Plants Root Interference Area, a Benefit to the Microbial Community

Aurica Breica BOROZAN¹, Sorina POPESCU¹, Oana-Maria BOLDURA^{2*}

¹Faculty of Horticulture and Forestry, Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timisoara, Romania

²Faculty of Veterinary Medicine, Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timisoara, Romania

^{*}Corresponding author, e-mail: oanaboldura@gmail.com

Bulletin UASVM Horticulture 74(1) / 2017

Print ISSN 1843-5254, Electronic ISSN 1843-5394

DOI:10.15835/buasvmcn-hort:12302

Abstract

Part of byproducts synthesized by plants through photosynthesis reach the ground, where are creating selective microenvironments for micro-flora and associations of plant – microorganisms, being beneficial for plant growth. In this paper, the effect of the root interference area of vines and herbaceous plants and of radicular exudates from vine rhizosphere on microbial community was studied by estimating the present microbial population. Also, by molecular studies, the diversity of microbiological community was assessed. The microbiological study of biological samples was performed by classical and molecular methods. The biological material was represented by microbial communities from rhizosphere and phyllosphere areas of two varieties of vines White Romanian Tămâioasă and Black Romanian Tămâioasă cultivated in a soil that has never been chemically treated. Overall, bacteria had a significant presence in soil samples taken from the interference area of vines and herbaceous plants roots. Actinomycetes quantitatively dominated the root interference area of herbaceous plant with variety White Romanian Tămâioasă. The range of actinomycetes species and leaves microflora was found to be reduced. In this study we have shown that significant growth of microorganisms occurs in the interference area of vine with other herbal plants as a result of the cumulative effect of radicular exudates and that the surrounding microbial community differs among varieties, areas of interference and also is strongly influenced by the levels of tillage and fertilization of soil.

Keywords: *genetic diversity, microbial community, plants plant root interference area*

INTRODUCTION

Plants are associated with diverse epiphytic microflora which may represent a protective barrier of plants against pathogens, but also a microenvironment that in certain conditions may favor their development (Blakeman and Fokemma, 1982; Hallmann *et al.*, 1997; Gray and Smith, 2005; Lindow *et al.*, 2003). The studies conducted by Martins *et al.* (2013) revealed numerical but also qualitative differences in the bacterial population present in different organs of vine plants, however

the existence of common bacterial genera was also signaled.

Such as in the case of plants, the soil microbial population is heterogeneous. As it can be noticed from the studies mentioned in this work, soil area of interest for many researchers is the rhizosphere.

The plants through the root system, release in the soil large amounts of metabolites formed in the most amount of water soluble compounds, which are an important source of nutrients for organisms present in the rhizosphere (Jensen,

1996; Broeckling *et al.*, 2008). Root exudates exert a chemo tactical effect on microorganisms, followed by selection and root colonization by microorganisms that are fast growing and more competitive (Nihorimbere *et al.*, 2011).

The soil macroflora, represented by the roots, together with microflora develops an intense activity, beneficial both for plants and for microorganisms (Badri *et al.*, 2009; Prashar *et al.*, 2014). (Beerling and Bernese 2005; Taylor *et al.* 2009).

Foster (1988), said that the number of rhizobacteria may reach to 10^{10} and 10^{12} bacterial cells / g soil, from where they can reach other in other living environments on other media nutrients as saprophytes or contaminants.

Many studies have reported higher numbers of bacteria in the area influenced by the root system, compared to the free of roots soil, localized mainly at the base and at the tips of the root system (Tesařová and Řepová, 1984; Olsson *et al.*, 1987; Chin A Woeng *et al.*, 1997; Watt *et al.*, 2006; Dennis *et al.*, 2008).

Microorganisms in the plants influenced area are bacteria, fungi, algae, protozoa (Raaijmakers and Weller, 2001).

In terms of microbial diversity, it was noticed that in the area of plants influenced soil, especially dominate the proteobacteria and actinobacteria (Suzuki *et al.*, 2006; Singh *et al.*, 2007), which are the most studied. Microorganisms are influenced by an accumulation of abiotic and biotic factors that lead to numerical and qualitative variations, observed by Grayston *et al.* (1998). Regarding the importance of microorganisms association with roots, more research shows the vital role they play in the degenerative processes of soil (Pate *et al.*, 2001; Taylor *et al.* 2009) biogeochemical cycles, plant protection against some pathogens, the synthesis of antibiotics, toxins, surfactants, organic compounds (Compant *et al.*, 2005; Haas and Defago, 2005) and promoting plant growth by producing specific nutrients, either it is about phytohormones, macro and microelements (Kent and Triplett, 2002; De Werra *et al.*, 2009).

From the most studies is appearing the beneficial effect of microorganisms on plants and on soil microhabitats, but also the contribution of plants to changing chemical, physical and biological properties of soil s which could be a bridge to sustainable agriculture and can help to eliminate

the shortcomings caused by the use of chemicals. Although the rhizosphere of plants (usually crop plants) is studied, there are many unknown things or contradictory discussions. Studies related to vine plants, in the direction of interest to us are reduced, and existing ones are of recent date which makes us think that this will be a topic that will awaken interest toward the microbiology of the two varieties of romanian traditional vines, well adapted to local environment conditions and still present in the landscape of rural areas in Banat, with grape which has a special flavor, but is more sensitive to disease attack.

The studies in this paper are a first step to microbiological and molecular investigates the microorganisms in a soil that has never been chemically treated, occupied by the two varieties of vines, whose roots intersect with plants nearby, either cultivated or spontaneous, but also of the phylloplane. Microbial association of plants can influence even the finished product, which is the wine (Verginer *et al.*, 2010; Nisiotou *et al.*, 2011).

Modern analysis of bacterial communities requires development and application of molecular genomic tools and by this the field of microbial ecology is undergoing unprecedented changes. Molecular approaches enable the interrogation of the structural and functional diversity of environmental microbial communities and reveal that the genetic and metabolic diversity studies have been somehow superficial in the area of the most abundant organisms of the Earth, the Prokaryotes (Muyzer, 1999).

For diversity evaluation at a molecular level, two types of VNTR (Variable Number of Tandem Repeats) molecular markers systems: ISSR (Inter simple sequence repeats) and DAMD (directed amplification of minisatellite region DNA) are recommended (Claes, 1997). Inter simple sequence repeats (ISSRs) are dominant markers located between microsatellite sites in the genome, are often highly polymorphic at the species level and require no prior sequence information (Przyborowski *et al.*, 2013). Minisatellites are tandem repeated DNA regions of genomes, many of which showed high levels of allelic length variation due to differences in the number of repeated units. These loci are highly informative genetic markers that have been used extensively in many areas of genetics. Heath *et al.* (1993) described a technique, called Directed Amplification of Minisatellite DNA

Tab.1. Samples collected in this study

	TA - White Romanian Tămâioasă		TN - Black Romanian Tămâioasă	
Soil from rhizosphere - SR	SR 1	a1 b1	SR 2	a2 b2
Soil from roots interference area - SI	SI 1	a3 b3	SI 2	a4 b4
Plant phyllosphere - Ph	Ph 1	c1 c3	Ph 2	c2 c4

Tab. 2. Soil samples used in molecular analyses

Sample code	Vine specie	Soil sample
A	Black Romanian Tămâioasă (TN)	SI 2
B	Black Romanian Tămâioasă(TN)	SR 2
C	White Romanian Tămâioasă(TA)	SI 1
D	White Romanian Tămâioasă(TA)	SR 1

DNA isolation and purification

Region (DAMD) to direct the PCR mediated amplification of minisatellite DNA region. DNA markers can be used to assess the genetic structure and to estimate the level of genetic diversity and differentiation of populations.

MATERIALS AND METHODS

The biological material was represented by samples collected from two varieties of vines: White Romanian Tămâioasă (TA) and Black Romanian Tămâioasă (TN) according to Table 1. The soil samples were collected from vines rhizosphere - **SR**, and from the interference area of vine roots with two herbaceous plants: *Chelidonium majus L.* and *Phaseolus vulgaris L.* - **SI**. The phyllosphere samples - **Ph**, were represented by leaves collected from the two vines. Beside above described abbreviation, a1, a2, a3, a4, c1 and c2 represent bacterial samples whereas, b1, b2, b3, b4, c2 and c4 represent actinomycetes samples.

Over the years the studied soil was organically fertilized and annually tilled in the case of TA variety or untilled or fertilized for several years in the case of TN variety. Soil samples were taken from 0-20 cm depth.

The abundance of microorganisms in the rhizosphere and phyllosphere, differentiated in two groups, bacteria and actinomycetes, was assessed by culture on specific nutritional culture

media. Soil bacteria were isolated on Topping culture media and phyllosphere bacteria were isolated on Agar nutrient. Actinomycetes were studied on Gause culture media (Stefanic, 2006). The incubation temperature was 28°C and the incubation time was 48 hours for bacteria and 7 days for actinomycetes. The humidity has also been determined from the soil samples.

Microorganisms in the soil samples were analyzed by molecular and classical methods. The samples that were submitted to molecular assay are presented in Table 2.

DNA was isolated and purified from approximately 100 mg of soil sample using "FastDN SPIN Kit for Soil" (MP Biomedicals) commercial Kit, following the producer described procedure.

The used kit was adapted to ensure an efficient isolation for bacterial and fungal DNA from soil samples.

The quality and quantity of isolated DNA was assessed by spectrophotometric measurement using a UV-VIS NanoDrop 8000, (Thermo Scientific) spectrophotometer.

The concentration of DNA in suspension was equalized by dilution to 50 ng/μl.

PCR amplification of soil DNA using ISSR and DAMD molecular markers systems

ISSR and DAMD molecular markers were chosen for the genetic diversity in soil bacterial communities' assessment (Tab. 3).

Tab. 3. Sequence of primers used for diversity evaluation

1		UBC820	(GT) ₈ C
2		UBC834	(AG) ₈ YT
3	ISSR	UBC853	(TC) ₈ RT
4		A 12	(GA) ₆ CC
5		A 21	(CA) ₆ AC
6		URP6R	GGCAAGCTGGTGGGAGGTAC
7	DAMD	URP9F	ATGTGTGCGATCAGTTGCTG
8		M13	GAGGGTGGCGGCTCT

PCR was carried out in a final volume of 25 µl containing 100 ng of DNA template. The composition of amplification mixture was carried out following the producer instructions for GoTaq Green Master Mix (Promega, USA). The reaction was performed on a DNA Engine Peltier Thermal Cycler (MJ Research, U.S.A.) and the PCR program consisted of a first denaturing step for 5 min at 95°C, followed by 45 cycles of denaturation at 95°C for 45 sec, annealing at 48°C- 55°C for 45 sec and extension at 72°C for 2 min, the final step of extension at 72°C for 5 min., according to literature data.

The resulting PCR products were run on 1.8 % agarose gels in TAE buffer at room temperature at a constant voltage of 100 V for 90 minutes. The PCR products were visualized and photographed under UV light (PhotoDocumentation System, UVP, England). The obtained data were analyzed with VisionWorksLC software.

The dendrogram was assessed from a set of variables by using DendroUPGMA, software. The program calculates a similarity matrix and transforms similarity coefficients into distances and makes a clustering using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm.

RESULTS AND DISCUSSIONS

The results of microbiological determinations are illustrated in Figure 1. By analyzing the bacterial populations of the vine plants and from soil there were observed similarities between phyllosphere and rhizosphere samples, as suggested by Martins *et al* (2013), whom revealed the existence of interaction between bacteria present in the soil and the epiphytes ones and Zelenev *et al.* (2005), that have revealed that in the rhizosphere area,

oligotrophic bacteria dominate, but there are also copiotrophic microorganisms.

In conformity with our studies the microorganisms from analyzed biological samples differs depending on the collection area and the type of soil. The highest number of bacteria and actinomycetes was recorded in the sample **SI 1**, and the lowest in the sample **SI 2** (Fig. 1).

Measurement of the number of bacteria and actinomycetes by the classical method, pointed out significant differences between the soil samples analyzed, with the exception of the soil sample b3., which differs through a the large number of actinomycetes

There are studies showing that the distribution of microorganisms in the zone of radical influence is uneven, but also the microbial community is numerically fluctuating, because the compounds released by plant roots diffuses into the ground at different distances from the root surface (Gupta and Mukerji., 2002), but due to the cell death or lysis intervenes (Semenov *et al.*, 1999). Distance to the plant roots and depth of soil sampling are among the factors that influence the number of bacteria and actinomycetes. Microorganisms are progressively reduced, as the soil samples are collected at greater distances towards the root and the bigger depths (<http://agriinfo.in>).

Regarding the sites of the root area, populated by microorganisms, some authors (Maloney *et al.* 1997; Paul *et al.*, 2010) showed an increased number of oligotrophic bacteria in the root peaks area, compared to the root base.

Bacteria and actinomycetes from phyllosphere were lower compared to those isolated from soil. The number of bacteria was higher in the samples c1 and c3, and actinomycetes recorded higher values in the sample c2 and c4 (Fig. 1). According

Fig. 1. Evolution of bacteria and actinomycetes from biological samples studied by classical method
 Legend: a1-a4-soil bacteria, b1-b4- soil actinomycetes c1-c2-phylosphere bacteria; c3,c4-phylosphere actinomycetes

Fig. 2. DNA fingerprint obtained using ISSR primers: **A 12, UBC 844, A 21, UBC 820, UBC 853**: 1 – soil sample A, 2- soil sample B, 3- soil sample C, 4 – soil sample D, M - Molecular weight marker - GeneRuler Express DNA Ladder (*Fermentas*).

to McCarthy and Williams (1992), the population of actinomycetes is influenced by humidity, temperature, pH, plants, and organic matter.

The extracted DNA was of acceptable quality in order to be used in PCR analysis, however consequent purification was necessary since it was considered that some of detected inhibitors can negatively affect the results.

The selected molecular markers systems ISSR (5 primers) and DAMD (3 primers) yielded a DNA fingerprint of good quality in terms of amplified sequences number and well defined PCR product (Fig. 2, Fig. 3). The selected molecular systems provided enough data for the bacterial

communities' diversity to be scored and a dendrogram to be developed on the basis of data collected by PCR analysis (Tab. 3).

DAMD primers yielded a reduced number of amplicons, compared to ISSR primers, but overall they exceeded in terms of polymorphic bands percentage (Fig. 2, Fig. 3), fact that enables us to confirm that microsatellite regions are more abundant than the minisatellite ones in the genome of soil bacterial communities. Those differences are easily predicted to occur because of their different sequence and DNA target specificity. Nevertheless, when those two sets of data are combined the result is more accurate than in the case of using

Fig. 3. DNA fingerprint obtained using DAMD primers: **URP 6R, M 13, URP 9F**: 1 – soil sample A, 2- soil sample B, 3- soil sample C, 4 – soil sample D, M - Molecular weight marker - GeneRuler Express DNA Ladder (*Fermentas*).

Tab. 3. Genetic distance matrixes as resulted from DendroUPGMA software.

	A	B	C	D
A	0	0.659	0.664	0.681
B		0	0.678	0.814
C			0	0.696
D				0

Tab. 4. Genetic similarity as resulted from DendroUPGMA software.

	A	B	C	D
A	1	0.341	0.336	0.319
B		1	0.322	0.186
C			1	0.304
D				1

Fig. 4. Genetic diversity Dendrogram of *soil samples* created by DendroUPGMA program using data from 8 VNTR molecular markers systems

a single marker system. From data resulted from the analyses of PCR results, as were given by the analysis software, a binary matrix for each individual soil sample was developed. This matrix consisted of scores (1 for present and 0 for absent)

for each of the four soil samples DNA fingerprint obtained by using the eight primers. The binary matrix was used in assessing the similarity matrix and the genetic distance matrix using the Jaccard similarity coefficient (Tab. 3; Tab. 4).

The genetic distances are used to approximate the genetic divergence between analyzed samples. Analyzing the obtained matrix it can be observed that the soil bacterial samples are very closely related, as expected all the obtained values were above the medium threshold. The minimum value of 0.659 was obtained in this case is between samples A and B. The higher value of 0.814 is recorded between samples B and D.

Considering a medium value of 0.5 the data are interpreted function of approaching to the mentioned extremes. All the coefficients are below the medium value. The highest value of 0.341 is recorded between samples A and B, and the lowest of 0.186 is recorded between samples B and D. Considering the recorded data it can be noticed that soil sample D is less similar with the others three. Similar, the genetic distance matrix revealed that individual D very distant from soil sample A and B, but related to sample C.

On the basis of resulted matrices an UPGMA dendrogram was designed (Fig. 4). The similarity coefficients were used to group the samples in clusters. The length of the clusters was dictated by the genetic distance coefficients. The dendrogram illustrates the genetic relationship among studied microbial communities soil samples. Dendrogram analyses revealed that the four samples are grouped in two different major clusters. The first cluster contains only the soil sample D, being the most distinct among them as it is clustered separately. In the second major cluster the most distinct sample is C and the more related, grouped individually, are the samples A and B that were collected from the same vine variety roots interference area. Although the samples C and D are collected from the same vine variety roots interference area, those are not grouped in the same cluster, still it can be considered closer to the sample D. In the case of sample D we can conclude that the herbaceous plants bacterial root symbionts are influencing the soil bacterial communities.

CONCLUSION

Soil microflora was large compared to that present in phyllosphere. The variation in amount of microflora from studied biological samples may be due to a complex of abiotic and biotic factors, and the interaction between them, plus the tillage of soil, distance from the root, root

exudates. Studies by classical methods, made separately on the two microbial groups (bacteria, actinomycetes), are highlighting the abundance of bacteria and actinomycetes in the sample **SI 1**. In some studies (mentioned above), it appears that microorganisms can be found in abundance at the root tips. According to the molecular analyzes the microbial communities from TA rhizosphere is different from the other analyzed samples.

REFERENCES

1. Dayakar B, Tiffany W, Van Der Lelie D, Jorge V (2009). Rhizosphere chemical dialogues: plant-microbe interactions, Current Opinion in Biotechnology 20(6): 642-650.
2. Beerling DJ, Berner RA (2005). Feedbacks and the coevolution of plants and atmospheric CO₂. Proc Natl Acad Sci USA 102:1302-1305.
3. Blakeman JP, Fokemma NJ, (1982). Potential for biological control of plant diseases on the phylloplane. Ann. Rev. Phytopathol., 20:167-92.
4. Broeckling Corey D, Broz A, Bergelson J, Manter D, Vivanco J (2008). Root exudates regulate soil fungal community composition and diversity. Appl. Environ. Microbiol., 74, 738-744.
5. Woeng C, TFC, De Priester W, Van der Bij AJ, Lugtenberg BJJ (1997). Description of the colonization of a gnotobiotic tomato rhizosphere by *Pseudomonas fluorescens* biocontrol strain WCS365, using scanning electron microscopy. Mol Plant Microbe In 10: 79-86.
6. Claes R (1997). Mini- and Microsatellites, Environmental Health Perspectives, Vol 105, Supplement 4.
7. Compant, Brion D, Jerzy N, Christophe C, Essaid Ait Barka (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol., 71, 4951-4959.
8. De Werra P, Péchy-Tarr M, Keel C, Maurhofer M (2009). Role of gluconic acid production in the regulation of biocontrol traits of *Pseudomonas fluorescens* CHA0. Appl. Environ. Microbiol., 75, 4162-4174.
9. Dennis PG, Miller AJ, Clark IM, Taylor RG, Valsami-Jones E, Hirsch PR (2008). A novel method for sampling bacteria on plant root and soil surfaces at the microhabitat scale. J Microbiol Meth 75: 12-18.
10. Foster RC (1988). Microenvironments of soil microorganisms. Biol. Fertil. Soils, 6, 189-203.
11. Gray EJ, Smith DL (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37: 395-412. doi: 10.1016/j.soilbio.2004.08.030.
12. Grayston SJ, Wang SQ, Campbell CD, Edwards AC (1998). Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem., 30, 369-378.
13. Gupta R., Mukerji KG, (2002). Root exudate-biology. In: Mukerji K.G., Manoharachary C., Chamola BP, eds.

- Techniques in mycorrhizal studies. Dordrecht, The Netherlands: Kluwer Academic Publishers, 103-131.
14. Haas D, Défago G (2005). Biological control of soilborne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbiol.*, 3, 307-319.
 15. Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997). Bacterial endophytes in agricultural crops. *Can J Microbiol* 43: 895-914. doi: 10.1139/m97-131
 16. Heath DD, Iwama GK, Devlin RH (1993). PCR primed with VNTR core sequence yields species specific patterns and hypervariable probes. *Nucleic Acids Res.*, 1993, 21: 5782-5785.
 17. Jensen E, (1996). Rhizodeposition of N by pea and barley and its effects on soil N dynamics. *Soil Biol. Biochem.*, 28, 65-71.
 18. Kent AD, Triplett EW (2002). Microbial communities and their interactions in soil and rhizosphere ecosystems. *Ann. Rev. Microbiol.*, 56, 211-236.
 19. Lindow SE, Brandl MT (2003). Microbiology of the phyllosphere. *Appl Environ Microbiol* 69: 1875-1883. doi: 10.1128/aem.69.4.1875-1883.2003.
 20. Maloney PE, Van Bruggen AHC, Hu S (1997). Bacterial community structure in relation to the carbon environments in lettuce and tomato rhizospheres and in bulk soil. *Microb. Ecol* 34: 109-117.
 21. Martins G, Lauga B, Miot-Sertier C, Mercier A, Lonvaud A, Soulas M-L (2013). Characterization of Epiphytic Bacterial Communities from Grapes, Leaves, Bark and Soil of Grapevine Plants Grown, and Their Relations. *PLoS ONE* 8(8): e73013. doi:10.1371/journal.pone.0073013.
 22. McCarthy AJ, Williams ST (1992). Actinomycetes as agents of biodegradation in the environment – a review. *Gene*. 115, 189-192.
 23. Muyzer G (1999). Genetic fingerprinting of microbial communities – present status and future perspectives. *Methods of microbial community analysis. Proceedings of the 8th international symposium on microbial Ecology.* Atlantic Canada Society for Microbial Ecology, Halifax, Canada.
 24. Nihorimbere V, Ongena M, Smargiassi M, Thonart P (2011). Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotechnol. Agron. Soc. Environ.* 15(2), 327-337.
 25. Nisiotou AA, Rantsiou K, Iliopoulos V, Cocolin L, Nychas GJE (2011). Bacterial species associated with sound and *Botrytis*-infected grapes from a Greek vineyard. *Int J Food Microbiol* 145: 432-436. doi: 10.1016/j.ijfoodmicro.2011.01.017.
 26. Olsson S, Baath E, Soderstrom B (1987). Growth of *Verticillium dahlia* Kleb hyphae and of bacteria along the roots of rape (*Brassica napus* L.) seedlings. *Can J Microbiol* 33: 916-919.
 27. Pate JS, Verboom WH, Galloway PD (2001). Co-occurrence of *Proteaceae*, laterite and related oligotrophic soils: coincidental associations or causative inter-relationships? *Aust J Bot* 49:529-560.
 28. Dennis P, Miller A, Hirsch P (2010). Are root exudates more important than other sources of rhizo deposits in structuring rhizosphere bacterial communities?. *FEMS Microbiol Ecol* 72, 313-327.
 29. Pratibha P, Kapoor N, Sachdeva S (2014). Rhizosphere: its structure, bacterial diversity and significance, *Reviews in Environmental Science and Bio/Technology*, 13(1):63-77.
 30. Jerzy PA., Sulima P, Kuszewska A, Załuski, Kilian DA (2013). Phylogenetic Relationships between Four *Salix* L. Species Based on DArT Markers, *Int J Mol Sci.* 2013 Dec; 14(12): 24113-24125.
 31. Raaijmakers JM, Weller DM (2001). Exploiting genotypic diversity of 2,4-diacetylphloroglucinol producing *Pseudomonas* spp.: characterization of superior root colonizing *P. fluorescens* strain Q8r1-96. *Appl. Environ. Microbiol.*, 67, 2545-2554.
 32. Semenov AM, van Bruggen AHC, Zelenev VV (1999). Moving waves of bacterial populations and total organic carbon along roots of wheat. *Microbiol Ecol.*, 37, 116-128.
 33. Samar S, Ladha JK, Gupta RK, Bhushan L, Rao AN, Sivaprasad B, Singh PP (2007). Evaluation of mulching, intercropping with Sesbania and herbicide use for weed management in dry-seeded rice (*Oryza sativa* L.). *Crop Prot.*, 26, 518-524.
 34. Ștefanic G (2006). Metode de analiza a solului (biologica, enzimatica si chimica), Probleme de agrofitotehnie teoretica si aplicata. Institutul de cercetare-dezvoltare Agricola Fundulea, XXVIII, 5-25.
 35. Suzuki M, Michiko T, Takashi T, Satoshi W, Shinpei M, Junshi Y, Naoki K, Shoshi K, Hiromi N, Satoshi M, Naoko KN (2006). Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. *Plant J.*, 48, 85-97.
 36. Taylor LL, Leake JR, Quirk J, Hardy K, Banwatts SA, Beerling DJ (2009). Biological weathering and the long-term carbon cycle: integrating mycorrhizal evolution and function into the current paradigm. *Geobiology* 7:171-191.
 37. Tesařová, Řěpová A (1984). A microbial biomass of a grassland soil as estimated by the fumigation and direct counting methods. *Folia Microbiol* 29: 189-192.
 38. Verginer M, Leitner E, Berg G (2010). Production of Volatile Metabolites by Grape-Associated Microorganisms. *J Agric Food Chem* 58: 8344-8350. doi: 10.1021/jf100393w.
 39. Watt M, Hugenoltz P, White R, Vinall K (2006). Numbers and locations of native bacteria on field-grown wheat roots quantified by fluorescence in situ hybridization (FISH). *Environ Microbiol* 8: 871-884.
 40. Zelenev VV, van Bruggen AHC, Semenov AM (2005). Modelling wave-like dynamics of oligotrophic and copiotrophic bacteria along wheat roots in response to nutrient input from a growing root tip. *Ecol Model* 188: 404-417.
 41. <http://agriinfo.in/?page=topic&superid=5&topicid=155>
 42. <http://genomes.urv.cat/UPGMA/index.php>