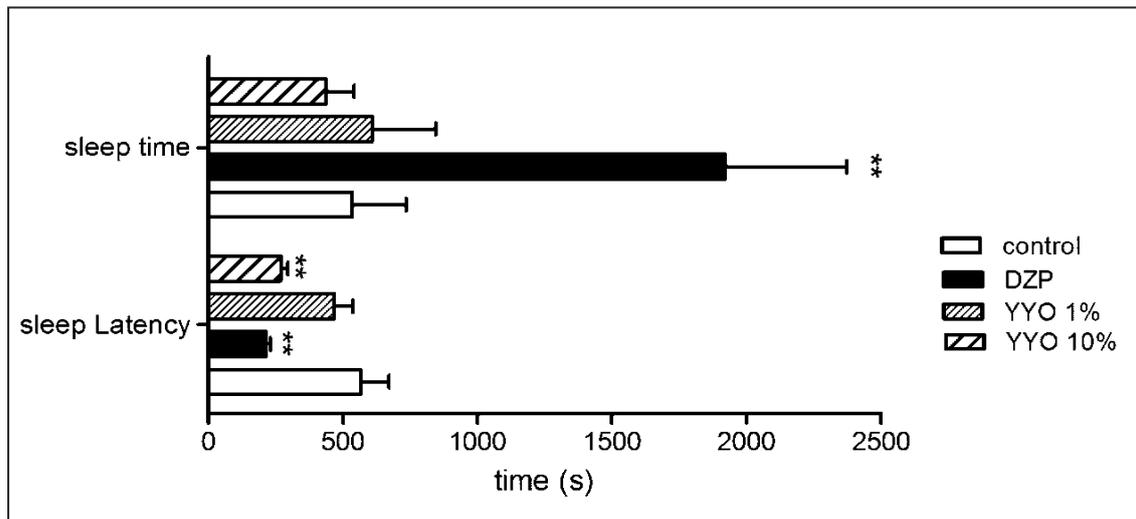


Fig. 2. The effect of YYO exposure on the pentobarbital sleep test. The sleep latency and sleep time were recorded. Values represent the mean  $\pm$  SE ( $n=6\sim7$ ), \*\*  $p<0.01$ . One-way ANOVA followed by a post hoc Duncan test was used.

### 3.3 The effect of YYO on the social behaviors after acute odor exposure

In the test phase 1, neither YYO nor DZP treatment affected the social interaction time of the test mice with the empty cage ( $F(2, 25) = 0.41, p>0.05$ ) or the stranger 1 ( $F(2, 25) = 0.88, p>0.05$ ) (Fig. 3, a). In the test phase 2, YYO treatment significantly affected the social behaviors of mice (social interaction time with stranger 1 :  $F(2, 25) = 0.88, p>0.05$ ; social interaction time with stranger 2:  $F(2, 25) = 3.36, p < 0.05$ ). Compared with the control treatment, YYO significantly increased the social interaction time of the test mice with the stranger 2 ( $p<0.05$ , Fig. 3, b). DZP treatment still had no significant effect on the social behavior of mice during the test phase 2 ( $p>0.05$ ).

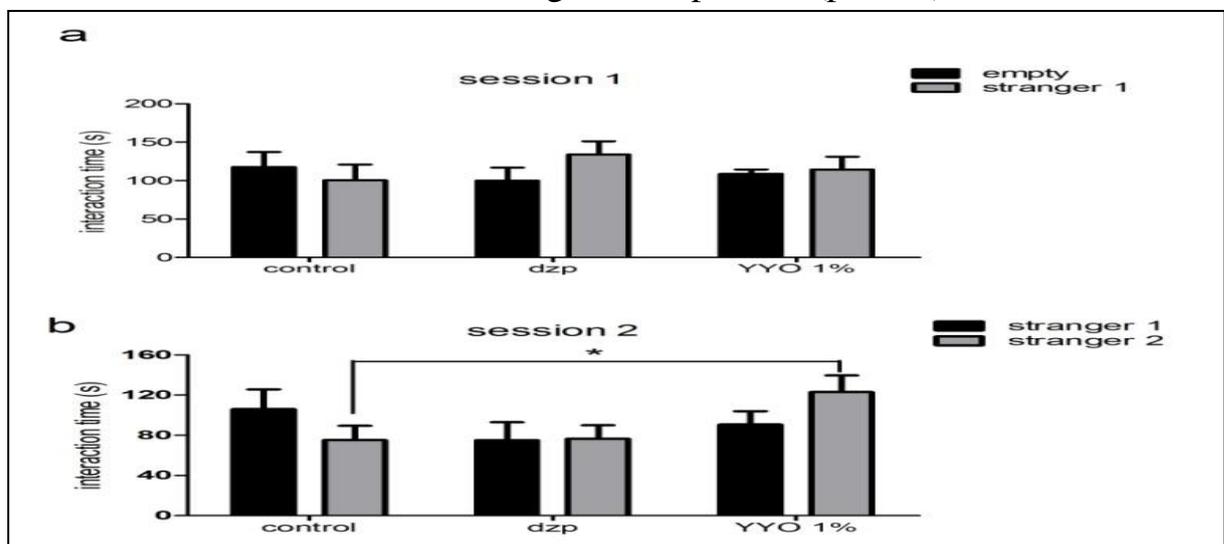


Fig. 3. The effect of YYO on the social behaviors after acute odor exposure in the three-chambered social task. a, The amount of time sniffing the empty cage or the cage with the stranger 1; b, The amount of time sniffing cages with stranger 1 or stranger 2. Values represent the mean  $\pm$  SE (n=9~10), \*  $p < 0.05$ . One-way ANOVA followed by a post hoc Duncan test was used.

#### 4. Discussion

YYO is usually used in anti-depression or anti-anxiety formula in aromatherapy. People believe that the fragrance could bring pleasantness, help relax and have some sedative effect in humans. However, there were only two studies report the effect of YYO inhalation on reducing blood pressure and heart rate functions in humans. In this study, YYO was proved to be effective to affect the anxiety and social behaviors of the mice.

In the open field test, acute administration of DZP could significantly increase the total distance of mice. The total distance was an indicator of the spontaneous activity. The YYO exposure treatment did not affect the total distance. It indicated that the effect of YYO on the anxiety behaviors in the EPM test might due to the anxiolytic effect rather than the effect on the motor ability of the mice. The action mechanism of YYO might be different from that of DZP.

Mice are typical social animals and social behavior is very important for maintaining social class status and selecting spouses. Many mental illnesses, such as bipolar disorder, depression, schizophrenia, obsessive compulsive disorder, anxiety disorder and autism could block social behavior and social cognition. Many anti-anxiety drugs could increase the social behavior of mice in social models. Previous studies had found that antagonists of mGluR5 (Spooren et al., 2000) , 5-HT<sub>2C</sub> receptor (Dekeyne et al., 2008) and NK1 receptor (Ebner et al., 2009) could increase the social behavior of mice in social models. In the three-chambered social approach task, the social ability of the mice was evaluated by comparing the social interaction time of the test mice with the empty cage and the stranger 1 in the test phase 1. The social preference of the mice was evaluated by comparing the social interaction time of the test mice with the familiar individual (stranger 1) and the new individual (stranger 2) in the test phase 2 (Moy et al., 2004). YYO could increase the social interaction time of the test mouse with novel stranger 2 in test phase 2, which indicated that YYO exposure might raise the social preferences to new individuals during socialization.

Many nature essential oils such as lavender oil (Shaw et al., 2007), lemon oil (Komiya et al., 2006) and orange oil (Lehrner et al., 2005) were believed to lead anxiolytic effects through the sedative effect. In the OF test of this study, acute oil exposure did not affect the total distance of the mice. These results indicated that there was no significant sedative effect of YYO exposure. To confirm this point of view, a pentobarbital sleep test was proceeded. No sleep time enhance effect was observed after acute YYO

exposure. These results indicated that the anxiolytic effect of YYO exposure was not work through sedative effect. A study from Komori et al. (2006) also showed that the YYO exposure did not affect the sleep time induced by pentobarbital sodium (50 mg/kg), which is consistent with the results of this study. Interestingly, Tsuchiya et al. (1991) pointed out that the essential oils that could alter the sleep behavior induced by pentobarbital sodium in normal mice could not work in olfactory-injured mice. This study indicated that the sedative effect of the essential oils might work through the olfactory pathway. The odorant molecule binds to the olfactory receptor and then initiates a series of signal transduction. The chemical composition and concentration of the essential oil might affect the signal intensity. The mechanism of the essential oils that had been reported to prolong the sleep time was mostly related to the gamma aminobutyric acid (GABA) system. These essential oils could increase the content of GABA in the brain or reduce the content of glutamate (Koo et al., 2003; Komori et al., 2006). The action mechanism of YYO might differ from this.

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# Development of the production of aromatic oil crops in Bulgaria

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## Abstract

The production and collection of raw materials from essential oil-bearing crops has long-standing traditions in Bulgaria. Kazanluk rose, lavender, white oregano, basil, clary sage, and salvia are important aromatic and ornamental plants. These plants produce essential oils, used in medicine, cosmetics, pharmaceuticals and food industry. Essential oils usually have a wide range of bioactivity due to the presence of active ingredients that have a different mode of action. After the extraction, mainly by distillation, the essential oils contain a variety of volatile molecules such as terpenes and phenolic derivatives of aromatic and aliphatic components. The aromatic oil bearing plants in Bulgaria are presently subject to phytochemical attention due to their biological and chemical diversity. The current review summarizes the development of the industrial rearing of aromatic and medicinal plants in Bulgaria.

## 1. Production of oil-bearing rose in Bulgaria

### 1.1 Historical data

The rose belongs to class Magnoliophyta, subclass Rosales, genus *Rosa*. In the genus *Rosa* there is an incredibly diverse variety of over 200 species (Rusanov et al., 2009), of which 95 originating from Asia and 18 - from North America and the rest, from Europe. Interesting is the fact that no representatives of the *Rosa* family have been found in the southern hemisphere of the Earth. Rose oil production in the world today takes place mainly in Bulgaria, Turkey, Russia, Ukraine, Georgia, China, Iran, India, Egypt, Algeria, and France. Bulgarian rose oil obtained from flowers is the benchmark for the highest quality.

The Kazanluk oil-bearing rose (*Rosa damascena* Mill. f. *trigintipetala* Diesk.) is an emblematic culture for Bulgaria with an important agricultural and economic significance. It is originating from the group of bush-like roses created in the ancient Middle Eastern countries - Babylon, Persia, and Syria. One of the taxonomic classifications considered that the Kazanluk rose probably originated from hybridization between *Rosa centifolia* and *R. gallica* (Topalov and Irinchev, 1967; Topalov, 1978; Topalov, 1989). Staykov and Kalaydzhev (1980) define the Kazanluk oil-bearing rose as a population of forms of the *Rosa damascena* Mill. It has been found that forms of Kazanluk

oil-bearing rose with differed in the number petals, color, the number of stamens, the habitus and the number of flower buds.

The Kazanluk oil-bearing rose passes through the different influence of climatic and geographic factors to reach its present appearance. During the long cultivation in the region of Kazanluk, the rose has changed significantly, having become more abundant with petals. These facts determine its separation on its own - *Rosa kazanlika* V. T., and Bulgaria is reasonably considered to be its second homeland. First more significant plantations were established around Kazanluk, where the rose finds favorable conditions for development and becomes a valuable industrial culture, and later all the valley began to be called the Rose Valley.

Registered climatic changes in recent years have led to the establishment of new rose-growing regions out of the Rose Valley, such as the village of Mirkovo (Western Stara Planina, the Pirdop Valley), the town of Bratzigovo (low slopes of Rhodopes).

### ***1.2 Climate conditions in Bulgaria***

The climate conditions of the Rose Valley proved to be more favorable for the cultivation of roses. The traditional centers from the beginning of the 20th century are the localized between Kazanluk and Karlovo, which represent 83% of the rose plantations. The air humidity, cloudiness, and temperature in May and June contributed to obtaining roses yielding high percentage oil. The soils in the Rose Valley are cinnamon forest and sandy soils. The importance of this fact is that they do not retain water long after rainfall. The average quantity of precipitations is 653 mm/m<sup>2</sup> with a maximum in May – 82 mm/m<sup>2</sup>. The average annual temperature is 11.4oC. The areas favoring the rose plantations are mainly in the valley of the rivers Tundzha (Kazanluk) or Stryama (Karlovo), between Stara Zagora and Chirpan and partly in the Rhodopes around Peshtera (Staikov et al., 1975). During the last 2-3 years, new plantations of rose around the town of Strelcha (Pazardzik district) and the village of Zelenikovo were developed, where the planting areas have grown twice in 2015-2017.

### ***1.3 Genotypes of oil-bearing roses grown in Bulgaria***

Breeding process of oil-bearing crops is carried out at the Institute of Roses and Essential Oil Crops - Kazanluk.

**Kazanluk oil-bearing rose (*R. kazanlika*)** is “The Queen” of the essential oil crops in Bulgaria. Its importance is determined by the extremely high quality of rose oil, produced after the rose flower processing. It has a delicate, elegant, beautiful and lasting aroma. For years, Bulgarian rose oil has glorified our country everywhere, and today it is an indispensable ingredient of first-class perfumes. Besides in addition to the production of aromatic substances, Kazanluk rose was also used as raw material for the production of food, wine and vinegar, tea and medicines. Today, the petal leaves are used in the food industry for the production of jams, jellies, and liqueurs. In the

pharmaceutical industry, Kazanluk rose is involved in the production of drugs for eyes, stomach, skin and dental diseases (Dimitrova, 2014). Also, rose products are known for their anti-sclerotic and stimulant effects on humans. In medicine, the rose flowers traditionally serve as a laxative and applied in homeopathy against fever (antipyretic effect). The essential oil is the primary and most valuable product that comes from the flowers of the Kazanluk rose, and its content is about 0.03 - 0.05%. One kilogram of rose oil could be obtained from 2-3 tons rose flowers (up to 5 tons at unfavorable years).

**White rose** (*Rosa alba* L.) is a perennial shrub with a less branched stem and a height of 2-2.5 m (Atanasova and Nedkov, 2004). It is a stronger and more durable plant and successfully grows on barren and dry soils. The flowers are large, white to creamy or pale pink. The scent is nice. The number of leaflets varies greatly. The most widely distributed forms are with 20-40 leaves in one flower. There are also beehive shapes with a reduced number of 5-10 petals in flower. The white rose gives twice the flowers of the Kazanluk rose, but with a higher percentage of stearoptene content. It grows limited in the Kazanluk region, but it is present in the plantations because of its resistance to fungal diseases. White rose significantly more flowers per hectare but with reduced oil content compared to *R. kazanlika*. A high geraniol content characterizes *R. alba*, and the values of citronellol and neroll are respectively 23.73 % and 6.78 % (Nedkov, 2005).

**Rosa gallica** contains a high percentage of essential oil - 0.083%, but the average flower yield is low. Oil posses a low content of 2.46% of neroll and 4.14% of citronellol but compared to other roses has the highest geraniol content of 38.09%. It is resistant to rust (Nedkov, 2005).

**Rosa centifolia** - the flowers are large, yielding is high (5300-7000 kg/ha), but with a low percentage of essential oil - 0.015-0.04 % (Nedkov, 2005).

#### ***1.4 Composition of the essential oil from the oil-bearing rose grown in Bulgaria***

Bulgarian rose oil is a complex product that is impossible to obtain chemically. It contains over 180 ingredients, divided into two main groups - a liquid part called the eleoptene (the volatile part of the oil) and stearoptene (solid component) (Nikolov, 1977; Kovats, 1987). The stearoptene ratio depends on the essential qualities of rose oil - density, refraction, polarization and hardening point. The quality of rose oil depends on genotype and is significantly affected by environmental and climate conditions. The primary sources for the industry are the flowers of the species *Rosa kazanlika* V. T. and *Rosa alba* L. Traditional Bulgarian cultivars Svezhen and Iskra have been developed by hybridization and selection, while the cultivars Eleyna and Janina, through the chemical and radiation mutagenesis. All of the mentioned cultivars exhibited resistance to critical negative temperatures and increased resistance to rust diseases and black leaf spots.

Flowers yield varies from 4 700 to 10 070 kg/ha. The composition of the essential oil (Table 1) shows a discrepancy in citronellol content between different species and cultivars with a range of 13.18-25 %, geraniol 3.09-7.59 %, neroll 9.25-38.09 %. With the most volatile oil content is the *Rosa gallica* – 0.083%, followed by *R. kazanlika* -0.03-0.05 %.

**Table 1. Composition of essential oil and indicators for species of oil rose grown in Bulgaria, (according to Atanasova and Nedkov, 2004).**

Rose oil composition and indicators	Cultivars of oil bearing rose grown in Bulgaria							
	<i>R. kazanlika</i>	<i>R. alba</i>	<i>R. gallica</i>	<i>R. centifolia</i>	<i>cv. Svezhen</i>	<i>cv. Iskra</i>	<i>cv. Janina</i>	<i>cv. Eleyna</i>
Essential oil content, %	0.030-0.050	0,22	0,083	0.015-0.04	0,054	0,047	0,045	0,052
Flower weight, g	2,39	2,76	1,71	2,87	2,37	2,39	2,4	2,43
Flower Yield kg/ha	5130-10070	7000-10000	4700	5000-7000	7320	6040	5620	6670
Citronellol (20-34%)	25	23,73	13,18	15,61	23,04	19,7	21,09	20,16
Neroll (5-12%)	8,37	6,78	2,76	3,09	7,59	8,37	6,07	7,19
Geraniol (15-22%)	16,78	2,76	38,09	9,25	18,87	17,57	16,61	15,34
Phenylethanol (0.1-0.3%)	0,2	0,25	0,13	0,21	0,25	0,23	0,21	3,64
Hydrocarbons (18-25%)	26,2	22,05	22	25,07	23,9	21,7	19,67	20,5
Paraffins, %	23,05	21,28	29,52	33,34	18,3	24,37	14,32	15,75

The Bulgarian rose oil has a yellow color with a greenish tint and a strong aroma. It is extracted from the flowers by water distillation. Eleoptene is the basic component of the oil that determines the aroma of the oil. It contains citronellol, phenylethyl alcohol, geraniol, neroll. The relatively higher content of citronellol and geraniol and the lower content of phenylethyl alcohol in the Kazanluk Rose, compared to other oil roses, determine the unique smell of Bulgarian rose oil. Stearoptene occupies a smaller proportion (15-23%) of the essential oil composition. Bulgarian rose oil contains mainly eicosane. It is due to the fixing properties of Bulgarian rose oil and its balancing function in perfume compositions.

A valuable product in the process of rose oil manufacturing is the rose concrete, produced in the country since 1906. It has a red-orange color, wax-like consistency, and typical rose aroma. It is obtained by extracting petroleum ether from flowers. One kg of rose concrete comes from about 400 kg of rose flowers. It is used in cosmetics as an effective medicine for skin diseases.

From the rose concrete could be yielded up to 60% rose absolute, which is a reddish-colored liquid with a rose aroma and composition including the main ingredients of the oil - phenylethyl alcohol (about 70%), citronellol, geraniol, and neroll.

Rose water is a secondary product during the manufacturing. It has a pleasant aroma (contains 0.2 % to 1.1 % essential oil), making it also suitable as a raw material for the pharmaceutical and cosmetic industry. Rose water is an active cosmetic for cleansing and toning as well as refreshing and softening the skin. It acts anti-inflammatory, and it is widely used to relieve allergic skin rashes. Rose water is also indispensable for eye irritation, regulation of gastric

activity and it has a mild laxative effect. Processed rose flowers after distillation can be applied as an organic fertilizer, particularly suitable for fertilizing rose gardens.

### ***1.5 Genetic analysis of Kazanluk Rose in Bulgaria***

The study of the genetic resources of Kazanluk Rose in Bulgaria is significant for the characterization of the polyphenol composition. The genetic homogeneity of roses from different regions of the Rose Valley, the village of Mirkovo and the town of Bratzigovo has been confirmed (Ginova, 2015). The results proved that differences in phenotypic and metabolic traits are due to the influence of climatic conditions in the area (Rusanov et al. 2009). Similarly, studies with RAPD, AFLP, and SSR markers did not detect polymorphism among the *R. damascena* plants grown in Isparta, Turkey (Agaoglu et al., 2000; Baydar et al., 2004). In Bulgaria, available genetic resources of the oil-bearing rose have been characterized by molecular markers (Rusanov et al. 2009; Ginova, 2015). Their results show a shallow level of biodiversity in the Kazanlak rose, and it is explained by prolonged clonal selection. Microsatellite analysis of *R. damascena* varieties demonstrated that: (1) the old European Damask roses and oil-bearing roses cultivated in different geographic regions have identical microsatellite profiles and originate from a common ancestor; (2) the oil rose cultivation is based on very narrow gene pool; (3) genebanks maintain a large number of accessions that show identical genotypes; and (4) the long-term vegetative propagated Damask roses possess high somatic stability of the microsatellite loci (Rusanov et al. 2009).

### ***1.6 Rose oil production and market***

The historical chronicles in the past have spoken of Bulgaria as the "country of roses." The name of our country now is almost emblematic associated with the Bulgarian rose oil, characterized by very high quality and as the most expensive. Its international market price varies from 3000-4000 euro/kg to 5000-6000 euro per kilogram (in 2017). With an annual production of 5-6.5 tones (2016-2017), Bulgaria today occupies about 40% of the world market share of rose oil. Almost 99% of the output is export. With its unique composition of the rose oil, Bulgaria is currently the world market leader regarding quantity and quality, followed by Turkey. Moreover, 90 % of the rose flowers are processed to rose oil production, 5-6% to rose water, 3-4% to rose concrete and only a small amount used by the food industry for the production of jams and liqueurs.

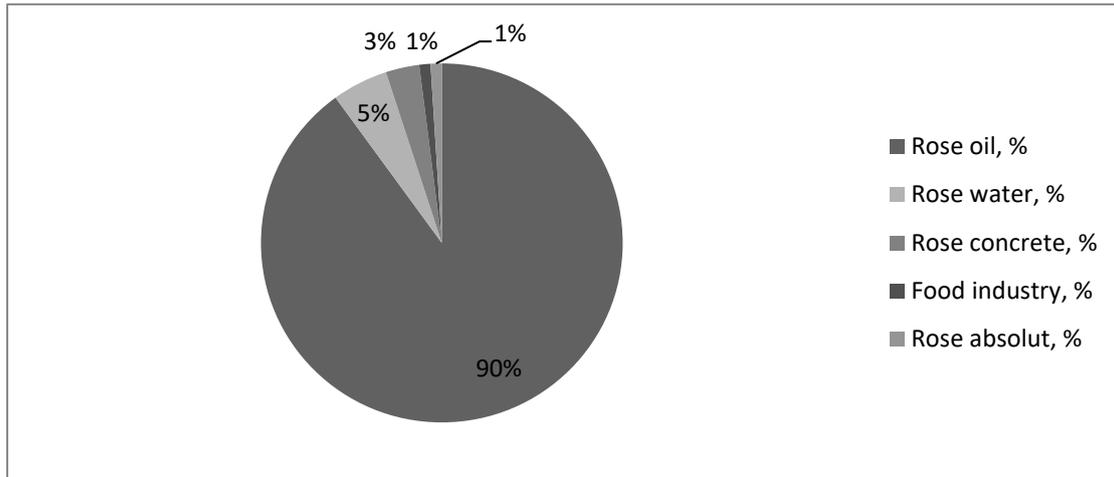


Figure 1. Products in rose flower processing, according to Terziev, 2006.

The area of rose-bearing oil in Bulgaria increased over the past ten years. Reduction of production is observed in years when renewing declining area of old plantations. The total area in 2017 is nearly 34498 ha - 15 % more on an annual basis and 25 % more compared to 2009 (Fig. 2). The annual production of Bulgarian rose oil during decade ranged between 870 and 2000 kg according to the annual report of the Ministry of Agriculture and Food in Bulgaria. The growing investor interest is dictated by the steady growth in the prices of rose oil and the cost of the rose flowers. In the quest for rapid planting, not always certified planting material is used. In some farms, *Rosa centifolia*, which has bigger flowers but less oil content, is planted.

Over 30 companies and more than 50 distilleries produce and trade with rose oil. The expectation is that the total production in the future to exceed 30 tons. Theoretically, the production potential of the country is twice bigger.

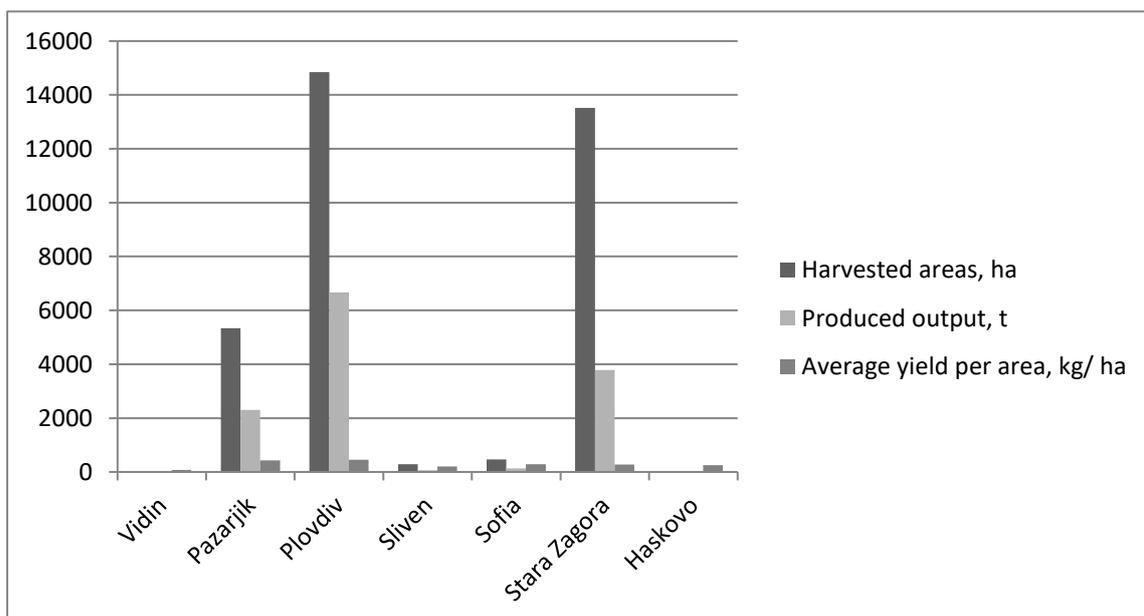


Figure 2. Distribution and allocation of rose growing areas (ha), produced output (t) and average yield per area (kg/ha) according to Ministry of Agriculture and Food in Bulgaria (2009).

Rose industry in Bulgaria involves 65 000 employees, as most of them are seasonal workers. The harvesting of rose flowers starts in May, and it is the most labor consuming process in the rose industry. These are also the most decisive factors influencing the overall production and price of the fresh rose flower as well as rose oil.

The relatively constant level of the world consumption of rose oil over the years makes the rose market highly competitive for both traditional and emerging producers. The diversification of the rose flower products could provide new opportunities for the industry as rose jam, liqueur, and dry flowers for tea.

## **2. Production of lavender and lavender oil in Bulgaria**

### ***2.1 Cultivars of lavender and growing conditions in Bulgaria***

Lavender is one of the most common essential oil-bearing crops in the world, grown for essential oil, for dry lavender flower, and decorative purposes.

At the beginning of the 20th century, other essential oil-bearing plants - lavender, peppermint, balm, basil, and others were introduced to the country. Progressively, a whole branch of plants bearing essential oils and medicinal plants became of industrial significance. Manufacturing refineries have been built for the extraction of oils, plant extracts and biologically active substances. It is widely used in cosmetics, pharmaceutical, perfume industry, plant protection in organic production, suppression of methane production from farms (*L. latifolia*) (Zheljazkov et al., 2013).

In Bulgaria, lavender is widely spread. Due to cross-pollination and seed propagation, there is significant biodiversity resulting in plant morphology oil quality. Old plantations established by seeds propagated material are low-yielding, and with low oil quality and quantity. Lavender is a perennial plant, and it grows for 25-30 years, so the choice of the place is critical to creating high-yield industrial crops. One of the critical factors for high productivity of lavender is the soil pH (the best are alkaline and neutral soils).

Lavender breeding is conducted towards the improvement of the content of essential oil and linalyl acetate. As a result of the lavender breeding research in Bulgaria, seven cultivars have been created of with five by hybridization as Karlovo, Sevtopolis, Hemus, Druzhba, Jubileyna and two through chemical mutagenesis as Raya and Hebar (Topalov, 1979; Staikov and Boyadzhieva, 1989; Raev and Boyadzhieva, 1988). The high content of linalyl acetate is an indicator of the excellent quality of the lavender oil. Linalyl acetate content in the lavender oil extracted from different Bulgarian cultivars was found to be to 50%, comparing to the standard (up to 30% of linalyl acetate). Moreover, of great importance for the quality of the essential

oil is the ratio between linalyl acetate and linalol, which for the Bulgarian lavender oil is 1:0.7. Linalol in the Bulgarian cultivars is within the range of the standard (from 22 to 34%).

### ***2.2 Composition of the oil extracted from different lavender cultivars***

Cultivars grown in our country have different applications (Table 2). Among them, Hebar demonstrates the highest yield (7550 kg/ha), with the essential oil content of 2.22%, and with more than 60% of linalyl acetate content (Nedkov et al., 2005; Zagorcheva et al., 2016). With the lowest yield is cultivar Jubileyna, suitable for dry flower production - 5600 kg/ha, with an essential oil content of 2.86% and 55% of linalyl acetate content (Nedkov et al., 2005).

**Table 2. Oil Composition and yield from different lavender cultivars (Atanasova and Nedkov, 2004).**

<b>Oil Composition from different lavender and lavandin cultivars</b>				
<b>Cultivar</b>	<b>Flower Yield kg / ha</b>	<b>Essential oil content, %</b>	<b>Linalylacetate, %</b>	<b>Application</b>
Hemus	5600	1.6	61.2	Essential oil
Jubilee	5540	1.9	52.8	Dry flower production
Sevtopolis	6250	2.1	49.2	Essential oil
Druzhba	6370	1.9	52.8	Essential oil
Karlovo	6900	1.3	76.9	Dry flower production
Hebar	7550	2.3	43.5	Essential oil
Raja	6370	2.8	34.7	Essential oil

### ***2.3 Lavender oil production and market***

Until recently, France was the leading producer of lavender oil in the world. However, due to long-standing problems related to the spread of diseases and pests, after 2006 the country gradually ceded leadership to Bulgaria. Since the 70s of the 20th century the areas in Provence - the emblematic region of the crop, have periodically been affected by decline, caused by the bacteria *Stolbur* phytoplasma (*Stolbur*). The bacteria are not subject to treatment, and the only measures that can be taken are prevention – fight against the vector (cicada *Hyalesthes obsoletus*), and uprooting of the infected plants (Greibenicharski, 2016).

The first Bulgarian varieties were created in the 60s of the previous century at the Institute of Rose and Essential Oil Cultures in Kazanluk. The planted areas in the country have increased more than three times since 2008 reaching nearly 9600 ha in 2017 (Figure 3 A). Although crop yields are highly dependent on weather conditions every year, a distinct increase in the average values is observed (Greibenicharski, 2016).

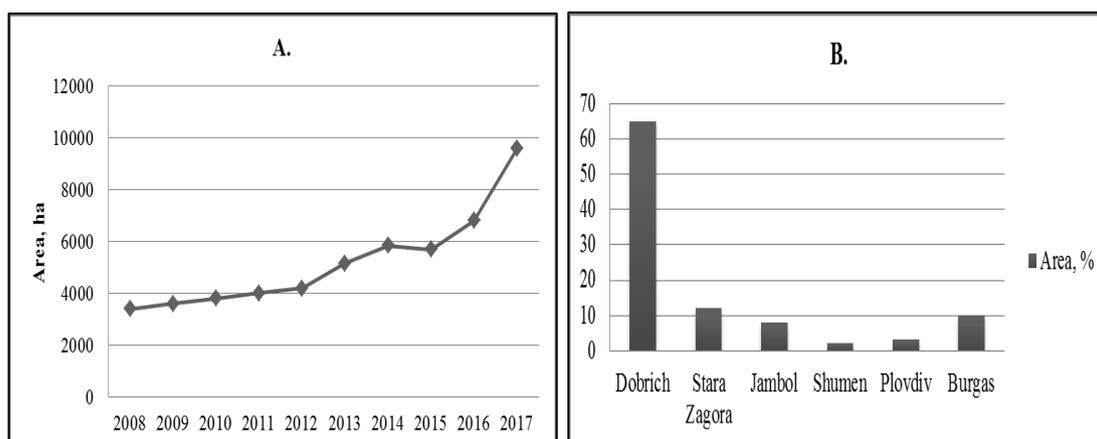


Figure 3. A. Lavender cultivation areas (ha) and B. distribution area by districts in Bulgaria (2016-2017), (according to InteliAgro, 2017)

The increase in the green mass productivity is mainly due to the spread of the crop on more fertile soils in Dobrudzha and Thrace, where the growth and formation of clusters are considerably more intense (Fig. 3 B) (InteliAgro, 2017).

Dobrich (Dobrudzha) has gradually become the most significant production region in the country, overtaking traditional Southern central areas. Prospects for high yield attract many grain producers to include lavender growing in their business. The number of farms in the industry reaches 1600-four times more than in 2017. Only for a year - between 2016 and 2017, the number of producers in Dobrich region doubles, to reach the 640 farms. The technology for flower processing by steam distillation is not expensive, which allows small and medium-scale farms to produce essential oil.

Lavender is among the most popular organically grown crops in Bulgaria which is also encouraged by the relatively slight difference with the conventional technology. Almost half of the areas in the country are organically grown, two-thirds of them being in a period of transition (Grebenicharski, 2016). Since 2015, the growth rate of exceeded by 16%, and the number of organic producers increased by 50%.

Regardless of Bulgarian traditions in the pharmaceutical and cosmetic industry, the domestic consumption of lavender essential oil plays a rather supplementary role, and the retail sales are symbolic. In this situation, nearly the whole production is for export. France is the primary market, with almost half of all trading for period 2012-2015 and more than threefold increase compared to the previous four years (Grebenicharski, 2016). Other fast-growing destinations are the United States, Germany, China, and Japan. The growth areas and production in Bulgaria will inevitably lead to pressure on prices, especially when young plantations reach their full productivity. Increasing the competitiveness of the sector requires that the future support policies shift their emphasis to mechanisms encouraging investments.

### 3. Production of Mint oil in Bulgaria

### 3.1 Cultivation of mint in Bulgaria

Two forms of mint - Black or English mint (*Mentha piperita* f. *rubescens*) and white or French mint (*Mentha piperita* f. *palescens*) are grown in Bulgaria. Black peppermint is widely spread in Bulgaria, giving a high yield, but with essential oil, weak in esters and menthol. The white peppermint yields 30000 kg/ha of green leaf, 3000-5000 kg/ha of dry leaves, about 90 kg/ha of essential oil with an average mint content of 37% (Topalov, 1989; Nedkov et al., 2005).

For many years in our country, grown *Mentha piperita* population was characterized by high oil quality, but it had some disadvantages such as rust sensitivity and an extended vegetation period. These disadvantages have been overcome with the establishment of cultivars Tundgzha and Zeffir, both with excellent indicators for quality (Table 3).

**Table 3. Economic indicators for mint cultivars (Atanasova and Nedkov, 2004)**

Cultvar	Yield, kg/ ha	Oil content kg/ha	Menthol, %
Tundgzha	3000-5000	80-90	32
Zeffir	2500-5500	90-120	58

In medicine, both the oil itself and the pure menthol have some applications and drug preparations. Menthol causes contact with nerve endings feeling cool, acting painless, vasodilating, irritating the mucous membrane of the intestine and stomach and enhanced peristaltic (Dimitrova, 2014). Menthol is part of some antiseptic medicines, for the treatment of cardiovascular diseases (validol), anti-migraine preparations, nasal drops, inhalation mixtures, toothpaste, etc. Dry mint leaves find broad applications in the herbal tea compositions with tonic effect on the nervous system (Atanasova and Nedkov, 2004).

### 3.2 Mint production and market

The yields of dry leaf and the mint essential oil depend on the specific soil and climatic conditions and the applied technology (Nedkov et al., 2005). Both Bulgarian cultivars Tundgzha and Zeffir have approximately same indicators regarding yield per hectare (Table 4).

**Table 4. Comparison of Yields on the first and second year of cultivation (Nedkov et al., 2005).**

Yield (kg/ha)	First year of cultivation	Second year of cultivation
Fresh mass	8000-10000 (15000)	30000-45000
Dry leaf	800-1000 (1500)	3000-5000
Dry stalk	2000-2500	8000-10000
Essential oil	30-50	70-90

Over the last decades, the European market has turned to a mint leaf for the production of tea, alone or combined, that act refreshingly products and

drugs for nervous remedy. Accordingly, mint production was oriented towards a more extensive production of dry mint. Cultivation of mint requires specific knowledge and experience. The culture is moisture-friendly, and has requirements for soil, heat, and light, as it does not tolerate colds and shading. Mint investment is tempting, lucrative enough, but also requires relatively large planting costs.

### **1. Production of *Melissa officinalis* oil in Bulgaria**

Lemon balm (*Melissa officinalis* L.) is naturally growing along streams and forests in Bulgaria. During the period 1978-1982, more than 130 accessions of lemon balm were studied and selected for high contents essential oil, vitamin C, caffeic acid, folic acid, and saponins. The main components in the essential oil from balm are citronellal, citronellol, caryophyllene, and geraniol. The plant has a strong citrus aroma. The popularity of the lemon balm has grown significantly over the last years. The planting area reached in 2017 over 20000 ha as a result of the market changes (Nedkov et al., 2005).

Production up to 30% is coming from the Dobrich region, where more than 60 farmers have declared cultivation first in 2016, and others 90 in 2017 (InteliAgro, 2017). The remaining 70% of the areas are distributed in the South Central Region and Eastern Bulgaria - Plovdiv, Stara Zagora, Shumen, Yambol, Sliven, and others.

Prices for export of melissa oil have been stable over the last three years - 2000 euros/kg. In the domestic market, traders bought the oil at about 3000 USD in 2015 and between 3300-3900 USD/kg in 2016 and 2017.

### **2. Production of medical chamomile in Bulgaria**

Chamomile is another booming essential oil crop in Bulgaria, widely used as well as a herb.

Medical chamomile (*Matricaria chamomilla* L.) is an annual plant of the Asteraceae family. The coloration of the essential oil can identify two types of chamomile - chamazulene type with intense blue coloration and bisabolol type with yellowish-brown coloration. In our country is grown Lazur cultivar, whose yields reach 1140-1200 kg/ha. The essential oil yield is 0.8% with blue color and chamazulene content 14-18%.

In 2016, following several years of the relatively constant area, chamomile plantations increased almost three times. The interest in culture continues, and the area increases by 80% in 2017, surpassing 11000 ha, cultivated in about 120 farms. The increase in 2016 is due to the Plovdiv district, where the number of producers expanded, and the plantations increased three-fold, according to SFA – area applied for direct payments. Meanwhile, the crops in Dobrich have increased seven-fold in 2017, making the region the second most abundant producer. Stara Zagora and Varna are also joining this trend. The price of chamomile oil varies between 800 and 1000 euros/kg (export) and about 1500 euros for certified organic products that are used in medicine (Nedkov et al., 2005).

### 3. Production of White oregano in Bulgaria

The genus *Origanum* includes 13 species and subspecies that are not clearly defined botanically. Selection is directed to increasing the content of essential oil in the upper part during flowering. Gas chromatographic analyzes show the main components: carvacrol, cymene, cineol, terpineol, alpha- and beta-pinene (Tab. 4). White oregano (*Origanum vulgare*) has a high quantity of polyphenols, tannins, anthocyanins, and flavonoids – all of which make it an excellent anti-cancer and anti-aging essential oil. White oregano is also known as a spice for meat and fish cans. The specific spice character of the aroma is due to the thymol and carvacrol, which make up 47-53% of its oil.

The essential oil contained in the entire fresh plant is approximately 0.5 to 0.8 %. Inflorescences are three times richer in oil than leaves (up to 2%). In medicine, it is used as an antiseptic and sedative remedy. Oregano soothes coughing in acute bronchitis, used in the stomach and intestinal disorders, and also in nervous excitement (Popova T., 2016).

**Table 4. Economic indicators for white oregano cultivars (according to Atanasova and Nedkov, 2004).**

Economic indicators	Cultivars of white oregano		
	Hebros 43	Hebros 55	Hebros 64
Oil Yield, kg/ha	36.2	32.7	37
Oil content on the 3rd year, %	1.1	2.4	2.4
Carvacrol, %	67.7	61.8	67.4
para-Cymene, %	11.1	8	8.8
Cineol+terpineol, %	4.5	4.3	6.5
α- and β-pinene, %	5.5	5.8	4

### 4. Production clary sage oil in Bulgaria

*Salvia sclarea L.* is grown in Bulgaria for the production of oil and serves as an excellent honey plant. Two cultivars Thrakiyika and Boyana have been developed in the Institute of Rose Essential and Medical Cultures – Kazanluk (Atanasova and Nedkov, 2004).

The cultivar Thrakiyika has branched stem and dark-violet colored flowers. The yield of inflorescences in the second year is an average of 14800 kg/ha. It contains an average of 0.45 % essential oil. The oil yield is 40-50 kg/ha with 81.56 % linalyl acetate and high perfume grade.

Boyana cultivar has been selected with broom type of inflorescences, and the petals are pale pink. The yield of inflorescences in the second year is an average of 8150 kg/ha. It contains an average of 0.38% essential oil. The oil yield is 30-35 kg/ha (Atanasova and Nedkov, 2004).

Linalyl acetate has anti-inflammatory, analgesic and antihypertensive effect. It is good on skin as it reduces skin inflammation and heals rashes. It also helps to balance natural oils in the skin, acting well on both dry and oily

skin making it look beautiful. The oil can be used directly or mixed with carrier agents like almond oil for complete absorption and to achieve better results.

Maximum oil content for clary sage is obtained when all of the flowers have reached maturity. Like most of the oil-rich plants of Lamiaceae family, the maximum oil content of clary sage is achieved at full flowering, because the calyces contain the essential oil per unit area (0.50-1.20 %). *Salvia sclarea* oil was found to be rich in linalyl acetate (65-68 %), linalool (16-17 %), acetic acid and sclareol (0.9-1.7 % in different plant organs) (Atanasova and Nedkov, 2004).

The yield of sclareol from plant material as well oil yield are critical for the producers to make a profit. The costs of oil production are high because the crop is in the land for ten months for farming.

The average yields in Bulgaria are 8000-10000 kg/ha fresh or 1800-2000 kg/ha dry leaves and about 200-300 kg seed per hectare in the seed plots. The maximum yields are 25000 kg of fresh weight per hectare or 5800 kg of dry leafy dough and 500-600 kg of seed per hectare. In the first year yields were lower (15).

### **5. Production of basil in Bulgaria**

Basil (*Ocimum basilicum* L.) cultivation directions are for extracting essential oil and for dry drugs. The content of essential oil in the plant is 0.3-0.4 % and in the dry drug mass is around 1.2-1.5%. The oil is rich in methyl chavicol or linalool (up to 50%), cineol, eugenol, geraniol, myrcene, pinene and other terpenes, tanning substances (up to 5%), mineral salts, organic acids and others. Ordinary basil is used in the pharmaceutical and perfumery industry, and as a spice in the culinary industry, in the canning, and the meat processing industry.

Essential oil and dried drugs have application in both formal and traditional medicine. Basil oil has a proven sedative, antimicrobial, and anti-inflammatory action.

In our country is grown mainly in Southeastern Bulgaria - Plovdiv, Stara Zagora, Yambol districts. The yield of fresh raw material can reach 30-40 t (average 15-20 t/ha), and essential oil - 120 kg/ha (average 60-100 kg/ha) (Atanasova and Nedkov, 2004).

### **Conclusion**

Current review elucidates the biodiversity of oil-bearing crops and their long-term traditional application in Bulgaria. The fertile lands of the country preconditioned the cultivation of 120 different agricultural crops. Climate conditions allow valuable production of some essential oil plants. Except for *R. kazanlika*, other essential oil crops do not have high demands on soil and climatic conditions. The growing of oil-bearing rose is not limited only in the Rose Valley. But because of its specific climatic conditions, produced rose oil has unique chemical composition. It is a standard of quality, which means that

the price on the international market is determined annually besides the demand and the quality of the Bulgarian rose oil. In this respect, Bulgaria has not reached the potential of growing essential oil crops.

Cultivation of aromatic plants is supplemented with the establishment of refineries in these regions. In recent years, for example, many farms have been created for the production of essential oil crops. The technology for flower processing by steam and water distillation is not expensive, which allows small and medium scale farms to produce oils. Last few years production capacity has increased considerably with investments from the government or foreign investments in the sector.

Bulgarian essential oils have applications in cosmetic, modern medicine and pharmaceuticals, food industry, plant protection. Their cultivation is suitable in low productive regions of the country as alternative cultures.

The main problem is the lack of labor, especially in rose-growing and rose harvesting. The challenge for the production of essential oils in our country is the introduction of varieties with higher content of valuable ingredients. Developing programs for stimulation planting and training of farmers is necessary based on mechanization of the production process and safety control of diseases and pest.

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# Evaluation of rural landscape recreation quality in metropolis: Case study in Shanghai

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## Abstract

The evaluation of rural landscape recreation quality is now widely recognized as an important phase for rural landscape planning and management, the rural landscape in metropolis now plays a more important role in the aspects of recreation and ecology than before. The purpose of this research is to construct an evaluation method of rural landscape recreation quality in metropolis mainly by combining AHP (analytic hierarchy process) method and VRD (vector resemblance-degree) method together. Firstly, the evaluation index system was constructed according to AHP method, including four levels, namely, target level A, rule level B (rural landscape ecology quality B1, rural landscape aesthetics quality B2, and rural landscape facilities quality B3), index level C (11 indicators), and index level D (26 indicators). Then, the weight of each index was calculated by VRD method objectively. Finally, the value of each index of rural landscape recreation quality was confirmed. Results of applying evaluation method of rural landscape recreation quality to a case study in Songjiang district in Shanghai showed that the rural landscape recreation quality among 10 towns of Songjiang district were greatly different from each other, the recreation quality of rural landscape was directly correlated to rural landuse types, natural landuse and agriculture landuse were the fundamental elements for rural recreation. Aesthetics quality was positively correlated with ecological quality. This methodology can be applied to other areas in order to rank and explain the quality of rural landscape for recreational purpose.

**Key words:** Rural landscape; landscape evaluation; recreation quality; Shanghai

## 1. Introduction

The term 'landscape' has been used with many different meanings over years, including nature, territory, geographical area, the environment, a system of systems, habitat, backdrop, environment, and the surrounding area (Pastor et al. 2006). In the 18th century, landscape was used to describe the natural landscape as a painting term, the meaning of which was a painting to portray natural landscape of internal land. Later on, it referred to the painting objective, namely natural landscape and rural landscape.

In terms of geography, landscape is an integration of nature, geography and earth's surface vision, such as urban landscape and agricultural landscape. In landscape ecology, landscape is defined as a region of high spacial

heterogeneity, which is composed of mosaics (ecosystem), and repeats in similar forms (Forman and Godron 1986).

As to ecology, landscape represents scale units in ecological system. Whereas, for landscape architecture, landscape is considered as the complex geographic entity linking nature and culture in all scales, which combined science and technology with art, including the relationship between human and environment (Simonds 1990; Che 2003).

According to landscape attributes which are affected by human, landscape can be classified into two major types. The human landscape includes residential areas, roads, railways, and other human-made elements (Blankson and Green 1991). And the biological landscape (De Agar et al. 1995; Bailey 1996; Bernert et al. 1997) includes climate, soils, vegetation, fauna, water and landforms. While, landscape components can be grouped into three major categories according to the attributes of landscape: (1). physical---landforms, soil surface, watercourses, et al. (2). biotic---natural and spontaneous vegetation, fauna. (3). human activities in natural landscapes (Pastor et al. 2006).

Six features of landscape can be summarized finally. First, landscape is a special structure of topography, vegetation, land-use and human living pattern. Second, it is a heterogeneous mosaics of interactive ecosystem. Third, landscape is a regional whole system integrating human activities and land. Forth, landscape is an extended structural level based on ecosystem. Fifth, landscape is some pix in remote sensing images. Last, landscape is a scenery, whose aesthetics value is decided by nature and culture (Berg and Wintjes 2000; Marc 2004; Claval 2005).

Rural landscape is one of the important landscape types appearing early and distributes broadly in the world, possessing special landscape behaviors, forms and intensions. It is a regional assembling pattern from scattered rural houses to town areas providing food production and other services to citizens. Furthermore, compared with urban landscape, it is also a region with rough land utilization, small population densities and distinct rural characteristics (Wang and Liu 2003; Claval 2005).

The main difference of rural landscape between other landscapes is its agricultural production economic function, rough land-use, and there is domestic rural culture and life styles in countryside. Rural landscape mainly includes rural agriculture landscape (farmlands), rural residential landscape (villages and towns) and natural landscape (forest, water, wetland, grassland, et al.).

According to human productivity, rural landscape can be classified into three phases, namely rural landscape in agriculture period, rural landscape in industry period and rural landscape in post-industry period. The rural landscape in different countries varies in different phases due to the imbalance of development throughout the world. For developed countries such as most

European countries and north America, they generally have been entering into post-industry period. Most developing countries including China, however, are in their industry period and some poor regions are still in agriculture period (Bastian 2000; Bills and Gross 2005).

### ***1.1 Values of rural landscape***

The values of rural landscape include production value, ecology value, aesthetics value, travel value and spirit value.

Production value: human beings obtain their basic life materials from agriculture directly, so the rural landscape is firstly based on the production function of agriculture.

Ecology value: as the fundamental fact of rural landscape, the ecology value of rural landscape presents many aspects, such as ecological diversity, landscape abundance and harmony of all kinds of ecological elements.

Aesthetics value: it is assumed that rural landscape has an intrinsic or objective beauty, while it is a subjective response of the observers (Shuttleworth 1980). It includes land-use forms, natural phenomena, birds songs, sunsets, farmer's work, et al.

Travel value: rural environment is not only the site for people to work and live, but also the objective of recreation for benefits of tourists.

Spirit value: rural landscape represents the happiness, sadness, hope and pursuits of farmers and embodies human being's history from our ancestors to our generations. It represents the memories which imbed inners of human. It is human being's nature to exploits the original spirits (Berg et al. 2000; Zhou and Chen 2005; Wang 2006).

### ***1.2 Features of existing rural landscape evaluation methods***

The process of landscape evaluation is currently recognized as a powerful, interdisciplinary and environmental research method (Pastor et al. 2006). According to the differences of rural landscape evaluation objectives, there are at least three rural landscape evaluation approaches.

The first approach, known as rural landscape comprehensive evaluation, has been used in many rural landscape evaluation studies to measure the rural landscape functions which provide agricultural products, ecological services and tour resources as well as maintaining ecological balance and improving local culture value (Gómez-Sal et al. 2003; Xie 2004). The general purpose is to assess social, economic, ecological and aesthetic functions of rural landscape appropriately, to identify problems that might exist in the present and to point out potential developing opportunities (Mendoza and Prabhu 2000; Pannell and Glenn 2000). Some researchers proposed an integrated, ecosystem-based methodology for sustainable environmental planning and management, involving ideas of the ecosystem approach to plan (Armitage 1995) and the agro-ecosystem analysis approach (Altieri 1987; Conway 1991).

The second one, rural landscape, is restricted to its visual properties, including human-made elements and physical and biological resources

(Daniel et al. 1983; Amir and Gidalizon 1990). The rural landscape evaluation is defined as “the comparative relationships between two or more landscapes in terms of assessment of visual quality” (Laurie 1975). Rural visual landscape evaluation can be applied to rank or explain the scenic beauty of landscape and can enrich the decision-making process for rural landscape integrated management purpose and for rural landscape recreational planning.

Two main approaches are applied to evaluating rural visual landscape: direct method and indirect method. The direct method compares the scenic preferences of members of the public for landscapes in order to reach a consensus (Arthur et al. 1977; Briggs and France 1980). The indirect method evaluates the landscape on the basis of the presence and/or intensities of designated features (Fines 1968). The indexes include ecological, formal aesthetic, psychophysical, psychological and phenomenological ones (Daniel and Vining 1983). Finally, the method of rural visual landscape evaluation can be divided into five categories: direct models, models to predict public preferences, indirect models, mixture models and economic evaluation models.

The third approach takes account of sustainable land use of rural landscape. Sustainable land use evaluation emerged in the 1990s, which paid attention to the social, economic and ecological benefits. Evaluation for sustainable land use is regarded to extend land feasibility evaluation at the temporal scale. Gómez-Sal et al. (2003) took into account five aspects: the ecological, productive, economic, social and cultural. Peng et al. (2006) made a contribution to synthetic evaluation at both temporal and spacial scales, by combining landscape ecology with sustainable land use of rural landscape, landscape ecological evaluation for sustainable land use,.

Rural landscape can be used for natural, agricultural, social and recreation purposes. Whereas, Metropolitan rural landscape is close to downtown which generally has relatively high density populations. It is deeply affected by metropolis from land use form and function to lifestyles and culture et al. The main functions of metropolitan rural landscape are natural preservation, ecological maintenance and rural recreation. More and more urban people desire to get close to rural landscape for recreation purpose. Therefore, the evaluation method of metropolitan rural landscape recreation quality is different from other rural landscape recreation quality assessment.

Rural landscape recreation quality evaluation can provide significant knowledge for rural landscape recreation planning and management afterwards, and it also plays an important role in sustainable development of the rural landscape in metropolis even the whole metropolis. The main aim of this study is to construct the evaluating method of rural landscape recreation quality in metropolis, providing scientific foundation for further planning and management as well as significant basement for rural landscape sustainable development.

## **2. Methods**

### *2.1 Indicators selecting principles*

The function of rural landscape can be divided into ecology, production, living, recreation and culture et al. Recreation function of rural landscape cannot be independent from other indicators of rural landscape, but it firmly depends on the quality of ecology, nature and agriculture. The evaluation of rural landscape recreation quality must be multi-criteria and multi-dimensional.

Many indicators have been developed to measure the quality of rural landscape recreation. In a survey about measuring the quality of rural areas for recreation, it is important to understand that recreation is a common definition, identifying several forms of recreation (Goossen and Langers 2000).

The evaluation of rural landscape recreation quality requires indicators for different aspects considered. The relationship structure among indicators needs to be known, and they have to be placed in a hierarchy. Once a series of precise and sufficient indicators are conformed to each evaluative aspect, they can be used as a tool to evaluate the system.

For the economic evaluation and prioritization of sustainability indicators in agriculture, a conceptual framework of criteria collection was presented by Pannell and Glenn (2000). Goossen and Langers(2000) classified recreation quality into utilization quality and perception quality. Utilization quality is defined as fitness for use, while perception quality refers to the quality of the environment people notice or experience (such as the beauty of the landscape or tranquility) when engaging in recreational activities. Tranquility, accessibility, water quality and nuisance values are the most important quality indicators. Müller et al. (2000) took a conceptual research for deriving environmental indicator sets, ranging from political target hierarchies and sustainable management strategies to holistic protection concepts such as process protection, resources preservation, ecosystem health and ecosystem integrity. Mendoza and Prabhu(2000) indicated that multi-criteria methods are effective tools that can be used as structured decision aiding to evaluate, prioritize, and select sets of criteria and indicators for sustainable management. Gómez-Sal et al (2003) took into account the independence indicators as different evaluative dimensions and constructed their hierarchical relationships. Then, the multidimensional conceptual models for assessing landscape values was carried out.

To sum up, evaluation indexes of rural landscape recreation qualities are chosen as the following principles:

**1. Multi-criteria:** Although compared with all levels of rural landscape assessment, rural landscape recreation quality evaluation considers more about recreational values of rural landscape. Rural landscape recreation is a certain part of various functions of rural landscape, the recreational content and quality of rural landscape is fully based on the physical, aesthetics, social, cultural and ecological aspects of rural landscape. Therefore, the rural

landscape recreation quality is decided by various components of rural landscape, and multi-criteria is an inevitable choice for indicators. Multi-criteria method is an effective tool that can be used as structured decision aiding to evaluate, and select sets of criteria and indicators for evaluation and management (Mendoza and Prabhu2000).

**2. Multi-objectives:** Rural landscape recreation quality is an objective of rural landscape, however, the recreation indexes are composed of various aspects of rural landscape, such as ecology, visual, social and other man-made elements. So, it is necessary to take into account various factors of rural landscape related to the recreation quality of rural landscape.

**3. Regional dominance:** For any evaluation process, the selection of evaluation indexes must be based on the dominant factors, because in different regions the attributes of ecology, aesthetics, society, culture and economy are different, consequently resulting in different emphases in evaluation. Dominant and typical indexes that are consistent with local conditions must be chosen to make an appropriate evaluation (Peng et al. 2006).

**4. Relative independence:** Factors related to the recreation quality of rural landscape usually correlate with each other. So index selection must be independent to avoid repetitive evaluations for the recreational function.

**5. Hierarchical:** It is very important to classify various indexes into certain layers and construct their hierarchical relationships in orders of different scales. The indexes belonging to different hierarchical layers cannot be mixed.

### ***2.2 Evaluation index system construction***

Firstly, methods of analytic hierarchy process (AHP) to filtrate evaluation index are applied and evaluation index system is constructed (Liu et al. 2003). Then, vector resemblance-degree (VRD) method is adopted, and sample data of systematic capacity index to confirm index weight is used (Jiao and Yang2006). Finally, the method of multi-objective linear function is adopted to calculate comprehensive evaluation standard values and index values of all levels (Song et al.1999; Xie 2004).

The evaluation index of rural landscape recreation quality is decomposed of several concrete targets which can be called 'Rule'. The second layer of evaluation index is obtained by adopting analytic hierarchy process (AHP), optimizing and synthesizing original projects of evaluation index system. The, third and fourth layers of evaluation index can be confirmed in turn.

Target layer is rural landscape recreation quality evaluation.

The second layer is rule layer, including three aspects of rural landscape recreation: ecology quality, aesthetics quality and facility quality.

Rural landscape ecology quality analyses the rural landscape as an ecosystem taken into account the degree to which the landscape maintains basic ecological processes and the environmental services that it provides.

Undoubtedly, ecosystem is the ultimate foundation for the sustainability of rural landuse and rural landscape recreation. However, ecology is not usually considered as a differentiated system, sometime it can be said that the ecology system is “the forgotten aspect” in the rural landscape recreation quality assessment. It includes ecological stable capacity and environmental recreation value.

Rural landscape aesthetics quality mainly focuses on the rural landscape visual property, including man-made elements, such as historical scenic sites, cultural features and traditional knowledge (heritage value.), physical and biological resources (Daniel and Vining1983; Amir et al. 1990). Direct method and indirect method were combined to assess the quality of rural landscape aesthetics (Briggs and France 1980).

Rural landscape facilities quality takes into account rural area infrastructure and recreational facilities, is it convenient to access recreational locations, or is it can provide more recreational locations to travelers. Thus, it includes both the convenient degree of rural recreational facilities and amount of recreational facilities.

The three evaluative dimensions (ecology quality, aesthetics quality, and facilities quality) have enough capacity for rural landscape recreation quality assessment, because they include most of the elements contributing to the values of the rural landscape recreation quality. However, other more abstract dimension, such as the ethic or politic, is not correlated with the quality of rural landscape recreation directly, so it can be ignored.

The third layer is the index layer C, namely each evaluation rule is concretely decided by different factors. The fourth layer is the index layer D, each evaluation factor is expressed by concrete index. The detailed evaluation indexes system of rural landscape recreation quality is shown in Fig. 1.

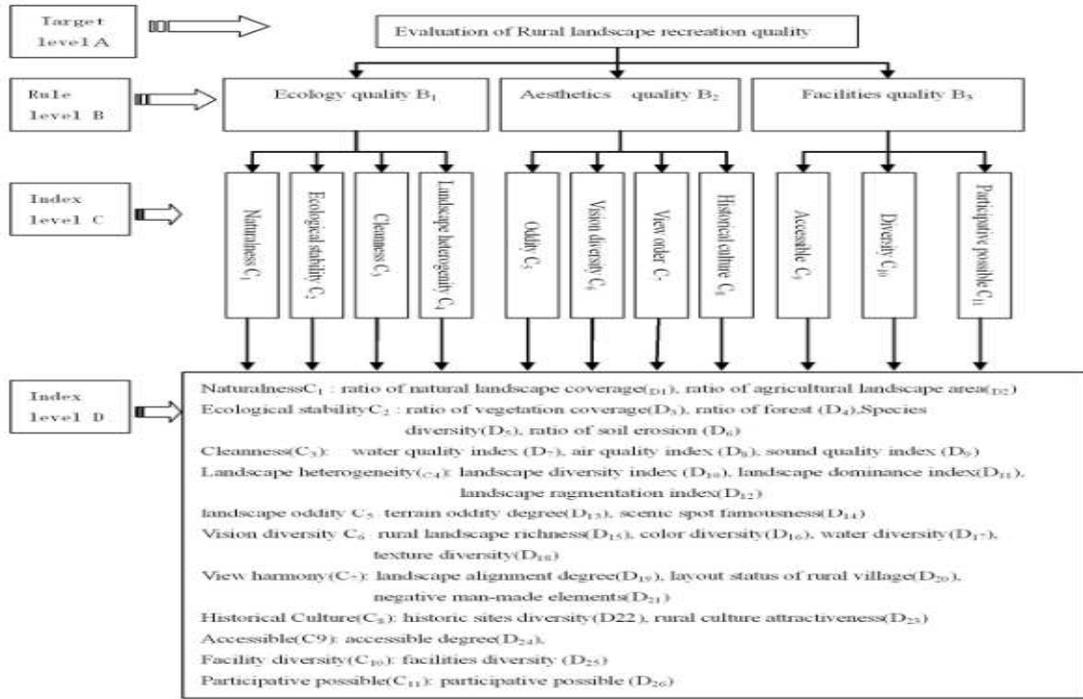


Fig. 1 Evaluation indexed system of rural landscape recreation quality

The calculating methods for original values of level D indexes are shown as follows:

1. Ratio of natural landscape coverage (D<sub>1</sub>):

$$R_n = S_n / S_t$$

Where,  $R_n$ : ratio of natural area coverage;  $S_n$ , all of natural landscape area;  $S_t$ : total of research area .

2. Ratio of agricultural landscape area (D<sub>2</sub>):

$$R_a = S_a / S_t$$

Where,  $R_a$ : ratio of agricultural landscape coverage;  $S_a$ , all of agricultural landscape area;  $S_t$ : total of research area.

3. Ratio of vegetation coverage (D<sub>3</sub>):

$$R_v = S_v / S_t$$

Where,  $R_v$ : ratio of vegetation coverage;  $S_v$ : all of vegetation area;  $S_t$ : total of research area .

4. Ratio of forest (D<sub>4</sub>):

$$R_f = S_f / S_v$$

Where,  $R_f$ : ratio of forest coverage;  $S_f$ : all of forest area;  $S_v$ : all of vegetation area .

#### 5. Species diversity (D5):

$$H = -C \sum_{i=1}^n P_i \log_2 P_i$$

Where,  $P_i$  is the proportion of the number of species  $i$  in total of all species, and  $n$  is the number of species.  $C=1$ .

#### 6. Ratio of soil erosion (D6):

The degree of soil erosion is divided into five grades according to “soil erosion classification standard (SL190- 96), China”, namely, very slight erosion, slight erosion, medium erosion, serious erosion, very serious erosion.

$$R_e = S_e / S_t$$

Where,  $R_e$ : ratio of soil erosion;  $S_e$ : the soil erosion area above medium erosion grade (including medium erosion, serious erosion and very serious erosion),  $S_t$ : total of research area .

#### 7. Water quality index(D7):

Water quality is divided into five grades according to Chinese national standard of “environmental quality standard for surface water (GB3838-2002)”, the first grade is used as standard value.

#### 8. Air quality index (D8):

Air quality index is divided into three grades according to Chinese national standard of “Ambient air quality standard (GB3095-1996)”, the first grade is used as standard value.

#### 9. Sound quality index (D9):

Sound quality index is divided into five grades according to Chinese national standard of “Noise Standard of Environmental Noise of Urban Area (GB3096-93)”, the first grade is used as standard value.

#### 10. Landscape diversity index (D10):

Landscape diversity is the abundance and complexity of patch type in the landscape, mainly considering the number of patch types, and the area proportion of different patch types.

$$H = -\sum_{i=1}^n P_i \ln(P_i)$$

Where,  $P_i$  is the proportion of the rural landscape type  $i$  in patch type  $i$ , and  $n$  is the number of patch types.

#### 11. Landscape dominance index (D11):

$$D = H_{\max} + \sum_{i=1}^n P_i \log_2 P_i$$

$$H_{\max} = \log_2 n$$

Where, D is landscape dominance index,  $P_i$  is the proportion of area of rural landscape type i in area of all rural landscape, n is the number of all rural landscape types,  $H_{\max}$  is the landscape maximum diversity index.

#### 12. Landscape fragmentation index (D12):

Landscape fragmentation indicates the degree of division in the landscape, reflecting the area heterogeneity of landscape patches.

$$FN_1 = (N_p - 1) / N_c$$

Where  $N_p$  is the total numbers of landscape patches in all patch types and  $N_c$  is the total area in the landscape.

#### 13. Terrain oddity degree (D13):

Terrain oddity degree is the degree of how terrain changes, or how different it is from flat terrain. The terrain types are divided as follows: 1.almost flat; 2.slightly wave; 3.hill; 4.some mountains and 5.mountains dominate the scene.

#### 14. Scenic spot famousness (D14):

Scenic spot famousness refers to the famous degree of rural landscape, such as: historical tree (more than 100 years old), national protecting plant or animal, historical site: ancient building, ancient tomb, et al., typical traditional rural village. According to Chinese national standard of “landscape and famous scenic standard (GB50298—1999), it is divided into five degrees: super national grade, first national grade, second national grade, third national grade, and ordinary.

#### 15. Rural landscape richness (D15):

Rural landscape types includes woodland, old rural villages, agriculture, special recreational area, water et al., in a certain area with more types of rural landscape and more beautiful landscape vision. It is divided five grades: <2, 2-4, 4-6, 6-8, >8

#### 16. Color diversity (D16):

Color is very important for vision. Color diversity includes color types, color contrast and color compatibility. Color types: one color, two colors, three or more colors; Color Contrast: weak, certain, clear. Color compatibility: weak certain, clear. The color diversity can be divided into five grades, from worse to better, by integrating all attributes of color.

#### 17. Water diversity (D17):

Water is a significant element for rural aesthetics. Usually the rural landscape artistic quality is affected by water deeply. There are two factors of water diversity, one is water types, mainly including: river, stream, lake/reservoir, marsh, sea et al.

Another is amount of water, it can be assessed by ratio of amount water: <5%, 5-10%, 10-15%, 15-20%, >20%.

18. Texture diversity (D18):

Rural landscape texture appears smooth, undulating, rough, mountainous, rugged, et al. More changeable the texture, more beautiful the vision.

19. Landscape alignment degree (D19):

It refers to landscape orderly degree, usually, mess landscape makes negative visual impression.

20. Layout status of rural village (D20):

Rural village is noticeably obvious landscape in the rural landscape, referring to rural village space disperse patterns.

21. Negative man-made elements (D21):

It refers to man-made landscapes unharmonious with natural or traditional rural landscape, such as industries, power lines, wasteland, polluted area, dustheap et al.

22. Historic sites diversity (D22):

Historic sites refer to ancient and valuable sites, such as ancient house, temple, old town, traditional garden, ancient tomb et al.

23. Rural culture attractiveness (D23):

It refers to rural landscape abundant degree, such as traditional festival, nationality life styles, nationality religions, local special culture et al.

24. Accessible degree (D24):

It is easy to access rural landscape for recreation; it is decided by the traffic condition. It can be divided into five degrees: very difficult (>2hours) difficult (1.5-2hours), medium (1-1.5hours), easy (20-60'), very easy (<20').

25. Facilities diversity (D25):

The diversity of man-made facilities for recreation, such as: rural park, DIY centre, rural club, playing field, rural museum, folk-custom holiday village, ancient and traditional village, camp area. It can be divided into five degree: <2, 2-4, 4-6, 6-8, >8

26. Participative possible(D26):

It refers to the opportunities that tourist can take part in the travelling process, for instance, working in farmland, boating, fishing, swimming, bicycling et al..

All indexes of level D can be divided into quantitative indexes and qualitative indexes.

For quantitative evaluation indexes, national first grade environment standard is used for standard values of ratio of soil erosion (D6), water quality index (D7), air quality index (D8), sound quality index (D9). Other quantitative evaluation indexes are confirmed by the standard of maximum value (positive polarity index), minimum value (negative polarity index), or middle value of certain index in the similar region around the country. They are ratio of natural landscape coverage (D1), ratio of agricultural landscape area (D2), ratio of vegetation coverage (D3), ratio of forest (D4), species diversity (D5), landscape diversity index (D10), landscape dominance index (D11), landscape fragmentation index (D12), accessible degree (D24), facilities diversity (D25), participative possible (D26).

The qualitative indexes are confirmed by the expert consultation method(panel), they are: terrain oddity degree (D13), scenic spot famousness (D14), rural landscape richness (D15), color diversity (D16), water diversity (D17), texture diversity (D18), landscape alignment degree (D19), layout status of rural village (D20), negative man-made elements (D21), historic sites diversity (D22), rural culture attractiveness (D23). Firstly, each index is divided into five grades, namely A, B, C, D and E. The quotiety of each grade is 1.0, 0.8, 0.6, 0.4 and 0.2. Secondly, each expert of panel (ten people) makes the score for each qualitative index. Finally, the value of each index is calculated according to the following formula:

$$V = \sum_{i=1}^D Q_i / N$$

Where, V is the qualitative indexvalue;  $Q_i$  is the quotiety value of ith index made by each expert; N is the number of experts; D is the number of indexes.

### ***2.3. Confirmation of Evaluation Index Weight***

It is crucial to provide scientific and rational evaluations to confirm the weight coefficient of each evaluation index in the process of comprehensive evaluation (Jiao and Yang2006).

The weight of each evaluation index was calculated by VRD method. It makes use of sample data of systematic capacity index to calculate index weight, so it is objective and practical, avoiding the personal ingredients.

Steps of weight-calculation by VRD method are showed in Fig. 2.

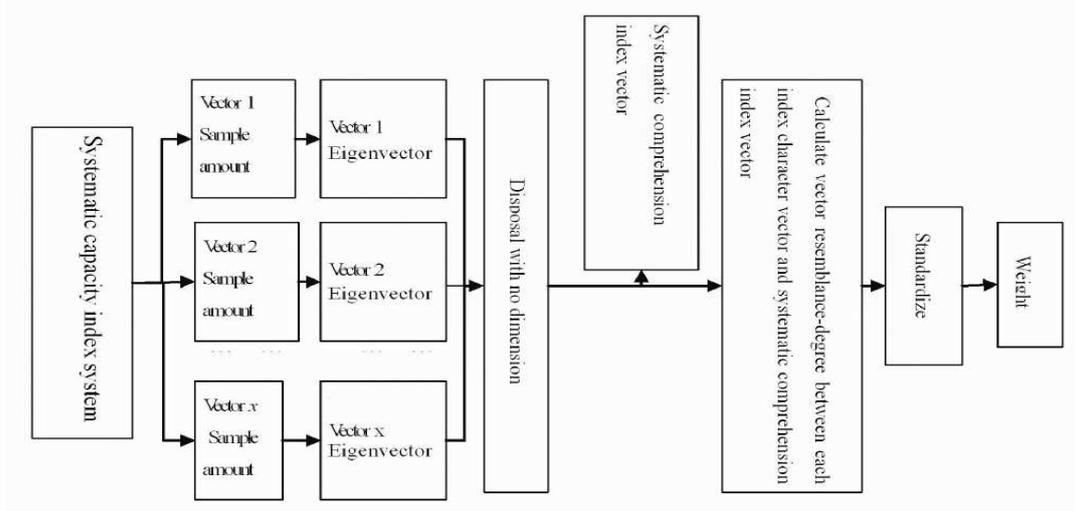


Fig. 2 Processes of weight-calculation by vector resemblance-degree

**The details are listed as follows:**

1. Set up systematic comprehensive index vector

Supposing systematic index system has n capacity indexes, and the systematic comprehensive index vector  $M = [m_1, m_2, \dots, m_n]$  was set up according to known data of each index under the standard situation.

2. Take samples of each index

The number of sample (Z) should be enough to reflect the system situation ( $z \geq 4$ )

3. Calculate eigenvector of each index sample

Supposing the Kth eigenvector of index sample is:  $X_k = [x_1, x_2, \dots, x_z]$   
 $x_j$  ( $j=1, 2, \dots, z$ ) is the index value of zth sample in the system

$$X' = \begin{cases} 1.0 - (q_1 - x) / \max \{q_1 - m, M - q_2\}, & x < q_1 \\ 1.0 & , x \in [q_1, q_2] \\ 1.0 - (x - q_2) / \max \{q_1 - m, M - q_2\}, & x > q_2 \end{cases} \quad (4)$$

4. Dispose comprehensive index vector and index eigenvector with no dimension.

Evaluation index is usually divided into maximum index (positive polarity index), minimum index (negative polarity index), middle index and inter scale index.

To maximum index x, let

$$X' = (x - m) / (M - m) \quad (5)$$

where M, m is separately the maximum and minimum value of index X.

To minimum index, let

$$X' = (M - x) / (M - m) \quad (6)$$

To middle index x, let

$$x' = \begin{cases} 2(x-m)/(M-m), m \leq x \leq (M+m)/2 \\ 2(M-x)/(M-m), (M+m)/2 \leq x \leq M \end{cases}$$

(7)

Then, x can be turned into the maximum index with no dimension through formula (5) (6) , (7) .

5. Calculate the resemblance-degree between eigenvector and systematic comprehensive index vector of each index.

Making use of formula (4) to calculate resemblance-degree between  $X_k$  and  $M_k$ , namely,  $\gamma_k$  is the Kth resemblance-degree between index vector and systematic comprehensive index vector. It reflects the contributing extent of the index to whole system efficiency.

6. Confirm weight distribution of each index

Standardize the resemblance-degree  $\gamma_k$  between eigenvector and systematic comprehensive index vector of each index, then the weight  $W_k$  of each index can be obtained.

Four samples were chosen in Shanghai for calculating indexes by VRD method, and all indexes' weights of rural landscape recreation quality were confirmed as follows.

Rule layer B: B1(0.35) , B2 (0.48) , B3(0.17) ;

Index layer C: C1(0.14), C2(0.33), C3(0.28), C4(0.25), C5(0.22), C6(0.35), C7(0.24), C8(0.19), C9(0.26), C10(0.53), C11(0.21)

Index layer D: D1(0.57), D2(0.43), D3 (0.24), D4 (0.27), D5(0.25), D6(0.24), D7(0.38), D8(0.35), D9 (0.27), D10 (0.35), D11(0.34), D12(0.31), D13(0.45), D14(0.55), D15(0.38), D16(0.21), D17(0.28), D18(0.13), D19(0.37), D20(0.31), D21(0.32), D22(0.68), D23(0.32), D24 (1.0), D25(1.0), D26 (1.0)

#### ***2.4. Comprehensive Evaluation Model***

Method of multi-objective linear function is used for reflecting regional rural landscape situation from different aspects by each single index in the evaluation index system of rural landscape recreation quality. Comprehensive evaluation should be carried out to develop from index layer to target layer in order to reflect the whole situation. The following are the detail steps:

The formula of index layer C

$$F = \sum_{i=1}^M (W_i \times D_i)$$

Where, F is the value of certain factor in index layer C;  $W_i$  is the value of ith index in index layer D;  $D_i$  is the weight of ith index in index layer D; M is the number of indexes in index layer D.

The formula of rule layer B

$$I = \sum_{j=1}^N (F_j \times C_j)$$

Where,  $I$  is the evaluation value of certain factor in rule layer B;  $F_j$  is the evaluation value of  $j$ th index in index layer C;  $C_j$  is the weight of  $j$ th factor in index layer C;  $N$  is the number of indexes in index layer C.

The formula of complex evaluation

$$O = \sum_{t=1}^T (I_t \times B_t)$$

Where,  $O$  is the complex evaluation value;  $I_t$  is the evaluation value of  $t$ th factor in rule layer B;

$B_t$  is the weight of  $t$ th factor in rule layer B;  $T$  is the number of factors in rule layer B.

The evaluation results can be gained according to the formulas listed above. Referring to the various grouping methods of comprehensive indexes, evaluation standard of rural landscape recreation quality was established as Table 1.

**Table 1 Evaluation standard of rural landscape recreational quality**

Value of integrative evaluation	> 0.75	0.45 ~ 0.75	0.35 ~ 0.45	0.25 ~ 0.35	< 0.25
Criterion of evaluation	Excellent	Better	Ordinary	Worse	Worst

### 3. Study area and materials

#### 3.1 Study area

Shanghai is well known as the biggest metropolis and the financial, economic center in China. Its total area is 63,400 Km<sup>2</sup>, and its population is more than 24 million. Its population density is highest in China. The study area, Songjiang district is located in the south-west of Shanghai, the total area of which is 604.67 Km<sup>2</sup>, among which 72.54% belongs to rural area, and 12.09% is water area. Sheshan Mountain, located in Songjiang district, is the only mountain in Shanghai, the highest altitude of which is 89.8m. The upriver of Huangpu River, known as the mother river of Shanghai, flows through Songjiang district too. Songjiang county, established in Tang dynasty, A.D.751, has a history of more than 1300 years. Its climate belongs to sub-tropic zone. The typical vegetation is ever-green. Annual average temperature and rainfall are 17.9°C and 939.2mm.

The whole district is composed of 10 towns, Sijing, Jiuting, Dongjing, Xinqiao, Maogang, Xinbang, Shihudang, Yexie, Sheshan and Chedun. The location map of Songjiang district is shown in Fig. 3.

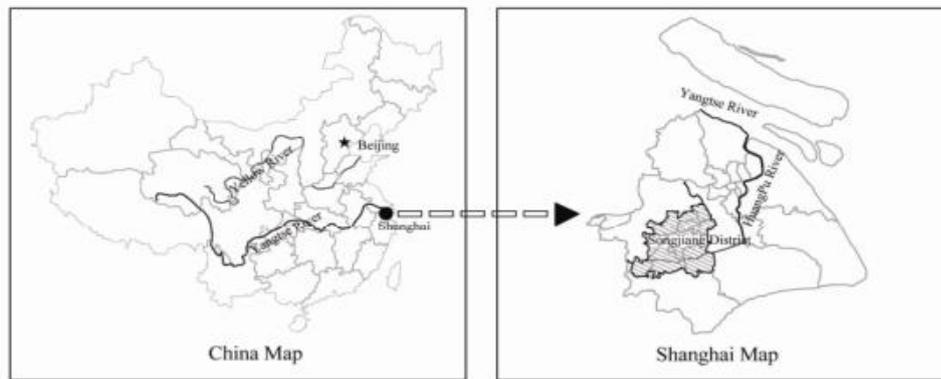


Fig. 3 Location map of Songjiang district in Shanghai, China

Songjiang district is a typical suburb area of metropolis, this area exhibits a notable richness of situations owing to their abundant rural land use types and potential rural landscape recreational values. Actually, more and more urban people spend their weekend and holidays spontaneously there, but the knowledge about the rural landscape recreation quality there is still rare.

### **3.2 Materials**

Based on ERDAS 8.4 software, landsat 5 TM image and Spot image on Sept.15th, 2005, were syncretized and interpreted with reference to field reconnaissance on Oct., 2005, land use digital map of Songjiang in 2009 (scale 1:10,000).

Consequently, the study area was classified into 11 relatively homogeneous landscape units: forest land, crop land, grass land, agricultural land, water area, orchard land, nursery land, greenhouse land, waste land, residential land, industry land. On the basis of the land use classification map from the remote sensed image, the value of the follow 11 indexes—ratio of natural landscape coverage (D1), ratio of agricultural landscape area (D2), ratio of vegetation coverage (D3), ratio of forest coverage (D4), landscape diversity index (D10), landscape dominance index (D11), landscape fragmentation index (D12) --were measured using landscape analysis software, Fragstats2.0.

The values of fellow indexes--ratio of soil erosion (D6), water quality index (D7), air quality index (D8), sound quality index (D9)- were calculated according to the statistical yearbook of Songjiang in 2005.

Species diversity (D5), accessible degree(D24), facilities diversity (D25), participative possible (D26) were calculated according to the investigation during Oct. 2th, 2005 to Oct. 15th, 2005 with reference to the flora of Shanghai in 1999 and tourism yearbook of Songjiang in 2005.

Other values of indexes, Terrain oddity degree(D13), scenic spot famousness(D14), rural landscape richness(D15), color diversity(D16), water diversity(D17), texture diversity(D18), landscape alignment degree(D19), layout status of rural village(D20), negative man-made elements(D21) were

confirmed with the expert consultation method, 10 experts were invited as the member of panel.

#### 4. Results

The values of rural landscape recreation quality of 10 towns in Songjiang district were shown in Table 2, according to the methodology of rural landscape recreation quality evaluation.

**Table 2 Values of rural landscape recreation quality of 10 towns in Songjaing district**

	Ecology quality	Aesthetics quality	Facilities quality	Comprehensive evaluation
Sijing	0.312	0.498	0.511	0.435
Jiuting	0.301	0.152	0.415	0.249
Dongjing	0.482	0.489	0.367	0.466
Xinqiao	0.211	0.323	0.427	0.301
Maogang	0.921	0.814	0.325	0.768
Xinbang	0.765	0.712	0.356	0.670
Shihudang	0.819	0.732	0.611	0.742
Yexie	0.745	0.639	0.561	0.663
Sheshan	0.915	0.842	0.833	0.866
Chedun	0.254	0.358	0.412	0.331

The ecology qualities of rural landscape recreation in Songjiang, Maogang (0.921), Sheshan (0.915), Shihudang (0.819), Xinbang (0.765) are top four. The value of Yexie (0.745) is relative higher, whereas, the value of Chedun (0.254) and Xinqiao (0.211) is relatively lower.

Maogang, Xinbang, Shihudang and Yexie are located in the river-side of Huangpu River, and 70% drinking water supply for Shanghai for daily usage comes from these areas. In order to protect drinking water of Shanghai, 500m forest corridor in width along both sides of Huangpu River has been constructed as a core protective area, and 2000m rural land in width along both river sides of Huangpu River is managed as a buffer zone. Therefore, Maogang, Xinbang, Shihudang and Yexie belong to ecological agriculture area. The agricultural types and scales of those four towns must be consistent with the aim of ecological protection of Huangpu River. Any pollution is forbidden. The social aims of those four towns are cleaning production, ecological protection, rural landscape tourism, and sustainable development.

Sheshan Mountain, the only mountain in Shanghai, is located in Sheshan town, the altitude of which is only 98.8m above sea level, but the average altitude of other areas in Shanghai is 3.5m above sea level. So the ecological value of Sheshan town is relatively higher than other areas of Songjiang District. Meanwhile, Shenshan Mountain is a national forest park and national tourism zone. The vegetation and habitat represents natural features of Shanghai. And the biodiversity is the highest in Shanghai.

Chedun and Xinqiao is the industry zone of Songjiang, large number of rural land has been transferred to industrial land. Agricultural land, industrial land and residential land are mixed together. The environment has been polluted since 1990s, and environmental quality is being degraded. The main types of agriculture are aquaculture, nursery, and greenhouse.

Sijing, Jiuting and Dongjing are managed as residential area, 55% of rural land is used as residential land that is mixed with a few industry land. The ecological qualities of those three towns are degraded along with the population growing, but the average quality of ecology is medium.

In terms of aesthetics quality, the values of Sheshan (0.842), Maogang (0.814), Shihudang (0.732) and Xinbang (0.712) are top four, which are followed by Yexie (0.639). The values of Jiuting, Xinqiao, Chedun are relatively lower too.

The aesthetics value of Sheshan town is the highest, and the reason is that Sheshan Mountain is the only mountain in Shanghai, the sceneries of which are composed by mountain, water, agriculture, rural village and forest. Furthermore, there are many historic landscapes such as famous Catholic Church. As the biggest church in Eastern Asia, it is more than one hundred years old and has astronomical observatory.

As mentioned, Maogang, Shihudong, Xinbang and Yexie belong to ecological agriculture area, through which Huangpu river flows, the types of landuse is diverse, such as cropland, orchard, rural village, forest, water, wetland. The beautiful rural landscapes are composed of diversity water types, abundant vegetation, good agriculture environmental quality, harmonious landscape pattern and landscape texture.

Jiuting is residential area, Xinqiao and Chedun are industry areas, agriculture landuse of those three towns is becoming less and less, the quality of environment is degraded, the types of rural landscape are mess, industry and residential landscape is the domestic landscape. Therefore, the aesthetics of rural landscape is very much degraded.

Although, similar to Jiuting, Dongjing and Sijing are residential area too, great number of rural landuse has been protected, only 30% land is residential landuse, the rural landscape pattern and historical culture are better than that of Jiuting, therefore, the aesthetics quality of those two towns is also better than that of Jiuting.

In terms of facility quality, Sheshan town is highest with value of 0.833. Shihudang, Yexie, Sijing are at better level, their values are 0.611, 0.561 and 0.511, respectively, while others are at normal levels. All traffic is in good condition and the reason is that Songjiang is located at the outskirts of Shanghai metropolis, resulting good infrastructure. As a national tourism zone, Sheshan has rural clubs, playing fields, rural museums, folk-custom holiday villages and camp areas, so the facilities in Sheshan are the best. Besides of good traffic conditions, Shihudang, Yexie and Sijing have some facilities of

rural tourism, such as traditional rural villages, holiday villages, rural clubs, et al. For residential and industry towns, such as Jiuting, Dongjing, Xinqiao and Chedun, the rural recreational facilities are rare because of the descendance of rural landscape. The recreational facilities in Maogang and Shihudang are relative less, because these two towns are located in the upriver of Huangpu River. The main function of these two towns is to protect drinking water source, thus the rural recreational types of those two towns are ecological recreation and ecological education.

As for comprehensive quality of rural landscape recreation, the comprehensive quality value of rural landscape recreation of Sheshan (0.866) is the highest. It is excellent because there are wonderful ecology, clean environment, fluctuant topography, natural vegetation, diversity landscape types, abundant historic culture, and good facilities of recreation. However, with the rural recreational development, more and more high-density villas have been constructed around Sheshan Mountain in recent years, seriously damaging the rural landscape in Sheshan and thus needing to be controlled.

The second one is Maogang (0.768), still at an excellent condition. The comprehensive quality of Shihudang (0.742) is very close to excellent condition. Xinbang (0.670) and Yexie (0.663) are at better levels. The similar features of those four towns are agriculture as main landuse, lower proportion of industry land, good environmental situation, various type of rural landscape and harmonious landscape pattern.

The comprehensive quality of Dongjing is at better level, but the value (0.466) is relatively lower, and closes to Sijing (0.435) at the ordinary level. Both of them belong to residential area. The residential density is lower, so the environment of rural landscape is relatively better.

The comprehensive qualities of Xinqiao (0.301) and Chedun (0.331) are worse because more than 60% landuse is residential landuse or industry landuse. The proportion of rural landuse is lower. Rural landscape has been damaged. Natural vegetation is rare. Many rivers and soils have been polluted and the rural landscape features have disappeared.

The comprehensive quality of Jiuting (0.249) is the worst. More than 80% land is used as residential land and industry land. The 20% agriculture landuse is greenhouse or aquiculture. The rural landscape is fragment, and the rural culture has been lost.

The results of recreation quality evaluation of rural landscape shows that, the recreation quality of rural landscape is correlated to landuse types directly. Natural landscape and agricultural landuse are the fundamental elements for rural recreation. Aesthetics quality is correlated with ecological quality positively, and the rural landscape recreation planning and management must be based on the evaluation of rural landscape recreation quality.

## **5. Discussion**

The AHP method and VRD method were combined together to estimate rural landscape recreation quality in this paper. So the index weight was confirmed more objectively comparing only AHP method using. This methodology can be applied to other areas in order to rank and explain the quality of rural area for recreation. It provides insight into how to obtain the index and confirm the weight and make the comprehensive evaluations of rural landscape recreational quality. Policymakers have an instrument to measure potential and actual values of special areas for rural recreational activities. The information supplied by the results of evaluation can enrich the decision-making process that has to plan and construct for recreational facilities.

Among the three dimensions of evaluation indexes, ecology quality is primary foundation for rural landscape recreation. The original ecological environment should be protected and maintained. The aesthetics quality is a significant aspect for rural landscape recreation. The facility quality is a basic qualification for rural landscape recreation purpose.

For rural landscape recreation planning, the planning principles must be based on the assessment of rural landscape recreation quality. The planning should follow the following principles: scenario—focusing on features, intensifying characteristics, diversification and variability, creating high personalization; Process—exploring by visitors' themselves, looking for the most beautiful sceneries and feeling rural atmosphere; Identification—establishing visual identification system by using local materials, picking up local symbols; Convenience—creating convenient, clean, pure rural landscape; Economy—relying on ecological landscape, building up beautiful rural landscape, and focusing on the application of landscape economy and ecological technology.

The metropolis plays an important role in the world, usually acting as the regional center of economics, politics, or culture. The rural landscape and function of metropolis differ from other rural landscapes. The recreation functions of rural landscape metropolis is more important for metropolitan residents than for others. Thus, it is important to be aware of the rural landscape recreation quality so as to provide significant data for further rural landscape recreation planning and management.

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# The sustainability of farming enterprises in Bulgaria<sup>2</sup>

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## Abstract

In this paper, we suggest a practical and holistic approach to assessing the sustainability of farming enterprises under the conditions of EU CAP implementation in Bulgaria. First, a specific framework for assessing the sustainability of farming enterprises is proposed, including a system of principles, criteria, indicators, and reference values. After that, a positive endorsement is made for the suggested framework in terms of evaluating the overall, governance, economic, social, and environmental sustainability of farming enterprises of different juridical types, sizes, production specializations, and ecological and administrative locations. Additionally, relevant factors of sustainable farming in Bulgaria are identified. In conclusion directions for further research and improvement of managerial practice are suggested.

## Introduction

The issue of the sustainability of farming enterprises is one of the most topical for researchers, farmers, investors, administrators, policymakers, interest groups, and the public at large around the globe (Andreoli and Tellarini, 2000; Bachev, 2010; Bachev and Petters, 2005; Candido et al., 2015; EC, 2001; FAO, 2013; Fuentes, 2004; Häni et al., 2006; OECD, 2001; Rigby et al., 2001; Sauvenier et al., 2005; UN, 2015; VanLoon et al., 2005). Nevertheless, in Bulgaria there are only a few comprehensive studies on the sustainability of farms at the current stage of development (Bachev, 2017, 2018).

Most of the assessments of agricultural sustainability undertaken are at industry, national, or international level and the important farm level are usually missing. Simultaneously, there are many systems that locate an individual 'parcel' of land as the lowest level of sustainability assessment ignoring the important links with the sustainability governance. Estimates of farm and agrarian sustainability are often unjustifiably equalized while agrarian sustainability has a larger dimension. In most cases a holistic approach is not applied, and the purely economic, purely ecological, and purely social aspects of farm development are assessed independently from one another. In most of the available frameworks there is no hierarchical structure or systematic organization of the aspects and components of farm sustainability, (pre)determining a random selection of sustainability indicators.

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The critical governance functions of farms, the costs associated with this governance (transaction costs), and the relations between different aspects of farm sustainability are mostly ignored. Nevertheless, very often the level of managerial efficiency (adaptability) of the farm predetermines its overall level of sustainability independently of its productivity, or its social or ecological responsibility. The majority of proposed frameworks for assessing sustainability apply a universal approach to ‘faceless’ farms, without taking into consideration the specificity of individual holdings (type, resource endowment, specialization, stage of development) and the environment in which they function (competition, institutional support and restrictions, environmental challenges and risks). What is more, most of the proposed systems cannot be practically used by farms and managerial bodies, since they are difficult to understand, calculate, and monitor.

In this paper, a holistic approach for assessing the sustainability of farming enterprises in Bulgaria is proposed, and the overall, governance, economic, social, and environmental sustainability of farms of different juridical types, sizes, production specializations, and ecological and administrative locations assessed, and factors of sustainable farming identified.

### **1. Framework for assessing the sustainability of farming enterprises**

Farm sustainability is the ability of a particular farm to exist over time, maintain its governance, economic, ecological and social functions in the specific socioeconomic and natural environment in which it operates and evolves. It has four aspects that are equally important and have to be taken into account. Managerial sustainability - a good or high absolute and comparative standard of efficiency in the organization of activity and relations, a high level of adaptability to evolving socioeconomic and natural environment according to the specific preferences and capabilities of the farm owners. Economic sustainability - a good or high level of productivity in utilization of natural, personal, material, and financial resources, an acceptable level of economic efficiency and competitiveness, and adequate financial stability. Social sustainability – a good or high level of social responsibility regarding farmers, workers, other agents, communities, and consumers, contribute to the conservation of agrarian resources and traditions, improve the welfare and living standards of farm households, and encourage the development of rural communities and society as a whole. Ecological sustainability - a good or high level of ecological responsibility and be associated with socially desirable conservation, recovery, and improvement of natural environments and nature as a whole, respect animal welfare and other socially determined standards related to nature.

The hierarchical levels, which facilitate the formulation of a system for assessing the sustainability of farming enterprises, include well-determined and selected principles, criteria, indicators, and reference values (figure 1).

Principles - the highest hierarchical level associated with the multiple functions of agricultural enterprises. They are universal and represent the state of sustainability, which is to be analyzed according to the four main aspects. Criteria - more precise than the principles and are easily linked to sustainability indicators, representing a resultant state of the evaluated farm when the relevant principle is realized. Indicators -quantitative and qualitative variables of different types, which can be assessed according to the specific conditions of the evaluated farms allowing the measurement of compliance with particular criteria. The set of indicators provides a representative picture of farm sustainability in all its aspects. Reference Values - desirable levels for each indicator according to the specific conditions of the evaluated farming enterprises assisting sustainability assessment and giving guidance for achieving (maintaining, improving) farm sustainability.

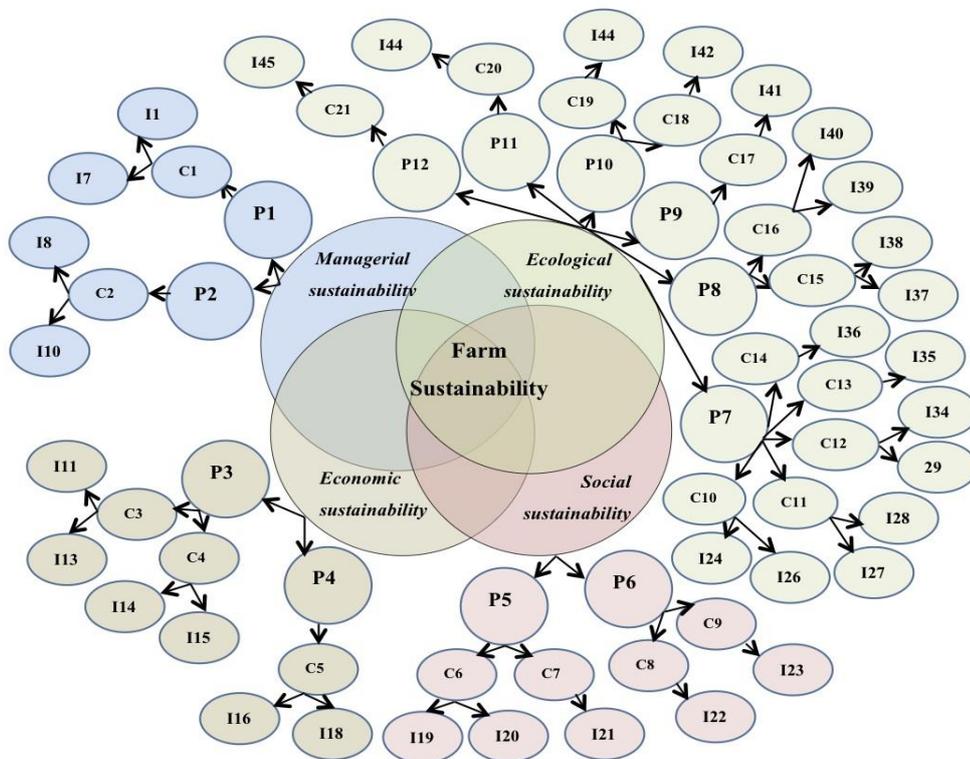


Figure 1. System for the Assessment of Sustainability of Farming Enterprises in Bulgaria  
Source: Bachev, 2017

Specific for the Bulgarian farming enterprises principles, criteria, indicators, and reference values were selected by leading experts in the area and include 12 principles, 21 criteria, and 44 indicators with relevant reference values. A number of criteria were used in selecting sustainability indicators: relevance to reflecting aspects of sustainability; discriminatory power in time and space; analytical soundness; intelligibility and synonymy; measurability,

governance and policy relevance; and practical applicability. A survey of managers of “typical” 190 market-oriented farms of different juridical types, sizes, specializations, and ecological and geographical locations was carried out in 2016 with the assistance of the National Agricultural Advisory Service and professional associations which identified representative farming enterprises.

The sustainability level of individual farming enterprises was based on estimates made by enterprise managers for each indicator according to four qualitative levels: high/higher or better than average for the sector/region; similar/good; low/lower or worse than average for the sector/region; negative/unsatisfactory/unacceptable. This approach was the only feasible way of getting the necessary data, since the levels for most governance and social indicators are practically known only by farming enterprise managers (satisfaction of activity, acceptable income, inputs supply alternatives). Precise data for certain ecological indicators are not available as some can only be gathered through costly laboratory tests. The official typology was used to classify the surveyed farming enterprises according to: juridical type, production specialization, geographical and administrative region, and ecological location. In addition, every manager determined the size and ecological location of farming enterprise. The qualitative estimates for individual farming enterprises were then quantified and transformed into sustainability indices for each indicator (SI(i)) using the following scales: 1 for high; 0,66 for good or average; 0,33 for low; and 0 for unsatisfactory or unacceptable. Equal weighting was given to each principle of a particular aspect, to each criterion of a particular principle, and to each indicator of a particular criterion. Four levels of sustainability of farming enterprises were distinguished by the experts: high - 0,84 to 1; good - 0,5 to 0,83; low - 0,22 to 0,49; and unsustainable - 0 to 0,21.

## **2. Sustainability Level of Farming Enterprises**

A multiple indicator assessment of the sustainability level of the surveyed farming enterprises indicates that the index of overall sustainability for the holdings is 0,55 - this represents a good level of sustainability of Bulgarian farms (figure 2). The highest levels are shown in the indices of environmental (0,61) and social (0,57) sustainability, while indices of governance (0,52) and economic (0,5) sustainability are significantly lower. Improvement in these two areas appears critical to maintaining a good level of farming sustainability

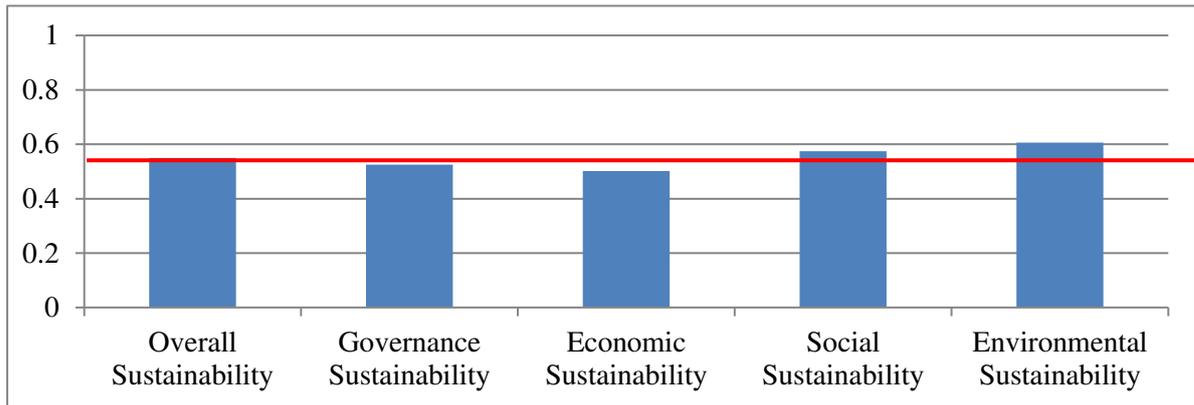


Figure 2. Indices of Overall, Governance, Economic, Social, and Environmental Sustainability for Bulgarian Farming Enterprises

*Source: farm manager survey, July 2016*

Analysis of individual indexes for the sustainability principles, criteria, and indicators allows identifying components contributing to diverse aspects of sustainability. For instance, good governance and economic sustainability are both relatively low - the index of governance efficiency is 0,49 and the index of financial stability is 0,47 (figure 3). Similarly, it is clear that despite overall environmental sustainability being relatively high, the index of preservation of agricultural lands (0,52) and the index of preservation of biodiversity (0,56) are relatively low. In depth analysis of individual criteria and indicators further specifies the elements that enhance or reduce a farming enterprise's sustainability. For instance, insufficient comparative governance efficiency and financial capability (figure 4) is determined by a combination of low comparative efficiency in the short-term inputs supply in relation to alternative organizations (0,28), unsatisfactory profitability of an enterprise's own capital (0,41), and low overall liquidity (0,48) (figure 5). Similarly, low values on the indices of preserving agricultural land and preserving biodiversity are determined by insufficient application of recommended irrigation norms (0,46), high soil erosion by water (0,55), and a reduced number of wild animals on farm territory (0,53).

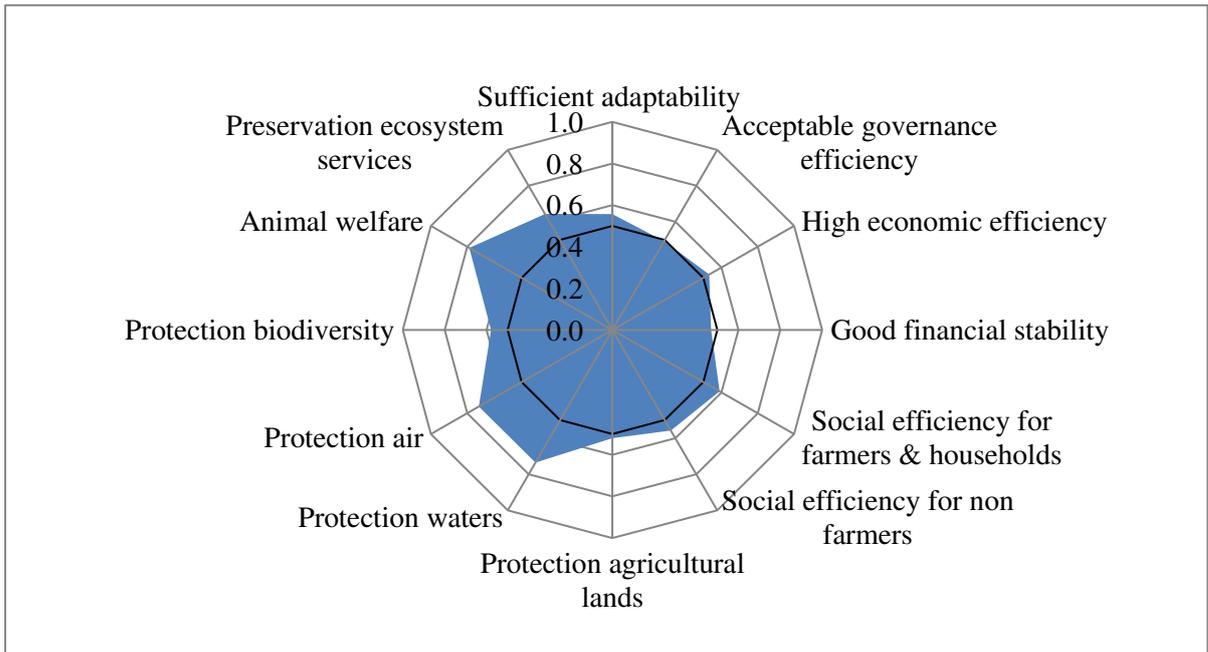


Figure 3. Indices of Sustainability of Bulgarian Farming Enterprises for the Main Principles  
 Source: farm manager survey, July 2016

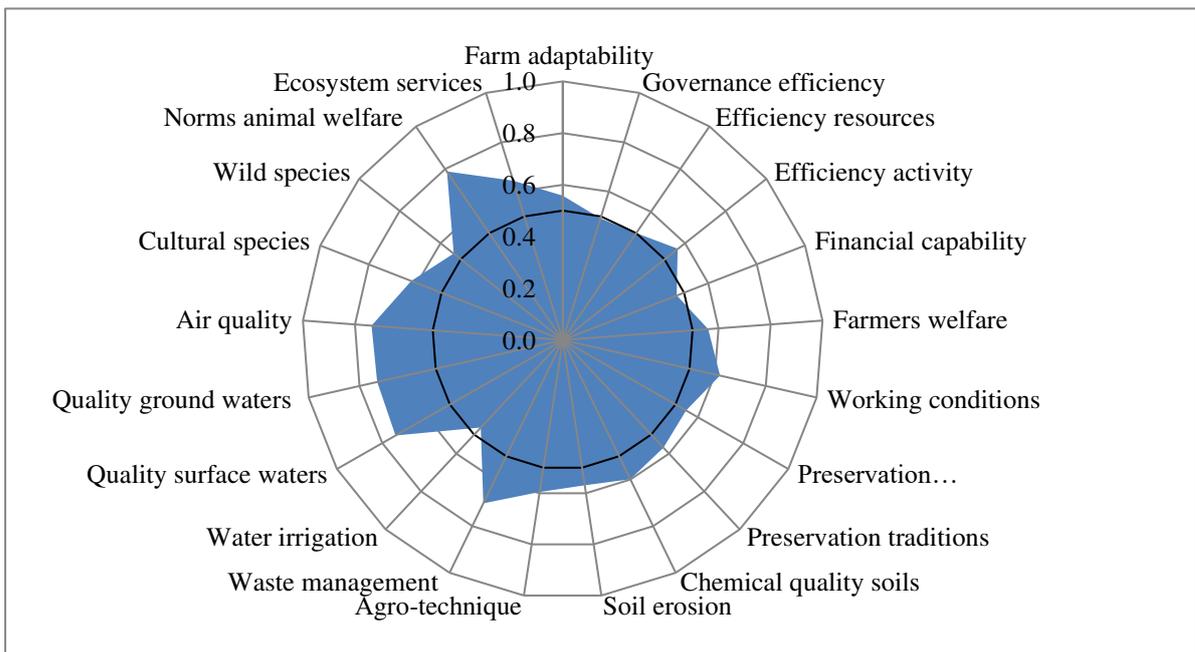


Figure 4. Level of Sustainability of Bulgarian Farming Enterprises for Individual Criteria  
 Source: farm manager survey, July 2016

Low values for indicators help identify specific areas that require improvement through changes in management strategy and/or public policy. For instance, despite the overall adaptability of farms being relatively high (0,56), the adaptability of farms to changes in the natural environment

(climate, extreme events) is relatively low (0,5). Therefore, effective measures must be put in place to improve this through education, training, information, ameliorative agricultural techniques, restructuring of production, different crop varieties, and technological and organizational innovation.

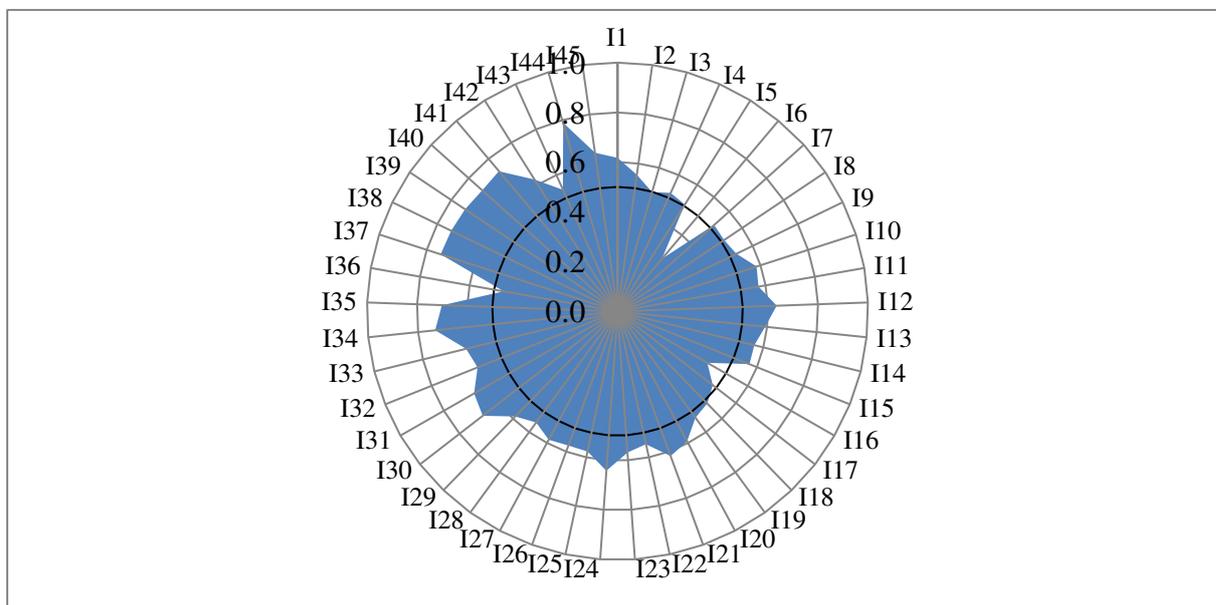


Figure 5. Indicators\* for Assessing the Sustainability of Bulgarian Farming Enterprises  
*Source: farm manager survey, July 2016*

I1-Level of Adaptability to Market Environment; I2-Level of Adaptability to Institutional Environment; I3-Level of Adaptability to Natural Environment; I4-Comparative Efficiency of Supply and Governance of Labor Resources; I5-Comparative Efficiency of Supply and Governance of Natural Resources; I6-Comparative Efficiency of Supply and Governance of Short-Term Inputs; I7-Comparative Efficiency of Supply and Governance of Long-Term Inputs; I8-Comparative Efficiency of Supply and Governance of Innovation; I9-Comparative Efficiency of Supply and Governance of Finance; I10-Comparative Efficiency of Governance of Marketing of Products and Services; I11-Land productivity; I12-Livestock Productivity; I13-Level of Labor productivity; I14-Rate of Profitability of Production; I15-Income of Enterprise; I16-Rate of Profitability of Own Capital; I17-Overall Liquidity; I18-Financial Autonomy; I19-Income per Farm-Household Member; I20-Satisfaction of Activity; I21-Compliance with Working Standards; I22-Contribution to Preservation of Rural Communities; I23-Contribution to Preservation of Traditions; I24-Nitrate Content in Surface Water; I25-Pesticide Content in Surface Water; I26-Nitrate Content in Ground Water; I27-Pesticide Content in Ground Water; I28-Extent of Air Pollution; I29-Number of Culturally Important Species; I30-Number of Wild Species; I31-Respect of Animal Welfare; I32-Preservation of Quality of Ecosystem Processes; I33-Soil Organic Content; I34-Soil Acidity; I35-Soil Salinification; I36-Extent of Wind Erosion; I37-Extent of Water Erosion; I38-Crop Rotation; I39-Number of Livestock per ha of Farmland; I40-Nitrogen Fertilizer Norms; I41-Phosphorus Fertilizer Norms; I42-Potassium Fertilizer Norms; I43-Application of Good Agricultural Practices; I44-Type of Manure Storage; I45-Irrigation Rate

The high levels of certain indicators show the absolute and comparative advantages of Bulgarian farms in terms of sustainable development. At the current stage of development, the most prominent of these include: respecting animal welfare standards; preserving surface and ground water quality through limiting contamination with nitrates and pesticides; preserving air quality;

implementing good agricultural practices; reducing livestock per unit of farmland; ensuring acceptable labor conditions; comparative satisfaction from farming; optimal productivity of livestock; good adaptability to the market (prices, competition, demands); and comparative efficiency of governance of marketing of products and services.

There is significant variation in the level of sustainability of farming enterprises of different types and locations (figure 6). Only those holdings predominately for subsistence and mixed livestock production have low sustainability. Good governance and economic and social sustainability are particularly low for subsistence farms (figure 7). Mixed livestock farms tend to have low levels of governance and economic and environmental sustainability and only marginal social sustainability.

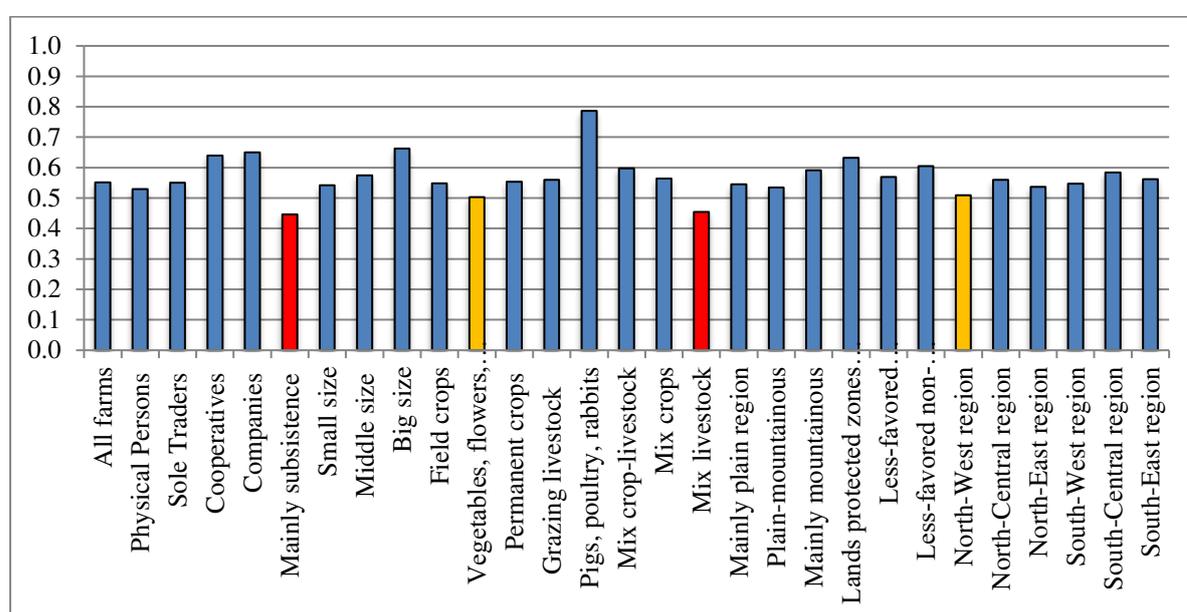


Figure 6. Index of Sustainability of Bulgarian Farming Enterprises of Different Types and Locations

Source: farm manager survey, July 2016

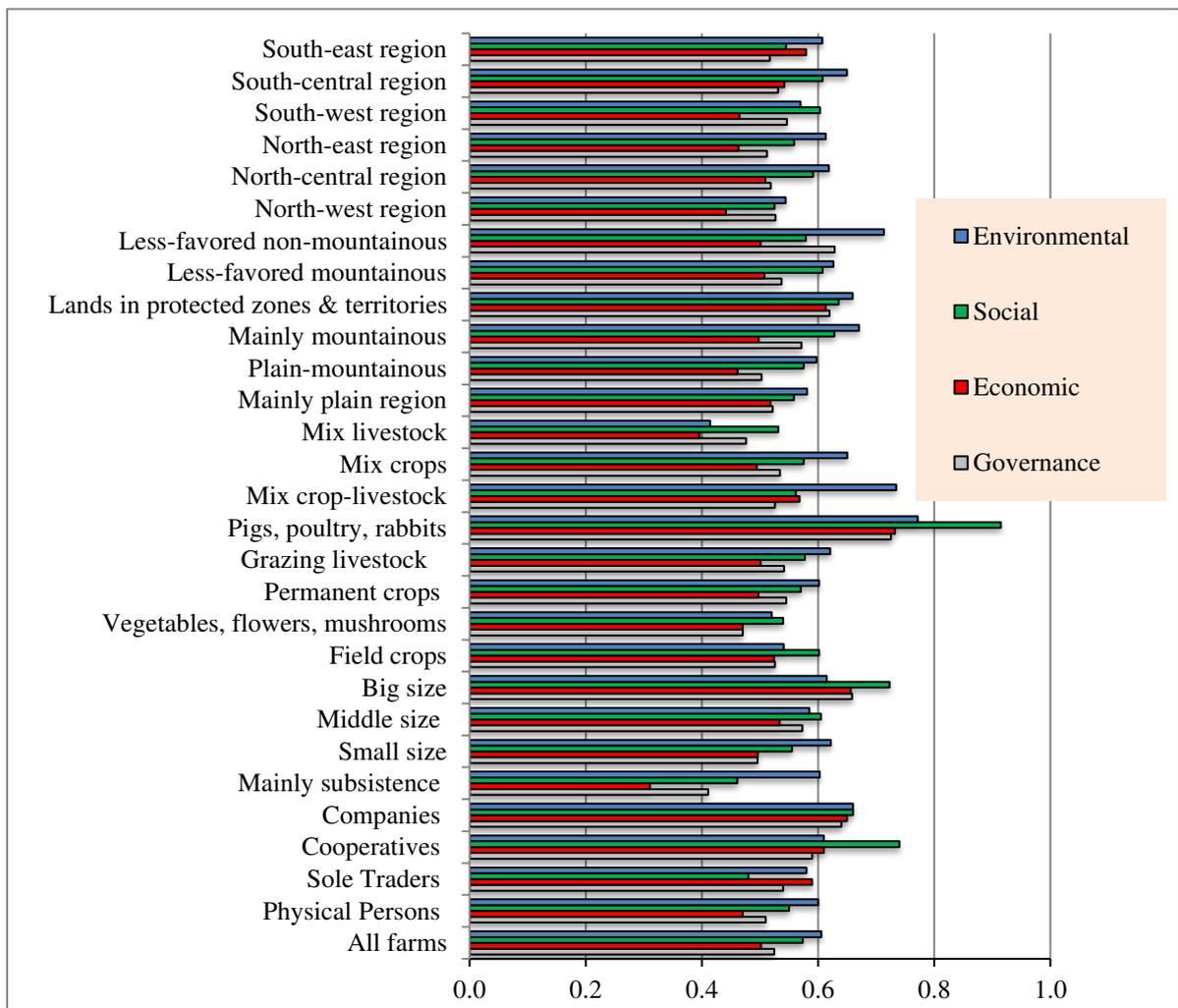


Figure 7. Levels of Governance, Economic, Social, and Environmental Sustainability of Bulgarian Farming Enterprises of Different Types and Locations

Source: farm manager survey, July 2016

Holdings specializing in vegetables, flowers, and mushrooms had an overall sustainability of 0,5, with low levels of governance and economic sustainability (0,47). They had a relatively poor level of social sustainability (0,5) and environmental sustainability (0,52). Physical person enterprises had an overall sustainability level of 0,53, with low economic sustainability (0,47) and marginal social sustainability (0,5) and governance (0,51) (in contrast to good environmental sustainability). Farms located in the northwest region of the country had an overall sustainability of 0,5, with low economic sustainability (0,44) and not particularly good social (0,52), environmental (0,54), or governance (0,53) sustainability. Effective measures need to be undertaken to improve all aspects of sustainability among these enterprises.

Small farming enterprises (0,496), those specializing in mixed (0,49) and permanent crops (0,498), and situated in plain-mountainous (0,46), mountainous (0,498), the northeast (0,46), and the southwest (0,46) regions had low economic sustainability. Consequently, the overall sustainability of these farming enterprises is marginal and effective measures need to be

undertaken to increase economic sustainability. Sole traders had low social sustainability (0,48) and appropriate measures need to be introduced to improve this aspect through training, stimulation, regulation, and support. Companies, cooperatives, and large farming enterprises had high levels of overall sustainability with high levels of governance, economic, social and environmental sustainability. Holdings specializing in the production of pigs, poultry, and rabbits had the highest level of sustainability, with very good levels of governance, economic, social, and environmental sustainability. They are also the only type of enterprise to have a high level of social sustainability (0,9). Farming enterprises with land in protected zones and territories and located in non-mountainous regions with natural handicaps had high levels of overall sustainability. Farming enterprises in non-mountainous regions with natural handicaps had reasonable levels of governance and environmental sustainability and low levels of economic and social sustainability. Those in the south-central region had relatively good levels of environmental and social sustainability, but poor governance and low economic sustainability. Farms with a mixture of crops and livestock had reasonable levels of environmental, but a lower level of governance sustainability.

### **3. Farming Enterprises with Different Levels of Sustainability**

The sustainability of farming enterprises as a whole and of different juridical types, operational sizes, product specializations, and ecological and geographical locations do not give a full picture of the state of all holdings in Bulgaria. Evaluation of the sustainability of individual farms indicates that there is great variation in farming enterprises in terms of sustainability.

The largest proportion of farming enterprises had a good level of sustainability (just above 68%) and just under 2% had a high level of sustainability (figure 8). 30% of agricultural holdings had a low level of sustainability (almost 27%) or were unsustainable (a little more than 4%). Companies made up the greatest share of farming enterprises with a good (88%) or high (6%) sustainability level. Next are cooperatives, of which 77% had good sustainability and 8% were highly sustainable, and sole traders of which three-quarters had good sustainability levels. Physical persons had the smallest number of holdings with good sustainability (65%) and less than 1% were highly sustainable. More than a third of latter had low sustainability (29%) or were unsustainable (5%). A quarter of sole traders and 15% of cooperatives had low overall sustainability, while only 6% of companies were low sustainable.

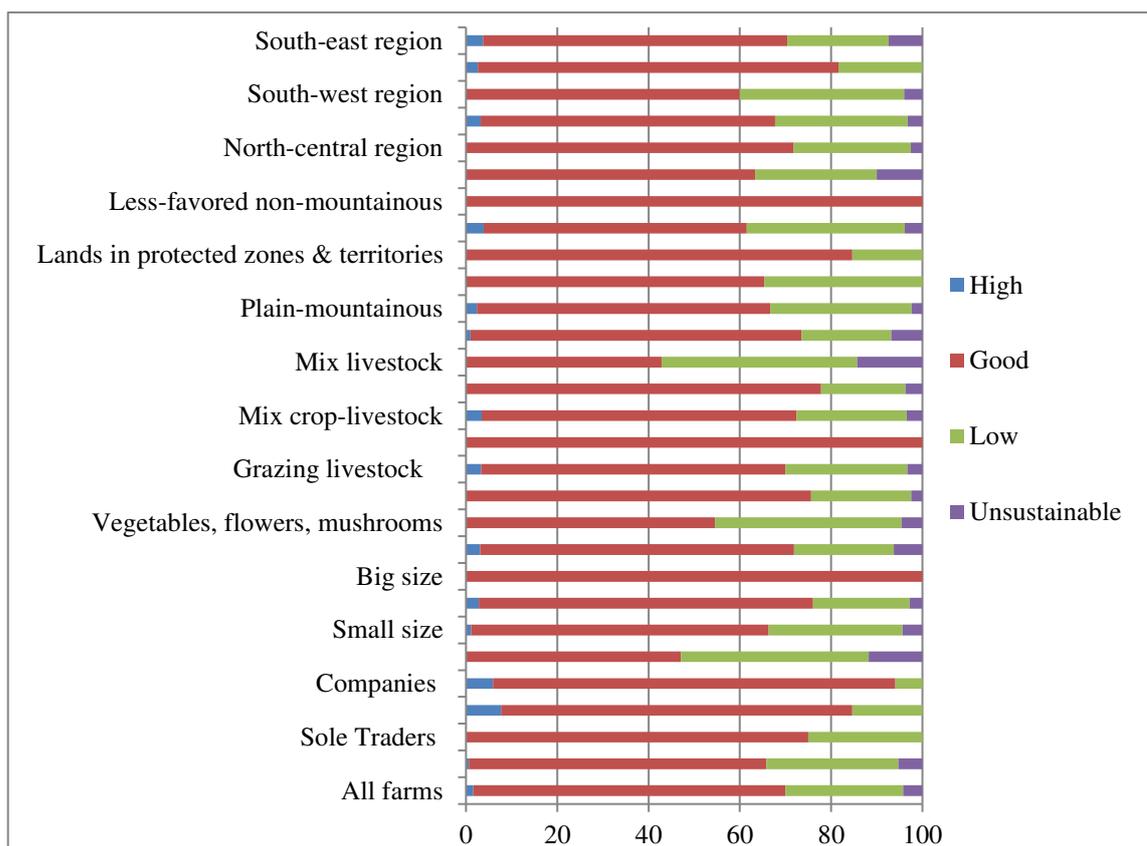


Figure 8. Structure of Farming Enterprises of Various Types and Locations with Different Levels of Overall Sustainability in Bulgaria (percent)

Source: farm manager survey, July 2016

While all farming enterprises of large size had a good level of sustainability, more than a half of holdings predominantly for subsistence had low sustainability (41%) or were unsustainable (12%). Likewise, around a third of farming enterprises of small size and 24% of those of medium size had low sustainability or were unsustainable. In terms of specialization, holdings with good or high sustainability can be ranked as follows: pigs, poultry, and rabbits (100%); mixed crops (78%); permanent crops (76%); mixed crops and livestock (73%); field crops (72%); and grazing livestock (70%). In contrast, the majority of holdings specializing in mixed livestock had low sustainability (43%) or were unsustainable (14%). A good proportion of farming enterprises specializing in vegetables, flowers, and mushrooms also had low sustainability (41%) or were unsustainable (4%). The greatest number of farming enterprises with good or high sustainability were located in non-mountainous regions with natural handicaps (100%), followed by those with land in protected zones and territories (85%), in plain regions (74%), in the south-central region (82%), in the north-central region (72%); and in the southeast (70%) region. 40% of holdings in the southwest region, 37% in the northwest region, and 32% in the northeast region had low sustainability or were unsustainable. The northwest region had the greatest number of

unsustainable farming enterprises (one tenth). A large number of holdings in mountainous regions with natural handicaps (38%), 35% of located in mountainous regions, and one third in plain-mountainous regions had low overall sustainability or were unsustainable

Data on the spread of farms by type and with diverse levels of sustainability has to be taken into account when forecasting the number and importance of holdings of each kind and modernizing public (structural, sectorial, regional, environmental) policies to support agricultural producers of different types, subsectors, ecosystems, and regions of the country.

Analysis of the structure of farming enterprises with different levels of sustainability broken down by aspect gives important information about the long-term sustainability of farms and highlights issues for improvement. Good governance and economic sustainability are of prime importance for the survival of farming enterprises in the short and medium terms, particularly with the rapidly changing socioeconomic and natural environment. Maintaining and improving sustainability in these two areas is critical to maintaining the long-term sustainability of these enterprises. 40% of the surveyed farming enterprises showed poor governance (35%) or a lack of managerial sustainability (4,5%). The comparative governance efficiency (capability) for the supply of labor, land, finance, etc. and/or marketing of produce in these farming enterprises is lower than other feasible organization forms and the adaptability to evolving socio-economic, institutional, and natural environments is inadequate. 42% of all farming enterprises had low economic sustainability (34%) or were unsustainable (8%). Their economic and financial efficiency of activity and resource utilization is low and does not correspond to the requirements of modern management and market competition.

Farming enterprises with good or high levels of governance included: companies (94%), cooperatives (77%), large-sized (89%) and medium-sized (75%) holdings, those in pigs, poultry, and rabbits (100%), permanent (63%), mixed (63%) and field (63%) crops, in mixed crops and livestock (62%), located in non-mountainous regions with natural handicaps (100%), with land in protected zones and territories (77%), in plain regions (63%), in mountainous regions with natural handicaps (62%), in the north-central (67%), the southeast (63%), the northwest (60%), and in the southwest (60%) regions.

Farming enterprises with poor governance included: sole traders (50%), physical persons (45%), holdings predominantly for subsistence (65%), small size (49%), in vegetables, flowers, and mushrooms (50%), in plain-mountainous regions (48%), and in the northeast and south-central regions (45%). This means that a considerable proportion of Bulgarian farming enterprises have levels of governance that are inadequate when faced with

contemporary socioeconomic, institutional, and natural challenges - they need to modernize or they will cease to exist over the medium term.

Farms with good or superior economic sustainability included: companies (88%); cooperatives (85%), and sole traders (63%). A significant proportion of farming enterprises had high economic sustainability (18% of companies and 12,5% of sole traders). All enterprises of large size had good economic sustainability highlighting the comparative economic advantages of operating on a large scale. Farming enterprises with good or high levels of economic sustainability included: a considerable number of holdings of medium size (66%), in pigs, poultry, and rabbits (100%), mixed crops and livestock (66%); field (59%) mixed (59%), and permanent (59%) crops; with land in protected zones and territories (77%), located in plain regions (63%), mountainous regions with natural handicaps (62%), the southeast (78%), the south-central (66%) and the north-central (62%) regions.

The greatest number of farming enterprises with low economic sustainability were found among: physical persons (48%), predominantly for subsistence (88%), in mixed livestock (57%), grazing livestock (47%), vegetables, flowers, and mushrooms (45%), in mountainous (54%) and plain-mountainous regions (45%) regions, in the northeast (58%) and the southwest (52%) region. A significant proportion of these holdings are currently economically unsustainable: almost one tenth of physical persons, 29% of farms with mixed livestock, a fifth of located in the northwest and 12% in the southwest region, 18% of predominantly for subsistence, 9% of specialized in vegetables, flowers, and mushrooms; 9% of small size; and 7% in plain-mountainous regions. This indicates that a large number of farming enterprises have limited economic sustainability or are economically unsustainable and most likely will cease to exist in the near future unless effective measures are taken to improve their economic sustainability.

The majority of farming enterprises surveyed (77%) had good (71%) or high (6%) levels of social sustainability. Despite this, holdings with low social sustainability are numerous (18%), and one in ten is unsustainable in social terms. This demonstrates that the social efficiency of enterprises for farmers, communities, and society as a whole does not meet contemporary requirements and standards.

A considerable number of cooperatives showed good levels of social sustainability (77%) and the rest (23%) had a high level of social sustainability. The share of companies with good (82%) or high (12%) social sustainability was also impressive (only 6% of them had low social sustainability). A significant proportion of physical persons also had good (67%) or high (4%) social sustainability. Despite this, one in five had low social sustainability and 7% were unsustainable. Sole traders had the highest proportion of enterprises with low social sustainability at around 38%. The level of social sustainability increases with farming enterprise size. One third

of enterprises of large size had high social sustainability and 56% had good social sustainability; the share of those with low social sustainability was 11%. 72% of medium-sized enterprises had good or high social sustainability; almost one fifth had low social sustainability (15%) or were unsustainable (4%). Contrary to the traditional view, the enterprise type with the largest proportion of farms of low social sustainability or unsustainable were among semi-market (18% unsustainable) and one quarter of small-sized enterprises (4% unsustainable). The largest number of farming enterprises with good or high social sustainability was seen in specialized in pigs, poultry, and rabbits (50%), field crops (81% and 6%), and mixed crops (74% and 7%). 37% of farms specializing in vegetables, flowers, and mushrooms had low social sustainability (32%) or were socially unsustainable (5%), 29% of holdings specializing in mixed livestock had poor social sustainability (including 14% socially unsustainable). Farming enterprises with good or high social sustainability were located: in mountainous regions and in protected zones and territories (85% and 8%); in the southwest region (92% and 4%); in the south-central region (79% and 8%); and in the north-central region (62% and 11%). Those with low social sustainability or that were unsustainable were located: in plain regions (21% and 8%); plain-mountainous regions (19% and 5%); in the northwest region (23% and 10%); in the southeast region (22% and 7%); and in the northeast region (26% and 3%).

These data show that a good proportion of Bulgarian farming enterprises have low social sustainability or are socially unsustainable. This compromises their medium and long-term sustainability and effective measures need to be put in place to improve the incomes and labor and living conditions of farmers and farm households; their importance for the preservation of rural communities and traditions also needs to be recognized.

The environmental sustainability of the majority of the surveyed farming enterprises was good (69%) or superior (9%), a considerable proportion having low eco-sustainability (18%) or were unsustainable (4%). These figures suggest that the ecological efficiency of a large number of farming enterprises does not meet contemporary norms and standards for the conservation and protection of land, water, air, biodiversity, ecosystem processes, and animal welfare.

A reasonable number of companies (18%), physical persons (9%), and cooperatives (8%) displayed a high level of environmental sustainability and the majority a good level (59%, 68%, and 69% respectively). Despite this, many holdings had low environmental sustainability (24%, 18%, and 23% respectively) and one in twenty physical person enterprises were environmentally unsustainable. All of the sole traders surveyed maintained a good level of ecological efficiency. Farming enterprises with a good or high level of ecological sustainability included: holdings predominantly for subsistence (76% and 12%), small size (71% and 10%), and large enterprises

(67% and 11%). The greatest number of holdings with a low or unacceptable standard of ecological sustainability was found among large and medium-sized enterprises. The proportion of strongly environmentally sustainable farming enterprises is significant among specialized in crops and livestock (21%), grazing livestock (17%), mixed (11%) and permanent (7%) crops. All enterprises specializing in pigs, poultry, and rabbits, most in mixed crops (81%), and three quarters in crops and livestock and permanent crops had good environmental sustainability. At the same time, a considerable proportion of farming enterprises specializing in vegetables, flowers, and mushrooms had low ecological sustainability (32%) or were ecologically unsustainable (14%), also in mixed livestock (29% and 14%) and field crops (31% and 3%). The share of environmentally unsustainable enterprises is also considerable among specializing in permanent crops (7%).

All farming enterprises located in non-mountainous regions with natural handicaps had good environmental sustainability as did most with land in protected zones and territories (93%). The largest number of enterprises with a high level of ecological sustainability were found in plain-mountainous and mountainous regions (12%) and mountainous regions with natural handicaps (7,7%). At the same time, the greatest fraction of enterprises with low ecological sustainability or that were ecologically unsustainable were found in plain-mountainous (26%) and plain (25%) regions, as well as in mountainous regions with natural handicaps (19%). The largest number of enterprises with a high or good level of ecological sustainability were found in the north-central (3% and 87%) and the south-central (18% and 63% accordingly) regions. Regions of the country where enterprises with low ecological sustainability or unsustainable were found included: the southwest (28% and 4%), the northwest (17% and 10%), the southeast (26% and 0%), and the northeast (23% and 3%). These data indicate that a significant number of Bulgarian farming enterprises have a low level of ecological sustainability or are environmentally unsustainable - this compromises their overall long-term sustainability. Effective measures need to be undertaken to improve their ecological efficiency through training, information campaigns, stimulation through policy, and regulations and sanctions.

#### **4. Factors of Farming Enterprise Sustainability**

Diverse social, economic, market-related, ideological, and personal factors stimulate or restrict the activities of farming enterprises in terms of sustainable operation and development. According to the managers surveyed, factors that could increase the quality of enterprise governance include: available information; official regulations, standards, and norms; access to advisory services; professional training for managers and hired labor; personal convictions and satisfaction; the positive experiences of other farms; innovation; financial capability; private contracts and agreements; and the registration and certification of products and services. Factors encouraging

farming enterprises to improve economic sustainability include: market demand and price; direct state subsidies; market competition; financial capability; participation in public support programs; possibility of benefitting immediately; possibility of benefitting in the near future; tax preferences; possibility of benefitting in the long term; and integration with buyers of farm products. Factors encouraging the enhancement of social sustainability for the greatest number of farming enterprises include: personal convictions and satisfaction; social recognition of individual contribution; immediate benefits for other people and groups; regional community initiatives and pressure; access to advisory services; European Union policy; and existing regional problems and risks. Factors encouraging environmental sustainability include: problems and risks existing at the global scale; official regulations, standards, and norms; existing regional problems and risks; and European Union policies. These incentives need to be examined in relation to the modernization of public policy and the establishment of programs for sustainable development for Bulgarian farming enterprises.

This survey has found that current public policies only weakly affect the quality of governance among Bulgarian farming enterprises. National and European Union mechanisms of regulation and support that successfully increase governance in the enterprises include: professional training and advice; obligatory standards, norms, rules, and restrictions; modernization of agricultural holdings; and establishing produce organizations (figure 9). Instruments that could impact the economic sustainability of the majority of the surveyed enterprises include: direct area-based payments; national top-ups for products and livestock; modernization of agricultural holdings; green payments; support for semi-market farms. The impact that national and European policies have on the social and environmental sustainability of Bulgarian farming enterprises is relatively weak. Instruments that could augment the social sustainability of the majority of farming enterprises include: strategies for local development; the provision of services to residents of rural areas; restoration and development of residential areas; and stimulation of rural tourism. The most important actions to improve the environmental sustainability of farming enterprises include: green payments; support for organic farming; obligatory standards, norms, rules, and restrictions; and agro-environmental payments.

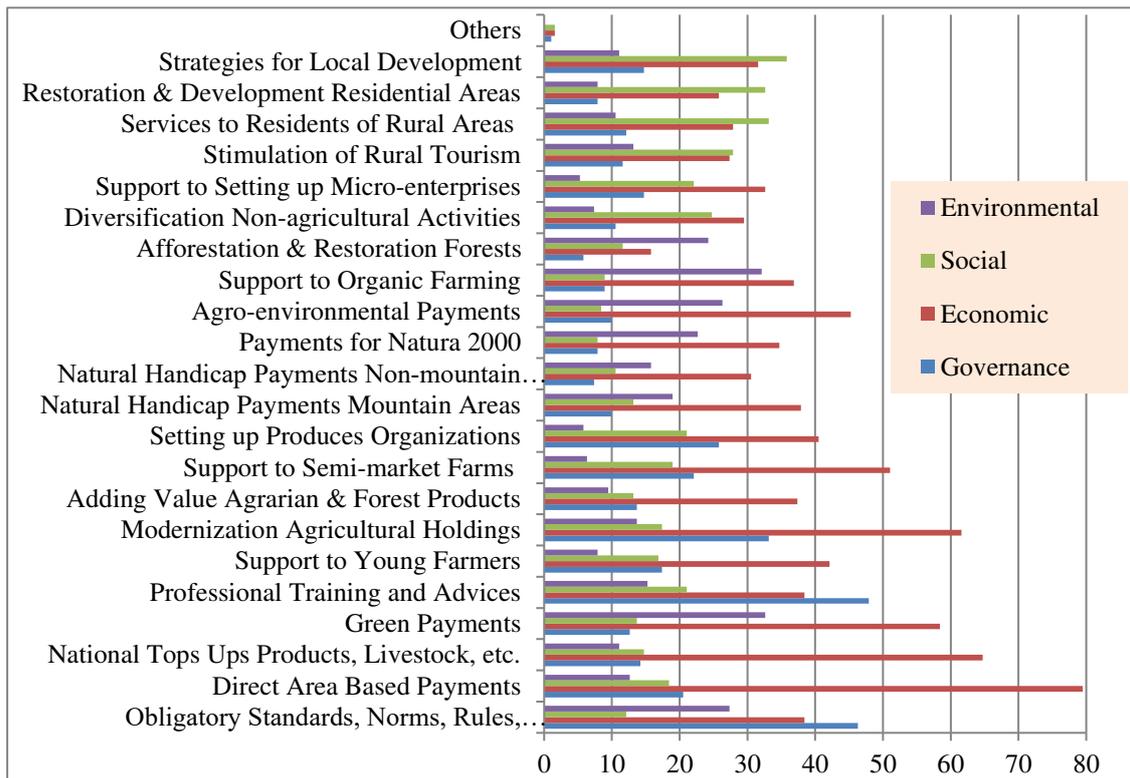


Figure 9. Public Policies Most Affecting the Sustainability of Farming Enterprises in Bulgaria (percent)

Source: farm manager survey, July 2016

There is a difference between individual instruments of public policy and impact on the sustainability of farming enterprises of different types and locations. Mechanisms and instruments with the greatest impact in improving the sustainability include: 1) Obligatory standards, norms, rules, and restrictions in terms of the governance of big enterprises and the environmental sustainability of enterprises specializing in pigs, poultry, and rabbits. 2) Direct area-based payments to improve the economic sustainability of: sole traders, cooperatives, companies, holdings of small size; enterprises specializing in pigs, poultry, and rabbits, mixed crops, and permanent crops; and located in non-mountainous regions with natural handicaps, with land in protected zones and territories, in mountainous regions, mountainous regions with natural handicaps, and in the southwest and south-central regions. 3) National top-ups for products and livestock to improve the economic sustainability of: companies, holdings predominantly for subsistence, and specializing in grazing livestock; in mountainous regions, with land in protected zones and territories, and located in the north-central and southwest regions. 4) Green payments to improve the economic sustainability of enterprises located in mountainous regions, with land in protected zones and territories, and in the southwest region. 5) Professional training and advice for large enterprises. 6) The modernization of agricultural holdings to improve the economic sustainability of: sole traders and companies; in mixed livestock and

mixed crops; and in mountainous regions and in the north-central and south-central regions. 7) Support for semi-market farms and the establishment of produce organizations to improve the economic sustainability of semi-market holdings. 8) Natural handicap payments to farmers in mountainous areas to improve the economic sustainability of farms in such areas. Data on the real impact of individual mechanisms and instruments of public support on different aspects of sustainability among farming enterprises need to be taken into account when seeking to improve policies and programs supporting agricultural sectors and enterprises of diverse types and locations.

### **Conclusion**

The holistic framework suggested offers the possibility of improving the quality of assessment, analysis, and management of sustainability in individual farming enterprises of different types. This includes important aspects, principles, criteria, and indicators of their governance, economic, social, and environmental sustainability. New perspectives on governance, relevant indicators, and data drawn from farming enterprises have been incorporated into a hierarchical framework offering a comprehensive analytical approach that is easy to understand and use in everyday managerial practice. The latter needs to be further discussed, experimented, adapted to specific operational conditions, and improvement made according to farming enterprises of different types, subsectors, geographical regions, and ecosystems, as well as the specific needs of decision-makers at various levels.

The overall sustainability of Bulgarian farming enterprises is at a good level, with superior levels of environmental and social sustainability, but borderline low levels of governance and economic sustainability. There is also a great variation in the sustainability of farming enterprises of different types and locations, and the factors for the improvement of various sustainability aspects.

Keeping in mind the importance of the holistic assessment of farming enterprise sustainability and the enormous benefits for farm management and agrarian policy making, this type of study needs to be expanded. That requires close cooperation between all interested parties and the participation of farmers, agrarian organizations, interest groups, research institutes, experts, and local and state authorities. The precision of estimates has to be also improved through the incorporation of other relevant information from field tests and surveys, the expertise of relevant professionals, and statistical and other data.

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# Research on the Landscape of Shanghai Rural Settlements

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## Abstract

In this paper, Shanghai rural settlements are divided into five modes such as villages along the water pattern, spread-type settlements pattern, water separated from the settlements pattern, water round pattern and the edge of the lake pattern, based on the relationship between the water and the settlements from the perspective of sociology, geography, landscape ecology. On this basis, we have a comprehensive and in-depth analysis of the landscape architecture on three levels: the relationship between landscape waters and settlements, the internal structure and scale, and the surrounding environment of the rural settlements. In conclusion, give a general idea of the current problems and characteristics of the Shanghai rural landscape.

**Key words:** rural settlements; landscape; space structure

The rural settlements refer to the collective name of the rural life and the developmental residential areas[1,2], while the rural settlement landscape is the sum of the landscape elements of the human settlements, including various building elements, structures, water bodies, roads, green spaces and other material elements.

At present, the research on rural settlement landscapes is divided into two major categories. One is based on the research perspective of landscape ecology, and the research on the changes of scale, quantity, spatial distribution of landscape patterns according to GIS and digital processing theory [3-6]. The other one is the research based on architecture, geography and other disciplines, focusing on the historical changes of landscape, architectural decoration, spatial characteristics and other aspects of the study [7-17]. Both focus on the data analysis of the status quo, but have little reference to planning and design. While China is in a critical period of new rural construction, how to build a sustainable living environment with cultural heritage, local characteristics, and ecological health is currently an important research direction.

From the perspective of landscape design and planning, this paper analyzes and interprets the rural settlement landscape in Shanghai. Through the qualitative classification and comparison, the settlement landscape with Shanghai's characteristics is extracted and summarized, including the road system that are closely connected with the settlements, the corridor water system, the greening environment, the cultural center and other open spaces,

and excavate rural settlements with local characteristics in Shanghai. By studying their landscape patterns and distinctive elements, explore a new path to new rural construction with Shanghai's characteristics.

## 1 Objects and Methods

### 1.1 Objects of study

This study takes the typical representative villages and towns of Shanghai suburbs as research objects. The main investigate targets are: Qianwei Village, Sanxing Village, Zhongjiu Village Shuyuan Town, Zhuanghang Town, Zhonghua Village Langxia Town, Xiunan Village Nanqiao Town, Xinfeng Village Zhonggu Town, Qianqiao Village and so on forth.

These villages and towns are typical agricultural areas, farmlands basically concentrated, and the characteristics of the agricultural industry are obvious, and the rural dwellings and village landscape have their own unique characteristics and they are located in different districts of Shanghai with different geography, economy, history, and culture, possessing fairly strong typicality.

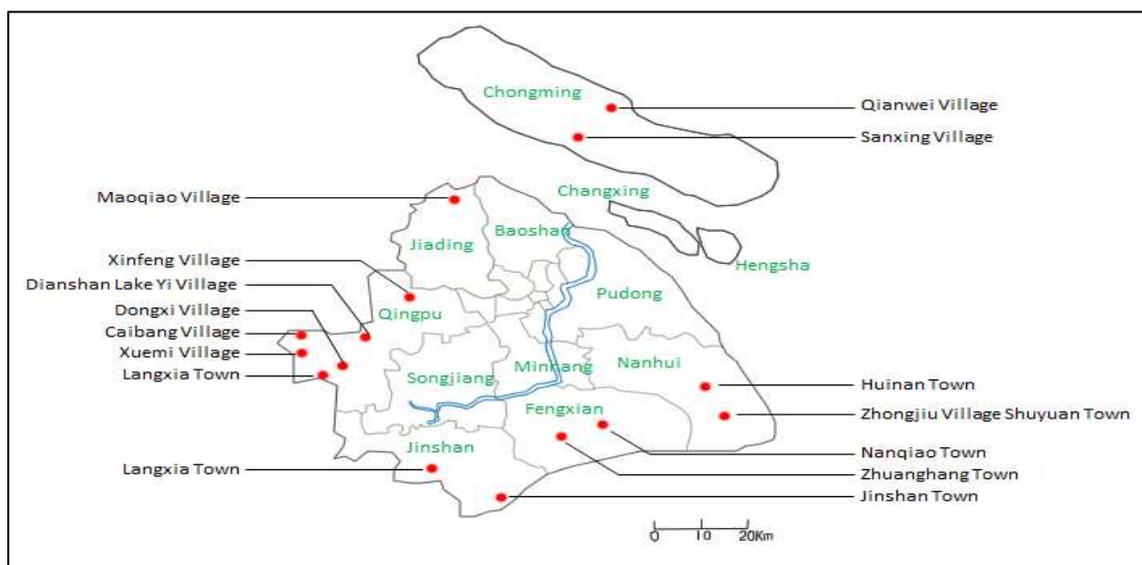


Fig. 1 The distribution of typical rural settlements in Shanghai

### 1.2 Methodology

Mainly use of remote sensing techniques, site investigation and photographic recording methods to find typical villages and conduct investigation and analysis

#### 1.2.1 Data Extraction of Geographic Information System

Firstly, through the data of the Geographic Information System, the suburbs of Shanghai are classified according to the aspects of water body form, land use, and other indicators, so that to conduct screening;

#### 1.2.2 Contrast classification method

To compare and classify similar rural settlement patterns and landscape elements, so as to find typical representatives, and summarize and refine the optimization model.

## 2 Classification of rural settlement patterns in Shanghai

This paper focus on the study of the rural settlement pattern. Mainly through three levels of classification analysis. The first level is the overall landscape relation, and classification is based on the location and encircling relationship of water systems and settlements.

The second level is the classification based on the morphological structure of the settlements themselves. It mainly studies the degree of building aggregation within the settlements, the scale and position of the road system and the public space therein.

The third level is to discuss the surrounding greening environment, which is mainly the greening around settlements, greening along the river, the relationship between public green space and its surrounding.

### 2.1 Overall landscape-cluster relationship

As it is located in the flood plain of the Yangtze River Delta, as well as the estuary of the East China Sea, thus Shanghai has many rivers and dense water networks. Therefore, in the classification of the overall landscape-cluster relationship, it is mainly based on the location and encircling relationship of water systems and settlements and is divided into five major categories as shown in table 1, namely, Along the water, Irregular scattering, Away from water, Water system encircling, and Lacustrine margin.

**Table 1 the style and characteristic of the rural settlements**

Settlement Pattern	Characteristic	Legend
Along the water	Settlements are distributed along the water and are basically in accordance with the form of water body.	
Irregular scattering	Settlements distribute surrounding individual small water system	
Away from water	The water system is generally distributed on one side of the settlement, usually outside, as a natural barrier to ensure the safety of the settlement.	
Water system encircling	The entire rural settlement is surrounded by water and is enclosed inwards. It is more closed and safe.	
Lacustrine margin	Rural settlements in large lakes, distributed along the edge of the land	

### 2.1.1 Settlements classification

#### 2.1.1.1 Along the water type

Settlements along the water is a typical structure type of settlements with a relatively long history. In the investigation, the Jinze Ancient Town,

Zhuanghang Town, and the Yi Village of Dianshan Lake are typical examples of this type of settlements.

Such settlements are usually prosperous due to the convenient transportation and trade on water, and they had become declining due to the decline of trade on water. As a result, these settlements present an obvious status along the distribution of water, and most of the streets facing the water are shops.

Since the waterside directly serves as a place for trade, or where the residents directly access to water source, the hydrophilicity of the waterfront is very strong, and the distance from the building to the water is very short, usually between 5 and 10m, therefore the waterfront as an important place of life has relatively few greening layers and areas, usually just a row of willow trees, and mainly hard pavement.

Among them, Zhuanghang Town (Fig. 2), Jinze Ancient Town (Fig. 3) and North Santang Village (Fig. 4) are typical of this type of village.



Fig.2 Master plan of Zhuanghang Village

Fig.3 Master plan of Jinze Ancient Town

Fig.4 Master plan of North Santang Village

#### 2.1.1.2 Irregular scattering type

Settlements distribute surrounding individual small water systems. The shape and area of the water system itself is small, but the number is large and most of them are not connected. It is an independent small pond. Most of the settlements are distributed around these small ponds. A relatively small number of households constitute settlements themselves, usually a dozen. Examples as shown in figures below.



Fig.5 Settlements on the boundary of Baoshan District and Jiangsu Province

Fig.5 Settlements on the boundary of Baoshan District and Jiangsu Province

Fig.5 Settlements on the boundary of Baoshan District and Jiangsu Province

#### 2.1.1.3 Away from water type

Water systems are generally distributed on one side of the settlements, usually on the outside, and there are often relatively more layers of greenings between the settlements and the water system as a separation. Water systems

and greening together as a natural barrier, settlements have clear boundaries with the outside world to ensure their safety. Among which, settlements in Sanwei Village, Qianwei Village, Nanhui District, Jinshan District and so on forth belong to this type.

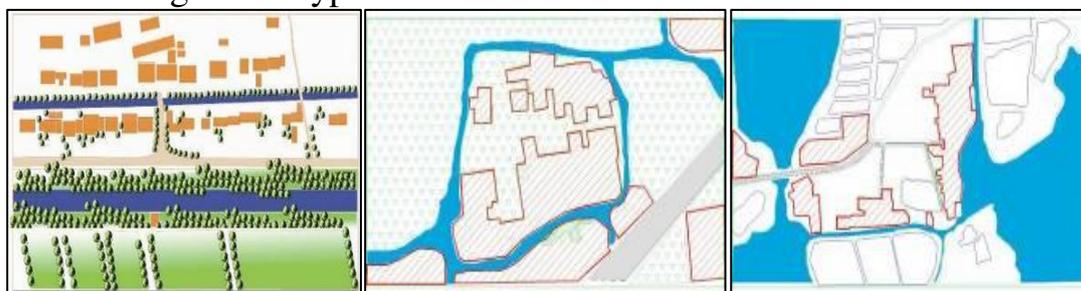


Fig.8 Master plan of Qianwei Village      Fig.9 Master plan of Yi Village in Dianshan Lake      Fig. 10 Master plan of Dongxi Village

#### 2.1.1.4 Water system encircling type

The entire rural settlements are surrounded by water and is enclosed inwards. It is relatively closed and safe. Such settlements have a nature water system that surrounds them as a barrier, nearly circular, and the settlements were clustered together on the island. Typical villages such as Yi Village in Dianshan Lake.

#### 2.1.1.5 Lacustrine margin type

Rural settlements are located in large lakes and are distributed along the edge of the land. Settled on the edge of the island or the peninsula, it is scattered in small patches near the water. With easy access to water, the land near water can be reclaimed for fish-farming, and the middle area of the land can be fully used. Typical villages such as Dongxi Village.

**Table 2 the general structure of the water system and rural settlements**

No.	Settlement Pattern	Shape and scale of water system	Connectivity and functionality	Distribution of settlements	No. of households in settlements	Greening situation
1	Along the water	Wide, about 10-15m	Strong connectivity, for trade and living	Banded distribution along water	Relatively large, above 100	Relatively low
2	Irregular scattering	Irregular shape, small area	Almost no connectivity, mainly for production and living	Distribute surrounding individual small water systems	Around a dozen	Fair
3	Away from water	5m	no connectivity, separation for safety	on one side of the water	20-50	Relatively high
4	Water system	Water encircling	Connected to the outside	Be surrounded	Depends on the area of	Relatively high

	encircling		world through water system,	inside by water	the island	
5	Lacustrine margin	water system is lake	Strong connectivity with outside	along water	Around a dozen	Strong ecotype

## 2.2 Settlements structure

The settlement structure mainly studies the morphological structure of the settlement itself, including the forms and distributional structures of the interior architectures, road systems, and the scale and location of public spaces and other places.

### 2.2.1 Settlements monomer

First of all, the architectural style. The native buildings in the suburbs of China are usually the two-storey, three-room buildings with black roofs and gray walls. However, due to the rapid development of urbanization, most of rural buildings tend to be the same as urban buildings, and because of the relatively large gap between the rich and the poor, different financial situations and aesthetic characteristics determine the style of houses in each household. As a result, local Chinese-style architecture and Western-style architecture are often mixed as shown in Fig. 11-14. There is no uniform style and planning, and it looks rather messy. The more representative of two relatively extreme architectural styles: one is a single-storey cottage with three rooms, very traditional and simple and unsophisticated but age-old with high degree of damage, while at the same time there are many small individual two or three storeys western-style villas around, which is colorful and outstanding.

Second is the colour and volume of architecture. Followed by the color and volume of the building, due to the differences in style, the color and volume of the building are also different. The color of the rural buildings in the suburbs is relatively simple, and the roofs are mostly black or brown, and the walls are gray or yellowish. The color of the new building is obviously richer, such as blue-gray, white, pink, sky blue, reddish-brown and so on forth. Most of the glass windows also use colored plexiglass.

Because of the difference in color and style, it will inevitably lead to a large difference in volume. Buildings with two rooms, three rooms, four rooms, as well as one storey, two storeys, and three storeys are all exist together.



Fig. 11 One-storey classical architectures



Fig. 12 Two-storey joint uniform architectures



Fig. 13 Two or three storeys unique architectures



Fig. 14 Western-style Villas

## 2.2.2 Settlements structure

### 2.2.2.1 Single-row settlements

The single row of settlements lined up along narrower water surfaces, and no one else lived within 50 to 100 meters front and back of the settlements. Most of these settlements are near water, and on one side is a water system, and on the other side is open farmland. The width of the water surface is about 3-5m. The waterside is planted with *Metasequoia*. Examples are shown in Fig.15 and Fig.16.

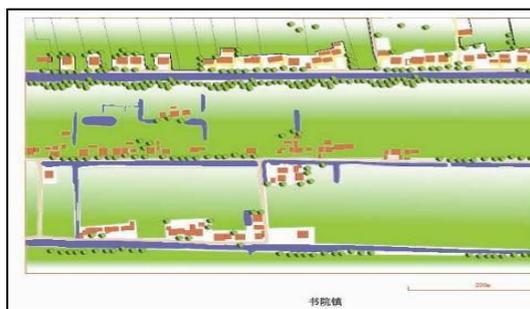


Fig. 15 Master plan of one-row rural settlements on the border of Shuyuan Town

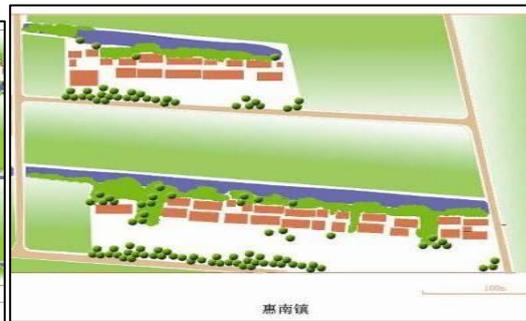


Fig. 16 Master plan of one-row rural settlement in Huinan Town

### 2.2.2.2 Multiple-row settlements

Multiple-row settlements are more compact than single-row settlements. There are other settlements within 50m at the front and back of the settlements, and the population density is relatively high. Although most of the suburban rural settlements do not have a unified overall planning, some unwritten conventions and rules have caused the settlements to be aligned in the front and back, with equal spacing, and thus they do not appear particularly random and messy.

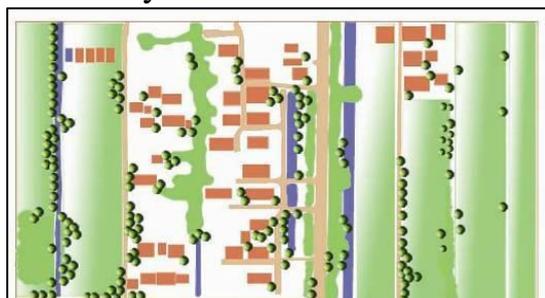


Fig. 17 Master plan of Sanxing Village multi-row

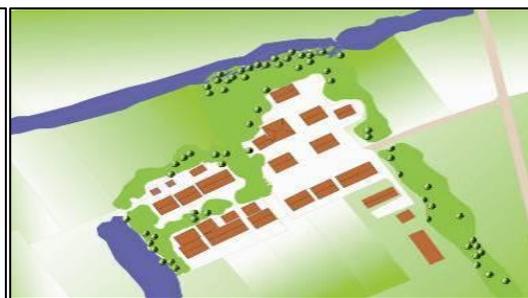


Fig. 18 the rural settlements on the border

### 2.2.3 Intention map of rural settlements in Shanghai

According to the distribution characteristics and data of multiple settlements in rural areas of Shanghai, the relatively regularized plane intention map of the multiple-row settlements of the following villages can be drawn.

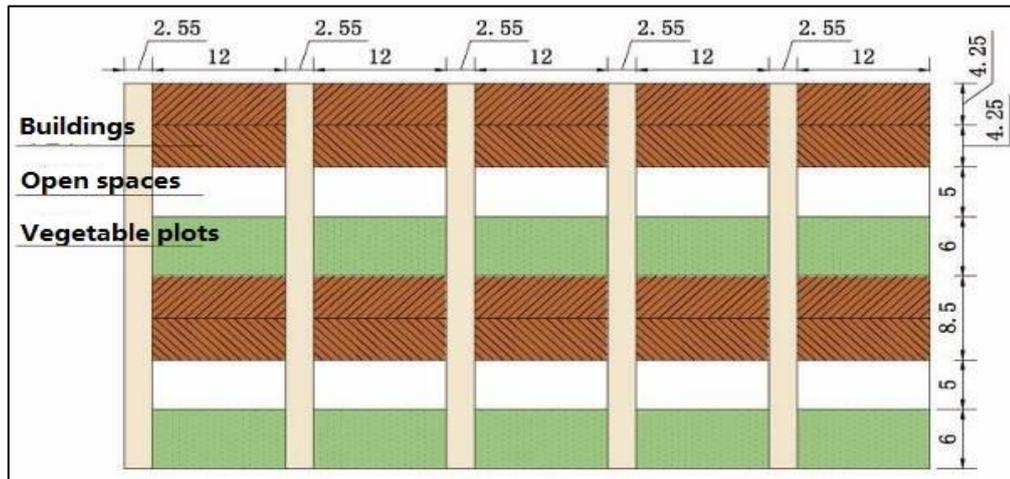


Fig. 19 The ideal mode of the multi-row rural settlements

### 2.2.4 Public space in settlements

As far as rural areas in the suburbs of Shanghai are concerned, the number and area of public spaces are not too large. A wide range of large public spaces seldom can be seen, and there are fewer places for exercise. Religious sites, as a large public space, can produce a certain degree of cohesion and influence, but the number is limited, and the frequency of use is usually low. In the face of today's increasingly aging population, the necessity and functionality of public space is getting higher and higher. The public spaces referred to here include public communication centers, cultural centers, fitness centers, and welfare centers.

### 2.3 Greening environment

According to the characteristics of the distribution of green space in rural settlements in Shanghai, greening includes the following types:

#### 2.3.1 Entrance greening

Mainly divided into three categories: 1. settlements directly connected to the main road; 2. settlements and the outside world have a natural water system as a barrier, the bridge as the main channel linking internal and external; 3. between settlements and the outside world is a large area of farmland.

In the first case, settlements are directly linked to major roads. This type of situation is generally less frequent. However, due to the acceleration of municipal construction and the transformation of many suburban roads, it

sometimes undermines the original features of settlements and enables settlements to directly connect with roads. Then, the entrance space is relatively narrow, and it generally plant a large piece of bamboo as the main vegetation, and some giant trees, such as Metasequoia and Fragrant Plum, are planted around the site, together to separate the interior and exterior spaces. Meanwhile, the entrance can be covered to ensure safety.

In the second case, water systems act as barriers between settlements and outside. Such settlements have a unique geographical advantage and generally have higher security and privacy. Some of the water systems around the settlements are natural, and some are artificial. Natural water systems generally have strong landscape efficacy, relatively clean water, and rich aquatic species on the shore. Due to the limited scale of artificial water systems, the water system is more turbid and the plant species are less. However, due to the presence of water systems, both of them have variety types of plant, trees brushes, grasses and aquatic species. The overall landscape efficacy is strong. In addition, since such entrances usually use bridges as the main channel, the importance of the green landscape of the bridge naturally goes without saying. Among them, there is an entrance bridge where two rows of Metasequoia are densely planted on both sides, forming a long and narrow see-through landscape, forming a relatively closed landscape effect which is quite artistic. Landscape examples are shown in Fig.20 to Fig.22.



Fig. 20 water landscape of the entrance with rich greening  
 Fig. 21 The water belt between settlement and road  
 Fig. 22 The Metasequoia on both sides of the entrance bridge

The third situation is that there is a large area of farmland between settlements and the outside world. This kind of settlement is also relatively common. From the main entrance, it is a large area of farmland which is first introduced into the eyes, and a settlement after the farmland. This kind of settlement is also more intimate because it is in the innermost part. Meanwhile, a large area of farmland provides an open field of view. However, such entrance greening is not particularly landscape rich.

### 2.3.2 Settlements center greening

Settlements center greening, that is, the center greening area inside settlements. In the investigation, there was a discovery in Yi Village of Dianshan Lake. It is composed of an entire single piece of woodland. It is

mainly a dense forest of Camphor which forms a square array embedded in the settlement patches to form a rich landscape element.

### 2.3.3 Courtyard greening

Almost all of the rural settlements in Shanghai have green courtyards, but mainly for vegetable cultivation, and there are few trees and shrubs. Usually there is a small piece of land in the corner of the courtyard, as a vegetable garden, and there will usually be one or two trees such as Camphor, Metasequoia and so on. The fruit trees are mainly loquat trees, peach trees, jujube trees, and the shrubs are mostly sweet-scented osmanthus. The overall species is relatively simple.



Fig. 23 Different landscape in the courtyard of settlements

### 2.3.4 Riverside greening

The riverside greening has the most abundant types of landscape in the rural settlements. Due to Shanghai's unique geographical location, there are extensive water systems, and water systems around settlements are also abundant. Usually there are one or two ponds around the settlements, and the pond side is usually planted with rich greening, such as mulberry, locust trees, Broussonetia, fragrant plum, Metasequoia and so on forth. On the one hand, greening along the waterside can block wind and fix soil, and on the other hand it can conserve water sources and preserve water quality and cleanliness.

### 2.3.5 Farmland greening

The types of farmland greening are comparatively simple and basically consist of two to three rows of shelterbelts as a green isolation belt between farmlands. The tree species is relatively simple, mainly fragrant plum and Metasequoia, when the array is relatively wider or longer it appears spectacular. Sometimes there are also single tree or three or five trees clustered in farmland, which is also very artistic.



Fig. 24 Different landscape on the farmland

Generally speaking, large areas of greening are mainly distributed in the northern part of the settlements and near the pond side close to the settlements as shown in Fig. 25. There are sometimes small patches of greening inside the settlements, and at the front and back of the houses, there are sporadic distributed Metasequoia and fruit trees.

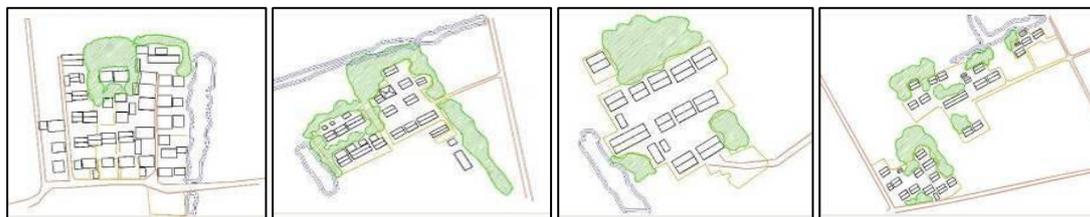


Fig. 25 The plants distribution around the settlements

Greening mainly has the following functions: 1. Windshield to protect houses, as greening is mainly distributed in the northern part of the settlements, so that the invasion of northwestern wind towards the entire settlements can be weakened in winter; 2. Water conservation, the greening at the waterfront is mainly to prevent water and soil loss around the pond, as well as clean water source; 3. Protect the territory, dense greening can be used as an effective barrier, divide the land between settlements and settlements, as well as can reduce the interference of outside; 4. Beautify the environment, induce the scenery at the front and back of the house full of seasonally change.

### **3 Conclusion and discussion**

#### ***3.1 Landscape features of rural settlements in Shanghai***

##### *3.1.1 Harmonious co-existence between man and nature, respect for nature*

The villagers in the suburbs of Shanghai are best adhering to the ancient ancestors' spirit of "harmony between man and nature and respect for nature". Most of the villagers in Shanghai's rural villages live in harmony with nature, observe the laws of nature, respect nature, build water conservancy, and dredge water systems to suit local conditions and develop agriculture.

##### *3.1.2 Water township emotion*

Bridges, flowing water, and households which are almost universally recognized as the impression of water towns in Yangtze River Delta areas.

##### *3.1.3 The collision and integration of multiculturalism*

As an international metropolis and a colony of Shanghai culture, Shanghai is full of collision and integration of multiculturalism. Since ancient times, because of the convenience of the river port advantages, Shanghai became the center of economy and trade, merchants all around the world have stayed.

Most of the farmers in the suburbs of Shanghai live along the water, not only for the needs of life and production, but also for commercial trade, the side facing the river, is mostly for shops. Besides, the population of Shanghai

is diverse, people of different regions, different cultures and different levels gathered together.

### ***3.2 Existing problems of the rural landscape***

#### *3.2.1 limited excavation of indigenous cultural elements*

As far as the development of rural cultural tourism in the suburbs of Shanghai is concerned, the heterogeneity and characteristics of the landscape are not high. Most of the rural tourism shows a high degree of convergence not only from the overall layout, settlement style, landscape features, or tourism projects. How to excavate the local landscape cultural characteristics and rational layout, inheriting history and combining modern construction has become a very critical issue.

#### *3.2.2 Few ecological concerns about sustainable development*

How to protect native plants, traditional settlement culture and simple spirit. Behind our blind development and utilization, we should give more consideration to future generations, the fresh air, pure water, green forests and beautiful and rustic rural landscapes that are necessary for them.

#### *3.2.3 Lack of recreation and entertainment*

With the increase in people's economic income, rural construction is also moving towards a high level and quality development. The original muddy path was replaced by the wide asphalt road and the low bungalows were also replaced by small villas. However, as people's material living standards have improved, we have also found that the leisure and entertainment in the new countryside is obviously lacking. Therefore, in the construction of a new countryside, we must integrate more of the original natural landscape into living environment.

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## **Economic Viability: Sustainability and Safety through Human Values and Economic Modes**

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### **Introduction**

Society expects much of its agriculture – to produce food and raw materials in enough quantity and good quality, to keep natural resources, to provide lovely countryside, to solve social problems giving job and income to less privileged groups, to contribute to economic growth, and etc. And to do it immediately and do it permanently. To achieve these goals, the society uses a wide range of sanctions (regulation, standards, monitoring) and incentives (direct payments, subsidies, tax reductions). The results are not very encouraging. Food crises, high price volatility, lack of agri-business in some regions and deficiency of farm workers in other, low quality (even dangerous) products, hard (often impossible) entry, ineffective distribution of wealth, reluctance of young people to carry on with the family farming, and etc. negative effects could be seen all over the world. Moreover, all this happens after high spending of public funds.

A question came naturally – how to achieve the goals mentioned above in more reasonable and effective way? Once Adam Smith has said: As every individual ... pursuing his own interest he frequently promotes that of the society more effectually than when he really intends to promote it (Smith [1776], 1994). We do believe in this idea. We are also agreed with the continuation: I have never known much good done by those who affected to trade for the public good (ibid.)

The goal of this paper is to present, discuss and proof the idea for sustainable agriculture which produces high quality products without significant public support. Natural human values needed for it, as well as proper economic mechanisms are described after a field study<sup>3</sup> on the problem.

### **On the term**

Having so high expectations on its agriculture the society gives it various names – multifunctional, ecological, organic, sustainable, circular. A new one (even the idea is not new) is already in action – resilient (Resilience Alliance). Sustainable agriculture is the most popular of all these terms. Its definition is under intensive debate. Some of the early understandings are already set aside. The new are not enough persuasive and also criticised.

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<sup>3</sup>The Bulgarian National Science Fund has supported this research

According to Bachev, understanding of sustainability as an approach in farming is not always useful in the direction for changes in agriculture. For sustainability is important to assess the impact of external for the agroecosystems factors – the institutional environment like social standards, limitations, social support etc., development of markets (e.g., new alternative channels for ecological products) and current macroeconomic environment. Finally, it is necessary to assess the specific socio-economic environment and available specific nature resources for the particular agroecosystem. Definition of agrarian sustainability has also another weakness. It is often understood in politics as ability for covering requirements of external criteria, e.g. measures for economic sustainability with financial indexes either for covering quantity goals while ignoring the quality of production or measures for covering of ecological requirements only because of high political sensibility Bachev (2018).

If we rethink the sustainable for us personal values, e.g. traditions, belief, feelings, we easily discover that they are sustainable - independent from availability or lack of support (public, financial and etc.); they are kept independent of the end result (financial or other); people have willingness to spread these values to next generations. Thus, these personal or social values survive, they are resilient and sustainable. That is why some authors (Terziev and Radeva 2018), prefer to use in their work the term economic viability, in order to differentiate from mentioned above weaknesses in the conception of sustainability.

Viability: Forgotten meaning of term interest

Adam Smith's view on the creative role of personal interest is widely applied in economic theory. But it is transformed and limited (mainly by neoclassical school) to two binding characteristics of the economic agents: a) rationality (in economic sense), and b) profit maximization behaviour. Moreover, both become critical assumptions for all economic models. And they continue to play the same role even today after the critical work of leading economists on bounded rationality, non-market behaviour, institutional structure of production, asymmetric information, and other areas. An old truth that custom not competition, governs much of the economic world (Mill [1848], 2004) is forgotten.

Human interest could be oriented to various values. After an intensive study on alternative Bulgarian farmers, an economic view on viability of a farm, including five dimensions, was formulated (Radeva, 2017):

- ability for providing of satisfied level of earnings and personal satisfaction. As we know from Herbert Simon, people normally seek for options (or economic results), which are “sufficiently good” and don't seek by all means the best alternatives or options, leading to maximization (Simon, 1961);
- ability for self-sustain existence (relative financial independence). We

believe that an economic unit or an alternative agrosystem could be sustainable, when it is able to survive and evolve without subsidies, tax concessions or other public financial support and as well under low level of dependence on bank financing;

- engagement to practiced activity or project (e.g., crop cultivation, restoring of agrosystem and etc.). When such an activity like crop cultivation is practiced, which carries satisfied earnings and satisfaction, to what extent it is fulfilled with engagement to its future development and work in this direction;
- ability for transfer the generated till today viability to next generations. This could happen when interconnection and cooperation with local communities is realized, when models for sustainable way of work and living are created, with training, sharing, employment creation, building of philosophy for healthy living and spreading the ideas to young people and their families;
- keeping of ecological and ethical principles as care for the earth and care for people while preserving, rebuilding and regenerating of natural and human resources.

**Table 1. Economic viability of Bulgarian alternative farmers**

Ability for providing of satisfied level of earnings and personal satisfaction
53% - positive economic results; 88% - personal satisfaction.
Ability for self-sustain existence
10 years in business on average; 76% - work without subsidies or other governmental support; 87% - work without bank credits.
Engagement to practiced activity
73% - very high engagement by reasons other than financial results;
Ability to transfer achievements to next generations
53% - young family people; 50% - demonstration of new model of farming and life; 19% - support for local development.
Application of ecological and ethical principles
87% - no use of chemical; 75% - predominantly manual labour; 75% - volunteer labour; 47% - use only own seeds;

38% - usage of renewables from all energy sources.

*Source: Radeva 2017*

From a mainstream economics point of view these people are not rational – do not seeking the best alternative in money term. Also, their behaviour is not profit maximising – again in money term. But obviously they are very interested in what they are doing. They produced healthy products, protect environment, save resources, and do not spend public funds. That is what modern society expects from agriculture. They carry out their business based on customs and traditions, on ideas even dreams. And they survive. That is what we call viability.

Viability: Way to achieve

The leading Bulgarian author in the field of sustainability emphasized the high importance of non-ignorance of the economic aspects when determine the level of sustainability, because even the most ecological farm in the world cannot be sustainable for long-term, if not able to self-sustain economically (Bachev, 2018). He is right, of course. Let us see now how vital Bulgarian alternative farmers cope with the problem.

We continue our work with a survey on the modes these farmers develop and use to govern their transactions. We follow the understanding that a variety of governance modes exists. These are (Bachev and Terziev, 2018):

- internal organisation (a farm) is the basic governance mode. Farmers use it to manage technologies choice, resources usage, and activity organisation;
- market modes – various decentralized initiatives governed by price movements and market competition. Examples are: exchange of resources, products and services directly through the market; various trade contracts; buying and selling production and ecosystem services, etc. Farmers use these modes because of the benefits of specialization and free exchange;
- private modes - a set of initiatives, arrangements, codes, partnerships and etc. forms to surmount difficulties and save cost of market exchange;
- public modes - local, national, and transnational interventions in order to introduce structure in economic agents behaviour;
- hybrid forms – public-private partnership, guild inspection and licensing of private initiatives, and etc. mixed mechanisms born to avoid disadvantages of the market, private and public modes.
- and finally - institutions. These are rights and obligation, and mechanisms for their enforcement. Institutions could be: a) formal - law, regulations, standards, court decisions, etc., or b) informal – behavioural

norms coming from tradition, culture, ethic, and etc.

Deeply were investigated twelve types of transactions for:

- land supply;
- labour supply;
- machines and mechanical services;
- technologies and knowledge;
- transport;
- veterinary medicine services;
- seeds and sow materials;
- energy and fuel;
- long term assets;
- bank financial products;
- other financial resources;
- marketing of the products.

The results obtained from the survey could be summarised and analysed as:

a) internal organisation. Farmers use this mode by various reasons. They apply specific technologies and produce specific products. Seeds with guaranteed quality for the more of them could not be found on the market. Often own production is the only chance. Also highly specific is the needed knowledge. Even sometimes it is an individual secret. Such knowledge could not be supplied from outside or it is very expensive. To follow ecological (organic) principle farmer need of a proper agricultural land, for a long period of time. Obviously using own land is the only solution. Alternative farmers hate bureaucracy – administrative or those of big organizations, including banks. That is why they prefer to mobilize own financial resources (or those of relatives and friends) instead of applying for credit or public financial support. Special case here is Crowdfunding. By its economic characteristics it is more close to internal organisation. We could conclude that alternative farmers internalize within the farm some of their input transactions in order to cope with the problems of high specificity (labour and knowledge), high uncertainty (land and seeds), or to save directly transaction costs (financial products);

b) market mode. Farmers are not able to produce machines, fuel or vaccines. The only option is to buy these resources from the market. It is important to mention here that these are rare transactions for alternative farmers – 87% of them never use any veterinary medicine services, 33% apply only manual operations (no machines), 75% - predominantly manual labour, 13% do not use any fossil fuel, energy for 25% of the rest come from renewable sources. The situation with output transactions is different. All of interviewed farmers declare that they produce to sell. Market is a natural canal in this case. But they use it in specific way as it will be shown below.

Summary: market mode is preferred for standardized resources (low uncertainty), and final goods (high frequency);

c) private modes. This governance mechanism requires strong organization, enforcement power, and (more often) huge financial resources. Bulgarian alternative farmers are small and relatively poor. They have no capacity to initiate and realize sustainable private modes. But some other – have it. For example – national and international organizations for voluntary labour. In fact, 89% of hired labour in alternative farms is voluntary. Applicants are selected and chosen by trusted organizations which know the exact needs of the farmers. Obviously private mode is expensive and impossible at that moment for Bulgarian alternative farmers. They could only joint existing mechanisms of this type;

d) public modes. The results of our survey show that this is the most undesired governance mode. 73% of the farmers have never received subsidy (80% of them declare that they do not want it), no one trust in government standards, only 8% have registered themselves officially as organic producers, even they are in fact. The situation is the same for hybrid modes. Farmers of our group have neither willingness nor power to initiate such forms;

e) institutional modes. The situation here is contradictory. From one side, farmers do not like formal institutions. Problems in registration of the land, in access to water for irrigation or to electricity, in communication to local and central administration have faced 84% of the farmers. On the other hand, they use intensively informal institutions. Their business is based mainly on reputation and trust. As it was mentioned above, they use market for some of their input and for the most of their output transactions. But this usage is in special way. They insist that they need of unique resources (seeds and labour for example) and produce unique products. Try to avoid competition and to replace it by stable, long term contacts with suppliers and buyers built upon full confidence and best faith. Thus alternative farmers solve the problems with high specificity and uncertainty, with information asymmetry and hostile official institutional framework.

All our findings on governance modes are summarized in table 2.

**Table 2. Governance modes used by Bulgarian alternative farmers**

Transactions	Reasons to use or not use of governance modes				
	Internal	Market	Private	Public, hybrid and formal institutions	Informal institutions
Land supply	High uncertainty			Lack of capacity	
Labour supply	High specificity		High specificity		Need of trust and confidence

Machines, mechanical services		Low specificity& uncertainty			
Technologies, knowledge	High specificity				
Transport		Low uncertainty			
Veterinary medicine		Low uncertainty			
Seeds and sow materials	High uncertainty				
Energy and fuel		Low uncertainty			
Long term assets		Low specificity& uncertainty			
Bank financial products	High transaction cost				
Other financial resources					
Marketing of the products		High frequency			Need of trust and confidence

*Source: Authors' survey*

**Conclusion: We, the economists**

Farmers could contribute to all society development and could cover all expectation of the general public only following their own interest. Human interest is not a simple term (profit or utility) that could be easily calculated. It is a mysterious set of values, believes and customs. The economist could say little, or even nothing, on human interest. Other sciences are needed for studying and explaining this phenomenon. Economists have to keep their eyes open for the achievements of these sciences.

But a wide field of work is in front of us. We have to build economics models incorporated other sciences findings on human nature, to show the economic agents (farmers in this case) proper modes for organisation of their business activities, and to propose the authorities administrative measures which will support creative behaviour in effective way.

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**The straw organic fertilizer workshop in the  
container:  
Research and application of integrated technology  
for distributed straw cleaning and  
fertilizer fermentation**

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**Abstract**

To reutilize rice straw immediately during the agricultural production process, an actinomycete named JSD-1, which was classified as *Streptomyces griseorubens*, has been isolated from soil and rotten straw. Genome sequencing was carried out using MiSeq platform to further investigate the lignocellulose-degrading mechanisms. After obtaining its draft genome, the key lignocellulolytic genes such as multicopper oxidase, exo-1, 4- $\beta$ -glucanase, endo-1,4- $\beta$ -glucanase, and  $\beta$ -xylosidase were identified and characterized. Quantitative real-time PCR revealed that expression levels of all analyzed enzymes were significantly and generally upregulated during the whole cultivation, indicating that they all contributed to the biodegradation. Then the composite inoculants JFB-1 was developed by the combination of JSD-1 with other bacteria, which could decompose straw much more effectively. Moreover, a system equipment with the property of portable and sealed has been developed to realize the integration of straw collection and fermentation. High ventilation volume in the equipment was supplied to maintain aerobic fermentation of bacterial complex for effective degradation of straw, which could shorten the composting cycle to 15 days compared to above 3 months of traditional composting. Mature compost can be used as organic fertilizer and nutrient soil, both of which have the ability of promoting plant growth and preventing plant disease.

Key words: straw degradation, *Streptomyces griseorubens*, bacterial complex, system equipment, organic fertilizer

**1. Introduction**

According to incomplete statistics, over ten billion tons of straw had been produced recent years all over the world and almost one billion tons of straw was produced in China per year. Rice straw, which accounted for 31.6%

totally, had the largest amount among crop straw [1]. As the structure of rice straw is mainly cellulose and hemicellulose encrusted with lignin and covered by pectin, the degradation and utilization of rice straw were relatively difficult compared with straw of other crop (Parr et al., 1992; Gao et al., 2002). Mass of rice straw were usually eliminated through burning, which might cause great fire and bring serious threats to environment. Nearly 90% straw had been used improperly, which meant great resource waste and environmental pollution. Another approach of dealing with rice straw was mixing it into the soil. As the existence of soil microorganisms, it was easier for the lignocellulose components to transform into compost. However, it usually took a long time to decompose and impoverished the soil easily. A large number of studies have shown that compost straw contains abundant plant nutrients [2-5] and presents long and stable manurial effect. In addition, it can promote the formation of granular structure and increase the water retention, heat preservation, gas permeability and fertility in soil. Some microorganisms in compost also have biocontrol effect [6]. Moreover, it can be made into potted matrix, which can greatly ease the environmental pressure caused by peat exploitation [7].

At present, in China the traditional composting method is time and labor consuming. The composting process produced a large amount of harmful gase and stench, which is harmful to the environment. Compared with the traditional composting, machine composting is more efficient. There are many kinds of modern composting facilities abroad, but their prices are very high. In contrast, some composting devices has been developed in China, however, they varies in quality. Lacking monitoring system and the long fermentation period may lead to uncontrollable process and unsatisfactory quality of the compost. Therefore, it is urgent to develop a set of equipment with clean, short-time and controllable fermentation process to improve the production quality.

Our team in Shanghai Jiaotong University has been committed to the prevention and control of agricultural environment pollution, and clean utilization of agricultural waste. The integrated cleaning and fermentation technology of distributed straw has been popularized and applied in Chongming district, Shanghai. A high efficiency complex microbial fertilizer and an optimized fermentation process for straw degrading have been developed. In order to achieve ‘turnning the waste into wealth ‘, an auxiliary machinery controlled by cloud platform was also developed to shortening the composting cycle and improve the compost quality. This approach will be of guiding significance to achieve the goal "the comprehensive utilization rate of straw will reach over 85% by 2020" which was put forward by the ministry of agriculture in China.

## **2. Materials and methods**

### ***2.1 The determination of straw degrading enzymes***

Xylanase activity and Pectinase activity were detected with the method of Shimizu and Kunoh [8] and Mnamiyamat et al. [9]. Ligninase activity was estimated with the method of Song et al. [10]. The actual activities of straw degrading enzymes including carboxymethyl cellulase (CMCase), filter paper cellulase (FPase), xylanase, pectinase, laccase (Lac), lignin peroxidase (Lip) and manganese peroxidase (Mnp), were all measured under certain conditions. The enzyme liquid was taken from fermentation liquid of straw degrading process at the 5th day.

### ***2.2 Genome sequencing and functional annotation***

Genome of *S. griseorubens* JSD-1 was extracted using the TIANamp Bacteria DNA Kit (Tiangen Bio Co., Ltd.). Genome sequencing was performed by Illumina MiSeq 2×250 bp platform with insert sizes of 300, 360, and 700 bp paired-end as well as 3 and 8 kb mate-paired libraries. Assembly of all sequence reads applying Newbler 2.8 assembler resulted in a draft genome map. Glimmer 3.0 was used to predict open reading frames (ORF) with BlastP annotation. The functional annotation was determined with KEGG, COG, and SwissProt databases.

### ***2.3 Expression levels detected by qRT-PCR***

Total RNA of *S. griseorubens* JSD-1 was extracted using RNAPrep Pure Bacteria Kit (Tiangen Bio Co., Ltd.). Culture was sampled every 24 h within 7 days. The reverse transcription PCR for cDNA was performed followed by quantitative real-time PCR (qRT-PCR) with SYBR Green dye method according to the manufacturer's recommendations of SYBR® Premix Ex Taq™ GC (TaKaRa Bio Co., Ltd.). Cycling parameters of qRT-PCR reactions were programmed with an initial step of 30 s at 95 °C following 40 cycles consisting of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, and finally with an extension at 72 °C for 15 s. The relative quantification of these genes was analyzed with the  $2^{-\Delta\Delta C_t}$  method. Technical triplicates were performed for each biological replicate, and the average values were used for quantification. Herein, 16S rRNA was used as internal control to normalize the relative transcription levels of the analyzed genes.

### ***2.4 Development of system equipment***

The system equipment for straw degradation was produced by The 711th research institute of China Shipbuilding Industry Corporation.

## **3. Results and discussion**

### ***3.1 Isolation and characterization of JSD-1***

After the enrichment, the population of cellulose-degrading microorganisms had dramatically increased. By the selection of filter paper agar plates and the other selective medium, one streptomycete strain was isolated finally. The morphology of JSD-1 on Gause 1 medium plate was shown in Figure 1. The morphology of JSD-1 through optical microscope (40×) is shown in Figure 1b. It was shown clearly, after 8 days of inoculation,

that long spores chain with 1-3 loose, open and spiral loops were formed on ISP2 medium at 30°C.



Fig.1 Morphological characters of JSD-1 through microscope

### 3.2 The determination of straw degrading enzymes

The activities of cellulase, xylanase, pectinase and ligninase were all detected. From Figure 2, different sizes of hydrolysis zones were shown on the plates. Therefore, the activities of xylanase, cellulase and ligninase were clearly presented as a result of the hydrolysis of carbon substrate. Moreover, all enzymes activities are listed on Table 1 in detail, but the values were not measured under optimum conditions.

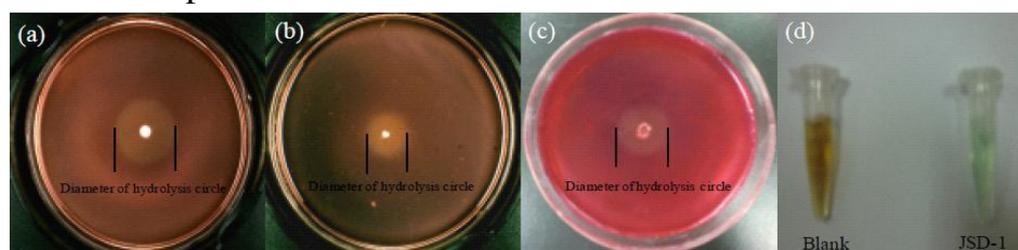


Figure 2. (a) A colony and clear halo formed by JSD-1 on cellulose Congo-red agar plate. (b) Plate assay for demonstration of the xylanase activity. (c) Plate assay for demonstration of the pectinase activity. (d) Liquid assay for demonstration of the peroxidase activity.

**Table 1. The activity of straw degrading enzymes**

Isolate	CMCase (U/ml)	FPase (U/ml)	Xylanase (U/ml)	Pectinase (U/ml)	Lac (U/L)	Lip (U/L)	Mnp (U/L)
JSD-1	59.19	31.68	4.27	3.66	50.00	16.12	13.23

### 3.3 Genome sequencing and bioinformatics analysis of *S. griseorubens* JSD-1

A total number of 6,432,848 reads including 2209-Mb clean data were generated, which represented a 263.0-fold average coverage of the whole genome. The assembled genome consisted of 2 scaffolds and 246 contigs. The N50 length of contigs was 53,294 bp, and that of scaffolds was 7,563,100 bp. Finally, we obtained the draft genome of *S. griseorubens* with a single linear chromosome of 8,463,223 bp and an average GC content of 72.42 %. Analysis of the genome revealed that it contained 6 rRNA operons, 66 tRNA genes, and 7159 protein-coding sequences (CDS). Among these CDSs, 4587 proteins could be assigned to clusters of orthologous group (COG) families (Table 2). This whole genome shotgun project has been deposited at GenBank under the accession number JJMG00000000.

**Table 2 Genome features of *S. griseorubens* JSD-1**

Attribute	Value
Genomesize(bp)	8,463,223
DNAcodingregion(bp)	6,623,064
GCcontent(%)	72.42
Numberofreplicons	1
Totalgenes	7159
rRNAgenes	18
rRNAoperons	6
tRNAgenes	66
Proteincodinggenes	7159
Geneswithpredictedfunction	4587

### 3.4 Differential expression responds of the analyzed enzymes

To further investigate expression responds of these lignocellulolytic enzymes when induced by various carbon sources such as glucose or rice straw, qRT-PCR was carried out. Relative transcription levels of the analyzed genes when cultured with rice straw were generally improved compared with those of glucose. And, expression of glucanases was particularly upregulated with the stimulation of rice straw. Generally, all enzymes exhibited their greatest expression levels at the 4th or 5th days during the whole cultivation.

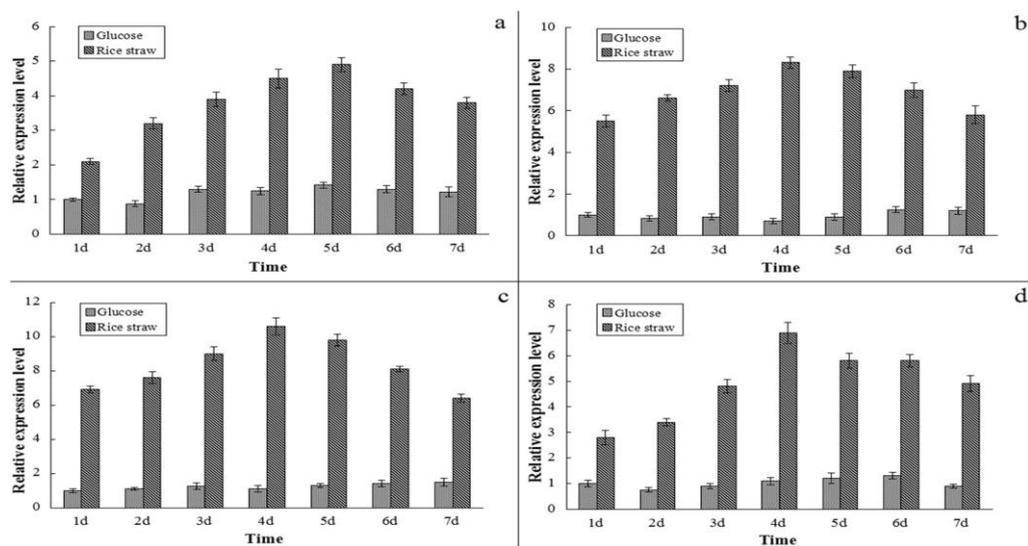


Fig. 3 Expression responds of the analyzed enzymes with the addition of different carbon sources.

a Multicopper oxidase, b exo-1,4-β-glucanase, c endo-1,4-β-glucanase, d β-xylosidase

### 3.5 Straw composts with composite inoculants JFB-1 and their effects on soil carbon and nitrogen contents and enzyme activity

Based on the isolated 15 high-efficient cellulose degrading bacteria in the laboratory, the composite inoculants (JFB-1) which can effectively degrade crop straw were screened, and the effects of straw composts with the composite inoculants on soil carbon and nitrogen contents and enzyme activity were studied. The results showed that the composite inoculants can accelerate straw decomposition for 1-2 d during single fermentation period,

and the organic matter contents in straw composts reached 403.5-515.1 g kg<sup>-1</sup>, while the ratio of carbon and nitrogen decreased from 10.53 to 15.30. The pot experiments found that the application effects of rice straw composts are generally better than those of corresponding asparagus straw composts. Compared with the control compost of rice straw, when the application amount of rice straw compost using the composite inoculants was 150 g kg<sup>-1</sup>, the contents of soil organic matter and total nitrogen increased 33.5% and 7.3%, and soil urease and cellulase activities increased 16.7% and 30.8%, respectively. Compared with no fertilization treatment, the application of straw composts can improve soil microbial community structure, and increase microbial diversity indices. When the application amount of rice straw compost using the composite inoculants was 100 g kg<sup>-1</sup>, the biomass of common Chinese cabbage cultivated for 30 d increased 46.4% compared to the control compost of rice straw.

### ***3.6 Development of system equipment***

A system equipment with the property of portable and sealed has been developed to realize the intergration of straw collection and fermentation. High ventilation volume in the equipment was supplied to maintain aerobic fermentation of bacterial complex for effective degradation of straw, which could shorten the composting cycle to 15 days compared to above 3 months of traditional composting. Each single device has the dispose capacity of about 150 tons of straw. On-line monitoring and remote real-time intelligent control of the fermentation process has been developed. These system equipments have been applied in 4 demonstration bases in Shanghai.



Fig. 4 System equipment for straw fermentation. (a) first generation equipment, demonstrated in Chongming Zhongxin Taisheng Farm, Shanghai, (b) second generation equipment, demonstrated in Pujiang Green Valley Practice Base, Shanghai, (c) third generation equipment, demonstrated in Chongming Lvru fruit and vegetable Specialized Cooperative(Maqiao Base), Shanghai, (d) fourth generation equipment, demonstrated in Chongming Xiangnong Specialized Cooperative.

### ***3.7 Demonstration application of organic fertilizer and growing media products***

The composting cycle could be greatly shortened by using high-efficient straw degradation composite inoculants (JFB-1) and high ventilation volume in the equipment. Meanwhile, high temperature above 60°C could be arrived during the composting, which could kill weed seeds, pathogens and other harmful substances in compost. The colour of mature compost became dark brown in the end of fermentation. The water content and pH of mature compost was determined as 29% and 7.7, respectively, which totally meet national standards in China. Organic matter was determined as 70.8%, which was 1.6 times than that of national standard. Instant nitrogen, phosphorus and potassium was determined as 6.64%, which showed good fertility. The number of effective viable bacteria which arrived at  $2.58 \times 10^9$  CFU/g was 10 times than that of national standard. In addition, the straw organic fertilizer sourced from mature compost contained a lot of useful actinomycetes, which could effectively inhibit soil borne disease, had great significance to improve crop quality and yield.

#### 4. Conclusion

An efficient rice straw degrading strain isolated from compost treated soil, was identified and designated as *Streptomyces griseorubens* JSD-1. Mechanisms of lignocellulose biodegradation during composting was primarily revealed based on its genomic information. Besides, its application to compost was also analyzed. The investigation demonstrated the scientific background for the correct evaluation of its utilization in composting and mechanisms of improving the soil quality treated with compost. In addition, a potential approach to utilize crop straw was provided as well.

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# Study on the Spatial Structure of Shanghai Urban Agriculture Tourism

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## Abstract

Urban Agriculture Tourism, is a combined production of both urban agriculture industry and civic tourism demands. And its core content for the research is the united industrial characteristics of tourism and urban agriculture, forming the tourism spatial structure of urban agriculture. This research, based on the survey in urban agritourism of both industry development situation and development mode in Shanghai, identifies 7 industrial clusters in Shanghai and analyses the influence on spatial structure by agritourism industry. As a result, space layout of urban agritourism in Shanghai shows spatial structure like ‘Circle’ and ‘Cluster’. ‘Circle’ structure reflects the variation of Shanghai Urban Agriculture Tourism intensity and forms, while spatial ‘Cluster’ reflects the cooperation of the agriculture tourist spots within areas and the development of tourism industrial clusters.

**Key words:** urban agriculture tourism; spatial structure; Shanghai

Since 1990s, urban agriculture has gained worldwide concerns and tourism has been developed into one of the biggest industries. While, as a new expanding form of tourism due to increasing market needs and goals of effectively developing urban agriculture, urban agriculture tourism becomes the research focus of different fields. Most of current studies focus on the analysis of agriculture tourism sites and industry organizations [1], and they are lack of the spatial organization forms of the agriculture tourism industry. Taken Shanghai as an example, this paper discusses spatial structure models of urban agriculture tourism within urban areas.

As the name suggests, urban agriculture tourism includes two core concept, urban agriculture and agriculture tourism. Literally, urban agriculture means agriculture within and around cities. Due to its development heavily dependent on urban markets, it mainly serves for cities, to directly meet the needs of cities as well as the life, ecological and leisure activities for citizens [2]. While, agriculture tourism, or rural tourism, is called as a tourism form targeted to ‘countryside landscape, rural culture and agriculture production’ [3]. Generally, these two definitions can be regarded as similar. The only difference is that the former one emphasizes industry characteristics of close connections between the agriculture and tourism, while the latter one refers to rural geographical location characteristics of this kind of tourism. This paper

applies the concept of ‘agriculture tourism’, to emphasize the attraction of agricultural production and its related tourism activities to tourists. Besides, from the aspect of regional space, the choice of industry location is the fundamental reason why different city spatial structures form.

### **1. Industry development and current spatial layout of Shanghai urban agriculture tourism**

Shanghai agriculture tourism started with Nanhui ‘Peach Blossom Festival’ and Baoshan ‘Citrus Festival’ in 1991, and entered into its start-up periods. Despite of the late beginning, it developed fast. Till 2009, more than 100 agriculture tourism attractions have been registered, 17 of which were national agriculture tourism demonstration sites [4]. The annual incomes of agriculture tourism could reach 1.5 billion yuan, and more than 11 million tourists were accepted per year (Tab.1).

**Tab.1 Shanghai agriculture industry development situation (2005-2009)**

	2005	2006	2007	2008	2009
Agriculture Tourism Income (billion yuan)	4.5	6	10	12	15
Agriculture Tourists Number (million)	2	4.0	7.9	8.5	11
Agriculture Tourist Sites Number	38	40	46	78	106
National Agriculture Tourist Exemplary Bases Number	5	6	14	16	17

This study analyzed data on tourism economy of 118 agriculture tourism sites in Shanghai, and conducted on-site research on nearly 30 large-scale sites. From the aspect of development levels of tourism economy, significant differences were existed in various agriculture tourism sites and tourism incomes of tourist areas in Shanghai. The agriculture tourism income of Taohua Village reached 0.2 billion yuan in 2009, taking up 13.3% of more than 100 agriculture tourism sites in the whole city. And the income during 23-day ‘Peach Blossom Festival’ occupied 30% of the annual incomes. On the contrary, the income of Yangyi Village, a national agriculture tourism demonstration site located in Nanhui, was only 3.109 million yuan, taking up 1.55% of annual tourism incomes of Taohua Village.

Besides, the spatial distribution of agriculture tourism in Shanghai is unbalanced. Taken 17 national agricultural tourism sites as examples (Fig.1), the spatial distribution is mainly focused on the south of Shanghai suburbs (Nanhui and Fengxian) and Chongming Island, and it initially shows a trend to form groups.

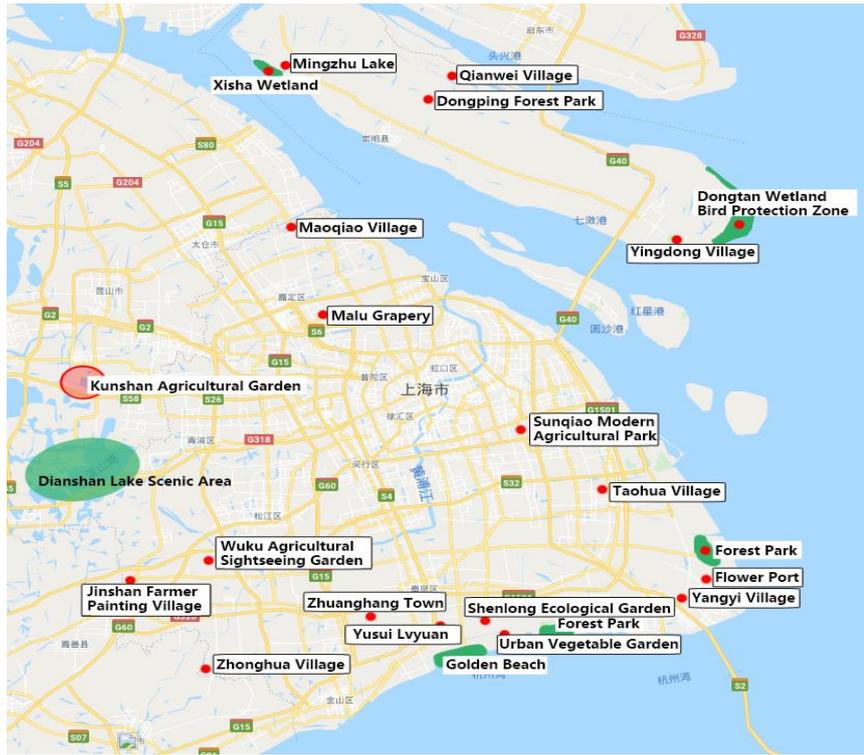


Fig.1 Demonstration sites map of Shanghai national agricultural tourism

Based on industry characteristics of urban agriculture tourism, this paper explores industry mechanism of Shanghai urban agriculture tourism spatial form characteristics and further studies the spatial structure models of Shanghai urban agriculture tourism, from three aspects, market demand and supply features, industry clustering and industry development types.

**2 Development characteristics of Shanghai urban agriculture tourism**

*2.1 Demand and supply characteristics of urban agriculture tourism*

Within the demand and supply relationship of tourism industry, tourism needs from tourist origins pull the development of tourism, while tourism resources and services supply of tourist destinations, development needs and policy supports of local economy push its development. ‘Pull and push’ force directs the development of tourism and locational choices of industry form spatial outcomes of its regional layouts.

To understand demand and supply characteristics, tourists status structures and satisfactions of Shanghai urban tourism, the research group carried out the questionnaire survey in agriculture tourism sites of Chongming (October, 2011) and Langxia (July, 2012). 480 questionnaires were sent out during two surveys, and 447 were taken back, among which 402 were valid. Based on the survey, market demand and supply characteristics of Shanghai urban agriculture tourism lied in several aspects.

1) Compared with urban tourism and scenic tourism mainly attracting foreign tourists, the attraction of urban agriculture tourism reflected

that agricultural production, life of peasants, rural life style attracted citizens from local and surrounding cities [5], and differences in tourism origins and destinations were mainly life style and environment, rather than history background, language and cultural identities.

2) Tourism destinations were agricultural areas around cities and tourism origins were mainly central metropolis and their surrounding large and medium-sized cities. Based on data from questionnaires, 72.5% of tourists in Chongming and more than 90% of tourists in Langxia Town were Shanghai citizens.

3) Tourism destinations were located around cities. Thus, the total tourism expenditures of citizens were relatively low. This study classified tourism consumption into catering, accommodation, tickets for scenic areas, transportation, agricultural productions and souvenirs. The biggest portion in Shanghai urban agriculture tourism consumption was catering (24%), while transportation and souvenirs were smallest (11% and 4%). The major concern of tourists was transportation cost.

4) Natural resources in Shanghai were quite scarce, while the industry scale extent and technological levels of urban agriculture were higher than those of other areas in China. Thus, urban agriculture tourism was mainly dependent on agriculture itself.

5) The urbanization rate of Shanghai was 88.86% in 2010 [6]. It has been one of the regions with highest urbanization rate. Since the improvement of urbanization levels occurred with the boost of constructing surrounding new rural areas, facilities and services levels of agriculture tourism in urban areas were higher than those in other areas, to increase the regional competitiveness of urban agriculture tourism.

## 2.2 Clustering development of urban agriculture tourism

The identification of industry cluster is based on two major industry characteristics industry scale and industry connections within clusters [7]. Industry scale refers to amounts of enterprises and population of tourists clustered within regions, while industry connections include industry relation chains and industry concentration degree [8]. Despite of unbalanced spatial distributions of Shanghai urban agriculture tourism and significant differences in development levels in tourism sites, the spatial layout of grouping cluster initially forms. This study collected data on characteristics, scale and economy of 118 Shanghai agriculture tourism sites and determined the scale extent, industry connection degree and concentration of these sites based on the identification path in Fig.2. 23 scaled agriculture tourism sites and 7 agriculture tourism clusters formed by them were finally confirmed (Fig.3).

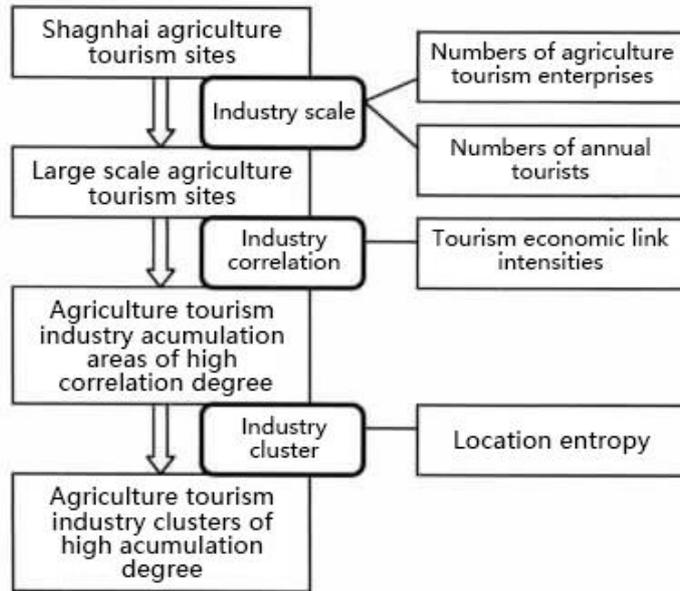


Fig. 2 The flow-chart for the identification of urban agriculture tourism cluster

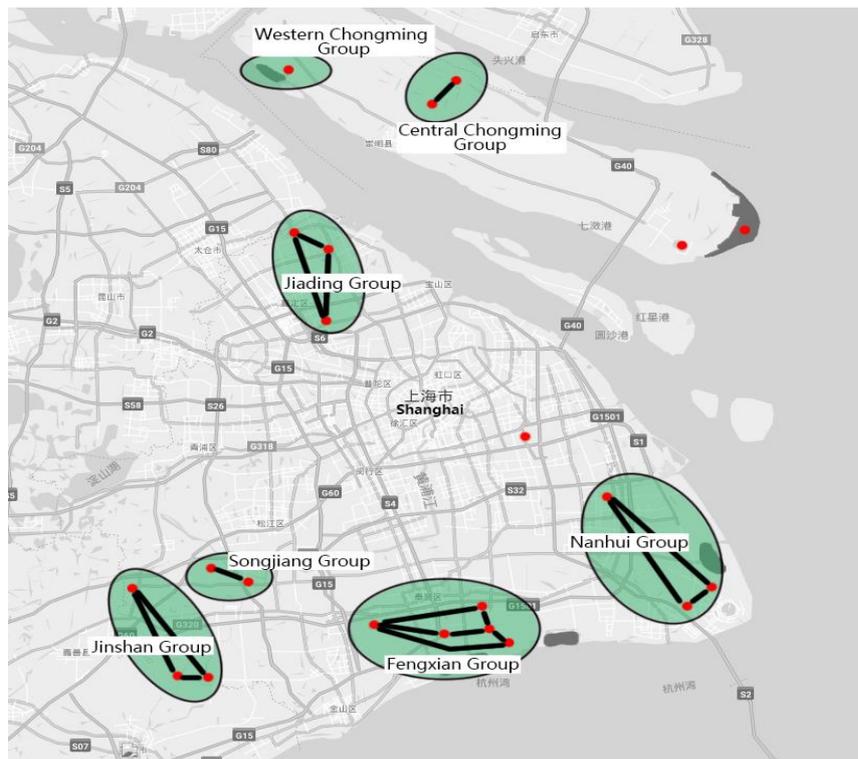


Fig.3 The distribution map of Shanghai urban agriculture tourism cluster

The clustering development of agriculture tourism can save industry cost, improve regional brand values and increase market competitiveness [9]. Within these 7 agriculture tourism clusters, scaled agriculture tourism sites ranged from 2 to 5. Together with other surrounding small agriculture tourism sites, they geographically clustered, and economically connected with each other. They shared resources and collaborated, to improve the attraction,

bearing capacity and vitality of whole tourism destinations. This study will not discuss the characteristics, advantages and specific identification methods of agriculture tourism clustering development because another study from this project ‘Research on Determination of Shanghai Urban Agriculture Tourism Clusters’ discussed this issue in detail.

### ***2.3 Development types of urban agriculture***

Currently, three classification ways are applied on development types in the study of Chinese agriculture tourism. The first one is based on agriculture tourism resource types, such as sightseeing orchards, aquatic sightseeing parks, planting sightseeing parks, and forest parks, et al. [10]. The second one is based on tourism activities, such as agriculture experiencing tours, rural leisure tours, natural ecological sightseeing tours, rural vacation tours and folk culture tours et al. [11]. The last one is based on operation subjects of agriculture tourism or operation ways. Shanghai agriculture tourism can be categorized into development mode of enterprises + peasants, mode of enterprises, mode of stockholding, mode of government + enterprises and mode of government + village committees + enterprises et al. [12]. Distinct characteristics of tourism resources direct to carry out tourism activities, and tourism needs guide the organization configuration of tourism resources. Thus, connected tourism needs, the classifications of agriculture tourism industry, based on tourism resources can better reflect core values of different types of industries. According this, Shanghai urban agriculture tourism was categorized into 5 development types (Tab. 2).

**Tab.2 List of the development types of Shanghai urban agriculture tourism**

Development Types	Main Characteristics	Typical Cases
Villages	Providing distinct food, accommodation and recreation activities, as well as agricultural experiences, sightseeing and technological demonstrations	Pandian Village, Yingdong Village and Qianwei Village
Agricultural Sci-tech Demonstration Parks	Base of agricultural technology demonstration and popularization, providing visiting, experiencing and learning agricultural knowledge	Sunqiao Modern Agricultural Park and Urban Vegetable Garden
Agricultural Scenery	Providing sightseeing, leisure and vacation, such as agricultural land, fishing, agricultural engineering	Peach Blossom Festival, Rape Flower Festival and Wuku Agricultural Sightseeing Garden
Agriculture Parks	City gardens with agricultural characteristics, such as botanic gardens and forest parks, generally changed from agricultural land	Donping Forest Park, Mingzhu Lake, Shenlong Ecological Park
Characteristic Agriculture	Scaled production base of distinct plants, aquatic products and animals, providing activities such as sightseeing, picking and tasting	Malu Grape Garden, Xianhua Harbor and Baizao Garden

It is found that scaled tourism sites within Shanghai urban agriculture tourism clusters have various types, such as Fengxian cluster containing 5 large-scale agricultural tourism sites, representing 4 development types:

villages (Pandian Village), agricultural sci-tech demonstration parks (Urban Vegetable Garden), agricultural sceneries (Shenlong Ecological Park), and characteristic agriculture (Yusui Green Garden and Baizao Park). It is the difference in the development types of tourism sites within clusters that allows various tourism sites provide tourists with different tourism opportunities, establish disparate cooperation among tourism sites, and further develop into industry clusters. On the other hand, the development modes between clusters are similar. Generally, tourism sites in the villages and towns are tourism reception sites, while other tourism attractions serve as tourism attractions.

### **3. Regional spatial structures of Shanghai urban agriculture tourism**

This paper furthered the study of spatial distributions of 23 scaled agriculture tourism sites discussed before. It is found that the industry characteristics of urban agriculture tourism directly influence the layout of industries in urban space, leading to two characteristics of Shanghai agriculture tourism spatial layout ‘Circle’ and ‘Cluster’ structures. ‘Circle’ is affected by demand and supply of agriculture tourism and locational choice of citizens to agriculture tourism, while ‘Cluster’ reflects the clustering development of agriculture tourism.

#### ***3.1 Layering structure of agriculture tourism space***

Taken Shanghai People Square as the city center, three driving time circles (0.5h circle, 1h circle and 2h circle) were drawn based on data on car timing distance from Google map and actual driving times (Fig.4). According to Shanghai quick road network layout structures, driving time-distance layers in Shanghai did not occur with standard concentric circles, while agriculture tourism spatial distributions in Shanghai generally coincided with these three timing layers, with the spatial structures of central layers ‘circle’ distribution.

##### ***3.1.1 Circling of spatial distribution***

Urban agriculture tourism is one of city’s short-distance recreation ways.

Traffic distance directly affects trips of urban residents through time and economic costs. Bao Jigang et al. thought that the recreational space around cities was distributed as layers and the tourism intensity basically declined concentrically [13], that is, on the basis of ignoring natural resources, the further the tourist sites around cities away from the city, the lower the intensities of tourism. Agriculture tourism attracts city residents depending on the rural landscape, lifestyle and agricultural production which are different from city’s living space. According to the size of Shanghai, agriculture tourism sites around 0.5h circle are basically located near the boundaries of the built-up areas of the city, only forming "urban farms". Considering the restrictions on urban land use in this region and high costs of land

opportunities, the number of agriculture tourism sites within this circle is low and their scales are small. Tourism attraction and bearing capacity are also relatively low. Thus, the circle with highest intensity of agriculture tourism in Shanghai appears near the 1h traffic circle and decreases outward by circles. Affected by dense agriculture tourism areas in Kunshan, Jiangsu Province, the number of agriculture tourism sites in the northwest of Shanghai is low and it tends to begin a clustering development.



Fig.4 The ‘Circle’ spatial structure of Shanghai urban agriculture tourism

### 3.1.2 Circling of tourism forms

The forms of agriculture tourism embodied in different agriculture tourism circles are also different. Agriculture tourism spots near 0.5h traffic circle is small-scaled and they have "urban park" landscape forms as well as recreation and ecological functions. Its tourism form is basically residents' short-lived recreation. Agriculture tourism sites near 1h traffic circle are densely distributed. The major one is leisure experience touring with various forms, such as countryside sightseeing, agricultural production and peasants' life experiencing et al.. The tourism sites are mutually influenced and they can be organized into a one-day tour (including weekend tours) tourism areas. Agriculture tourism sites near Shanghai's 2h traffic circle are mainly located in the western part of Chongming Island. Taken leisure vacation tourism as the main body, its tourism forms include tourism activities such as agricultural production and rural life experiencing, forming tourism areas for traveling multiple days (including long vacations). Its tourism status is equal to

provincial tourism sites, and tourism destinations include Shanghai and other provinces and cities.

### 3.2 Grouping structures of agriculture tourism space

The clustered development of urban agriculture tourism in Shanghai leads to the grouping spatial layout formation of agriculture tourism. Each industry cluster can correspond to one tourism space group. There are also Eastern Chongming group with a clustered development trend and Qingpu Dianshan Lake group. Therefore, currently Shanghai has formed nine large groups of agriculture tourism space (Fig. 5). Among them, tourists commonly regard Chongming Island (an ecological leisure island in Shanghai), and two groups in the west and central regions located in the 2h circle (and 2-day or long-distance tour areas) in Shanghai, as tourism destinations, which forms a bigger tourism group. In addition to the large-scale agriculture tourism sites within the cluster, small-scale agriculture tourism sites and other non-agriculture tourism sites also exist, such as Xisha Wetland on Chongming Island, Dongtan Migratory Bird Reserve, Golden Beach and Forest Park in Fengxian, and Dianshan Lake in Qingpu et al..

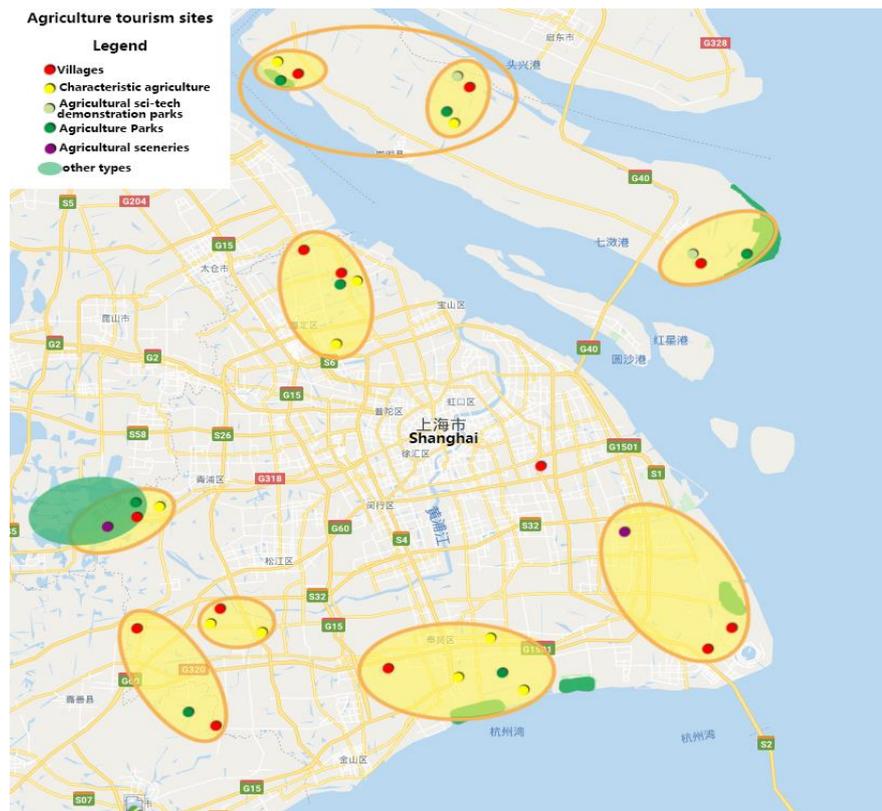


Fig. 5 The ‘Cluster’ spatial structure of Shanghai urban agriculture tourism

The compositions and types of urban agriculture tourism groups in Shanghai are in accordance with the characteristics of the cluster compositions formerly mentioned, that is, tourism sites within one group are different from with other. Their spatial connections are easy and they can complement each

other and develop collaboratively. Tourism sites of villages and towns are regarded as tourism receptions for most agriculture tourism groups and other types of tourism sites are taken as tourism attractions. Various festival activities are carried out based on agricultural resources, making the entire cluster an integrated tourism destination. Tourism sites and enterprise economy within cluster are closely tied and located in the same administrative district, which is easy to plan and manage the entire cluster. It promotes their coordinated development and takes advantage of clustering.

Based on questionnaire surveys of tourism destinations, given the similarities in the types of tourism sites in each group, each time a city citizens only choose one group as a tourism destination when they travel, which forms the tourism competitions between groups on the same circle. This competition leads to the self-optimization of tourism groups, including more vivid tourism images, more comprehensive tourism types, clustered development of industries, closer collaborations between tourism sites, more reasonable organizational forms and information platforms et al..

### 3.3 Spatial structure model of Shanghai urban agriculture tourism

As mentioned, spatial layout of Shanghai urban agriculture tourism is influenced by both tourists choosing tourism destinations and clustered development of industry, forming a ‘Circle + Cluster’ spatial structure model (Fig. 6). Three ‘circles’ of Shanghai agriculture tourism spatial layout reflect the discipline of diminishing concentric circles of tourism intensity and represent three kinds of tourism forms: residents' recreation, leisure experience tours (one-day tour), leisure vacation tours (multi-day tours). “Cluster” reflects the regional cooperation of Shanghai agriculture tourism sites and the clustered development of the tourism, as well as the types and compositions of agriculture tourism sites within clusters.

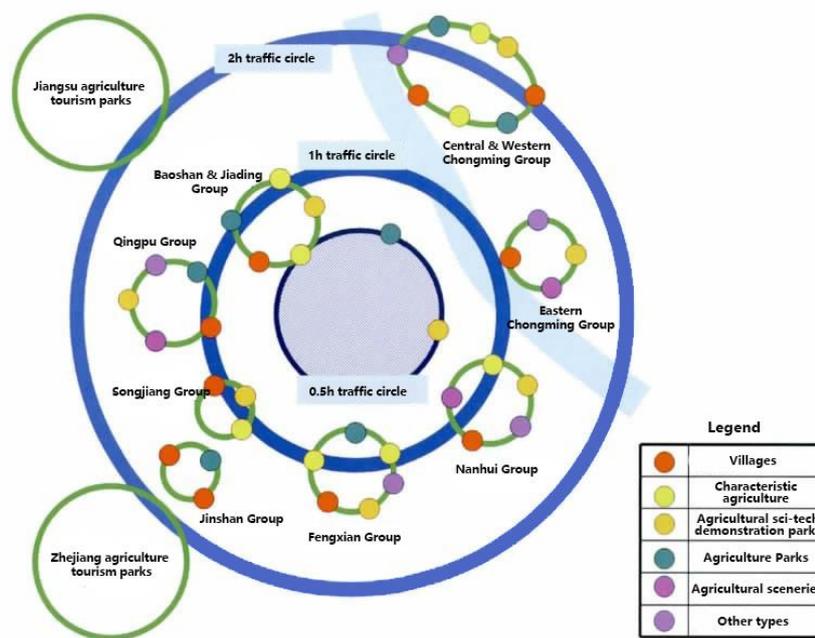


Fig. 6 The spatial structure model of Shanghai urban agriculture tourism

This spatial structure model will also classify the research on urban agriculture tourism space into three levels. The first level is on Shanghai's domain, that is, the research scope of this paper. The second one is the agriculture tourism grouping level, and agriculture tourism industry cluster research belongs to this level. The third one is research on specific agriculture tourism sites, that is, discussion on compositions of spatial elements and their spatial structures of various agriculture tourism.

#### **4 Conclusion**

Urban agriculture tourism is an industry combining urban agriculture and tourism. Its industrial development process spatially reflects the spatial structures and its changes. Thus, the spatial structure of “Circle” and “Cluster” of urban agriculture tourism in Shanghai is the location choice of urban agriculture tourism and the spatial results of industrial clustered development. Urban agriculture tourism planning should conform to this spatial structure characteristics. On the other hand, in terms of developing urban agriculture tourism sites, their positions in the spatial structures of urban agriculture tourism should be determined first, that is, the circle and grouping of agriculture tourism is judged by their locations. Appropriate forms and types of agriculture tourism are chosen on the basis of development types of agriculture tourism in current groups, to expand the dislocation advantages and avoid internal competitions within groups, thus increasing their attractiveness by agriculture tourism groups.

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# History, Challenge and Opportunity of Grape Wine in China

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## Abstract

Today, China is becoming one of the most important players in the world wine market. The fast growth of the consumption brings the great opportunity to all the wine producing countries, however, the entry of the imported wine also challenges on the local wine industry in China. This review aims to describe the development status of grape wine in China, by summarizing the history, cultivars, wine grape planting regions, wine production and consumption. The current issues and challenges of the Chinese wine industries are facing have also been stated and discussed.

**Keywords:** Grape wine; Chinese wine market; grape cultivars; challenge

## The history of grape wine in China

The production of Chinese wine can be traced back to 9000 years ago. The alcoholic beverages fermented from mixed sources (including wild grapes) were found in Jiahu archaeological site (c. 7000 BC). In literature, it was written that Chinese people have started to plant the grapevine and fermented it to wine before the Han dynasty (206 BC). In Tang dynasty (AD 618-907), the consumption of grape wine became more common and several Tang poets versified on grape.

The first modern winery (Changyu) of China was founded in 1892 in Yantai (Shandong province) by the overseas Chinese entrepreneur Zhang Bishi. However, the real development of wine industries occurs from 1949. Following the reformation and openness of new China, plenty international renowned wine grape cultivars have been introduced into mainland (mainly from Europe). After more than 60 years' development, now the total planting areas of wine grape reach 800 thousand Mu (equal to 53333 hectares), which account for 12.7% of total area of vineyard in China (Fig. 1). Red cultivars still take up the most of market due to the historical and Chinese cultural factors, the ratio of planting area of red cultivar to white cultivar is 4:1. Chinese wine industries heavily influenced by French wine culture, so that Cabernet Sauvignon is the number one cultivar according to its planting area (300 thousand Mu), following by Cabernet Gernischt, Merlot, Chardonnay, Italian Riesling, Cabernet Franc, Shiraz, Pinot noir.

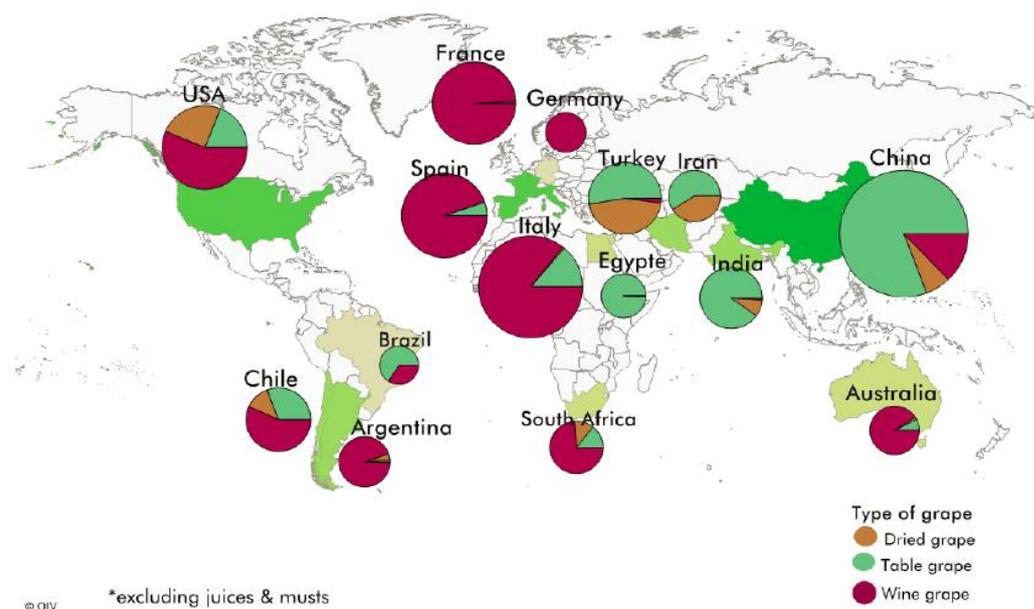


Fig. 1.

The composition of grapevine products of the main grape producing countries (Sourced from OIV)

### The wine production regions

Different from the table grape cultivars which spread out over the mainland, wine grape cultivars distributes mainly over the northern and northwestern regions of China. Apart from the factors of sunlight and the diurnal temperature, the weather of these regions is relative dry compared to other parts of China (e.g. hot and humid in Southeastern China). These make those regions more suitable for the growth of high quality *Vitis Vinifera* cultivars (e.g. Cabernet Sauvignon, Chardonnay, etc.), even though the extra labor will be needed to bury the vines with soil, in order to get them through the harsh winter. Table 1 and Fig.2 below present the information on main wine producing regions, climate types, cultivars, wine types and Table 2 presents the major wine brands in China.

Beyond that, the diverse landscape and vast territory bring the variable climate types to different parts of China, the intelligence and experience of Chinese people have make themselves isolate and cultivated the valuable wild grape species over the time, and created several new cultivars by crossbreeding. The fruit of several wild *Vitis* species have been used in wine-making in China, among which *V. amurensis* is the most valuable one. There are plenty of *V. amurensis* resources in Changbai Mountain. Tonghua Winery and Changbaishan Winery in Jilin Province and Yimianpo Winery in Heilongjiao Province have used *V. amurensis* berries for wine making for over 80 years. The wine made from *V. amurensis* has dark ruby colour, unique flavour, and is popular locally. As the most cold hardy species, *V. amurensis* is distributed in Jilin, Heilongjiang and Liaoning provinces in Northeast China,

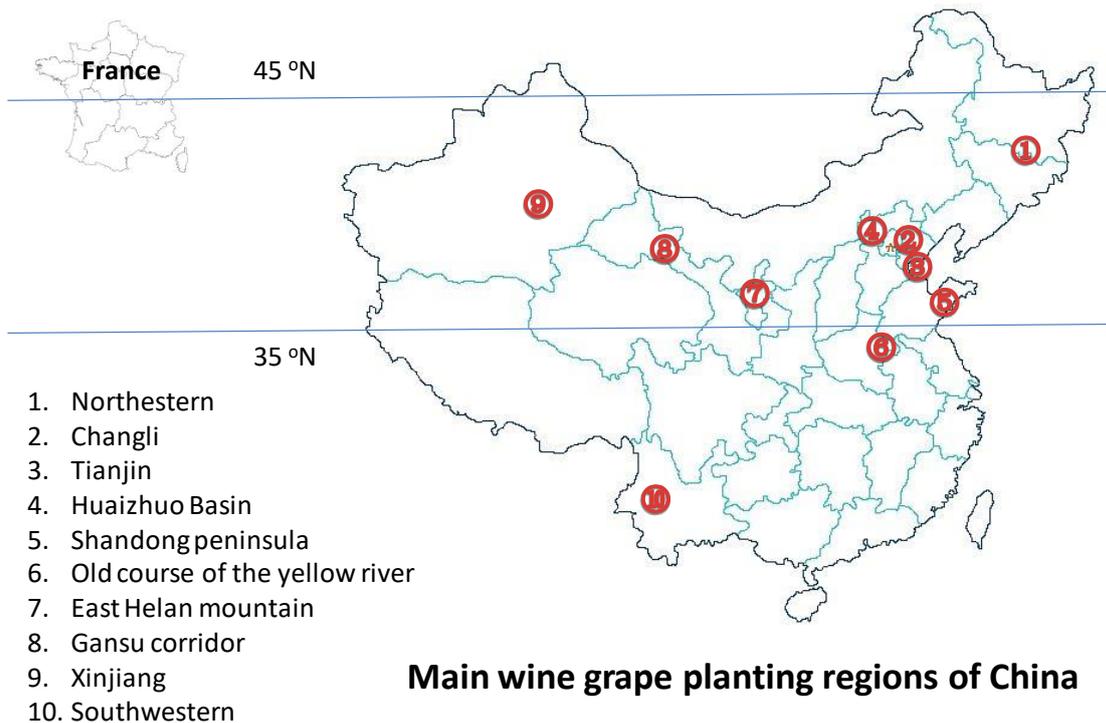
including Changbai Mountain, Da and Xiao Hinggan Mountains. Its distribution extends to the far eastern part of Russia and the Korean Peninsula. China has a long history in studying and using *V. amurensis* germplasm resources, and has acquired great achievements in its utilisation (Luo 2011). Jilin Province conducted research on domestic cultivation as early as 1957 (Lin 1982). Tonghua, Jilin Province has fermented *V. amurensis* wine since 1930's.

Opposite to the cold hardy *V. amurensis*, *V. quinquangularis* Rehd. is widely distributed in Guangxi province in Southern China. Local people have been using *V. quinquangularis* berries to make wine for a long time. In recent years, more selected clones from wild *V. quinquangularis* are being planted, and productive area in Guangxi has reached 5800 ha, and total annual production is 2100 tones (Zhu et al. 2006). In addition, Zaoyang County of Hubei Province and Danfeng County of Shaanxi Province also process the fruit (Wang 1993) into wine. Wineries in Feixian County of Shandong province and Zuogong County of Tibet use the local wild grapes for wine (Hu 1986).

**Table 1: The main wine producing regions in China and related information (Adapted from Ye 2012)**

<b>Region</b>	<b>Climate</b>	<b>Soil type</b>	<b>Wine grape area (10<sup>4</sup> Mu)</b>	<b>Wine grape cultivar</b>	<b>Production (10<sup>4</sup> Ton)</b>	<b>Wine type</b>
<b>Northeastern</b>	cold and semi-humid or humid climate	Black earth	6	Vitis amurensis and its hybrids	3.26	Vitis amurensis wine, ice wine
<b>Changli</b>	cold and semi-humid or humid climate	gravelly land, sandy land	10	Cabernet Sauvignon, Merlot, Chardonnay	7	dry red,
<b>Tianjin</b>	Semi humid continental climate	coastal saline alkali soil	6	Muscat, Cabernet Sauvignon, Merlot	5	dry red, dry white
<b>Huaizhuo Basin</b>	Semi humid continental climate	river sandy loam	7	Longan grape, Cabernet Sauvignon, Chardonnay	5	dry red, dry white
<b>Shandong peninsula</b>	Dry temperate continental monsoon climate	Brown forest soil	25	Chardonnay, Italian Riesling, Cabernet Sauvignon, Cabernet Gernischt, Carignan	23	dry red, dry white

<b>Old course of the yellow river</b>	Warm temperate continental monsoon climate	Sandy soil	4	Cabernet Sauvignon	3	Dry red
<b>East Helan mountain</b>	Temperate semi-arid climate	Gravelly and sandy land	8	Cabernet Sauvignon, Cabernet Gernischt, Merlot, shiraz, Chardonnay	4	Dry red, Dry white
<b>Gansu corridor</b>	Temperate arid/semi-arid climate	Sandy soil	5	Cabernet Gernischt, Cabernet Sauvignon, Pinot Noir, Merlot	2	Dry red, dry White, sweet wine, ice wine
<b>Xinjiang</b>	Continental arid climate	Gravelly soil, sandy soil, loamy soil	25	Cabernet Sauvignon, Cabernet Franc, Chardonnay, Italian Riesling	10	Dry red, dry white, sweet wine
<b>Southwestern</b>	Subtropical humid climate	Red earth	3	Rose honey, Merlot, Cabernet Sauvignon, <i>Vitis quinquangularis</i>	2	Dry red, wild grape wine, ice wine



**Fig. 2:** The major wine grape planting regions of China

**Table 2:** The major grape wine brands of China

Rank	Brand	Main wine producing regions
1	CHANGYU	Shandong, Xinjiang, Shaanxi
2	GREATWALL	Hebei, Shandong
3	DYNASTY	Tianjin
4	GRANDGRAGON	Shandong, Ningxia
5	MOGAO	Gansu
6	NIYA	Xinjiang
7	TONHWA	Northeastern
8	DRAGONSEAL	Hebei
9	YUNNAN RED	Yunnan
10	SHANGERI-LA	Yunnan

### **Wine consumption in China**

China is not a traditional grape wine drinking country even though it has a long history of grape wine fermentation. The liquor fermented from grains has played the major parts for thousands of years; several brands are becoming famous all over the world, such as Moutai. However, following the rise of economics of China after 1980s and the popularization of knowledge of grape wine, wine became more enjoyed by Chinese people due to the health benefit and its “symbol” for middle to high class of the society. In 2002, the year

average consumption in China is only 0.5 liter per person, however, sales of wine were already 1.2 billion US dollars. In the next 5 years from 2002, wine consumption is growing at a rate of 12 percent a year. In 2013, China became the world's biggest market for red wine consumption for the first time, overtaking France and Italy. Red wine drinkers in China increased their consumption by 2.75 times between 2007 and 2013 to 1.865 billion bottles. During the same period the amount drunk in France and Italy fell by, respectively, 18 percent and 5.8 percent. French drinkers consumed 1.8 billion bottles of red wine last year while Italians downed 1.7 billion.

Until today, 2017 datasets showed that the year average consumption in China is already 1.2 liter for person, and China has risen to become one of world's most important wine markets, offering both high growth potential and generous profit. The OIV statistics showed that the wine consumption of China in 2016 has reached 1.72 billion liters with the increase rate at 6.9%. From the table 3 and 4, we can see that there is a fast increase on the volume of imports in China.

**Table 3: Imports in terms of volume from 2012 to 2016 (In Million of hl, from OIV website)**

Nation	2012	2013	2014	2015	2016	2016/2012 Variation in %
Germany	15.4	15.3	15.4	15.3	14.5	-5%
UK	12.8	11.8	13.4	13.6	13.5	6%
USA	11.7	11.0	10.8	11.0	11.2	-4%
France	5.4	6.0	6.9	7.6	7.9	45%
China	3.9	3.8	3.8	5.6	6.4	62%
Canada	3.8	3.7	3.8	4.1	4.2	10%
Russia	4.9	4.9	4.7	4.0	4.0	-18%
Netherlands	4.0	3.6	3.8	3.8	3.8	-3%
Belgium	3.1	3.2	3.1	3.1	3.1	0%
Japan	2.6	2.6	2.7	2.8	2.7	4%
Sweden	2.0	2.1	2.3	2.2	2.2	7%
Switzerland	1.9	1.8	1.9	1.9	1.8	-3%
Denmark	1.9	2.0	1.9	1.8	1.8	-3%
Portugal	1.3	1.6	2.3	2.2	1.8	41%
Italy	2.8	2.7	2.8	2.8	1.7	-38%
Czech Republic	1.6	1.5	1.6	1.6	1.6	2%
Poland	1.0	1.0	1.1	1.1	1.2	23%

**Table 4: Imports in terms of volume from 2012 to 2016 (In billion of Euro, from OIV website)**

Nation	2012	2013	2014	2015	2016	2016/2012 Variation in %
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USA	3.9	3.9	4.0	4.9	5.0	27%
UK	3.9	3.6	3.6	3.9	3.5	-10%
Germany	2.4	2.6	2.6	2.5	2.5	1%
China	1.2	1.2	1.1	1.8	2.1	74%
Canada	1.5	1.5	1.5	1.6	1.6	4%
Hong Kong	0.8	0.8	0.8	1.3	1.4	74%
Japan	1.2	1.2	1.2	1.3	1.3	12%
Switzerland	0.9	0.9	0.9	1.0	1.0	6%
Netherlands	0.9	0.9	1.0	1.0	0.9	0%
Belgium	1.0	1.0	1.0	1.0	0.9	-8%

Currently, there is no doubt that France ranks the No. 1 wine export country to China (Table 5), other old world wine producing countries are also present in the list, such as Spain, Italy and Portugal. The wine from new world wine producing countries also doing well in the competition as they are excellent in innovation of viticultural and enological practices, as well as the marketing strategy. Previously, the imported wine is generally sold in relative high price as it is normally considered to be associated with high class. However, since 2014, the average price of imported wine has started to drop and becoming more reasonable (Table 5). The ability of consuming the grape wine still vary between different regions in China, and can be directly correlated with the economic status. From Table 6, we can see the top provinces/regions of the list are also the major players in the economics of China.

**Table 5: Average price of imported wine from Top 10 wine export countries (From OIV website)**

Rank	Nation	Volume (liter)	US dollar	Average price (dollar/liter)
1	France	191,029,411	965,444,756	5.05
2	Australia	79,384,687	542,542,756	6.83
3	Chile	60,143,147	209,385,748	3.48
4	Spain	72,070,433	142,086,620	1.97
5	Italy	25,788,979	114,273,276	4.43
6	USA	9,737,105	52,458,749	5.39
7	South Africa	9,607,153	34,786,293	3.62
8	Argentina	5,591,554	23,129,088	4.14
9	New Zealand	2,139,029	20,877,308	9.76
10	Portugal	6,800,792	18,979,108	2.79

**Table 6: Ranking of the province/region according to the consumption of grape wine in China (From OIV website)**

Rank	Province/region	Volume (liter)	US dollar
1	Guangdong	121,677,122	924,726,632

2	Shanghai	137,731,285	551,664,212
3	Beijing	38,073,133	144,930,343
4	Zhejiang	41,980,879	130,812,207
5	Fujian	42,891,492	107,395,178
6	Shandong	33,722,257	99,448,590
7	Tianjin	23,532,587	75,442,012
8	Jiangsu	16,001,304	51,739,521
9	Liaoning	4,472,633	27,391,779
10	Sichuan	4,587,731	17,049,115

### **Challenges and opportunities of Chinese grape wine industry**

The datasets regarding the production and consumption of grape wine in China showed the great potential as the average consumption of China is still much lower than the world average; considering the factor that huge population and fast growth economics of China, There is still a plenty of space for the consumption elevation. These bring the opportunity to both imported wine and Chinese local wine industry. However, a number of issues are still present in our industry even though a large proportion of wine consumed in China is produced locally at the moment.

#### **Industry regulation**

Currently, China lacks the unified organizations or governmental bureaus as other more established wine producing countries, for regulating the whole grape wine industry chain. Instead of that, the whole chain is split into several parts and supervised individually. For example, the viticultural part (establishment and management of the vineyard) is supervised by the governmental bureaus of agriculture and forestry, the winemaking process is supervised by bureau of quality inspection and the wine marketing is supervised by bureau of business, health, food and drug. Even though several associations have been created by local government in the wine producing region, however, without enough authorities given by Central government, the work they are allowed to implement is restricted on coordination, investment promotion and giving the advices to industry (Liu 2016). The lack of the unified regulation bureau will bring the negative impact on the wine producing region planning, policy making and associated scientific research.

The lag of creating the standards for quality control also limits the healthy development of the industry. The current standard (created in 1994) used in the wine industry is outdated, it uses a number of basic characters of wine and can only prove the product to be qualify to be called “wine” or not qualify. This standard was useful in the beginning of development of wine industry (Xi et al, 2008), however, it cannot provide more information and needed to be updated.

#### **Rising of the competition**

The competition from the imported wine is becoming one of the most important factors to our local wine industry. Beside those issues we have already mentioned in above sections, the tax exemption which will happen in next few years is the biggest challenge. As the key industry in many countries (France, Australia, New Zealand...), the wine industry get the subsidy from the national government to lower the cost of production, as well as on the international promotion. These add a lot of advantages on them when they compete with Chinese wine industry (Wang 2013).

### **Resource**

The quality of grape is globally recognized to be the most important factor to the final wine quality. Compared to other more established wine producing countries, China has a huge gap on scientific planning and effective guidance on the viticulture practice. Research showed that plasticity of grapevine make it easy to be influenced by the environmental factors (e.g. climate, soil, water, etc.), and result in the winemaking starting material in variable quality and characteristics. There are plenty of works have already been done extensively in those old wine producing countries to identify the suitable cultivars for specific regions, such as Pinot Noir in Burgundy (France), Riesling in northern Germany, Shiraz in South Australia. However, similar work has not been done in China. Due to the historical reason, China has strongly influenced by the wine culture of France. From the table 1 of the top wine producing regions, we can see the French cultivar, especially Cabernet Sauvignon, has been planted all over the mainland of China no matter if the weather is suitable or not. The homogenization of the product with variable quality makes them difficult to compete with the high quality imported wine (Liu 2016).

Other factors present also bring the instability. Vines in many Chinese vineyards of are still self-rooted instead of using disease resistant rootstocks; these make them difficult to survive from infection caused by the microorganism pathogen or insect (e.g. phylloxera). On the other hand, the wine industry prefer to focus on the winemaking process to the viticultural section, many of them do not have their own vineyard, but only purchase the grapes from the farmers of many small vineyards. However, these farmers have not been trained well with viticultural techniques and they are more of the pursuit driven, thus the volume is paid more attention than the quality.

### **Terrior and Flagship cultivar**

The word “Terrior” is very important in the world of grape and wine, it includes a number of factors (weather, soil type, water, wind, and all other environmental factors) which can describe a specific wine producing region together. Establishment and promotion of a “Terrior” can elevate the value of all the wine produced in this region, but not necessary on one single brand. There are several prerequisites are needed to succeed in establishment of a Terrior, including the good understanding of the specific wine region, well selected grape cultivar(s) as “flagship” to present the unique feature of this region, optimization of viticultural and enological practice on the selected cultivar(s), as

well as the strategic marketing plan and promotion. The successful cases are presented in the table 7.

**Table 7: The flagship wine and the producing region**

Nation	Region	Cultivar
France	Burgundy	Pinot Noir
Spain	La Rioja	Tempranillo
USA	Napa Valley	Cabernet Sauvignon
Australia	South Australia	Shiraz
New Zealand	Marlborough	Sauvignon Blanc
South Africa	Stellenbosch	Chenin Blanc
Argentina	Mendoza	Malbec

Recently, the wine producing regions in China have been integrated (Table 1), in order to copy other successful cases. However, there is still a long way for them to identify their own “flagship” wine in their specific Terrior as most of the common cultivars (especially the European cultivars) have been chosen and optimized to maximize their quality and value.

The use of Chinese wild grape could be a solution to avoid homogenization issue for future competition. China has rich resources of wild grape species, and some of them have already been used in winemaking, such as *Vitis amurensis* in Northeastern region due to its cold-resistant feature and special aromas; and *Vitis quinquangularis* in Guangxi due to its resistant to hot and humid weather. The wild grape species which survived from natural selection make them not only good material for directly use for processing, but also the potential genetic resources for breeding works. Successful case is the South African “Pinotage” which was crossed from the Pinot Noir and Cinsault, Cultivars or grape species from other part of the world with similar climate may be also considered, such as those American species (e.g. Muscadine) which can survive in hot and humid climate and is resistant to many diseases.

### **Wine grape breeding programs in China**

The development of grapevine industry for future competition cannot succeed without the scientific research and human resource training. Most established wine producing countries have the University departments or research organizations which continuously work on the grapevine related research, such as ISVV (Institut des Sciences de la Vigne et du Vin) in Bordeaux, France, Department of Viticulture and Enology of UC Davis in USA, University of Adelaide in Australia and Stellenbosch University in South Africa. Currently, there are more than 13 national and local grape research institutes involving in programs of breeding and genetic improvement of grapevines, and more Universities and colleges are engaging in education and research concerning of viticulture and enology in the major grape producing regions.

### **Northeast China**

*Vitis vinifera* grapevines in northern China need to be buried in winter for cold protection. This practice is laborious and is getting more and more expensive. This is indeed a limiting factor for the extension of local grape industry. Therefore, breeding cold hardy grape cultivars that do not require burying is a major goal in this region, and the same is true for the major viticulture areas in China (Yang et al. 1959; Pu 1960). *Vitis amurensis*, which is distributed mostly in northern China and can tolerate  $-40^{\circ}\text{C}$ , is a valuable resource for breeding cold-hardy grapes (Hu 1956).

At an early stage, the main objective of breeding wine grape cultivars in Northern China was to develop cold hardy red-coloured cultivars. A lofty goal is to develop new grape cultivars that can tolerate temperatures as low as  $-25^{\circ}\text{C}$  without burying. In 1951, the Pomology Institute of Jilin Academy of Agricultural Sciences initiated a breeding program of making crosses between *V. amurensis* and *V. vinifera* / *V. labrusca*. From around 15,000 hybrid seedlings, they selected ‘Gongniang No.1’ (*Muscat Hamburg* × *V. amurensis*) and ‘Gongniang No.2’ (*V. amurensis* × *Muscat Hamburg*). These cultivars were highly tolerant to cold stress, being able to survive through winter without burying (temperatures could be as cold as in the  $-20^{\circ}\text{C}$ 's). They had high brix, and the wines were evaluated as having good quality with some degree of *V. vinifera* alike. Since their release, these two cultivars were widely planted in Northeast China (He et al. 1981, 1990). In 1970's, these cultivars were used for making crosses with *V. vinifera* cultivars. A new high quality white wine cultivar named Gongzhubai (*Gongniang No.2* × *Golden Muscat*) was released from this effort. ‘Gongzhubai’ was obvious a better grape in terms of berry size, fruiting habit, berry flavor, and cold hardiness (Fang et al. 1993).

The Pomology Institute of Chinese Academy of Agricultural Sciences started research on cold resistant grape cultivars in 1951. They selected ‘Heishan’ from crosses of ‘Black Hamburg’ × *V. amurensis*, and ‘Shanmeigui’ from ‘*Muscat Hamburg*’ × *V. amurensis*, respectively. They could sustain temperatures as low as  $-26^{\circ}\text{C}$ , and showed no cold injury without burying. These selections had high Brix and low TA and were more suitable for winemaking than *V. amurensis* (Yang et al. 1959; Pu 1960). ‘Huapu No.1’ is a new wine grape hybrid cultivar of ‘*Zuoshan No.1*’ × ‘*White Malaga*’. It has strong cold and disease resistance, high yield, good wine quality. It was also used as a rootstock and grafting affinity with some table grapes was quite good (Wang et al. 2012).

In 1967, the Liaoning Agronomy College (in Xiongyue County) bred a cold resistant cultivar ‘Xiongyuebai’ by crossing ‘Longyan’ with a hybrid selection of ‘*Muscat Hamburg*’ × ‘*V. amurensis*’. This cultivar was suitable for making high quality white wine (Zhang 1987). Pomology Institute of the Liaoning Academy of Agricultural Sciences also released a white wine grape cultivar named ‘Xiongyuehong’, which was selected from a F1 hybrid crossed between *V. amurensis* with ‘Longyan’, a Chinese native grape cultivar.

At present, repeated-crosses and backcrosses to make F2 and F3 hybrids are strategy to screen progenies with superior wine quality in Special Plant and

Animal Institution of CAAS. In 1998, cold-resistant wine grape 'Zuohongyi' was selected from the hybrids of the female parent 79-26-58, which was the F1 hybrid between *V. amurensis* x *vinifera* and the male parent *V. amurensis* 74-6-83 (Lu et al. 2000). 'Zuoyouhong' was also selected from a hybrid of *V. amurensis* x *V. vinifera* and then backcrossed to *V. amurensis*. It was approved by the cultivar releasing committee of Jilin Province in 2005. The period from berry setting to harvesting was 119-128 days. It was considered as early ripening, very cold hardy and disease resistant, high yield cultivar (Song et al. 2005).

A cultivar named 'Beibinghong' [(*V. amurensis* x *V. vinifera*) F2 × (*V. amurensis* x *V. vinifera*) F2] was released in 2008. The Brix at maturity ranged from 17.6-25.8. It is used for making icewine and the Brix of the frozen fruit in early December was 35.2-37.0. This cold hardy cultivar is also disease resistant and high yield (Song et al. 2008). 'Xuanlanhong' (also called 'Zuohongsan'), derived from 'Zuoyouhong' × 'Beibinghong' and released in 2012, is another cultivar for dry red wine. The brix ranges from 16.2 to 21.8. It ripens in the end of September, and the period from blooming to harvesting is 137-145 days. Its cold hardiness is similar to 'Beta' rootstock (Song et al. 2012).

### **North China**

In 1954, Beijing Botanical Garden of the Chinese Academy of Sciences, made a cross of *V. amurensis* x Muscat Hamburg, and from which cultivars 'Beichun', 'Beihong' and 'Beimei' were selected and released. These Va x Vv hybrid cultivars are cold hardy (-25°C) and disease resistant with high yield and high brix. They do not need burial for winter protection (Yu 1959; Luo et al. 1990; Li 1983; Fan et al. 2010). In addition, the juice is bright-coloured and show good winemaking potential. Of all the hybrid cultivars, 'Beichun' is the most widely planted wine grape. Total cultivation areas of 'Beichun' reached 6600 ha over 30 counties in early 1980's. The Shandong Wine Grape Research Institute crossed *V. amurensis* x Sweet Water, a European cultivar, in 1964, and released a new cultivar 'Baotuhong' (Kong 2004). These cultivars are used for making wine or blend to enhance the colour of wines. In addition to cold hardiness, 'Beichun' is also resistant to fungal diseases and therefore it has been introduced to south China where the climate is warm but too humid to grow *V. vinifera*.

In recent years, the Beijing Botanical Garden crossed F1 hybrids of *V. amurensis* with European cultivars and released a new white wine grape cultivar 'Beiquan', which is cold hardy with excellent quality, high yield and disease resistance.

### **South Central China**

'Lingfeng' (NW196) was a superior individual selected by the Horticultural Institute of Guangxi Academy of Agricultural Sciences and Northwest A&F University from hybrids of a interspecies cross (cross combination No.88-110) made in Guangxi using *V. quinquangularis* 83-4-96 as the female parent and *V. vinifera* 'Muscat Rose' as the male parent. The cross was made in 1995-1996, and it was officially released in 2005. 'Lingfeng' has great adaptability, strong disease resistance and vigour, high fruit set, and

hermaphrodite flowers, and was suitable for domestic cultivation in the southern producing area. Experimental test plots have been established in Duan, Shanglin and Xingye counties of Guangxi Province. Another superior individual 2-1-3 (NW213) was also selected from the same cross. This individual was named as 'Lingyou' in 2005 after successful trials in Duan, Luo Cheng and Yulin counties of Guangxi Province (Huang et al. 2006).

### **Conclusion**

The wine industry of China is now in an important transition phase with challenges and opportunities. In order to actively response to the current issues and coming crisis, the whole industry chain needs to adjust the strategy and make the appropriate policy. These include: 1. Scientific planning to ensure the suitable cultivars by optimizing of the factors (cultivar, climate, soil condition and winemaking process) concerning the winemaking; 2. Improve and perfect the vineyard establishment and maintenance to control the quality of the winemaking starting materials; 3. Perform the brand guidance strategy to promote several "Territor" and a serious of leading brands; 4. Enhance the governmental support for scientific research for innovation on the high quality cultivar breeding and its associated viticultural and enological practice; 5. Build the wine cultural environment by adding the Chinese elements; 6. Improve the associated industries in whole industry chain, such as bottling, packaging, distribution, equipment, etc; 7. Policy making to increase the support, not only the financial subsidy, but also establishes the unified bureau to regulate the industry, in order to ensure the healthy and orderly development of wine industry of China.

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