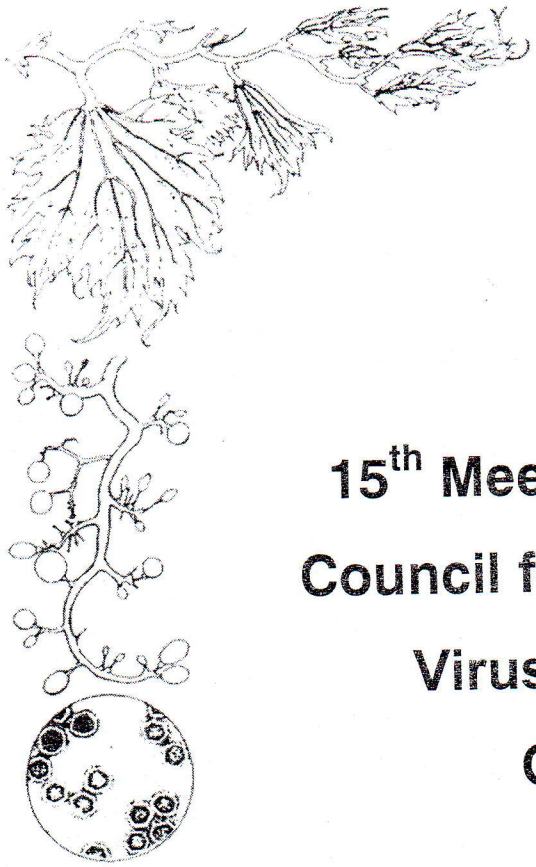


# 15th Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG)

**3-7 April 2006**

**Stellenbosch, South Africa**

## Extended abstracts

Although abstracts are published as received from authors, minor changes to the layout has been made. While every effort has been made to reproduce abstracts in their original form, SASEV regrets errors which may have arisen during the printing process.

ISBN 1-86849-318-0

**Acknowledgements:** Prof P.G. Goussard, Mr Abraham Vermeulen and Mrs R. Carstens photo's on cover page

# CORRECT CITATION

*Extended abstracts 15th Meeting ICVG*, Stellenbosch, South Africa, 3 – 7 April 2006, pg. - pg.

**Title:** 15th Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine – Extended Abstracts.

**Editor:** None - Published as received

**Publisher:** South African Society for Enology and Viticulture

**ISBN: 1-86849-318-0**

## SASEV BOARD

Dr I.A. Trautmann
Dr M. du Toit
Mr D. J. Smit
Mr S Joubert
Mrs R. Carstens
Dr V.A. Watts
Prof L.M.T. Dicks
Mr J.P. Marais
Mr J.H.M. van Zyl
Mr H.G. Tredoux
Mr J.L. Viljoen
Ms M. Fundira
Mr A. Schreuder
Dr P. van Rensburg

## ORGANISING COMMITTEE

Johan Burger (Stellenbosch University, Chair)
Roleen Carstens (ARC Infruitec-Nietvoorbij)
Maret du Toit (SASEV)
Mandi Engelbrecht (Stellenbosch University)
Dariusz Goszczynski (PPRI)
Gawie Kriel (Private)
Marietta Louw (Distell)
Tobie Oosthuizen (KWV Vititec)
Gerhard Pietersen (CRI, University of Pretoria)
Nico Spreeth (KWV Vititec)
Ilse Trautmann (SASEV)
Riël Tredoux (SASEV)
Melinda van der Ryst (SASEV)

## CONFERENCE SECRETARIAT

Mrs M. van der Ryst
PO Box 2092
Dennesig
STELLENBOSCH 7601
SOUTH AFRICA
Tel: +27 21 8093123
Fax: + 27 21 8896335
Email: sasev@arc.agric.za
Web: http://www.sasev.org

## ICVG COMMITTEE

MARTELLI, Giovanni.P. (Chairman)
BOVEY, Rene (secretary)
BELLI, Giuseppe
BURGER, Johan T.
FUCHS, Marc
GOLINO, Deborah A.
GUGERLI, Paul (Associate Secretary)
JOHNSON, Raymond
MAIXNER, Michael
NOLASCO, Gustavo
REZAIAN, Ali
RUMBOS, Iannis C.
De SEQUEIRA, Oscar A.
TANNE, Edna

# DEVELOPMENT AND EVALUATION OF A BULGARIAN GRAPEVINE GENEBANK

**I. Tsvetkov**[1*], A. Atanassov[1], M. Jankulova[1], V. Vulchev[2]., I. Todorov[3], S. Pandeliev[4], I. Malenin[2], K. Katerov[2],Y. Tsvetkov[5], T. Hvarleva[1], I. Genov[1], T. Dzambazova[1], I. Antonov[1], L. Marinov[1], E. Dimitrov[1], M. Panamska[1], G. Varadinova[1], K. Slavova[1], I. Atanassov[6], G. Shishmanov, V. Yaneva[8], N. Ivanova[9], P. Nikolov[10] & M. Laginova[10]

[1]AgroBioInstitute, 8, Dragan Tsankov Blvd., 1164 Sofia, Bulgaria, e-mail: ivantsvetkov@hotmail.com
[2]Institute of Viticulture and Enology, 1, Kala Tepe str., 5800 Pleven, Bulgaria
[3]Institute of Agriculture and Seedknowledge, 1, Prof. Ivan Ivanov str., 7007 Rousse, Bulgaria
[4]Agriculture University, 12, Mendeleev str., 4000 Plovdiv, Bulgaria
[5]Experimental Station of Viticulture, 5 Saedinenie str., 4490 Septemvry, Bulgaria
[6]Pepinier Consult AgroBioTech Ltd., Nayden Gerovo, obst. Saedinenie, Bulgaria
[7]Tsenko'95 Ltd., West Industrial Zone, 7100 Bijala, Bulgaria
[8]AMV-Agro Ltd., SPS Complex, Osvobozdenie Blvd., 4023 Plovdiv, Bulgaria
[9]Executive Agency for Variety Testing Field Inspection and Seed Control, 125 Tzarigradsko Shose str., 1113, Sofia, Bulgaria
[10]National Service for Plant Protection, 120, N. Moushanov str., 1330, Sofia, Bulgaria

## Introduction

Grapevines have been one of the most important crops of Bulgaria both from the economic and social point of view. The total area under vines is about 131 069 ha of which 110 505 ha yield wine grapes and only 20 564 ha table grapes. The range of **wine grapes** cultivated is presented by local and introduced varieties. Red wine varieties cover 52% of the total wine grape area. The most distributed varieties are "Pamid" (35%), "Merlot" (18%) and "Cabernet Sauvignon" (16%). White wine areas is occupied by "Rkatzitelli"- 25% from the total white wine areas, "Dimijat" (21%), "Chardonnay" (5%), "Muscat Otonel" (5 %) (1).

Regarding the 20 564 ha planted with **table grapes,** "Bolgar" leads the way representing 75% of them, followed by "Muscat Hamburg" (7%), "Cardinal" (5%), "Sultanina (1%)". Last, but not least, the new local table varieties such as "Velika", "Nadezda", "Hybrid VI-IV", "Muscat Roussenski", "Rusalka 1" and others are much appreciated in the local and foreign market. At the period lots of viticulture problems are still waiting for decision. Large part of the vineyards is too old and emergency needed to renew (65%). Development and maintenance of Bulgarian pre-basic grape genebank and a certification scheme related to the European Legislation for the marketing of material for the vegetative propagation of grapevines [Directives 68/193, 71/140, 77/129 and 02/11] appeared to be a promising step for solving the key problem for revitalisation of Bulgarian viticulture. The objective of the present study was the development and evaluation of a Bulgarian grapevine genebank trough preliminary field selection, ELISA tests for 16 viruses, bacterial analyses, DNA identification, *in vitro* micropropagation and establishment of nuclear pre-basic plants. This investigation was carried out within the framework of a previously reported grapevine selection programme of the AgroBioInstitute (ABI) (2).

## Materials and Methods

The mature cuttings from preliminary field selected plants placed on all parts of Bulgaria were used as a basic source for plant material development. A grapevine certification scheme based on Martelli *et al.* (1999) and on EEC Directives 68/193 and 02/11 was employed. ELISA analyses for 16 viruses were carried out as described by Voller *et al.* (1976) using DAS ELISA and RTA ELISA kits obtained from BIOREBA®. Bacterial analyses and DNA identification were based on modified protocols, reported by Genov *et al.* (2005) and Hvarleva *et al.* (2004). For grapevine clonal micropropagation a modification protocol based on Samson & Gasteran (1971) was used. The acclimatisation of grapevine *in vitro* plants was based on a modification of the protocol described by Babrikov *et al.* (2000).

## Results and Discussion

Results from ELISA tests indicated the high presence of viral diseases – totally 75.50% of the cuttings were infected. In opposite, only 7 % carrying crown gall disease. Thanks to the previous ABI selection programme, there are not find non- authentic ones after SSR molecular analyses. Healthy plants for the establishment of nuclear pre-basic stock were selected. Genetic and health status of the established ABI grapevine genebank up to 25.07.2005 were showed on Table 1. The genebank has been maintained on the three level (*in vitro*, greenhouse and field). Since 2004, there are two field replication of the genebank

placed both on ABI grapevine bases Bijala (North part of Bulgaria- Rousse region- Tsenko'95 Ltd.) and on village Nayden Gerovo (South part of Bulgaria- Plovdiv region- Pepinier- Consult Agorbiotech Ltd.).

Table 1. Development of ABI grapevine genebank /up to 25.07.2005/

| Virus Diseases | Authentity | Bacterial diseases |
|---|---|---|
| 1. Grapevine Fanleaf Virus (GFLV)<br>2. Arabis Mosaic Virus (ArMV)<br>3. Grapevine Leafroll assoc. Virus 1 (GLRaV-1)<br>4. Grapevine Leafroll assoc. Virus 2 (GLRaV-2)<br>5. Grapevine Leafroll assoc. Virus 3 (GLRaV-3)<br>6. Grapevine Leafroll assoc. Virus 6 (GLRaV-6)<br>7. Grapevine Fleck Virus (GFkV)<br>8. Grapevine Virus A (GVA)<br>9. Grapevine Virus B (GVB)*<br>10. Raspberry ringspot virus-ch strain (RpRSV-ch)<br>11. Raspberry ringspot virus- g strain (RpRSV-g)<br>12. Strawberry latent ringspot virus (SLRSV)<br>13. Tobacco ringspot virus (TRSV)<br>14. Tomato ringspot virus- Chikadee strain (ToRSV-Ch)<br>15. Tomato ringspot virus-peach yellow bud mosaic strain (ToRSV-PYBM)<br>16. Grapevine Bulgarian Latent Virus (GBulLV)** | 3 years preliminary selection on the base of ampelographic and economic characteristics | **Crown gall**<br>- Agrobacterium vitis<br>- Agrobacterium tumefaciens |
| **Methods** | | |
| **1. ELISA analyses**<br>- ELISA kits<br>BIOREBA® - Switzerland<br>* AGRITEST®- Italy<br>**AGROBIOINSTITUTE- Bulgaria<br>**2. Molecular diagnostics**<br>under the way | **1. Visual observation**<br>- Prof. V. Vulchev<br>- Prof. I. Todorov<br>- Prof. L. Radululov<br>- Prof. K. Katerov<br>- Prof. S. Pandeliev<br>**2. DNA identification**<br>- microsatellite /SSR/ markers:<br>VvUCH11; VvUCH29; VrZAG21; VrZAG47; VrZAG62; VrZAG64; VrZAG79; VrZAG83; VVS2 | **1. Isolation on semi-selective medium**<br>- Roy & Sasser<br>**2. Molecular diagnostic**<br>-PCR detection<br>**polygalacturonase gene*<br>**virD 2 gene*<br>**vir E 2gene*<br>**vitopine synthase gene* |

Except mentioned above companies, working under strict control, ABI has a long term contracts also with other leading Bulgarian nurseries such as AMV- Agro Ltd., PPK Korten, Zaychev & son Ltd. Genetic and health status of the genebank is presented on Tab.1. It was showed also methods and the names of breeders included in preliminary field selection of the mother plants. The genebank is already used for production of pre-basic and basic material in contribution with ABI first pre-propagation bases.

## References


Ministry of Agriculture and Forests (2004) Agrostatistics Bulletin ,1, 8-12.

Tsvetkov I. , Atanassov A., Jankulova M , Vulchev V., Todorov I., Pandeliev S., Katerov K., Atanassov I., Hvarleva T., Tsvetkov Y., Genov I., Dzambazova T., Antonov I., Marinov L., Dimitrov E., Panamska M., Varadinova G., Slavova K., Atanassov I., Shishmanov G., Yaneva V., Ivanova N., Laginova M., 2005. Grapevine Plant Genetic Resources- Improvement, Preservation And Biodiversity, Biotechnology & biotechnology equipment, Special issue, pp.13-21.

Martelli G., 1999. Infectious diseases and certification of grapevines. In: Options Mediterraneennes. Serie B: Studies and Research, No. 29, CINEAM (eds.), ISSN 1016-1228, Bari, Italy, pp.47-64.

Voller A., Bartlett A., Bidwell D., Clark M., Adams A., 1976. The detection of viruses by enzyme-linked immunosorbent assay (ELISA). J. Gen. Virol. 33: 165-167.

GENOV I., ATANASSOV I., TSVETKOV I., ATANASSOV A., 2005. Isolation and characterization of Agrobacterium strains from grapevines in Bulgarian vineyards and wild grapes V. vinifera ssp. Sylvestris, Vitis (in press)

HVARLEVA, T.; RUSANOV, K.; LEFORT, F.; TSVETKOV, I.; ATANASSOV, A.; ATANASSOV, I.; 2004: Genotyping of Bulgarian Vitis Vinifera L. cultivars by microsatelite analysis. Vitis, **43**, 27-34.

Samson C., Gasteran P., 1971. Techniques des multiplication de la Vigne. In: Science et Techniques de la Vigne, Vol. II, Paris, France

Babrikov D., Braikov D., Pandeliev S., 2000. Adaptation of in vitro obtained grapes. In: Viticulture with ampelography, Videnov & Son (eds.), Sofia, pp.233-237.

Vitis **45** (2), 97–101 (2006)

# Isolation and characterization of Agrobacterium strains from grapevines in Bulgarian vineyards and wild grapes, *V. vinifera* ssp. *silvestris*

I. Genov, I. Atanassov, I.Tsvetkov and A. Atanassov

AgroBioInstitute, Sofia, Bulgaria

## Summary

**Twenty strains of *Agrobacterium* spp. were isolated from grapevines derived from 9 different grape-growing regions in Bulgaria and from symptomless wild grapes, *Vitis vinifera* ssp. *silvestris*, naturally growing in forests of South-East Bulgaria. The isolated strains were examined by biochemical and pathogenicity tests and PCR analysis of chromosomal and plasmid-located genes. Two of the studied strains resemble non-pathogenic *A. tumefaciens*, but the others were determined as tumorigenic *A. vitis*. Only one out of 18 *A. vitis* strains, isolated from grapevines, was found to harbor vitopine Ti plasmid type. Surprisingly, all 4 *A. vitis* strains isolated from symptomless *V. vinifera* ssp. *silvestris* plants, proved to be tumorigenic. Thus, for the first time we report the isolation of pathogenic *A. vitis* strains from wild grapes. The strict correlation between the results from biochemical and virulence tests and PCR analysis suggests that the chosen PCR procedures can be used efficiently for direct PCR testing for the presence and tumorigenicity of *A. vitis* in grapevines.**

K e y w o r d s : *Agrobacterium*, grapevine, wild grapes, *V. vinifera* ssp. *silvestris,* pathogen strain, polymerase chain reaction.

## Introduction

Crown gall is one of the most important and widespread bacterial diseases of grapevines. It is caused predominantly by *Agrobacterium vitis,* although *A. tumefaciens* was also found occasionally in infected vines (Burr *et al*. 1998). *Agrobacterium* survives systemically in infected grapes (Lehoczky 1968). There is no effective chemical control and the preventive measures including early detection of the pathogen and propagation of disease-free planting material are the only way out (Burr *et al*. 1998, Burr and Otten 1999). In recent years the use of a non-pathogenic *A. vitis* strain F2/5 for biological control was proposed (Burr and Reid 1993, Burr *et al*. 1998). A number of *A. vitis* strains were isolated from various grape-growing areas in the world; they were further characterized for their tumorigenic capacity, T-DNA structure, physiological and biochemical properties. Representative strain collections were established and well characterized reference strains were selected (Burr *et al*. 1998, Burr and Otten 1999). Ti plasmid-located genes are responsible for the tumor formation in infected plants (Burr and Otten 1999 and references therein). Restriction maps, cloned fragments, and sequences are now available for the major types of *A. vitis* Ti plasmids. With regard to the Ti plasmid, three basic structures have been defined: the octopine/cucomopine type, the nopaline type and the vitopine type (Szegedi *et al*. 1988, Burr and Otten 1999, Szegedi 2003). Different primer pairs based on plasmid and chromosome DNA sequences are designed and PCR analysis was successfully applied for detection of *A. vitis*, prediction of strain tumorigenicity and T-DNA type (Winans *et al*. 1987, Eastwell *et al*. 1995, Haas *et al*. 1995, Louws *et al*. 1999, Szegedi and Bottka 2002).

The presence of wild grapes, *V. vinifera* ssp. s*ilvestris*, naturally growing as single plants in the forests of South-East Bulgaria was reported by Negrul (1968) and Katerov (1968). The occurrence of Crown Gall disease in Bulgarian vineyards was described for the first time by Valachev (1902). Malenin (1980) reported Crown Gall to occur in all grape-growing regions of Bulgaria. Besides these studies no systematic collection, differentiation and characterization of *Agrobacterium* strains isolated from Bulgarian vineyards and naturally growing wild grapes was performed so far.

Here we describe the isolation and characterization of *Agrobacterium* strains from different grape-growing regions of Bulgaria. Moreover, the isolation of pathogenic *A. vitis* strains from wild grapes (*V. vinifera* ssp. *silvestris)* is reported. The application of biochemical and PCR tests for strain differentiation and the determination of strain tumorigenicity are discussed.

## Material and Methods

B a c t e r i a l s t r a i n s : The donor grapevine plants are located in different grapevine growing regions and represent old native *V. vinifera* cvs like Mavrud and Dimiat, widespread cvs like Cabernet Sauvignon, Merlot and Chardonnay and locally selected cv. Velika. *V. vinifera* ssp *silvestris* plants were found in the forests of the Arkutino area and the Stranja Mountain, South-East Bulgaria. *V. vinifera* trunks and canes with typical crown galls were collected from vineyards in the main grape-growing regions of Bulgaria. Stem samples from wild *V. vinifera*

Correspondence to: Mr. I. Genov, AgroBioInstitute, 8 Dragan Tzankov blvd., 1164 Sofia, Bulgaria. Fax: +359-2-963-5408. E-mail: ivan_genov75@mail.bg

Vitis **45** (3), 125–130 (2006)

# Genetic diversity of *Agrobacterium vitis* strains, isolated from grapevines and wild grapes in Bulgaria, assessed by Cleaved Amplified Polymorphic Sequences analysis of 16S-23S rDNA

I. Genov[1)], I. Atanassov[1)], Y. Yordanov[2)], I. Tsvetkov[1)], and A. Atanassov[1)]

[1)] AgroBioInstitute, Sofia, Bulgaria
[2)] Sofia University "St. Kl. Ohridski", Department of Genetics, Sofia, Bulgaria

## Summary

**Nineteen tumorigenic *Agrobacterium vitis* strains isolated from commercial vineyards and wild grapes at different locations in Bulgaria were studied in relation to the Ti plasmid type and chromosomal background. The PCR analysis showed that all but one of the strains harbor an octopine/cucumopine type of Ti plasmid and one carries a vitopine type. The genetic diversity among the studied strains and 20 more *A. vitis* strains originating from different geographic regions in Europe, Asia, USA and South Africa was assessed by Cleaved Amplified Polymorphic Sequences (CAPS) analysis of 16S-23S rDNA region. The comparison of the obtained CAPS profiles and performed cluster analysis showed a high level of polymorphism among the studied strains distributed in totally 15 different groups within two main clusters. All Bulgarian strains are located in only three groups within one of the clusters. A high level of correlation between the chromosomal background and type of the carried Ti plasmids was found. The performed CAPS analysis demonstrated that all *A. vitis* strains isolated from wild grapes (*V. vinifera* ssp. *silvestris*) showed CAPS profiles identical with a number of strains isolated from commercial vineyards from different vine-growing regions in Bulgaria. A possible origin of this group of strains from an ancestral *A. vitis* strain, which initially inhabits wild grapes (*V. vinifera* ssp. *silvestris*) and later has been disseminated to cultivated grapevines is proposed.**

K e y w o r d s : *Agrobacterium vitis*, genetic diversity, 16S-23S rDNA, Cleaved Amplified Polymorphic Sequences markers, wild grapes, *Vitis vinifera* ssp. *silvestris*,

## Introduction

*Agrobacterium vitis* is the predominant causal agent of crown gall disease of grapevine (Burr *et al*. 1998). The pathogenic strains harbor Ti plasmid, which includes: T-DNA region that is transferred into plant genome, virulence genes responsible for packaging and transport of the T-DNA and genes involved in the synthesis and catabolism of opines. The Ti plasmids of *A. vitis* are classified into three main types: an octopine/cucumopine, a nopaline and a vitopine. Each Ti plasmid type has a specific genetic make-up of the T-DNA structure and is related to a particular type of opine synthase gene(s) (Szegedi *et al*. 1988, Burr and Otten 1999, Szegedi 2003). The opines metabolism of *A. vitis* is not exclusively linked to the Ti plasmids, since existence of non-pathogenic *A. vitis* strain F2/5 harboring an octopine utilization plasmid was reported by Szegedi *et al.* (1999). Following a genetic characterization of diverse pools of tumorigenic strains of *A. vitis*, Momol *et al*. (1998) demonstrate a high correlation between the chromosomal background and the type of Ti plasmids the strains carry. In recent years, the genetic diversity of *A. vitis* strains was evaluated mainly by assessment of the ribosomal DNA sequences (Bouzar *et al*. 1995, Otten *et al*. 1996, Momol *et al*. 1998, Argun *et al*. 2002). While the 16S and 23S genes of the ribosomal operon are highly conserved, the intergenic spacer region (IGS) between them is variable in *Proteobacteria* and thus suitable for analyzing closely related strains. Using "universal" PCR primers, regions of the ribosomal operon including the intergenic spacer could be readily amplified and characterized by Cleaved Amplified Polymorphic Sequences (CAPS) analysis. This approach could be easily applied to study very diverse pools of strains, providing high reproducibility of the results and reliable comparison of the obtained data within different experiments and laboratories.

In a previous study we reported the isolation of *Agrobacterium* strains from grapevines in Bulgarian vineyards and wild grapes and their physiological, biochemical and tumorigenicity characterization (Genov *et al.* 2006). It was demonstrated that tumor bearing grapevines, as well as symptomless wild grapes (*Vitis vinifera* ssp. *silvestris*), contain predominantly pathogenic strains of *A. vitis*.

In this study we determine the Ti plasmid type of 19 pathogenic *A. vitis* strains and apply CAPS analysis of rDNA region for assessment of their genetic diversity together with 20 other *A. vitis* strains originating from different countries. The correlations of the determined Ti plasmid type and chromosomal background, as well as the possible origin of the *A. vitis* strains isolated from the wild grapes are discussed.

## Material and Methods

P C R a n a l y s i s o f t h e s t r a i n s : The *A. vitis* strains used in our experiments are listed in the Table. Bacterial DNAs were isolated from overnight cultures using the rapid Triton X-100/sodium azide protocol

Correspondence to: Mr. I. Genov, AgroBioInstitute, 8 Dragan Tzankov blvd., 1164 Sofia, Bulgaria. Fax: +359-2-963-5408. E-mail: ivan_genov75@mail.bg

# ВИРУСНИ БОЛЕСТИ ПО ЛОЗАТА У НАС

**Ст.н.с. II ст. д-р Маргарита Янкулова, н.с. I ст. д-р Иван Цветков, ст.н.с. II ст. д-р Иванка Каменова, ст.н.с. II ст. д-р Виолета Кондакова, акад. Атанас Атанасов, проф. Васил Вълчев, АгроБиоИнститут в София.**

## GRAPEVINE VIRUS DISEASES IN BULGARIA

**St. n . s II st. d-r Jankulova M., n.s. I st. d-r Tsvetkov I., n.s. II st. d-r Kamenova V., n.s. II st. d-r Kondakova V., Akademic Atanassov A., Prof. Vulchev V., AgroBioInstitute - Sofia, Bulgaria.**

### SUMMARY

Progress in monitoring of grapevine virus diseases in Bulgaria during the period 1999- 2003 was presented. Totally, 1072 samples from two years previously breeding selected 86 grapevine varieties, 13 rootstocks and 20 hybrid forms from different vineyards in Bulgaria were analyzed for 8 economically important viruses: GFLV (Grapevine Fanleaf Virus), GBLV (Grapevine Bulgarian latent virus), ArMV (Arabis Mosaic Virus), AILV (Artichoke Italian Latent Virus), GLRaV 1 and 3 (Grapevine leafroll associated viruses 1 and 3) GFV (Grapevine Fleck Virus) and GVA (Grapevine Virus A). The results showed that total of 73.18 % of the ELISA tested Vitis vinifera vines and rootstocks were infected by one or more viruses. The most widespread virus was Grapevine flack virus (GFkV) (18.00%), followed by Grapevine leafroll associated viruses 1 and 3 (14.41 %) and Grapevine fanleaf virus (GFLV) (11.69%). There are not detected ArMV and AILV. Virus free varieties and rootstocks were included in AgroBioInstitute pre basic genebank after 5 in vitro propagation passages.

**KEY WORDS:** *grape, Bulgarian grapevine viruses, grapevine certification.*

Напоследък с интензивното създаване на нови лозови насаждения и изискванията на Европейската общност към здравното състояние на посадъчния материал, интересът към вирусните болести в тази култура значително нарасна. Директивите са категорични - насажденията се категоризират и точно се определят начините на отглеждане на лозите, предвидени за размножаване. Значителен е броят на вирусите или вирусно подобните болести по тази култура. Те са повече от 60 на брой, като непрекъснато се регистрират нови патогени. Вегетативното размножаване на лозата, пренасяне на резници от място на място, отглеждането ѝ при различни условия, допринасят тя да е изложена и на многобройни преносители - нематоди, листни и щитоносни въшки. Получаването на здрав посадъчен материал или сертифициран е дълъг и сложен процес, който включва предпазни, защитни и при необходимост оздравителни мероприятия. Кандидат майчините растения, използвани за размножаване, се контролират от упълномощени органи. Растенията трябва да отговарят на определения вид/сорт, като отсъстват каквито и да било болести.

Изследванията на вирусите по лозата след 1960 г. бяха съсредоточени главно върху нематодопреносимите вируси /Табл.1/, като провизорно те се разделят на европейски и американски. Изражданието или дегенерацията обикновено се свързват с "късовъзлието", което често се приема като синоним на Fanleaf или Reisigkrankheit, обединяващо нематодопреносимите инфекции. Симптомите могат да бъдат различни и зависят до голяма степен от сорта, подложката, климатичните особености, а също така и от щама на вируса. Така например от лоза с ярко жълти

*Таблица 1. Нематодопреносими вируси по лозата и техните вектори /по Martelli, 2003 /*

| Вируси | Вектори |
|---|---|
| **1.Причинители на израждането (degeneration), европейски вируси** | |
| 1.1. Късовъзлие (Grapevine fanleaf virus -GFLV) | Xiphinema index |
| 1.2. Арабисова мозайка (Arabis mosaic virus-ArMV) | X. diversicaudatum |
| 1.3. Лозов хромаво мозайчен вирус (Grapevine chrome mosaic virus - GCMV) | неизвестен |
| 1.4. Ягодов латентен пръстеновиден вирус (Strowberry latent ringspot virus -SLRV) | X. diversicaudatum |
| 1.5 Малинови пръстеновидни петна (Raspberry ringspot, virus - RRV) | Longidorus macrosoma Paralongidorus maximus, L.elongatus |
| 1.6. Артишоков италиянски латентен вирус (Artichoke Italian latent virus -AILV) | L.attenuatus, L. fasciatus |
| 1.7. Черни доматени пръстеновидни петна (Tomato black ring virus - TBRV) | L. elongatus, L. attenuatus |
| 1.8. Лозов латентен български вирус (Grapevine Bulgarian latent virus - GBLV) | неизвестен |
| **2. Причинители на закържавяването (decleine) (американски вируси)** | |
| 2.1. Прасковена розетъчна мозайка (Peach rosete mosaic virus - PRMV) | X.americanum, L.elongatus L.diadecturus, X.californicum,X.rivesi |
| 2.2. Доматени пръстеновидни петна (Tomato ringspot virus-ToRV) | X. americanum |
| 2.3. Тютюневи пръстеновидни петна (Tabaco Ringspot virus-TRV) | неизвестен |
| 2.4. Blueberry leaf mottle virus (BBLMV) | – |
| **3. Причинители с неопределен патогенитет** | |
| 3.1. Лозов тунизийски пръстеновиден вирус (Grapevine Tunisian ringspot virus - GTRV) | неизвестен |

*Таблица 2. Лози, инфектирани с вируса на късовъзлието Grapevine fanleaf virus - GFLV*

| Сортове | Общ брой | Заразени |
|---|---|---|
| **1. Бели десертни** | | |
| Армира | 12 | 3 |
| Болгар | 23 | 2 |
| Брестовица | 8 | 1 |
| Мискет Тракийски | 4 | 1 |
| Русенско едро | 6 | 1 |
| Супер ран Болгар | 9 | 1 |
| **2. Червени десертни** | | |
| Памид клон 2 | 3 | 1 |
| Палиери | 5 | 1 |
| Тича | 5 | 1 |
| **3. Червени винени сортове** | | |
| Гъмза | 23 | 4 |
| Мавруд | 136 | 11 |
| Мавруд кл. 1 | 44 | 2 |
| Мавруд кл.2 | 63 | 5 |
| Мелнишки Рубин | 6 | 3 |
| Мерло | 12 | 1 |
| Ранна Мелнишка /Мелник 55/ | 3 | 1 |
| Рубин | 38 | 3 |
| Сензо | 5 | 3 |
| Сторгозия | 3 | 1 |
| Шевка | 5 | 1 |
| **Подложки** | | |
| МК 4 | 6 | 2 |
| Общо : 21 | 419 | 49 (11.69%) |

листа изолирахме щам 862 на арабисовата мозайка /Arabis mosaic virus - ArMV/. Лози от сорт Юлски бисер в района на Поморие, показващи бледожълто хлоротично оцветяване по листата, бяха заразени с Лозовия латентен български вирус /Grapeveine Bulgarian latent virus - GBLV/. Същият вирус по Каберне Совиньон в Пловдивско или по Широка Мелнишка в Сандански е латентен. По листата не се наблюдават признаци, но лозите са по-слабо развити от здравите. В малко опитно насаждение в Института за защита на растенията в Костинброд през месеците май и юни на 2006 г. по листата на лоза от сорт Ркацители забелязахме слабо изразена мозайка, която по-късно изчезна. От тези листенца изолирахме Артишоковия италиански латентен вирус /Artichoke Italian latent virus - AILV/.

Американските нематодопреносими вируси предизвикват по европейските сортове, отглеждани в по-студени райони, вджуджен растеж, нисък добив и бързо загиване. Така например лозата от сорт Кардинал, в която доказахме наличието на Доматените пръстеновидни петна един "американски" вирус /Tomato ringspot virus-ToRSV/, щам 4 s, бе по-слабо развита от останалите и на следващата година загина.

В настоящата работа представяме резултатите от изследванията, направени през последните пет години първоначално в Института по генетично инженерство в Костинброд, по-късно преименуван в АгроБиоИнститут със седалище в София. Данните обхващат тестирането на 86 сорта, 13 подложки и 20 селекционни номера. Автентичните сортове, събирани от цялата страна, бяха подложени на предварително тестиране, преди тяхното ин витро или ин виво размножаване, необходими за т.нар. Генбанка. Развитите млади листенца се изследваха по метода двоен антитяло сандвич на ензимния имунологичен метод /DAS-ELISA/ за вирусите късовъзлие / Grapevine fanleaf virus -GFLV/, ArMV, GBLV и AILV. Тестове-

# VIRUS TESTING OF CERTIFIED GRAPEVINE PLANTING MATERIAL IN BULGARIA

I. Kamenova, I. Tsvetkov, A. Atanassov
AgroBioInstitute, Sofia, Bulgaria
Correspondence to: Ivanka Kamenova
Email: vkamenova@abi.bg

## ABSTRACT

*In accordance with the European Legislation and the recently developed national system for production of certified vine growing material, 1295 samples from grapevine varieties and rootstocks were checked for their virus status in the period of 2004-2006. The results obtained showed that total of 30.50% of the ELISA tested Vitis vinifera vines and rootstocks were infected by one (86.08%) or more viruses (13.92). The most widespread virus was Grapevine flack virus (GFkV) (23.3%), followed by Grapevine fanleaf virus (GFLV) (5.05%) and Grapevine leafroll associated viruses 1 and 3 (GLRaV 1 and 3) (3.34% and 2.78%), respectively. Grapevine Bulgarian latent virus (GBLV) (0.37%) was scarcely represented, while Arabis mosaic virus (ArMV), Grapevine leafroll associated viruses 2 and 7 (GLRaV 2 and 7), Grapevine virus A (GVA) and Grapevine virus B (GVB) were completely absent. The mixed infection showed eight virus combinations. The associations of GFkV + GLRaV 1 (8.3%) and GFLV + GLRaV 3 (8.3%) were the most widespread ones.*
*All tested for ArMV, GFLV and GBLV wild vines (Vitis vinifera ssp. sylvestris) were virus free.*

**Keywords:** Bulgarian grapevine viruses, grapevine genebank, certification

## Introduction

Viticulture is an important industry in Bulgaria and grapevines are grown on approximately 129,580 ha. Virus diseases on grapevine have a significant economic impact on plant growth, yield and fruit quality, as they can affect the graft compatibility, rooting capacity, winter hardiness, longevity of the vines and to lead to plant mortality (10). More than 53 viruses have been detected in *Vitis* (8, 9) and at least 8 viruses of them have been found in Bulgaria (6).

All known grapevine viruses are graft-transmissible. They are spread long distances through infected propagating material (buds/cuttings), or by the use of rooting cuttings from infected canes. Additionally, several grapevine viruses, classified as "*nepoviruses*", including *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Tomato black ring virus* (ToBRV), *Tomato ringspot virus* (ToRSV) and *Tobacco ringspot virus* (TRSV) are transmitted in short distances by certain species of nematodes.

The control of grapevine viruses is dependent upon the effectiveness of clean stock programs, where virus-free propagation material is used in nurseries and vineyards. Addressing the problem of the sanitary improvement of vegetative propagated crops, the EEC issued and acted Directive 68/193, complemented by Directive 00/11 containing provisions for the production of grapevine material free from a couple of virus diseases (fanleaf and leafroll). In Bulgaria, as a pre-accession country this Directive was applied promptly and a law concerning the production of "virus-free" and "virus-tested" certified seed and planting material was adopted in October 2000. A national system for production of certified vine growing material was established by the Ministry of Agriculture and Forestry with the help of FAMAD project to the German Society of Technical Assistance (GTZ).

Since 2000 the propagated grape materials are checked in AgroBioInstitute, Sofia by DAS-ELISA technique for a few prevalent viruses. This paper describes in short the results obtained from virus testing of certified grapevine planting material in Bulgaria for the last tree years.

## Materials and Methods

### Plant material

Grapevines were selected by their virology status from several vine-growing areas of Bulgaria in the period of 2004-2006. The surveys were based on the necessity to check and to select healthy grapevines with the aim to produce certified material by plant propagation (*in situ* or/in *in vitro*). Totally 1295 samples from *V. vinifera*, 77 rootstock samples and 69 samples from *V. vinifera ssp. sylvestris* were tested for some of the following viruses: *Arabis mosaic virus* (ArMV), *Grapevine Bulgarian latent virus* (GBLV), Grapevine fanleaf virus (GFLV), *Grapevine leafroll associated viruses* 1, 2, 3 and 7 (GLRaV 1, 2, 3 and 7), *Grapevine flack virus* (GFkV), *Grapevine virus* A (GVA) and *Grapevine virus* B (GVB).

### DAS - ELISA tests

DAS-ELISA detection of ArMV, GFLV, GFkV, GVA, and GLRaV 1 was performed with commercially available polyclonal antibodies, immunoglobulin G (IgG), and alkaline phosphatase-conjugated IgG (Bioreba, Reinach, Switzerland), according to manufacture's instructions. For GBLV, DAS-ELISA tests were conducted with polyclonal antibodies and

# CHARACTERIZATION OF GRAPEVINE ROOTSTOCKS USING MICROSATELITE MARKERS

T. Dzhambazova [1], T. Hvarleva[1], A. Hadjinicoli[2], I. Tsvetkov [1], A. Atanassov [1], I. Atanassov [1]
AgroBioInstitute, Sofia, Bulgaria[1]
Agricultural Research Institute, Nicosia, Cyprus[2]
Correspondence to: Theodora Dzhambazova
Email: teodoradjambazova@gmail.com

## ABSTRACT

*Twenty rootstock accessions from Bulgaria and Cyprus grapevine (Vitis spp.) collection were characterized through microsatellite analysis. The application of 14 highly polymorphic microsatellite markers revealed high genetic diversity (83%) and allowed accurate identification and discrimination of tested cultivars.*

*The dendrogram constructed on the base of microsatellite allele data demonstrated the genetic kinship between investigated rootstocks. The parentage of Bulgarian rootstock MK4, derived by cross between Bulgarian ancient cultivar Mavrud and Rupestris du Lot was proved.*

## Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest and important perennial crops in the world widely cultivated in subtropical and temperate climate. In the middle of XIX century the appearance and distribution of the most serious pest – grape phylloxera (*Daktulosphaira vitifoliae* Fitch) in Europe has destroyed two-thirds of the self-rooted grapevines in the European vineyards. Since that time, phylloxera has spread out in the most grape-growing areas all over the world. Phylloxera is native to the North America and the American *Vitis* species are resistant to this insect. Grafting the susceptible European grapevine *V. vinifera* cultivars to the resistant American rootstocks become the best solution to combat this pest and nowadays most of cultivated *V.vinifera* varieties are grown on rootstocks. Extensive research on grafting revealed that the rootstock exerted influence on several aspects of scion behavior. Because of different affinity of rootstocks to the cultivar scions the right choice of appropriate rootstock is of grate importance for the quality of grafted grape varieties. The false identity of rootstocks during the grafting and propagation of planting material may cause serious economic wastages since such failures could be identified 2-3 years after planting. European Community developed a uniform EU certification scheme, which aims to ensure varietals identity, purity and health status (16). Ampelography and ampelometry based on morphological differences between the varieties are traditional methods for cultivar identification. However the ampelographical description depends on environmental conditions, nutritional state and health status of the plant. The ampelographic criteria are hard to apply for rootstock varieties as only the basal part of the plant remains, once they have been used for grafting (2).

In last 15 years, the development of molecular markers as alternative method for cultivar identification has entered viticulture. Among them microsatellite markers became the most preferential and reliable tool for cultivar identification due to their high level of polymorphism, abundance in the genome, co-dominant nature and Mendelian manner of inheritance. SSRs have been extensively exploited in a number of countries for identification of cultivars, and characterization of grape genetic resources (1, 3, 5, 6, 11, 12, 13, 20, 21 22, 24, 26, 28, 29), verification of synonyms (1, 6, 13, 15, 22), parentage analysis (3, 26 and 4). The data about genotyping of rootstocks are very poor. Sefc et al. (24) analyzed and discriminated 19 grape rootstock cultivars with microsatellite markers. Four rootstocks included in Iranian grapevine collection were characterized by SSR (9). The application of microsatellite markers for genetic characterization of grapevine rootstocks will respond the demand of production of authentic and healthy planting material and high quality grape and wine respectively. The current study represents essential contribution of the analyses from 20 grapevine rootstocks accessions from Bulgarian and Cyprus collection by using 14 microsatellite markers. The obtained results were used to discriminate the investigated varieties, to identify the identical genotypes and verify the synonymous cultivars in both collections. The paternity analysis was used to prove the origin of Bulgarian rootstock cultivar MK4.

## Materials and Methods

The investigated grapevine rootstock cultivars represent 17 varieties cultivated in Bulgaria and Cyprus (**Table 1**). Seven accessions are obtained in the gene bank of AgroBioInstitute, Sofia and 13 accessions – from Agricultural Research Institute, Cyprus (**Table 1**).

Leaf samples were collected and held at -70ºC until DNA extraction. The cultivar Cabernet Sauvignon was used in the microsatellite analysis as a genotype for standardization of the results. Bulgarian cultivar Mavrud was included in the parentage analysis of the Bulgarian rootstock variety MK4.

# ПЪРВИ РЕЗУЛТАТИ ОТ ПРОУЧВАНЕТО НА ПРЕПАРАТА "СЕПТАМИН" В ЛОЗАРСТВОТО

Н. Танева[1], И. Цветков[2], Й. Цветков[1], С. Николова[3]
[1]Регионален център за научно-приложно обслужване, Септември
[2]АгроБио Институт, София
[3]Лесотехнически институт, София

**Резюме**

През периода 2005-2006 г. в РЦНПО гр. Септември е проведено изпитване на препарата "Септамин" върху облагородени вкоренени лозички произведени по картонажния метод и плододаващи лоза от сортовете Каберне Совиньон и Брестовица в концентрации 0.01 % и 0.02 %.

Препаратът "Септамин" представлява универсално дезинфекциращо средство с широк диапазон на действие и възможности за приложение в много отрасли. Доказано е, че той е ниско токсичен и екологично безопасен. До този момент не са провеждани експерименти за приложението на този препарат в земеделието. Целта на настоящото изследване е да се проучат възможностите за приложение на препарата "Септамин" в лозарството, като се проследи неговото влияние върху показателите на динамиката на растеж, добива и качеството при лозата.

Установено е, че третирането на лозите с препарата "Септамин" при концентрация 0.02 % влияе положително върху растежните и репродуктивните им прояви. Не се установиха остатъчни количества от препарата върху изследваното грозде и симптоми на нападение от мана (Plasmopara viticola) и брашнеста мана (Oidium tuckeri) върху третираните лози, което говори, че има и подтискащ ефект.

Ключови думи: дезинфекциращо средство, Септамин, лозарство.

# FIRST RESULTS FROM THE STUDYING "SEPTAMIN" APPLICATION IN THE VINECULTURE

N. Taneva[1], I. Tsvetkov[2], J. Tsvetkov[1], S. Nikolova[3]
[1]Regional Center for Scientific Extension Service, Septemvri
[2]AgroBioInstitute, Sofia
[3]University of Forestry, Sofia

**Abstract**

The effect of Septamin in concentration 0.01% and 0.02% over cultivated rooted vines, produced according to the cartoonage method and over fruit-bearing vines from the varieties Cabernet Sauvignon and Brestovitsa was tested in the period 2005-2006 in the Regional center for Scientific Extension Service - Septemvri.

Biochemical "Septamin" is a universal disinfectant with a wide range of application in all branches where carrying out of disinfection is necessary. It is economically effective due to high activity at low concentration of working solution. "Septamin" is low toxic and ecologically harmless. Until now, there are not any experiments for implementation of "Septamin" in agriculture. The aim of this study is investigation on the potential possibilities for utilization of "Septamin" in viticulture, related to grapevine growth behavior, harvest and quality.

It is established that the vines treatment with Septamin (0.02% concentration) influences positive their growth and reproduction.

No findings of residual Septamin in the examined grapes, no symptoms of mildew (Plasmopara viticola) and Oidium tuckeri on the treated vines.

This is to testify to an oppressive effect.

Key words: Viticulture, "Septamin", disinfectant

Journal of Mountain Agriculture on the Balkans, vol. 11, 5, 2008, (920-929)
Research Institute of Mountain Stockbreeding and Agriculture, Troyan

# ВЪЗМОЖНОСТ ЗА ИЗПОЛЗВАНЕ НА КОМПОВЕТ В-4 ЗА ОРГАНИЧНО ТОРЕНЕ НА ПЛОДОДАВАЩИ ЛОЗЯ

Й. Цветков, Н. Танева, Ив. Цветков

[1]РЦНПО – гр. Септември
[2]АБИ – гр. София

# POSSIBILITY OF USING KOMPOVET B-4 FOR NATURAL FERTILIZATION IN FRUCTI-FEROUS VINEYARDS

Y.Tsvetkov, N.Taneva, I.Tsvetkov

[1]AES, Septemvri
[2]ABI, Sofia

## РЕЗЮМЕ

Изследването е направено през периода 2002 -2004 г. в плододаващо лозе сорт Каберне Совиньон на РЦНПО – гр. Септември.

Проучено е влиянието на органичното торене с КОМПОВЕТ В-4 (отпадъчен продукт на „Биовет" АД- гр. Пещера) и оборски тор върху растежните и репродуктивните прояви на лозите. Почвата е алувиално-ливадна бедна на хранителни вещества и хумус с лек механичен състав .

Установено е, че торенето с КОМПОВЕТ В-4 влияе положително върху растежните прояви на лозите и води до увеличаването на показателите на родовитос и добивност. Средния добив от 1 лоза и от 1 da е по-висок спрямо контролата (V0) с 18-30%. Сравнително добри резултати се отчетоха и от вариантите с оборски тор и комбинирания NPK-тор, като разликите в добивите между тях са в границите на 3 до 11%.

КОМПОВЕТ В-4 може да се използва за органично торене на плододаващи лозя без да се налага

## SUMMARY

The examination was carried out in a fruit-bearing vineyard of the variety Cabernet Sauvignon of the AES[1)] Septemvri in the period 2002-2004.

The influence of the organic fertilization with KOMPOVET B-4 (waste product of BIOVET J.S.C. Peshtera) and the natural fertilization over the growth and reproductive activities of the grape vines were studied. The soil is of the alluvial-meadow type, poor on nutritive substances and humus, of light mechanical composition.

It was found that the KOMPOVET B-4 fertilization influences positive the growth activities of the grape vines and it caused increasing of the indices of fruitfulness and yield. The average yield from 1 grape vine and from 1da is higher towards the control variant ($V_0$) by 18-30%. Comparatively good results were reported in connection with the variants with natural fertilizer and with combined NPK-fertilizer; the yield differences between the above variants are limited within 3 to 11%.

KOMPOVET B-4 can be used for the purpose of organic fertilization of fruit-bearing vineyards (without necessity of

# ВЪЗМОЖНОСТИ ЗА ПРИЛОЖЕНИЕ НА БИОЛОГИЧНО АКТИВНИЯ ОРГАНИЧЕН ТОР КОМПОВЕТ В-4 ЗА ОТГЛЕЖДАНЕ НА ЛОЗИЧКИ ПО КАРТОНАЖНИЯ МЕТОД.

*Йордан Цветков*,Надка Танева*, Иван Цветков***
**- Регионален център за научно-приложно обслужване гр. Септември*
*** АБИ гр. София*

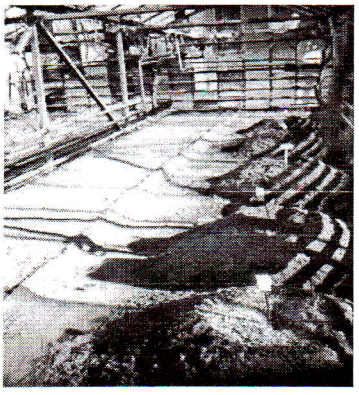
## Увод

Производството на лозов посадъчен материал по картонажния метод е най-прогресивния и икономически най-изгодният за създаване на нови лозя. За първи път той е изпитан в Института по селекция на лозата в Гайзенхаим, Германия (8,9). В Германия и Италия 45% от посадъчния материал се произвежда по този начин, а във Франция и Румъния се прилага от 1967-1969г. (3,6,10). Напоследък той се прилага и у нас, но производителите срещат големи трудности в подбора на най-подходящия за целта субстрат (1,2,3,4,5,7). Не на последно място стоят и неизяснените въпроси в сроковете на засаждане в субстрат и на открито. Какви са оптималните стойности на температурния, водния и хранителния режим по време на вегетацията, растителната защита и др. (3,4,5,7,8). Предимствата на този метод пред класическия се изразяват главно в ускореното формиране на лозите още първата година и плододаване на втората, което е икономически много изгодно. Премахва се риска от метеорологичните условия, отпада нуждата от плодородна земя за вкоренилище, отглеждат се много растения на малка площ за кратък период 50-60 дни с по-добри грижи, получава се по-висок процент първокласни лозички (6,7,8,10).

Целта на настоящето изследване е да се предложи субстрат с по-добри агрофизически свойства, оптимална запасеност и ниска себестойност, които да осигурят добър начален старт за вкореняване и развитие при подходящи условия и висок процент първокласни лозички годни за засаждане в края на м. май.

## Материал и метод

Опитите се изведени през периода 2003-2005г. в стоманено-стъклените оранжерии на РЦНПО гр. Септември. Субстратите бяха подготвени и хомогенизирани 30 дни преди употреба.



В схемата на опита са включени следните варианти: (сн.1)
$K_1$ (първа контрола) - оборски тор и наносна почва в обемно съотношение 1:1
$K_2$ (втора контрола) торф(Рекива-1С) и наносна почва в обемно съотношение 1:1
$V_1$ – Комповет В4 (Компост от "Биовет" АД Пещера) и наносна почва в обемно съотношение 1:1
$V_2$ – компост от гроздови дрожди и чепки и наносна почва в обемно съотношение 1:1
$V_3$ – торф (универсален –Германия) и наносна почва в обемно съотношение 1:1

Присадени бяха 1000бр. лозички от сорт Каберне Совиньон върху подложката $МК_4$ по утвърдена технология с двукратно парафиране с " Ребвакс" и РР 140 F, субстратно в сандъци с борови трици.

Засаждането се извърши в полиетиленови ръкави с размери 20/10/0,8см предварително напълнени със субстрат и полети с подгрята до $25^0$С вода, наредени в лехи с подово отопление покрити с полиетилен (сн.2)

# BULGARIAN ADDED VALUE TO ERA

## AGROBIOTECH PARK Ltd. – RELIABLE APPROACH TO THE APPLICATION OF BIOTECH PRODUCTS IN AGRICULTURE AND ENVIRONMENT PROTECTION

### START-UP AND CAPACITY BUILDING FOR THE PRODUCTION OF HIGH QUALITY PLANTING MATERIAL

**Elena Marinova[1], Ivan Tsvetkov[1], Violeta Kondakova[1], Evstati Dimitrov[3], Antoaneta Ginova[1], Ivanka Kamenova[1], Snezhana Milusheva[4], Petya Stoykova[1], Atanas Atanassov[1], Raycho Dimkov[2]**

[1]AgroBioInstitute, Agricultural Academy, 8, Dragan Tsankov Blvd., 1164 Sofia, Bulgaria
*Phone: +359 2 963 54 07; Fax: +359 2 963 54 08*

[2]Faculty of Biology, "St. Kl. Ohridski" Sofia University, 8, Dragan Tsankov Blvd., 1164 Sofia, Bulgaria

[3]AgroBioTech Park Ltd., 8, Dragan Tsankov Blvd., 1164 Sofia, Bulgaria

[4]Fruit Growing Institute, 12, Ostromila Str., 4004 Plovdiv, Bulgaria

**INTRODUCTION**

AgroBioTech Park Ltd. (ABTP Ltd.) has been registered on 09.10.2001 by AgroBioInstitute (ABI), National Centre of Agricultural Science (NCAS) (now Agricultural Academy) (AA), and "St. Kl. Ohridski" Sofia University (SU), namely Faculty of Biology (FB - SU). ABTP Ltd. is under the management of Prof. Atanas Atanassov *(E-mail: atanas_atanassov@abi.bg)* and Prof. Raycho Dimkov *(E-mail: ray@biofac.uni_sofia.bg)*, official representatives of both governmental institutions.

**Subject:** Development and application of biotech products in the agriculture and environment and consultations.

**Mission:** To create a modern research park in Sofia, Bulgaria with activity focused on plant biotechnology, biosafety and genomics.

ABTP Ltd. has been considered as a pilot project for Bulgaria and South-East European Region. It is expected to be a linking part to realize scientific achievements in all fields of agriculture, food industry and to solve problems of ecology and health care. The founding of ABTP Ltd. on the premises of FB - SU is of great benefit for the education of modern personnel capable to meet challenges and competition of the world science and business market.

**Main goals:**

• to develop independent, competitive and effective plant biotechnology in Bulgaria;

• to use global experience in plant biotech and the available know-how in order to promote further development of this scientific field;

• to promote technology transfer for improving the development of agriculture and solving local and regional problems in agriculture, ecology and health care;

• to serve as an incubator for establishing new start-up plant biotechnology and genomics companies;

• to be a center for improving biosafety and biodiversity and a regional center for educating public and private personnel in this area;

• to create an environment to attract investments for research development and application of local, regional and international organizations and biotech companies interested in Bulgarian

Vitis **48** (3), 115–121 (2009)

# Genetic diversity in native Bulgarian grapevine germplasm (*Vitis vinifera* L.) based on nuclear and chloroplast microsatellite polymorphisms

T. Dzhambazova[1], I. Tsvetkov[1], I. Atanassov[1], K. Rusanov[1], J. M. Martínez Zapater[2], A. Atanassov[1] and T. Hvarleva[1]

[1] AgroBioInstitute, Sofia, Bulgaria
[2] Instituto de Ciencias de la Vid y del Vino, (CSIC, Universidad de La Rioja, Gobierno de La Rioja), Logroño, La Rioja, Spain

## Summary

**Fifty one wild specimens collected in different areas in Bulgaria and nineteen native Bulgarian grapevine cultivars were genotyped with 7 nuclear and 5 chloroplast SSR markers. Based on the microsatellite allelic profile six wild samples, collected from the Danube Riverbank, were considered non *vinifera* genotypes. The genetic diversity for nuclear loci observed in the cultivated grapevines was comparable to that found in other cultivated collections. However, lower genetic diversity was observed in the set of wild samples. The dendrogram based on nuclear SSRs separated most of the cultivated grapevines from the wild samples. Four chlorotypes corresponding to previously determined chlorotypes A, B, C and D, were identified in the analyzed samples that occurred with different frequencies in groups of wild and cultivated plants. The most frequent chlorotype among wild samples was A, while it was C in the cultivated samples. The differentiation of Bulgarian grape chlorotypes in the context of differentiation of chlorotypes in Eurasian grape flora is discussed.**

K e y w o r d s : wild grapevines, native grapevines, microsatellite markers, chlorotypes, genetic diversity.

## Introduction

The Eurasian grapevine (*Vitis vinifera* L.) is one of ancient crops tightly linked to the human history. The fruits of the plant have been harvested for thousands of years and currently grapevine is the most widely cultivated fruit crop in the word. It is believed that the modern Eurasian grape cultivars (*V. vinifera* ssp. *sativa*) originated from the domestication of wild progenitors (*V. vinifera* ssp. *silvestris*) (Levadoux 1956). The place and period of primo-domestication events still remain uncertain. There are two main hypotheses that differ according to geographic locations and number of domestication events. The first hypothesis suggests the existence of a major domestication event occurred in the Neolithic time from a limited wild stock in the Near East (Olmo 1976). According to different archeological evidence, the roots of viticulture stretch back to about 5000-6000 BC on the territory of the Caucasus Mountains where native Neolithic populations discovered winemaking (McGovern 2003, McGovern *et al.* 1997). From this region the selected grapevines were spread to Egypt (ca. 3,000 BC) and later on to the Italian and Iberian peninsulas (ca 800 BC) (McGovern 2003). The second hypothesis proposes that domestication events occurred from multiple wild stocks and along the entire region of distribution of *V. vinifera* (Mullins *et al.* 1992). This hypothesis is supported by recent studies indicating the contribution of local wild grapevines in the development of current grapevine cultivars in Western and Central Europe (Arroyo-Garcia *et al.* 2002, Aradhya *et al.* 2003, 2006, Grassi *et al.* 2003).

Nuclear microsatellite markers have been extensively exploited for evaluation of genetic diversity and investigation of the genetic structure and relationships among grapevine cultivars in different collections, countries and populations (Thomas *et al.* 1993, Thomas and Scott 1993, Sefc *et al.* 1998, 2000, 2003, Lefort and Roubelakis-Angelakis 2001, Moncada *et al.* 2006, Almadanim *et al.* 2007, Stainer *et al.* 2008). However, the high level of polymorphism revealed by nuclear microsatellite markers hampers the determination of relationships between the DNA profile and the geographical distribution of grapevines. Chloroplast microsatellites are more suitable for this purpose due to the low rate of mutations and recombination in the chloroplast genome (Provan *et al.* 1999, 2001) as well as because of their maternal inheritance in grapevine (Arroyo-Garcia *et al.* 2002). The genotyping of grapevines with chloroplast SSR markers resulted in the identification of a few chlorotypes allowing to trace the frequency and geographical distribution of the studied genotypes (Arroyo-Garcia *et al.* 2002, 2006, Grassi *et al.* 2003, 2006, Imazio *et al.* 2005)

In the 19th and 20th century wild grapes (*Vitis vinifera* ssp. *sylvestris)* became close to disappearing because of a number of diseases and anthropogenic factors. Nowadays, wild grapevines exist in small populations in diverse natural ecosystems throughout Europe, the Mediterranean region of Northern Africa, the Middle East, and Western Asia (Ocete *et al.* 2002). In addition to the *sylvestris* populations, the existence of populations formed by naturalized rootstocks that are reproduced sexually giving rise to hybrids with higher genetic diversity than *Vitis vinifera* ssp *sylvestris,* have been recently reported (Arrigo and Arnold 2007).

Bulgaria is located near Transcaucasia, at the crossroads between Asia and Europe, within the area where primitive cultivars were disseminated from east to west. Bulgarian populations of wild *Vitis vinifera* ssp. *sylvestris*

Correspondence to: Dr. T. Hvarleva, AgroBioInstitute, 8 Dragan Tzankov blvd., 1164 Sofia, Bulgaria. Fax: +359-2-963-5408. E-mail: hvarleva@abi.bg

REVIEW DOI: 10.2478/V10133-009-0011-9 A&EB

# WINE METABOLITE PROFILING: POSSIBLE APPLICATION IN WINEMAKING AND GRAPEVINE BREADING IN BULGARIA

I. Atanassov, Tz. Hvarleva, K. Rusanov, I. Tsvetkov, A. Atanassov
AgroBioInstitute, Sofia, Bulgaria
E-mail: ivan_atanassov@abv.bg

## ABSTRACT

*Metabolite profiling of wine provides important information for characterization of grape and wine typicity and quality. A brief overview on wine metabolite profiling using mass spectrometry technology is presented. Possibilities for application of metabolite profiling for winemaking and grapevine breeding in Bulgaria are pointed out.*

**Keywords:** metabolite profiling, grapevine breading

Biotechnol. & Biotechnol. Eq. 2009, **23**(4), 1449-1452

## Introduction

The chemical composition of wine depends on a wide range of factors including grape variety, environmental conditions (climate, soil) and viticultural practices; yeast strains and oenological microflora; winemaking technologies and ageing processes. Wine composition was a subject in a number of metabolite studies during the last decade involving metabolite profiling and more recently extensive chemodiversity analysis. Most of the reported metabolite studies utilize mass spectrometry (MS) analysis following chromatographic separation, (2, 9, 16). The proton nuclear magnetic resonance ($^1$H NMR) profiling alone, or in parallel with the MS methods, has been also increasingly applied during the last few years, (22, 25). A large part of the reported studies on wine metabolite profiling are focused on characterization of volatiles and polyphenols, i.e. compounds substantially contributing to smell, test and therapeutic effects of wine. Here we present brief overview on wine metabolite profiling using mass spectrometry technology and discuss the possibility for application of metabolite profiling for winemaking and grapevine breeding in Bulgaria.

### Metabolite profiling of wine volatiles

The volatile composition of wine is the principal determinant of its aroma and substantially influences the entire organoleptic characteristics of wine. Over 800 volatile compounds have been identified in wines including terpenoids, phenols, alcohols, esters, aldehydes, ketones, lactones etc. In wines, they are present in wide concentration range, as only part of them is directly related to the wine odor. Many of the wine odor compounds are of the terpenic type (monoterpenoids, sesquiterpenoids, C13 norisoprenoids) and are important for wine varietal character and differentiation. At present the most widely used technology for analysis of wine volatiles is gas chromatography-mass spectrometry (GC-MS). The sample preparation of wine volatiles for GC-MS analysis employs different extraction techniques. Presently, wine volatiles analyses utilize several modifications of solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE) and liquid-phase microextraction (LPME) techniques, which have several advantages over the traditional liquid-liquid (LLE) or solid-phase (SPE) extraction procedures, (3, 20). Generally these procedures provide high enrichment factor, improved sensitivity, minimization of the amount of sample and consumption of harmful solvents.

The volatile composition of wine involves a mixture of volatile compounds of different origin, which could be most generally classified into three groups: volatiles originating from grapes – (varietal), compounds generated during alcoholic fermentation – (fermentative) and volatiles– compounds formed after metabolite transformations during ageing – (bouquet). Thus, the target or metabolite profiling studies could be the volatile composition of mature wine as well as the grape volatiles or those formed during winemaking process. A large part of the reported studies involves comparative metabolite profiling of volatiles extracted from: (a) wines from different grape varieties; (b) wines produced by different winemaking practices; (c) wines derived from the same grapevine variety grown under different environmental (soil and climate) conditions; (d) wine volatiles extracted at different time point of wine fermentation and maturation. Most of these studies resulted into identification of metabolite biomarkers – volatiles specific for the analyzed wine, group of wines or stage of wine processing. Such metabolite biomarkers could be further used for differentiation between wine varieties, quality control and monitoring of the stage of wine fermentation and ageing. A number of studies of wine volatiles include parallel studies of volatiles from the starting grapes, often extracted at different stages of grape development. The overall of such grape and wine volatiles profiling data provide possibility to follow interchanges of selected volatiles through the winemaking process and to use the obtained information for decision making. Some of the reported studies involving profiling and identification of grape and wine volatiles are outlined in **Table 1**.

### Metabolite profiling of wine polyphenols

Polyphenols play an important role in the organoleptic characteristics of wine, its aging attitudes and color stability. In addition polyphenols are the principal active ingredients

REVIEW DOI: 10.2478/V10133-009-0024-4 A&EB

# PHENOL COMPOUNDS - QUALITATIVE INDEX IN SMALL FRUITS

V. Kondakova, I. Tsvetkov, R. Batchvarova, I. Badjakov. T. Dzhambazova, S. Slavov
AgroBioInstitute, Sofia, Bulgaria
Correspondence to: Violeta Kondakova
E-mail: violeta.kondakova@gmail.com

## ABTRACT

*Over the past 10 years, researchers have become increasingly interested in polyphenols. The main reason for this interest is the recognition of the antioxidant properties of polyphenols, their great abundance in our diet, and their probable role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases (51). Furthermore, polyphenols, which constitute the active substances found in many plants, modulate the activity of a wide range of enzymes and cell receptors (1). In this way, in addition to having antioxidant properties, polyphenols have several other specific biological actions that are poorly understood.*

*Not all polyphenols are absorbed with equal efficiency. Knowledge of the bioavailability and metabolism of the various polyphenols is necessary in order to evaluate their biological activity within target tissues. The types and distribution of polyphenols in foods and the bioavailability of polyphenols are the topics of our knowledge. Several thousand molecules having a polyphenol structure have been identified in higher plants, and several hundred are found in edible plants. These molecules are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. These compounds may be classified into different groups as a function of the number of phenols that they contain.*

*This review aims to describe evidences for the effects of polyphenol consumption on health, and to specify which out of the hundreds of existing polyphenols are likely to provide the greatest protection in the context of preventive nutrition. If these objectives are to be attained, it is first essential to determine the nature and distribution of these compounds in fruits. Such knowledge will allow evaluation of polyphenol intake and enable epidemiologic analysis that will in turn provide an understanding of the relation between the intake of these substances and the risk of development of several diseases.*

**Keywords:** small fruits, phenol compounds, qualitative index

Biotechnol. & Biotechnol. Eq. 2009, **23**(4), 1444-1448

## Introduction

For many plant products, the polyphenol composition is much less known, knowledge is often limited to one or a few varieties, and data sometimes do not concern the edible parts. Some foods, particularly some exotic types of fruit and some cereals, have not been analyzed so far.

Berry fruits, such as bilberry (*Vaccinium myrtillus*), blackberry (*Rubus fruticosus*), blackcurrant (*Ribes nigrum*), blueberry (*Vaccinium corymbosum*), chokeberry (*Aronia melanocarpa*), cranberry (*Vaccinium macrocarpon*), grape (*Vitis vinifera*), raspberry (*Rubus idaeus*) and strawberry (*Fragaria x ananassa*) are a particularly rich source of antioxidants (13, 25, 27, 28, 30, 40, 50). Those compounds are mainly represented by vitamin C and polyphenols such as anthocyanins, phenolic acids, flavanols, flavonols and tannins. They are known as natural antioxidants and due to their high concentration and qualitative diversity, berry fruits are increasingly often referred as natural functional foods.

Phenolic compounds are the secondary metabolites of plant provided their normal growth and development. Many researchers reported that the fruits of berry plants which grow in a cold northern climate with a short vegetation season, without fertilizers and pesticides are marked by a higher content of polyphenols than the same varieties which grow in a milder climate. In addition to their protective functions, phenolic compounds /in particular- anthocyanins/ are responsible for the pigmentation of flowers, fruit and leaves (15, 16, 17).

Anthocyanins in berry fruits comprise a large group of water-soluble pigments. In the fruit, they are found mainly in the external layers of the hypodermis (the skin). In the cells, they are present in vacuoles in the form of various sized granules; meanwhile cell walls and flesh tissue practically not contain anthocyanins.

## Factors influencing on polyphenol content

Numerous factors other than variety may affect on the polyphenol content in plants. The content of phenolic compounds in berry fruits is determined by many factors too, such as the species, variety, cultivation, region, weather conditions, ripeness, harvesting time, storage time and conditions (3, 11, 19, 21, 31, 32, 37, 45, 47).

*Environmental factors* have a major effect on polyphenol content. These factors may be climatic (soil type, sun exposure, rainfall) or agronomic (culture in greenhouses or fields, biological culture, hydroponic culture, fruit yield per tree, etc). Exposure to light has a considerable effect on most flavonoids. The degree of ripeness considerably affects the concentrations and proportions of the various polyphenols (34). In general, phenolic acid concentrations decrease during ripening, whereas anthocyanin concentrations increase. Many

*Journal of Mountain Agriculture on the Balkans, vol. 13, 5, 2010, (1445-1463)*
*Research Institute of Mountain Stockbreeding and Agriculture, Troyan*

# МЕТАБОЛИТНО ПРОФИЛИРАНЕ НА МАЛИНА И КЪПИНА (*RUBUS* SP.)

И. Динчева[1], И. Баджаков[1], И. Цветков[1], В. Кондакова[1], Н. Стоянова[2]

*[1]АгроБиоИнститит – София, 1164*
*[2]Институт по земеделие – Кюстендил, 2500*
*E-mail: ivadincheva@yahoo.com, ibadjakov@gmail.com*

# METABOLITE PROFILING OF RASPBERRY AND BLACKBERRY (*RUBUS* SP.)

I. Dincheva[1], I. Badjakov[1], I. Tsvetkov[1], V. Kondakova[1], N. Stoyanova[2]

*[1]AgroBioInstitute – Sofia, 1164*
*[2]Institute of Agriculture – Kyustendil, 2500*

## РЕЗЮМЕ

През последните години се наблюдава засилен интерес към малина и къпина, което се дължи на изключително ценните им хранителни и лечебни свойства.

Те представляват богати източници на компоненти с висока антиоксидантна активност като фенолни киселини, флавоноиди, елагитанини, антоцианини и др.

Изследванията до момента показват, че антиоксидантите помагат на организма да намали увреждането на тъканите, ускоряват оздравяването и се противопоставят на инфекциите и стареенето.

Целта на проучването бе определяне на пълния спектър от биологично активни вещества в плодове и листа от малина и къпина чрез LC-ESI-DAD и GC-MS методи.

**Ключови думи:** малина, къпина, антиоксиданти, LC-ESI-DAD, GC-MS

## SUMMARY

In recent years, nutritious features of foods produced and consumed as well as their roles in disease prevention have been taken into consideration. In numerous studies has been reported that food habits are connected with many illnesses including cancer. Some components with anti-oxidative effects which are present in different quantities in berry fruits reduce the negative effects brought out by free radicals that serve as agents in those ailments. Additionally, doubts about artificial anti-oxidants which are being used in industry have increased the tendency in consumption of natural anti-oxidant sources such as berry fruits.

In our study, we focused on functional components of berry fruits and chromatographic methods of their detection particularly in raspberry and blackberry.

**Key words:** Berry fruits, antioxidants, LC-ESI-DAD, GC-MS

## УВОД

## INTRODUCTION

**ECOLOGY AND FUTURE, VOL. IX, No 3–4**
**Bulgarian Journal of Ecological Science**
**Sofia. 2010**

**Y. TSVETKOV*, I. TSVETKOV**, N. TANEVA*, N. KOVACHEV***
** Agricultural Experimental Station, Septemvri, Bulgaria*
*** AgroBioInstitute, Sofia, Bulgaria*

# Possibilities for Using of KOMPOVET B4 (Waste Product of Biovet Peshtera J.S.C.) for Natural Fertilizing of Fructiferous Hop Variety Perla

## Abstract

It was found that the compost can be used for the natural organic fertilization of the hop under the conditions of biological production with norms at the rate of 10 to 15 t/da in connection with the agrochemical soil analyses and without chemical fertilizers.

**Key words:** *Humulus lupulus*, hop fertilizing, natural fertilizer KOMPOVET B4

## Introduction

The hop fertilizing is a very important factor which determines the growth intensity of plants and the yield and the quality of the cones (Bavec et al., 1984; Biacardi, Wagner, 1989; Kisgeci et al., 1984). The intensive fertilizing systems with using of big quantities chemical fertilizers, preparations for growth stimulation and growth protection exercise a negative influence over the biological balance and over the ecological formed natural soil fruitfulness (Nikolov, Trendafilov, 1989; Matatko, Kopalova, 1989). The natural fertilizers lack puts the question about finding of appropriate substitutes for the purpose of soil fruitfulness maintenance in the hop plantations under condition of biological agriculture (Kisgeci et al., 1984; Matatko, Kopalova, 1989, Tsvetkov et al., 2004). According to some authors (Godovani, 1980; Milosta, Regilevich, 2006) from 10 to 15 kg nitrogen, 15-18 kg phosphorus and 10-15 kg potassium are dragged out every year together with the cones, steams and leaves.

The biological active natural fertilizer KOMPOVET B4 is produced by composting of waste tylosin spawn, plant sediment, marble waste slag, softwood bark and rice husks (our technology) with yearly production at the rate of 500 000 t with nutritive value which is no way inferior to the same of the natural fertilizer.

Our examination goal is to be determined the several KOMPOVET B4 fertilization levels influence over the agrophysical and chemical soil properties, leaves surface, yield and cones quality under the conditions of biological production of fructiferous hop variety.

## Material and Methods

The experiments were carried out in the period 2005-2006 in a hop vine plantation of the farm cooperative "Hmel" Town of Rakitovo. This was a model testing under field conditions on an area of 9 da with increasing fertilization norms. The variety Perla was used because of its widely representation in the region. The growth system is a system with high stems, distance between the rows at the rate of 3 m and distance between the plants – 0.8 m. 100 test plans are marked in 4 repeated samples (1 sample = 25 units). The soil is a very poor leached alluvial meadow soil with light mechanical structure humus poor (0.53%) and weakly acid reaction (pH = 5-5.4).

The agro-chemical soil analyses before the test setting showed very poor reserve of nitrogen 56 ppm and phosphorus ($P_2O_5$) 7.6 ppm, middle potassium reserve – 80 ppm, CaO – 150 ppm and MgO – 0.20 ppm, salts – 0.05 mmos. The soil tests are analyzed in the laboratory of Research Institute of Soil Science "N. Poushkarov", Sofia on the grounds of their approved and generally accepted methods.

The following fertilization samples were examined:

$V_0$ – control sample, not fertilized;

$V_1$ – control sample, not fertilized with B4, on the background $N_{20}$ $P_{20}$ kg/da;

$V_2$ – fertilized with KOMPOVET B4 – 10 t/da;

$V_3$ – fertilized with KOMPOVET B4 – 15 t/da.

# Soil management in the nursery of micropropagated rootstocks inoculated and non with biocontrol organisms

*By I. Tsvetkov, E. Markov, T. Dzhambazova, K. Petrov, N. Kuzmanov, K. Bashev, S. Stavrou*

*Edited by G.Tataranni and G. Montanaro*

Two different nursery managements were compared during the whole growing season. Specifically the fertilization and the soilborne pathogens control techniques routinely adopted by the SME were examined against those innovated by SITINPLANT. As a main result, a sustainable fertigation plan is proposed to reduce undue mineral elements application by matching plant's nutrients demand.

## PLANT MATERIAL AND SOME SITE CHARACTERISTICS

A total of 8,153 micropropagated rootstocks (GF 677, Myrobolan 29C, GiSeLa6® and OHxF selection 19-89) have been planted (planting distances 1.2×0.2m) in April 2009 in the nursery situated nearly Krushare village- Sliven region (Bulgaria).

**Soil and irrigation water characteristics.** The soil was calcareous (pH around 8,1) poor in organic matter, phosphorus and potassium. The water used for irrigation is good quality, with low salinity content (0.289 g/dm$^3$), rich of calcium (54 mg/l) and magnesium (17.3 mg/l).

**SITINPLANT management.**

● **Biocontrol agent inoculations**. A total of 4,896 rootstocks were treated twice with *Trichoderma harzianum* (T22 strain) and *Glomus intraradices* (separate and in combinations) at the beginning of the acclimatization stage and before transplanting in the nursery (April 2009). In the same field were planted also not inoculated plants of the same rootstocks.

● **Fertilization.** For the SITINPLANT management, were included only bio-agents and organic substances avoiding chemical pesticides and of synthetic fertilizers, which are leading to the reduction of microflora complexity in the soil and decreasing the overall plant quality. For the SITINPLANT management were distributed per each plant, by fertigation, 0.81 g N, 0.35 g K (34 kg/ha of N and 15 kg/ha of K) (Figure 1) . No phosphorus was distributed to enhance the efficiency of the mycorrhizae. These amounts were estimated, from previous results, on the basis of total dry weight and the mineral concentration, of the various mineral elements, in the different plant organs.

● **Soil-borne pathogens control.** Treatments with the antagonist microorganisms *Bacillus subtilis* and *Streptomyces lydicus* (Serenade ®- AGRA QUEST: Baccilus subtilis QST 713– 7.3 x 109 CPU/g) (Actinovate ®- MTS- Agro: Streptomyces lydicus WYEC 108 – 1.6 x 108 CPU/g) were applied in the soil twice (May and July) for all rootstocks in the SITINPLANT management.

● **Insects were controlled** by Telstar® 10 EK- FMC Ltd. was included three times (ones in May and twice in June), because of strong insect (arachnid) infection in all treatments

● **Water management.** Irrigation was carried out by drip irrigation system on the basis of evapotranspiration demand and rainfall. Each row was controlled separately and drippers were every 20cm a part (1.5 l/h discharge rate).

**SME management**

● **Fertilization** The SME empirically distributed about 2.8 g of N and K and 2.5 g of P per each plant (117 kg/ha of N and K and 104 kg/ha of P) (Figure 1).

# 13

# Grape Secondary Metabolites – Benefits for Human Health

Teodora Dzhambazova, Violeta Kondakova,
Ivan Tsvetkov and Rossitza Batchvarova
*AgroBioInstitute,*
*Bulgaria*

## 1. Introduction

Grapevine is one of the ancient crops linked with human history during the evolutionary development of man. The fruits and wine have taken part in daily life and ancient ceremonies of our predecessors and currently grapevine is one of the most widely cultivated fruit crop in the world. It is consumed fresh, dry or as beverage but the most popular consumption form in the world is wine. Recent decades researchers found another reason to praise grape products: its beneficial effect on health. In the late Eighties grape and wine have been in the spotlight because of the finding of so called 'French Paradox' that linked moderate red wine consumption to a lower incidence of cardiovascular disease. As an addition, epidemiological studies demonstrated the beneficial effect of moderate wine intake on the neurodegenerative process (Marambaud et al., 2005). These findings gave boost to numerous studies of grape and wine effects on health, revealing evidences about the protective effect of grape compounds against cancer and age-related disorders, such as certain neurological diseases and metabolic disorders. Moreover, scientists proved that some compounds of grape are implicated in important biological functions in the body such as antioxidant defense system, immunological regulation and anti-inflammatory processes. The main question is what makes grape so useful and healthy?

The answer is found in the fruit and wine constituents, i.e. grape secondary metabolites. The secondary metabolites are exclusively produced by plants and represent more than 30 000 different substances that give individual properties of plant species and define them as healthy, healing or even poisonous. Grape is rich in secondary metabolites which makes this fruit crop so popular among the scientific society. Different research groups have undertaken the study of grape metabolomics and revealed the rich profile of grape and wine, including polyphenols such as resveratrol, caffeic acid, catechin, quercetin etc., all of which present highly bioactive substances. In recent years the analyses of grape composition and its effect on human health led to the conclusion that resveratrol is one of the key grape substances responsible for the preventive and therapeutic abilities of wine. Recent study of Iriti et al. (2006) discovered another key substance in grape - melatonin, which is considered for one of the most powerful antioxidants involved in various physiological functions in human body (Srinivasan et al., 2006). It is noteworthy that the health promoting effects of grape and wine are due to the secondary metabolites presented in them.

# ВЪЗМОЖНОСТИ ЗА АЕРОБНО КОМПОСТИРАНЕ НА РАСТИТЕЛНИ ОТПАДЪЦИ ОТ РАСТЕНИЕВЪДСТВОТО И ДЪРВОДОБИВА

**[1]Любомир Н. Кузев, [2]Иван Цветков, [2]Росица Бъчварова, [3]Евлоги Марков, [3]Милена Стоичкова**

*[1]„Агроелит", гр. Пазарджик, [2]АБИ гр. София, [3]ИП „Н. Пушкаров" гр. София*

# OPPORTUNITIES FOR AEROBIC COMPOSTING OF PLANT RESIDUES FROM PLANT-GROWING AND WOOD INDUSTRY

**[1]Lubomir N. Kuzev, [2]Ivan Tsvetkov, [2]Rosica Bachvarova, [3]Evlogi Markov, [3]Milena Stoichkova**

*[1]"Agroelit", Pazardzhik, [2]ABI, Sofia, [3]Institute of Soil Science, Sofia*

**Abstract**

Composting experiments with plant wastes (fractional vine, rice husks and waste wood bark) with added inoculants' Cephalosporium Sp. were carried out during the period 2009/2010 in "Agroelit, Pazardzhik. Agrochemical properties, chemical composition, temperature regime, changes in the content of ammonia and nitrate nitrogen dynamics of the ratio of $CO_2$ / C of the biomass and amount of nutrients in mature composts were examined.

It was found that the physic-chemical composition of the investigated plant residues is suitable for aerobic composting. Adding Cephalosporium Sp. and marble flour increases the temperature of the compost, accelerates the degradation of cellulose and reduces the ammonia and nitrate nitrogen. Composts with vine supported higher temperature by 8-10°C than those with bark of conifers and rice husks. The total quantity of ammonia and nitrate nitrogen for the period in rice husks and peels is significantly higher than in composted vine.

The results show that all three types of compost are rich in nutrients. Composts vine has a high content of nitrogen and phosphorus than those of rice husks and bark. Composts from rice husks and bark have nearly twice higher content of potassium than the compost from rods. The ratio of N: P: K is favorable for growth and development of plants and shows that the composts can be used for intensive cultivation.

**Key words**: compost, inoculation, waste, agrochemical and physical indicators, biomass, ammonia and nitrate nitrogen.

## Увод

В резултат на преструктурирането на българското земеделие, през последните години се повишава стремежът за максимално използване на всички биологични ресурси от земеделието за торове (Бауман, 1978; Николов и Трендафилов, 1989).

В лозарството наред с основната продукция – гроздето, се получава и голям обем органични отпадъци – листа, лозови пръчки, гроздови чепки, люспи и семки. В България по статистически данни (Гецова и др., 1987; Николов и Трендафилов, 1989) всяка година се изгарят или изхвърлят след резитбата около 250 000 т. лозови пръчки, от оризопроизводството около 150 000 т. оризови люспи, от целулозните заводи – 350 000 т. кори от иглолистни трупи, за което се извършват трудови и материални разходи и се замърсява околната среда. Малко внимание е отделено досега на възможностите за рационално използване на тези растителни отпадъци за компости и торни средства в земеделието.

Извършени са значителни изследвания относно използването на дървесни стърготини, ленен пъздер, гроздови чепки и джибри, кори от иглолистни трупи и др (Бауман,1978; Николов и Трендафилов, 1989; Цветков,1991; Azhar et al., 1989; Bremner and Keeny, 1965).

В някои случаи, когато тези органични материали се внасят директно в почвата се установяват известни недостатъци като фитотоксичност, дългосрочна имобилизация на азота и високо съдържание на соли. До известна степен аеробното компостиране намалява много от тези недостатъци. Повечето цитирани публикации се занимават с началните и крайните физико-химически свойства на компостите, но известно внимание е отделено и на микробиологичните процеси, които оказват пряко въздействие върху качествата на компостите, използвани като торни средства.

*Journal of Mountain Agriculture on the Balkans, vol. 14, 6, 2011, (1331-1343)*
*Research Institute of Mountain Stockbreeding and Agriculture, Troyan*

# GC-MS DETERMINATION OF SECONDARY METABOLITES IN RED WINE FROM BULGARIAN WILD GRAPE (VITIS VINIFERA SSP. SYLVESTRIS)

I. Dincheva, I. Badjakov, I. Tsvetkov, T. Dzhambazova, V.Kondakova, R. Batchvarova

*AgroBioInstitute – Sofia, 1164*

## Abstract

A profound comparative Gas chromatography-Mass spectrometry (GC-MS) study of secondary metabolites in red wines from wild grape that grows nearby the Thracian sanctuary “Tatul” and tree popular cultivars - “Mavrud”, “Cabernet Sauvignon” and “Merlot” was performed. The analysis of four classes of secondary metabolites - phenolic acids, alcohols, stilbenes and flavanols revealed the rich profile of the wine from wild grape consisting in 22 compounds, most of which have tremendous beneficial effect on human health.

**Key words:** wild grape, secondary metabolites, GC-MS

## Introduction

Grapevine (*Vitis vinifera L.*) is one of the ancient crops tightly linked to human history since settlement of man during his evolutionary development. The fruits and beverages from grape were presented in daily life and ancient ceremonies of our ancestors and currently grapevine is one of the most cultivated crops worldwide. The history of cultivation of grapevine is linked to the discovery of wine and according to recent archeological data it dates back to the Neolitic period circa 8000 BP(This et al., 2006). In the early times, wine has been produced

# Growth and Mineral Uptake in Micropropagated Myrobolan 29C Plants Inoculated with Mycorrhizas and Bio-Control Microorganisms

I. Tsvetkov[a], T. Dzhambazova and R. Batchvarova
AgroBioInstitute
Sofia
Bulgaria

E. Markov
Institute of Soil Science
"Nikola Poushkarov"
Sofia
Bulgaria

S. Efstratios
Bulgarian Plant LTD
8800 Sliven
Bulgaria

G. Tataranni, G. Montanaro and C. Xiloyannis
Università degli Studi della Basilicata
Potenza
Italy

**Keywords:** mycorrhiza, antagonist, soil fertility, fertilisation, irrigation, plant defence

**Abstract**

**We compared the effect of biotisation techniques on growth and mineral uptake in nursery-grown micropropagated Myrobolan 29C plants, against conventional techniques. Biotisation techniques consisted of inoculation of mycorrhizas (*Glomus intraradices*) and bioagents (*Trichoderma harzianum*), moreover, the soil borne pathogens were controlled through antagonist microorganisms. Nutrients (N, P, K) were supplied based on plant demand for biotised plants whilst they were empirically supplied for the conventionally managed block. Biotised plants showed a double growth (total dry matter) compared to control plants (34 g/plant against 18 g/plant) despite a significant reduction of the amounts of nutrients supplied. A fertigation plan is proposed to reduce undue mineral elements application by matching the plant demand for nutrients.**

## INTRODUCTION

European as well as global agricultural environmental policies tend to promote limitation of chemical inputs during production processes (Dichio et al., 2012). Nursery plant producing protocols require application of chemicals for plant growth and defense increasing the environmental impact. It is unfortunate that residuals of such applications often have negative impact on soil resource, for example reducing soil micro-flora complexity (Schreiner and Bethlenfalvay, 1996).

Mycorrhizae are widely know to improve plant growth (Allen, 2007), in addition they could be associated with bio-control agents applied for control of certain pathogens (Harman et al., 2004). However, information on the impact of mycorrhizas as combined with bio-control agents inoculation on plant management at nursery scale is limited. Therefore, this study was undertaken to evaluate the use of mycorrhizas as combined with bio-control microorganisms on growth and mineral uptake of Myrobolan 29 C plants.

## MATERIALS AND METHODS

Trials were carried out at a commercial nursery located at Krushare - Sliven Region (Bulgaria). A total of 2,000 micropropagated Myrobolan 29C rootstocks were planted (1.2×0.2 m planting distances) in April 2009 on a calcareous soil (pH around 8.1). Soil was poor in organic matter (1.2 and 0.5% depth 0-30 cm and 30-60 cm, respectively). Irrigation water had low salinity level (0.289 g/dm$^3$ salt content), whilst it was rich in calcium (54 mg/L) and magnesium (17.3 mg/L).

To validate the effectiveness of mychorrhizas inoculation (biotised management), a 0.3 ha block (*B*-block) was differently managed concerning fertilisation and control of

[a] ivantsvetkov@hotmail.com

# GENETIC DIVERSITY AND RELATIONSHIPS OF INDIGENOUS AND NEWLY BRED BULGARIAN GRAPE CULTIVARS ASSESSED BY NUCLEAR AND CHLOROPLAST MARKERS

Teodora DZHAMBAZOVA[1], Ivan TSVETKOV[1], Ilian SIMEONOV[2], Miroslav IVANOV[2], Zdravko NAKOV[2], Violeta DIMITROVA[2], Ivan ATANASSOV[1] and Tzvetanka HVARLEVA[1]*

1 : AgroBioInstitute, 8 'Dragan Tsankov' Blvd., Sofia 1164, Bulgaria
2 : Institute of Viticulture and Enology, 1 'Kala tepe' St., Pleven 5800, Bulgaria

## Abstract

**Aim**: Assessment of the genetic diversity and relationships in a group of 31 Bulgarian grape accessions through microsatellite markers.

**Methods and results**: Thirty-one accessions, including 20 old rare local and 11 newly bred varieties were characterized through 13 nuclear and 5 chloroplast microsatellite loci. The genetic diversity (0.81±0.01) obtained for the investigated group of cultivars was comparable to those reported for other grapevine germplasms. The low PI value ($1.0x10^{-16}$) allowed proper genetic identification and determination of synonyms. Microsatellite analysis of the 31 accessions resulted in 26 unique genotypes and 2 groups of synonyms. Four cases of supposed synonymy with local Bulgarian and foreign cultivars were rejected. Three chlorotypes, B, C and D, were defined among the studied cultivars, with a prevalence of chlorotype C (62 %).

**Conclusion**: The high genetic diversity found in the set of old rare grapevines demonstrated their importance as a rich source of alleles for breeding. The pattern of chlorotype distribution observed among local varieties confirmed the previous results and supports the hypothesis of an Eastern origin of local Bulgarian cultivars.

**Significance and impact of the study**: The obtained results provide an important support for the preservation of grape biodiversity in Bulgaria as well as for the clarification of genetic relationships between local and foreign cultivars.

**Key words**: grapevine, microsatellites, genetic diversity, chlorotypes

## Résumé

**Objectif**: La diversité et les relations génétiques ont été étudiées dans un groupe de 31 cépages bulgares par des marqueurs microsatellites.

**Méthodes et résultats**: Trente et un échantillons, dont 20 anciens rares locaux et 11 nouvelles variétés produites ont été caractérisés par 13 microsatellites nucléaires et 5 microsatellites chloroplastiques. La diversité génétique (0,81± 0,01) obtenue pour le groupe de cépages étudiés était comparable à celles rapportées dans la littérature chez la vigne. La faible valeur PI (1,0x10-16) a permis l'identification génétique appropriée et la détermination des synonymes. L'analyse des microsatellites de 31 échantillons a révélé 26 génotypes uniques et deux groupes de synonymes. Quatre cas de synonymie supposée avec des cépages locaux bulgares et étrangers ont été rejetés. Trois chlorotypes, B, C et D, ont été définis parmi les variétés étudiées avec une prévalence du chlorotype C (62 %).

**Conclusion**: La grande diversité génétique observée parmi des vignes anciennes et rares a prouvé leur importance comme source d'allèles pour l'amélioration de la sélection. Le modèle de distribution des chlorotypes observés chez les variétés locales a confirmé des résultats déjà observés et soutient l'hypothèse d'une origine orientale des cépages locaux bulgares.

**Signification et impact de l'étude**: Les résultats obtenus ont un impact important pour la préservation de la biodiversité des vignes en Bulgarie ainsi que pour la clarification des relations génétiques entre les cépages locaux et étrangers.

**Mots clés**: vigne, microsatellites, diversité génétique, chlorotypes

*manuscript received 18th April 2011 - revised manuscript received 1st October 2011*

*Corresponding author : hvarleva@abi.bg



*J. Int. Sci. Vigne Vin*, 2012, **46**, n°2, 113-121
©Vigne et Vin Publications Internationales (Bordeaux, France)

*Bulgarian Journal of Agricultural Science, 18 (No 4) 2012, 545-556*
*Agricultural Academy*

# *ROSA DAMASCENA* MILL. - AN OVERVIEW FOR EVALUATION OF PROPAGATION METHODS

A. GINOVA*, I. TSVETKOV and V. KONDAKOVA
*AgroBioInstitute, 8, Dragan Tsankov blvd., BG - 1164 Sofia, Bulgaria*

## Abstract

GINOVA, A., I. TSVETKOV and V. KONDAKOVA, 2012. *Rosa damascena* Mill. - an overview for evaluation of propagation methods. *Bulg. J. Agric. Sci.,* 18: 545-556

*Rosa damascena* Mill. f.*Trigintipetala*, named Kazanlak oil – bearing rose is an emblematic plant species for Bulgaria with important economical sense. Its importance is determined by the extremely high quality of rose oil, which is obtained after distillation of rose flowers. The methods of propagation play a general role in maintenance and improving the quality of oil rose. Traditionally, the oil-bearing rose is vegetative propagated. In this review are considered the advantages and disadvantages of classical methods of propagation (by grafting, layering, cutting) and *in vitro* methods for propagation. Special attention is directed to the problems that accompany the use of these methods. The layering and grafting are not preferred for use, because their more time consuming and low multiplication rate. The cutting is the preferable way for oil rose propagation in Bulgaria. This method has high generative rate, but obtained plants are not always healthy. *In vitro* techniques are used for rapid multiplication with high rate, production of healthy and disease-free plants, but they are costly.

*Key words*: *Rosa damscena* Mill., classical methods of propagation, *in vitro* micro propagation, bioreactors
*Abbreviations*: MS - Murashige & Skoog Medium, QL- Quirin and Lepoivre Medium, BAP - 6-Benzylaminopurine, BA - Benzyl Adenine, NAA - 1-Naphthaleneacetic acid, IAA - Indole-3-acetic acid, $GA_3$ - Gibberellic acid, IBA - Indole-3-butyric acid, PGRs - plant growth regulators, Kin - Kinetin, TDZ - Thidiazuron, WPM – Woody Plant Medium, PVP –Polyvinylpyrrolidone, PVPP -Polyvinylpolypyrrolidone

## Introduction

*Rosa damascena* Mill. is a beautiful aromatic flower with immense horticultural importance, signification from economical and research point of view. The *Rosa* genus belongs to the family *Rosaceae* and has over 130 species (Cairns, 2001).

Roses are grouped into classifications based on the botanical characteristics: hybrid teas, grand floras, polyanthas, floribundas, miniatures, climbing, shrub, old roses, but only a few species are scented (Gudin, 2000). These include *Rosa damascena* Mill., *R. gallica* Linn., *R. centifolia* Linn., *R. bourboniana* Desportes., *R. chinensis* Jacq., *R. moschata* Herrm. and *R. alba* Linn.

The initially rose species used for oil production are: *Rosa damascena* Mill, *Rosa gallica* Linn., *Rosa centifolia* Linn. and *Rosa moschata* Herm. (Tucker and Maciarello, 1988). Hurst (1941) classified the today Damask roses into two types according to their flowering habit: summer Damask that blooms only in early summer, and autumn Damask that blooms during the autumn. Traditionally, highly scented summer Damask roses are cultivated in commercial rose gardens. There are several main prospects for production of Damask rose (*R.. damascena*) as rose water, attar of rose and other essential oils in the perfume industry (Lavid et al., 2002). It has application in pharmacology such as anti-HIV, antibacterial, antioxidant, hypnotic, antidi-

*E- mail: a.ginova@gmail.com*

**ЕКОЛОГИЯ И БЪДЕЩЕ, ГОД. XI, No. 3**
**Научно списание за селскостопанска и горска наука**
**Journal of Agricultural Science and Forest Science, vol. XI, No. 3**
**София. 2012. Sofia**

**ЛЮБОМИР КУЗЕВ*, ИВАН ЦВЕТКОВ**, РОСИЦА БЪЧВАРОВА****
* АГРОЕЛИТ ООД – Пазарджик
** Агробиоинститут, София
* E-mail: agroelitltd@abv.bg

# Използване на компости от растителни отпадъци при отглеждане на зеленчуков разсад

# Using Compost from Vegetable Waste in Growing Vegetable Seedlings

L. Kuzev*, I. Tsvetkov**, R. Bachvarova**
* AGROELIT LTD. – Pazardzhik, Bulgaria
** Agrobioinstitute, Sofia, Bulgaria

**Abstract**

In the period 2009–2010 under controlled conditions in the greenhouses of "Agroelit" Ltd. in the town of Pazardzhik were investigated the possibilities for using composts of plant waste for cultivation vegetable seedlings with the purpose of replacing the peat as a main component in the mixture for seedlings and to protect the environment from pollution.

The experiments were taken out of tomatoes sort "Jaguar" and cucumbers sort "Kalunga" in the following varieties:
$V_0$ – control – peat (enriched) and pearlite in exchanged correlation 3: 1;
$V_1$ – compost of rice husk and pearlite in exchanged correlation 3: 1;
$V_2$ – compost of vine-stocks and pearlite in exchanged correlation 3: 1;
$V_3$ – compost of grape barm and pearlite in exchanged correlation 3: 1;
$V_4$ – compost of cortex and pearlite in exchanged correlation 3: 1.

The tomato plants were cultivated for 6 weeks and the cucumber plants – for 4 weeks from the sowing day without additional feed. Agrochemical analysis of the substrata was held before the pricking in and in the end of the vegetation and biometry analysis for the growth activities of the plants.

It was settled that the investigated components could successfully be used as peat substitute for vegetable seedlings growing. They improve the nutrient regime and the received seedling has higher quality features (mass, height, thickness and leaves number) and a better activity ratio of the nutrient elements.

**Key words:** vegetable waste, compost, mixture for seedling, agrochemical analysis, biometry of seedling

Устойчивото управление на непрестанно растящите количества растителни отпадъци е едно от най-сериозните предизвикателства пред съвременното общество, а органичните торове са едно от средствата за поддържане и повишаване на почвеното плодородие. Те се използват широко и при приготвянето на торопочвени смески за отглеждане на разсад. През последните години освен класическите източници на органично вещество (торове от животински произход, торф, компости и др.) се употребяват и отпадъци от растениевъдството, целулозната, винодобивната, ленената, дървообработващата, каменообработващата и др. промишлености (Verdonek, 1988;

**ЕКОЛОГИЯ И БЪДЕЩЕ, ГОД. XI, No. 3**
**Научно списание за селскостопанска и горска наука**
**Journal of Agricultural Science and Forest Science, vol. XI, No. 3**
**София. 2012. Sofia**

**ЛЮБОМИР КУЗЕВ*, ИВАН ЦВЕТКОВ****
* АГРОЕЛИТ ООД – Пазарджик
** Агробиоинститут, София
* E-mail: agroelitltd@abv.bg

# Влияние на микоризната гъба *Glomus* spp. върху растежните и репродуктивни прояви на хидропонно отглеждани домати и краставици в оранжерии

# The Influence of the Mycorrhizal Fungi *Glomus* spp. over the Growth and Reproductive Activities of the Hydroponics Cultivation of Tomatoes and Cucumbers in Greenhouses

L. Kuzev*, I. Tsvetkov
* AGROELIT LTD. – Pazardzhik, Bulgaria
** Agrobioinstitute, Sofia, Bulgaria

**Abstract**

The aim of this research is to be valued the useful effects from the usage of *Glomus* spp. over the growth and reproductive activities of tomatoes and cucumbers, hydroponics cultivated in organic substratum in greenhouses.

During the period 10 August – 14 September and 10 August – 30 October in 2010 in the plastic greenhouse (Model 1987) was hold an examination with the mycorrhizal fungi (*Glomus* spp.) brought in the nests in doses of 5 g per plant for planting in organic substratum Compovet B-4 of tomatoes sort "Jaguar" and cucumbers sort "Kalunga". For control was used the same substratum treated with fungicides according to the perceived technology. The growth and reproductive activities of the plants were studied. It was found that the plants treated with the fungi grow faster and are considerably taller, with bigger stems and number of leaves. The phenophases beginning of flowering and first red fruit occurred respectively 4-5 days earlier compared to the controlled not treated plants. The general yield from the treated plants was respectively 300 and 500 kg/da higher for the tomatoes and cucumbers. There were not found any differences in the quality of the fruit and come down from root diseases plants.

**Key words:** hydroponics, organic substratum, mycorrhizal fungi

Биологичният метод за растителна защита намира все по-широко приложение при интегрираното и биологично производство (Карлие, Влахов, Атанасов, 2006; Каров, Андреев, 2000). Развитието на биологичния контрол като алтернатива вместо употребата на фунгициди, би могло значително да помогне оранжерийната индустрия да стане по-екологична и в същото време да ограничи загубите на реколтите, дължащи се на фитопатогенните коренови гъби от р. *Fusarium* и *Pythium* spp. при хидропонно отглеждане на зеленчуци (Dikand, Elad, 1999). Изгодните ефекти на спорите от микоризната гъба *Glomus* spp. са описани от редица автори (Dikand, Elad, 1999; Станчев, Левенсон, Бобошевска, 1998; Rao and Gowen, 1998).

У нас се знае много малко за ползотворните ефекти от употребата на микоризната гъба

**World Research Journal of Agronomy**
ISSN: 2320-3404 & E-ISSN: 2320-5644, Volume 2, Issue 1, 2013, pp.-011-014.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000009

# EFFECT OF BIOTIZATION ON GRAFTING SUCCESS OF THE PLANTS IN FRUIT NURSERY

**TSVETKOV I.[1]*, DZHAMBAZOVA T.[1], EVSTRATIOS S.[2], AND XILOYANNIS C.[3]**

[1]AgroBioInstitute, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria
[2]Bulgarian Plant LTD, 8800 Sliven, Bulgaria
[3]Universita della Basilicata, Via dell' Ateneo Lucano n.10., 85100 Potenza, Italy
*Corresponding Author: Email- ivantsvetkov@abi.bg

Received: November 05, 2013; Accepted: December 03, 2013

**Abstract-** One of the most important agrotechnical manipulations in the nurseries is grafting of the fruit tree varieties on the suitable rootstocks. The objective of our research was to observe the effect of different biotizations on grafting success of the plants in the fruit nursery. OHF 33 rootstock grafted with pear cultivar William displayed the best grafting rates reaching up to 61,40% in *Glomus intraradices*+ *Trichoderma harzianum* T22 variant. For comparison, the control variants in OHF 33 experimental plots showed grafting success values between 33,64 % and 40,60%. T-budding grafting of Myrobolan 29 were remarkably unsuccessful, showing 11,0 % as a best rate (again in *Glomus intraradices*+ *Trichoderma harzianum* T22 variant). In this case, except strong winter temperatures also graft incompatibility with plum cultivar Angelino may appear as a limited factor having in mind comparison with other rootstock/scion combinations. There are not observed statistically distinguishable differences between mycorrhizated and non-mycorrhizated variants inside of the GF 677/ Royal Glory experimental plots. In case of Gisela 6/cv. Van rootstock/scion combination we observed a successful grafting rate ranging from 25,67% (var. *Glomus intraradices*) to 44,44% (var. *Trichoderma harzianum* T22+ *Baccilus subtilis*). Best rates, in general, are obtained for *Trichoderma harzianum* T22 biotizated plants, which overrun the controls with 3% to 11%.

**Keywords-** Fruit nursery, Grafting, Mycorrhiza, *Streptomyces lydicus*, *Bacillus subtilis*, *Agrobacterium radiobacter*

## Introduction

Vegetative propagation of fruit species traditionally occurs by cuttings or grafting. In the latter case, farmers use suitable rootstocks to acquire tolerance for different types of abiotic and biotic stress, keeping the desired characteristics of the cultivar in the scion [1].

One of the most important agrotechnical manipulations in the nurseries is grafting of the fruit tree varieties on the suitable rootstocks [2]. When grafting is made in practice, we usually depend on our experience, and thus fundamental and important problems on grafting remain unsolved, especially with reference to the mechanism of grafting [3]. The first stage of the fusion in grafting begins with the contact of calluses which are formed on the cut surfaces of rootstocks and scions. These contacted calluses maintain cell division and proliferation, and consequently they tightly unite. As the second stage, the supply of nutrition and water through the calluses from rootstock to scion gives rise to the elongation of new shoots and the growth in thickness near the joint part. In this way, the fusion of rootstock and scion is completed [4]. Other important factors for successful grafting rate are air and soil temperatures, humidity and fertilization [5]. In general, the environmental factors have more influence over the grafting success in the countries with temperate climate (as Bulgaria), having in mind the strong lowering winter temperatures during January and February [6].

T-Budding is a preferable grafting method in most of the fruit growing species, providing better success rate and plant development. T -budding is performed when the rootstock bark is slipping during the early summer and the scion buds have matured and hardened [7] [Fig-1].

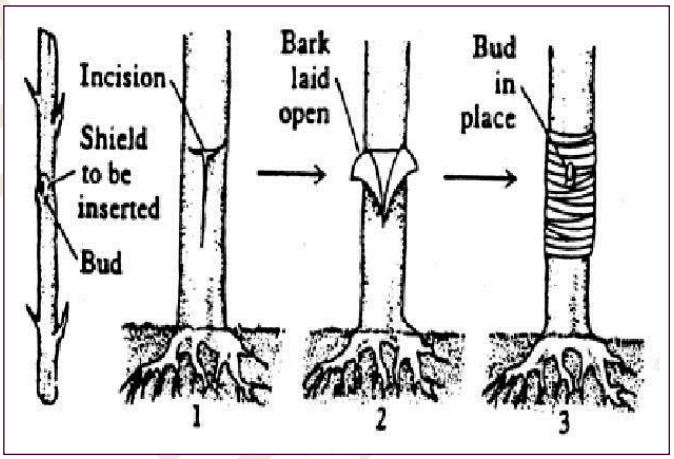



**Fig. 1-** The T-bud is the most common method used to bud fruit-growing variety scion onto rootstock. The shield is cut from the budstick and inserted into a T-cut on the stock.

Mycorrhizal fungi have been shown to increase plant uptake of water and mineral nutrient [8]. Until now, the influence of mycorrhizae inoculation on the grafting/budding success is not clarified [9]. The objective of our research was to observe the effect of different biotizations on grafting success of the plants in the fruit nursery.

## Materials and Methods

*In vitro* obtained and biotizated rootstocks were produced by SME Fitotechniki- Greece. They were planted in the Bulgarian project experimental field (Sliven region) on 16th of April 2009. Sliven is

Universal Journal of Plant Science 2(1): 23-26, 2014
DOI: 10.13189/ujps.2014.020104 http://www.hrpub.org

# Effect of Organic Substrates on the Growth and on the First Yield of Local Bulgarian Red Wine Variety "Mavrud"

**Ivan Tsvetkov*, Teodora Dzhambazova**

AgroBioInstitute, 8, DraganTsankov Blvd., 1164 Sofia, Bulgaria
*Corresponding Author: ivantsvetkov@abi.bg

Copyright © 2014 Horizon Research Publishing All rights reserved.

**Abstract** The aim of this study is to determine the effects of some organic substrates on growth characteristics and yield of local Bulgarian grapevine variety "Mavrud". In parallel, organic and conventionally cultivated experimental plants were used. Concerning growth behavior indexes, in generous, the plants cultivated by conventional agriculture methods showed similar results to organic agriculture variants in all observed parameters compared with the non-treated controls. New organic substrate Kompovet B4® appeared to be the most convenient, confirming the results from the first and from the second year of the experiments. That could be an encouragement for including this substrate in the further grapevine organic farming technologies. Regarding the excellent quality of planting material as well as the novel forced greenhouse plant production methods- the first berry clusters were formed at the second year, instead of the third one (usually, the grapevines start harvesting on third year after cultivation). First obtained fruits in organic farming experimental field have a typical variety cluster form and raisin shape.

**Keywords** Grape, Organic Farming, Growth Characteristics, Sustainable Agriculture, Cv. Mavrud

## 1. Introduction

Grape is a perennial crop that, compared to other crops, has relatively low nutritional needs and adapts well to marginal soils (Pongracz, 1978 [1]). These features make organic grape production a technically feasible, economically attractive and sustainable activity. About 70% of the worldwide organic grape production area was located in the EU-27, where Italy, France and Spain were the main producers (Eurostat, 2008 [2]). Bulgaria is one of the European countries with fast development of organic viticulture with already certified 513 ha and 1404 ha organic vineyards under the process of certification (MAF, 2011 [3]). Selection of quality grapevine varieties with high adaptation to the environmental conditions and tolerance to pests/diseases is very important both for organic and conventional viticulture (Tsvetkov et al., 2005 [4]). Organic vineyard requires specific fertilizers, training operations to facilitate pruning (a critical practice), spraying and harvesting (Guesmi et al., 2012 [5]). A significant part of organic livestock manure is directly applying in organic crops as fertilizer substrates that close the cycle of organic production (Sorensen &Rubaek, 2012 [6]). Farming systems without manure showed the severest loss in soil organic matter over time. Manure amendment to soils, as an attribute of mixed farming systems, is proven again to exert positive effects (Fließbach et al., 2007 [7]). Usually the animal production is concentrated in identified regions, different that crop production ones which made not cost effective to transport manure for utilization in other areas. For this reason, alternative organic substrates are used which very often are obtained by composting of residues from crop production and/or its relevant industries (Tsvetkov et al., 2010 [8]). The aim of this work is to study the effects of manure as a basic organic substrate and new certified one "Kompovet B 4"on growth behavior and yield of local Bulgarian grapevine variety "Mavrud". In addition, conventional managed control is also included.

## 2. Materials and Methods

### 2.1. Plant Material

Mavrud is an local red wine variety, cultivated only in Bulgaria from old times. It is area specific variety spread originally in the region of Assenovgrad at the food of Rhodopimountain. The wines produced from this variety have a deep ruby colour, enough tannin and acids in reserve and a typical pleasant flavour in which one can detect the taste of tendrils and blackberry. The wines develop perfectly when they are in contact with oak wood and

Universal Journal of Plant Science 2(2): 48-51, 2014
DOI: 10.13189/ ujps.2014.020204

http://www.hrpub.org

# *In vitro* Long-term Storage and Regeneration of Bulgarian Grapevine Variety "Velika" via Repetitive Somatic Embryogenesis

**Ivan Tsvetkov*, Teodora Dzhambazova, Violeta Kondakova, Rossitza Batchvarova**

AgroBioInstitute, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria
*Corresponding Author: ivantsvetkov@abi.bg

Copyright © 2014 Horizon Research Publishing All rights reserved.

**Abstract** Somatic embryogenesis being one of the *in vitro* techniques that produce high regeneration rates, serves for high frequency propagation, gene transfer and germplasm storage. The aim of this work is to study the possibilities for in vitro long-term storage of grapevine genetic resources via repetitive somatic embryogenesis. Thirteen years after induction from leaves explants embryogenic cultures of Bulgarian grapevine variety "Velika" are still propagating via repetitive somatic embryogenesis in frame of clusters keeping their embryogenic and morphogenic competence. Plantlents regeneration from cotyledonary stage somatic embryos showed a normal phenotype compared with shoot-tip propagated controls. The genetic fidelity of regenerated plants was assessed by DNA analysis using Inter Simple Sequence Repeat (ISSR) markers. The amplified loci showed no differences between mother and regenerated plants, confirming the genetic authenticity of in vitro stored and propagated plants.

**Keywords** Grape, Somatic Embryogenesis, ISSR analysis, in Vitro Regeneration, GENRES Preservation

## 1. Introduction

*In vitro* techniques are a convenient tool for vine breeding to overcome the difficulties in conventional breeding studies and obtaining new cultivars. One of these techniques is somatic embryogenesis. The term of somatic embryogenesis is described as embryo formation from somatic cell or tissue. These embryos are similar to zygotic embryos formed by fertilization. Somatic embryogenesis being one of the *in vitro* techniques that produce high regeneration rates, serves for high frequency propagation, gene transfer and germplasm storage (Tsvetkov *et al.*, 2005 [1]). Additionally, genotype specificity within species mainly limited the application of somatic embryogenesis as an effective tool for plant regeneration in *Vitis* sp. (Tsvetkov *et al.*, 2000 [2]). Different explants have been used for the induction of grapevine somatic embryogenesis, mainly anthers/ovules, but also tissues from vegetative organs (leaves, petioles, stems, internodes) (Martinelli and Gribaudo, 2001 [3]). Several strategies have been adopted in order to keep indefinitely the embryogenic competence of the cultures and thus obtain a long-term source of somatic embryos. The maintenance of embryogenic cultures has been reported to last even for several years, retaining their embryogenic competence (Martinelli and Gribaudo, 2009 [4]). *In vitro* culture techniques can lead to genetic variation in plants trough somaclonal variation (Neelakandan and Wang, 2011[5]). Flow cytometry of cells of grapevine plants, regenerated via somatic embryogenesis has shown that polyploidy somaclonal variation is produced during *in vitro* culture (Prado *et al.*, 2010 [6]; Acanda *et al.*, 2013 [7]).

## 2. Materials and Methods

### 2.1. Plant Material and Somatic Embryogenesis

Embryogenic cultures from *in vitro* leaf tissues were established and multiplied on the base of the protocol described by Tsolova and Atanassov (1996 [8]). The 13 years storage of the repetitive somatic embryogenesis were done by periodic transfer (every 3 months) to fresh NN medium (Nitsch and Nitsch, 1969 [9]) supplemented with 2 mg/l 2,4 D (2,4 dichlorophenoxyacetic acid), 1 mg/l IAA (indole-3-acetic acid), 1 mg/l BAP (benzylaminopurine) and 0,6 mg/l $GA_3$ (gibberelic acid) for embryo maintenance and multiplication. *In vitro* plantlets were regenerated on hormone-free ½ MS medium (Murashige and Skoog, 1962 [10]) from selected somatic embryos in cotyledonary stage of their development.

### 2.2. Inter Simple Sequence Repeat (ISSR)Analysis

*Bulgarian Journal of Agricultural Science, 20 (No 4) 2014, 849-855*
*Agricultural Academy*

# MYCORRHIZAL FUNGI *GLOMUS* SPP. AND *TRICHODERMA* SPP. IN VITICULTURE (REVIEW)

I. TSVETKOV*, T. DZHAMBAZOVA, V. KONDAKOVA and R. BATCHVAROVA
*Agrobioinstitute, BG - 1164 Sofia, Bulgaria*

## Abstract

TSVETKOV, I., T. DZHAMBAZOVA, V. KONDAKOVA and R. BATCHVAROVA, 2014. Mycorrhizal fungi *Glomus* spp. and *Trichoderma* spp. in viticulture (review). *Bulg. J. Agric. Sci.*, 20: 849-855

Mycorrhizal fungi's *Glomus* spp. and *Trichoderma* spp. are natural components of the healthy vineyard soils. They have the ability to improve nutrition and root resistance to attack by pathogens, and contribute positively to soil structure and stability. Implementation of practices that favors their propagation should be important aspects of viticulture management. Mycorrhizal inoculations of grapevines are proposed as a biotechnological alternative of conventional systems to manage soil sickness problem and to maintain improved grapevine plant growth and health. The conducted studies in this review showed the beneficial effects of mycorrhizal fungi *Glomus* spp. and *Trichoderma* spp. on grapevine growth/health status, plant nutrient uptake and soil fertility, as well on vineyard ecology in general.

*Key words*: *Glomus* spp., *Trichoderma* spp., viticulture, plant nutrient uptake, biocontrol

## Introduction

Currently, in grape nursery and wine production sectors there is a widespread usage of chemical pesticides to repress plant pathogens, and of synthetic fertilizers to stimulate plant growth. Such treatments lead to a reduction of microflora complexity in the soil and to declining of the overall plant quality. While tolerance of various pathogens to pesticides increases, the soil impoverishment determines a reduction of yield and quality. The negative effects of pesticides and synthetic fertilizers is the soil sickness phenomena, whose symptoms usually follow the replant of fruit tree crops such as apple, peach, cherry, apricot, grapevine, etc. One of the main reasons is related to the disappearance of mycorrhizal fungi from the soil, which, in association with other antagonist microorganisms, are known to increase the absorbing surface of the root system, causing the improvement of nutrient uptake and plant growth in general, and an increasing plant resistance to pathogens (Schreiner and Bethlenfalvay, 1995). In order to reduce negative environmental effects and to increase soil fertility, the policies at European and Worldwide level tend to limit the use of chemical substances for both fertilization and plant defense. There is a necessity of innovating plant production processes to produce plant more tolerant to common pathogens and more efficient in using the natural soil resources (mineral elements, water, etc) in all the phases of cultivation, from nursery to vineyard.

Last decade numerous studies regarding the impact and effects of organic *versus* conventional systems on soil microbial dynamic have been conducted (Mäder et al., 2000a; Ryan et al., 2000; Ryan and Graham, 2002; Purin et al. 2006; Freitas et al., 2011). The results show that mycorrhizal fungi are important for the good nutrient status of young host plants in the nursery phase and during acclimatization period. The other significant result is the bioprotective effect of mycorrhization against soilborne fungal pathogens (Vierheiling et al., 2008). Moreover, several fungal pathogens are considered as a principal cause of soil fatigue in vineyards (Nogales et al., 2008), thus the mycorrhizal inoculation of grapevines is proposed as a biotechnological alternative of conventional systems to manage soil sickness problem and to maintain improved grapevine plant growth and health.

## Mycorrhizal Fungi – *Glomus* spp.

*Glomus* spp. is the largest genus of arbuscular mycorrhizal (AM) fungi. All species form symbiotic relationships with plant roots. The AM symbiosis makes a significant con-

* Corresponding author: ivantsvetkov@abi.bg

*Bulgarian Journal of Agricultural Science, 20 (No 3) 2014, 666-669*
*Agricultural Academy*

# EFFICIENCY OF *PSEUDOMONAS SPP.* FOR BIOCONTROL OF THE POTATO CYST NEMATODE *GLOBODERA ROSTOCHIENSIS* (WOLL.)

Z. TRIFONOVA[1*], I. TSVETKOV[2], N. BOGATZEVSKA[1] and R. BATCHVAROVA[2]
[1] *„N. Poushkarov" Institute of Soil Science, Agrotechnology and Plant Protection, BG - 1331 Sofia, Bulgaria*
[2] *AgroBioInstitute, BG - 1164 Sofia, Bulgaria*

## Abstract

TRIFONOVA, Z., I. TSVETKOV, N. BOGATZEVSKA and R. BATCHVAROVA, 2014. Efficiency of *Pseudomonas spp.* for biocontrol of the potato cyst nematode *Globodera rostochiensis* (Woll.). *Bulg. J. Agric. Sci.*, 20: 666-669

The potential of *Pseudomonas spp.* as biocontrol agent against potato cyst nematode *Globodera rostochiensis* was tested in pot experiment under glasshouse conditions. The best control of nematode population was provided by the bacterial strains *Pseudomonas putida* 3 (2) and *Pseudomonas aurantiacea* 13 (2). The plant growing parameters in the treatments enhanced as compared with the inoculated with *Globodera rostochiensis.* The two bacterial strains significantly reduced nematode populations by 40.7% - 42.2 % compared to the control.

*Key words*: cyst nematode, biocontrol, bacterial strains

## Introduction

Plant-parasitic nematodes are among the most destructive plant pest, causing substantial economic losses to crops worldwide. The golden potato nematode *Globodera rostochiensis* (Woll.) causes severe yield losses of potato (*Solanum tuberosum* L.) in many regions of Bulgaria (Trifonova, 1995, 2000). Soil treatment with nematicides has been an established practice for the control of cyst-forming nematodes of the genus *Globodera*, though it is very expensive for the farming community. In recent years, we have been observing an increasing trend to use alternative methods of pest control based on replacing chemicals pesticides by natural compounds.

The results of more recent studies showed that in many cases, endophytic microorganisms have an important role in host protection against pathogens. According Hallmann et al. (1998) some endophytic bacteria are associated with beneficial effects such as plant growth promotion and bio control potential against plant parasitic nematodes. Most *Pseudomonas* spp. is free-living saprophytic organisms in soil whereas they play an important role in decomposition, biodegradation and the carbon and nitrogen cycles. Barahona et al. (2011) showed that three strains of *Pseudomonas fluorescens* possess biocontrol activity against fungal root pathogens as *Fusarium oxysporum* f. sp. *radicis-licopersici* and *Phytophtora cactorum.* Endophytes colonize the same root tissues as sedentary plant-parasitic nematodes. Therefore, this association of endophytic bacteria with nematodes throughout the nematode life cycle makes these bacteria excellent candidates for biocontrol strategies. Most research on the interaction of endophytic bacteria with nematodes has been conducted on root knot nematode (*Meloidogyne* spp.), but the association of other nematode species – cyst nematodes with the endophytic bacteria is also of great interest. Three *Pseudomonas fluorescens* strains inhibited invasion of the plant-parasitic nematode *Radopholus simillis* and *Meloidogyne* spp. in banana, maize and tomato roots. (Aalten et al., 1998; Ashoub and Amara, 2010; Siddiqui et al., 2001). One of the difficulties in developing rhizobacteria as a control measure is that the performance of these agents is highly variable.

The objective of this research was to determine the efficiency of different bacterial isolates against the potato cyst nematode *G. rostochiensis* applied to potatoes plants.

## Material and Methods

### Nematode population

The population of *Globodera rostochiensis* was obtained from soil samples collected from heavily infested potato fields in Smolyan region. The cysts of the nematode were pro-

* Corresponding author: zl.trifonova@abv.bg

CHAPTER 8

# New Approaches for Detection of Unique Qualities of Small Fruits

**Teodora Dzhambazova, Ilian Badjakov, Ivayla Dincheva, Maria Georgieva, Ivan Tsvetkov, Atanas Pavlov, Andrey Marchev, Kiril Mihalev, Galin Ivanov, Violeta Kondakova, Rossitza Batchvarova, and Atanas Atanassov**

## CONTENTS

8.1 Introduction ..... 187
8.2 Application of Omics Technology for Discovery of Unique Qualities of Small Fruits ..... 188
8.2.1 Genomics ..... 189
8.2.2 Proteomics ..... 191
8.2.3 Metabolomics ..... 192
8.2.4 Nutrigenomics ..... 195
8.3 Different Approaches for *In Vitro* Propagation of Small Fruits ..... 196
8.3.1 Case Study ..... 197
8.4 Berry Polyphenols: Processing and Food Matrix Effects ..... 201
References ..... 202

## 8.1 INTRODUCTION

The omics disciplines applied in the context of nutrition and health have the potential to deliver biomarkers for health and comfort, reveal early indicators of disposition to disease, and discover bioactive, beneficial food components. These technologies are aimed at unraveling the overall expression of genes, proteins, and metabolites in a functionally relevant context, and provide insights into the molecular basis of various fundamental processes involved in growth and development of plants and their environment.

Genomics is an entry point for looking at the other omics sciences. The information in the genes of an organism, its genotype, is largely responsible for the final physical makeup of the organism, referred to as the *phenotype*. The main purpose of the application of genomics is to gain a better understanding of the whole genome of plants. Agronomically important genes may be identified and targeted to produce more nutritious and safe food while at the same time preserving the environment.

Proteomics is known as protein *expression profiling*, whereby the expression of proteins in an organism resulting from a stimulus is identified at a certain time. Proteomics can also be applied to

# Mineral Uptake and Dry Matter Accumulation of Biotized GF677/ 'Royal Glory' Nursery Plants

I. Tsvetkov, T. Dzhambazova and R. Batchvarova
AgroBioInstitute
8 Dragan Tsankov Blvd.
1164 Sofia
Bulgaria

E. Markov
Institute of Soil Science "Nikola Poushkarov"
1080 Sofia
Bulgaria

E. Stavrou
Bulgarian Plant Ltd.
8800 Sliven
Bulgaria

**Keywords:** peach nursery, *Glomus intraradices*, *Trichoderma harzianum* T22, *Agrobacterium radiobacter* K84, plant mineral uptake, dry matter accumulation

**Abstract**

**The aim of this work is to study the influence of different mycorrhizal fungi and their further treatment in the nursery on plant mineral uptake and dry matter accumulation. Additional biotizations were preliminary done in some of the experimental treatments. The analysis of dry matter accumulation was performed twice in vegetation period (in May and October). Each sample was analyzed also for plant mineral uptake at six basic macro and microelements (N, P, K, Ca, Mg, Fe).**

**Generally, all additional mycorrhiza treatments have a positive effect on the dry matter accumulation. In *Trichoderma* experimental plots highest increase is observed in *Trichoderma* (BS)(TG) and *Trichoderma* (SL)(TG) treatments, which showed more than 55% dry matter accumulation rates. For comparison, *Trichoderma* non-treated increase rate is less than 10%. Similar results are obtained in *Glomus* and *Glomus+Agrobacterium* treatments where additional treated plots overrun non treated ones with 21.1% and 82.7% respectively. Concerning the mineral uptake, additional mycorrhiza application show advantages in all treatments.**

## INTRODUCTION

The beneficial effects of mycorrhiza fungi symbiotic association on the growth of plants are well known, and they may be involved in improving uptake of macro- and micronutrient, increasing plant resistance against biotic and abiotic stress, and beneficial alternations of plant growth regulators (Kung'u et al., 2008; Smith and Smith, 2011). Increased growth rates in mycorrhizal plants have most often been attributed to increased nutrient uptake. Mycorrhizas appear to increase nutrient acquisition, especially for elements that are immobile in soils, either because inorganic ions diffuse slowly (such as P, Cu, Zn, and Ca) or when (like both N and P) they are present in complex organic forms (Tinker and Nye, 2000). Mycorrhizal stimulation of nutrient uptake is attributed to (i) uptake by fungi from soil beyond the depletion zones that can develop around roots, (ii) production of degradative extracellular enzymes or organic acids by the fungi, and (or) (iii) the ability of fungi to translocate nutrients faster than they could diffuse through soil (Simard et al., 2002; Jones and Smith, 2004). Nowadays, mycorrhizal fungi have been widely used in agriculture, horticulture, and forestry programs, as well as for environmental reclamation, to increase crop yield and health and to limit the application of agrochemicals (Jiang et al., 2013).

While the artificial infection is effective in the nurseries, some problems have not yet been solved, such as the difficulty to produce a large amount of commercial pathogen-free product, the scarce knowledge on the host-fungus interactions, the likely competition with the soil borne fungi, the impossibility to sterilize the nursery soil (Lindermann and Davis, 2001; Bavaresco et al., 2011).

The aim of the paper is to study the influence of different mycorrhizal fungi and their additional treatment in the nursery on mineral uptake and dry matter accumulation of GF677/'Royal Glory' nursery plants.

# Nematode Diversity in Gf677/'Royal Glory' Nursery Plants Inoculated with Mycorrhizas and Bio Control Bacteria

I. Tsvetkov, T. Dzhambazova
and R. Batchvarova
AgroBioInstitute
8 Dragan Tsankov Blvd.
1164 Sofia
Bulgaria

S. Nedelchev
Faculty of Biology
Sofia University
"St. Kliment Ohridsky"
1164 Sofia
Bulgaria

**Keywords:** nematode diversity**,** peach nursery, biological control, *Glomus intraradices*, *Trichoderma harzianum* T22, *Agrobacterium radiobacter* K84

**Abstract**

**The soil-born nematodes and especially some groups of them are susceptible to different biological and chemical factors occurring in the environment. That is why investigations related to influence of mycorrhizal fungi including *Trichoderma harzianum* and *Glomus intraradices* and bio-control bacteria such as *Agrobacterium radiobacter* on soil structure and nematode population dynamics present a specific interest. The main results of this study showed that inoculation of GF677/'Royal Glory' nursery plants with mycorrhizas and bio control bacteria lead to reduction of the number of ectoparasite virus-transmitting phytonematodes while maintaining the density of semi-parasite and free leaving ones. Therefore, mycorrhiza and bio-control bacteria treatments could be recommended as a useful tool for biological control in peach nurseries.**

## INTRODUCTION

Interest and investigation of alternative methods and means for integrated control of nematodes has increased, including the possible exploitation of organo-biological products (Bileva et al., 2009). Most effective nematicides do not comply with current environmental demands and will have to be replaced by other means of nematode control with less impact on non-target organisms. Enhancing host resistance and/or tolerance by mycorrhizal fungi could be a promising alternative (Shoresh et al., 2010). Evidence exists that mycorrhiza-induced protection against root pathogens involves not only local but also systemic induced resistance, with reduction in root infection in mycorrhizal and non-mycorrhizal colonized parts of root systems, suggesting the existence of a signal-mediated phenomena acting at distance (Elsen et al., 2008; Hao et al., 2012). Plant nematodes and mycorrhizal fungi share plant roots as sources of food and space. The effects of both organisms occurring in one host on plant growth, on nematode numbers and on mycorrhizal colonisation, have been the subject of numerous studies, which have been reviewed at regular intervals (Pinochet et al., 1996; Borowicz, 2001; Hol and Cook, 2005; Hallmann and Sikora, 2011). The current consensus is that these agents may enhance host tolerance and increase resistance by slowing down nematode development.

The aim of our study was to determine the phytonematode species composition in GF677/'Royal Glory' nursery plants inoculated with mycorrhizas and bio-control bacteria.

## MATERIALS AND METHODS

In vitro grown GF 677 rootstocks were obtained from SME Fitotechniki- Greece. They were planted in the Bulgarian project experimental field (Sliven region). The rootstocks were grafted 5 months after planting with certified buds from peach variety 'Royal Glory' by T-budding (10 cm from the soil level, with one- bud scions) (Hartman et al., 1997). Before the start of the second vegetation period, additional treatments were done inside of the relevant experimental plots with the same mycorrhizal inocula used in the first stages of in vitro rootstocks production (Table 1). Mycorrhizal inocula were applied manually, as close to the plant roots as possible.

**Disease Note**

# FIRST REPORT OF *CUCUMBER MOSAIC VIRUS* INFECTING WATERMELON IN GREECE AND BULGARIA

**C.K. Xanthis[1], V.I. Maliogka[1], H. Lecoq[2], C. Dezbiez[2], I. Tsvetkov[3] and N.I. Katis[1]**

*[1] Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Plant Pathology Lab, P.O. Box 269, Thessaloniki 54124, Greece*
*[2] INRA, Unite Pathol Vegetale, UR407, F-84140 Montfavet, France*
*[3] AgroBioInstitute, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria*

In 2014, a total of 108 symptomatic watermelon samples were collected in Greece and analyzed by DAS-ELISA using polyclonal antisera (INRA, Montfavet) against the most common aphid-transmitted viruses. *Cucumber mosaic virus* (CMV) was detected in eight of 108 plants tested. Five of these plants collected at Lesvos were also infected with *Watermelon mosaic virus* (WMV), whereas three plants from Prohoma had single infections. Five samples with severe stunting and yellowing collected at Asenovgrad (Bulgaria) in 2013 and tested by DAS-ELISA for the above viruses hosted CMV and *Cucurbit aphid-borne yellows virus* (CABYV). To confirm CMV presence in watermelon, a nested RT-PCR assay was carried out first with the degenerated primers CMVup624a (5'-ATGGACAAATCTGRATC-3') and CMVdo1244a (5'-TGRTGCTCRAYGTCKACATGA-3') followed by CMVup624b (5'-GGACAAATCTGRATCTCCCAATGC-3') and CMVdo1244b (5'-TGCTCRAYGTCRACATGAAG-3') that amplify a 622 bp region from the viral coat protein gene. Total RNA (Chatzinasiou *et al*., 2010, method A) extracted from all ELISA- positive watermelon samples from both countries and from two healthy watermelon plants was used as template in nested RT-PCR. A product of the expected size was amplified from all serologically CMV-positive samples, but not from the healthy ones. The Greek CMV isolates from Lesvos (LN810059) and Prohoma (LN810060) showed 99% nucleotide sequence identity with potato (AB448694) and tomato (EF153734) CMV isolates from Syria and India, while the Bulgarian isolate (LN810058) showed 99% nucleotide sequence identity with an Indian cucumber isolate (JF279608). Phylogenetic analysis indicated that the Greek CMV isolate (LN810059) belongs to sub-group IA, while the Bulgarian isolate and a Greek isolate (LN810060) belong to sub-group IB. To our knowledge this is the first report of CMV infecting watermelons in Greece and Bulgaria.

Chatzinasiou E., Dovas C.I., Papanastassopoulou M., Georgiadis M., Psychas V., Bouzalas I., Koumbati M., Koptopoulos G., Papadopoulos O., 2010. Assessment of bluetongue viraemia in sheep by real-time PCR and correlation with viral infectivity. *Journal of Virological Methods* **169**: 305-315.

*Corresponding author*: N.I. Katis
E-mail: katis@agro.auth.gr

Received March 13, 2015
Accepted March 14, 2015

**Disease Note**

# FIRST REPORT OF *ZANTEDESCHIA MILD MOSAIC VIRUS* ON *ZANTEDESCHIA AETHIOPICA* IN ITALY

**D. Rizzo[1], A. Panattoni[2], L. Stefani[1], M. Paoli[1], B. Nesi[3], S. Lazzereschi[3], S. Vanarelli[1], P. Farina[1], M. Della Bartola[2], A. Materazzi[2] and A. Luvisi[2]**

*[1] Servizio Fitosanitario Regionale, Regione Toscana, Via Ciliegiole 99, 51100 Pistoia, Italy*
*[2] Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto, 80, 56124 Pisa, Italy*
*[3] Council for Agricultural Research and Economics, CRA-VIV, Via dei fiori 8, 51012, Pescia (PT), Italy*

Calla lily [*Zantedeschia aethiopica* (L.) Spreng] has become one of the most popular cut flowers worldwide. It has been reported as the natural host of various plant viruses, including potyviruses such as *Bean yellow mosaic virus* (BYMV), *Dasheen mosaic virus* (DsMV), *Turnip mosaic virus* (TuMV) and *Zantedeschia mosaic virus* (ZaMV). In 2005 a new potyvirus named *Zantedeschia mild mosaic virus* (ZaMMV) was identified in Taiwan (Huang and Chang, 2005). In 2012, 15 plants of Calla lily cultivated in Tuscan farms showed leaves with yellow spots and stripes, green islands and an unusual mild mosaic. Seventeen samples (15 symptomatic and two symptomless) were collected and assayed by ELISA for BYMV, DsMV, TuMV, ZaMV and potyviruses using antisera produced by DSMZ (Braunschweig, Germany) and LOEWE Biochemica (Sauerlach, Germany). Plants were positive for an anti-potyvirus group monoclonal antibody. Positive samples were assayed by reverse transcription-polymerase chain reaction for ZaMMV using total RNA extracted from leaves and specific primers for the coat protein gene (Wen-Chi *et al*., 2010). Amplicons of the expected size (792 bp) were obtained for 15 samples that reacted positively to the potyvirus antibodies, while no amplification was observed in symptomless samples. The sequence obtained from one ZaMMV amplicon (accession No. KF156666) had 99% nucleotide identity with the corresponding fragment of a reference ZaMMV isolate (GenBank accession No. AY626825.4). To our knowledge this is the first report of ZaMMV on *Zantedeschia aethiopica* in Italy.

Huang C.H., Chang Y.C., 2005. Identification and molecular characterization of Zantedeschia mild mosaic virus, a new calla-lilly-infecting potyvirus. *Archives of Virology* **150**: 1221-1230.

Wen-Chi H., Chin-Hsing H., Shu-Chuan L., Chun-I W., Ya-Chun C., 2010. Detection of four calla potyviruses by multiplex RT-PCR using nad5 mRNA as an internal control. *European Journal of Plant Pathology* **126**: 43-52.

*Corresponding author*: A. Materazzi
Fax: +39 050 2210559
E-mail: alberto.materazzi@unipi.it

Received March 17, 2015
Accepted March 24, 2015

*Bulgarian Journal of Agricultural Science, 22 (No 5) 2016, 745–751*
*Agricultural Academy*

# NEW PROTOCOL FOR *IN VITRO* PROPAGATION OF BERRY PLANTS BY TIS BIOREACTOR

L. GEORGIEVA[1*], I. TSVETKOV[1], M. GEORGIEVA[2] and V. KONDAKOVA[1]
[1] *Agricultural Academy, Agrobioinstitute, BG-1164 Sofia, Bulgaria*
[2] *Agricultural Academy, Institute of Mountain Agriculture and livestock, BG-5600 Troyan, Bulgaria*

## Abstract

GEORGIEVA, L., I. TSVETKOV, M. GEORGIEVA and V. KONDAKOVA, 2016. New protocol for *in vitro* propagation of berry plants by TIS bioreactor. *Bulg. J. Agric. Sci.*, 22: 745–751

The purpose of this study is to explore the possibility of optimizing the standard protocols for in vitro propagation of important berry crops: raspberry, cv. Polka and strawberry, cv. Tudla, using bioreactor system. The effectiveness of the two systems (solid media and liquid in TIS bioreactor, RITA® type) are evaluated in 2 stages – propagation (on MS based media supplement with 0.5 mg.l$^{-1}$ BAP, 0.05 mg.l$^{-1}$ IBA, 0.03 mg.l$^{-1}$ $GA_3$ and 30 g.l$^{-1}$ sucrose) and rooting (MS without hormones supplement with 30 g.l$^{-1}$ sucrose). At each stage are measured fresh and dry weights of the explants, while the rest continued their development. In solid medium strawberry plants multiplication ratio for 4 weeks was 1.9 while in liquid – 4.1. Respectively for raspberry this ratio was 3.2 and 6.7. Fresh mass of TIS plants were increased for both species, whereas in plants grown on solid medium was observed a much higher percentage of dry matter. This means that the high fresh weight of the plants in a liquid medium is primarily due to water accumulation. Root initiation of strawberry began about 3rd week and after 5th week all plants was rooted successfully in both liquid and in the solid medium. For the same time only few raspberry plants formed roots in the same condition. That is the reason we tested another 3 variants of rooting systems. All plants of the three variants are rooted successful within 4 weeks. Highest percent dry weight, where the plants transferred on solid medium from liquid one (variant 2). Rooted plants were successfully adapted in soil in a greenhouse. TIS is a promising alternative system of mass propagation for strawberries and raspberries. The different purposes of application of the bioreactor system requires further optimization of the growing medium and precise conditions of cultivation of each species and variety. Micropropagation for strawberry could be entirely in liquid medium. For raspberry there is a problem with hyperhydration when the plants stay more time in liquid medium. We suggest an optimized protocol for mass propagation of raspberries, which includes plant propagation in a liquid medium (TIS bioreactor) and rooting them in solid medium.

*Key words:* TIS bioreactor, *in vitro* propagation, strawberry, raspberry
*Abbreviations: TIS* – Temporary Immersion System; TDZ – Thidiazuron; MS – Murashige & Skoog, 1962; BAP – 6-Benzylaminopurine; IBA – indole butyric acid; $GA_3$ – Gibberellic acid; PTFE – Polytetrafluoroethylene

## Introduction

*In vitro* propagation has been proved as a highly efficient method for production of large quantities of true to type and free of diseases plants with minimum use of space and less initial plant material. However, the application of traditional micropropagation, using solid medium is today still limited in commercial production mainly due to its intensive labor input and thereby expensive (Welander et al., 2014).

As opposed to the traditional micropropagation, semi-automatic bioreactors use liquid media based on the temporary immersion principle. Temporary Immersion System (TIS) was created in the 80's (Harris, 1983). The principle of TIS technology is that plant material is immersed in liquid growth

**Corresponding autor*: lilk81@abv.bg

*Journal of Mountain Agriculture on the Balkans, vol. 19, 3, 2016, (197-211)*
*Research Institute of Mountain Stockbreeding and Agriculture, Troyan*

# Проучване възможностите за използване на диви лози (*Vitis vinifera* ssp. *sylvestris*) като лозови подложки

Иван Цветков[1*], Теодора Джамбазова[1], Мария Георгиева[2], Милена Баятова[1], Диян Георгиев[2], Виолета Кондакова[1]

*[1]Агробиоинститут, бул. Драган Цанков 8, 1164 София, България*
*[2]Институт по планинско животновъдство и земеделие, ул. "Васил Левски" 281, 5600 Троян, България*
**E-mail: ivantsvetkov@abi.bg*

# Explore the use of wild grapes (*Vitis vinifera* ssp. *Sylvestris*) as grapevine rootstocks

Ivan Tsvetkov[1*], Theodora Dzhambazova[1], Maria Georgieva[2], Milena Bayatova[1], Diyan Georgiev[2], Violeta Kondakova[1]

*[1]Agrobioinstitute, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria*
*[2]Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Str., 5600 Troyan, Bulgaria*

## РЕЗЮМЕ

Дивите лози са обект на проучване на повече от един век. В България се срещат повсеместно разпространени по брега на р. Дунав, крайбрежието на Черно море, поречието на р. Струма, р. Велека, р. Ропотамо, Западни Родопи, Пирин и др. (Негруль и др., 1965). Диворастящите лози се срещат във всички райони до 600-700 м. надморска височина. Терените, на които расте дивата лоза, са много разнообразни – от равнинни до хълмисти и планински (Ковачев, 1979, Катеров и др., 2004). Това е предпоставка за отбор на форми със стопански ценни качества, които имат важно значение за селекцията и практиката, като повишена устойчивост на ниски зимни температури и засушаване, на болести и

## SUMMARY

Wild grapes are being studied in more than a century. In Bulgaria meet ubiquitous along the Danube, the Black Sea coast, the Struma river valley, Veleka River, Ropotamo river, Western Rodopi mountain, Pirin mountain, etc. (Negrul et al., 1965). Wild grapes are found in all areas to 600-700 m altitude.

Terrains that grow wild grapes are very diverse- from flat to hilly and mountainous (Kovatchev, 1979, Katerov et al., 2004).

Thus is a prerequisite for selection of forms with economically valuable qualities that are important for the breeding and practice as increased resistance to low winter temperatures and drought, tolerance to diseases and pests, and

*European Journal of Sustainable Development* (2017), **6**, 1, 261-266 ISSN: 2239-5938
*Doi: 10.14207/ejsd.2017.v6n1p261*

# Future Challenges for the Breeders Struggling the Climatic Changes

Badjakov I.[1], Tsvetkov I.[1], Dincheva I.[1], Kondakova V.[1], Atanassov A.[2]

**Abstract**
The Climate Change is one of the major problems in the entire world. The limiting influence of harmful factors as climate and other environmental changes, it will be crucial to breed new varieties for improved resistance to abiotic and biotic stresses. Climate Change can be tackled through plant breeding and better use of Plant Genetic Resources. Generally, plant breeding is the art and science of genetically improving plants for the benefit of humankind. It is important to mention potential contribution of organically managed systems to climate change mitigation is identified in the careful management of nutrients and, hence, the reduction of N2O emissions from soils.
The application of biotechnological approaches, developing and implementation of new "omics" technologies and infrastructure construction on a high scientific level will help in building a strategy to overcome the damage of Climate Change.

*Key words: Climate Change, plant breeding, organic agriculture, plant biotechnology, "OMICS" technologies.*

## 1. Plant breeding in the context of the climatic changes

Nowadays one of the major problems widely recognized in the entire world is Climate Change. The Climate Change creates a serious threatening to push up the number of hungry people which is a result of negative impacts on food production. At the same time the growing global population will create an increased demand which will need to be produced on not much more land, using less water, fertilizer and pesticides (Fischer et al. 1994).
Looking at global issues we must be tackled correctly and in series with other global challenges of climate change as cleaner sustained energy and water security. Projections indicate that by 2030 the world will need to produce 50 percent more food, 50 percent more energy and 30 percent more fresh water and adapting to Climate Changes (Rosenzweig and Iglesias 1994). Some analysts of Climate Change explaining that if global temperatures do not increase more than 6°C over the next century, agricultural production can probably adapt to changes in mean global temperature using breeding, selection and management approaches.
The impact of Climate Change on production of various crops varies markedly depending mainly on the region, growing season, the crops and their temperature thresholds. To minimize the impacts of these climate and other environmental changes, it will be crucial to breed new varieties for improved resistance to abiotic and biotic stresses (Rosenzweig and Parry 1994). One of the major objectives for breeding research,

[1]AgroBioInstitute, Sofia, Bulgaria; www.abi.bg
[2]Joint Genomic Centre, Sofia, Bulgaria; www.jgc-bg.org;

*Journal of Mountain Agriculture on the Balkans, 2017, 20 (1), 227-250*
*Research Institute of Mountain Stockbreeding and Agriculture, Troyan*
ISSN 1311-0489 (Print)
ISSN 2367-8364 (Online)

# Предимства на микоризните гъби *Glomus* spp. за усвояване на хранителни вещества, биоконтрол и микробна екология при лозата

Иван Цветков[1*], Лилия Георгиева[1], Деница Цветкова[3], Виктория Михайлова[4], Диян Георгиев[2]

*[1]Агробиоинститут Агробиоинститут, бул. Драган Цанков 8, 1164 София, България*
*[2]Институт по планинско животновъдство и земеделие, ул. Васил Левски 281, 5600 Троян, България*
*[3]Лесотехнически университет, бул. Климент Охридски 10, 1756 София, България*
*[4]Химикотехнологичен и металургичен университет, бул. Климент Охридски 8, 1756 София, България*
**E-mail: ivantsvetkov@abi.bg*

# Benefits of the micorrhizal fungi Glomus spp. for grapevine nutrient uptake, biocontrol and microbial ecology

Ivan Tsvetkov[1*], Liliya Georgieva[1], Denitsa Tsvetkova[3], Victoria Michailova[4], Diyan Georgiev[2]

*[1]Agrobioinstitute, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria*
*[2]Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Str., 5600 Troyan, Bulgaria*
*[3]University of Forest, 10 Kliment Ohridski Blvd., 1756 Sofia, Bulgaria*
*[4]University of Chemical Technology and Metallurgy, 8 Kliment Ohridski Blvd., 1756 Sofia, Bulgaria*

## РЕЗЮМЕ

*Glomus* spp. е най-големият род от арбускуларните микоризни гъби (AM). Всички видове от този род имат способности да формират симбиотични отношения с корените на растенията. В зависимост от плътността, тази симбиоза може да има значителен принос за растежа и усвояването на хранителни вещества при растенията. Основният ефект на *Glomus* spp. при храненето е резултат от хифалното транспортиране на неподвижни минерални йони. Това е важно предимство, особено за бавно дифундиращи минерални йони като

## SUMMARY

*Glomus* spp. is the largest genus of the arbuscular mycorrhizal fungi (AM). All of the species of this genus has abilities to form symbiotic relationships with plant roots. Depends of the density, this symbiosis could makes a significant contribution to the plant growth and nutrient uptake. The major effect of *Glomus* spp. on the nutrition is result from the hyphal transport of immobile mineral ions. It is important advantage especially for the slowly diffusing mineral ions such as phosphorus. The AM mycorrhizal fungi

*Journal of Mountain Agriculture on the Balkans, 2017, 20 (1), 267-277*
*Research Institute of Mountain Stockbreeding and Agriculture, Troyan*
ISSN 1311-0489 (Print)
ISSN 2367-8364 (Online)

# Влияние на генотипа върху развитието на болестния процес при български популации от блатно кокиче (*Leucojum aestivum* L.)

Лилия Георгиева[1]*, Виолета Кондакова[1], Иван Цветков[1], Мария Стоянова[2], Мария Георгиева[3]

*[1]Агробиоинститут Агробиоинститут, бул. Драган Цанков 8, 1164 София, България*
*[2]Институт по почвознание, агротехнологии и защита на растенията "Никола Пушкаров", Шосе "Банкя" 7, 1080 София, България*
*[3]Институт по планинско животновъдство и земеделие, ул. "Васил Левски" 281, 5600 Троян, България*
**E-mail: lilka81@abv.bg*

# Influence of genotype on the development of the disease process in Bulgarian populations of summer snowflake (*Leucojum aestivum* L.)

Liliya Georgieva[1]*, Violeta Kondakova[1], Ivan Tsvetkov[1], Mariya Stoyanova[2], Maria Georgieva[3]

*[1]Agrobioinstitut, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria*
*[2]Institute of Soil Science, Agro-technology and plant protection "Nikola Pushkarov", 7 Shousse Bankya Str., 1080 Sofia, Bulgaria*
*[3]Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Str., 5600 Troyan, Bulgaria*

## РЕЗЮМЕ

Блатното кокиче е ценен, застрашен вид, от който се произвежда българското лекарство Нивалин®. Проведени са изкуствени заразявания в стерилни условия на *in vitro* луковички от 6 естествени български популации на блатно кокиче с 4 бактериални изолата от луковици със симптоми на гниене. Проследена е появата на симптоми на 3-ти, 6-ти и 9-ти ден след заразяването. Изчислен е процентът заразени луковички от всеки изолат. Резултатите показват,че проявата на вирулентност на патогена е в зависи-

## SUMMARY

Summer snowflake is a valuable, endangered species, from which the Bulgarian drug Nivalin® is produced. The artificial infections were carried out in sterile conditions on *in vitro* bulbs from six natural populations of Bulgarian summer snowflake with 4 bacterial isolates from bulbs with symptoms of decay. The symptoms were traced on the 3rd, 6th and 9th day after infection. The percentage of infected bulbs from each isolate was calculated. The results showed that the expression of virulence of the pathogen is dependent on the genotype of the plants,

*Journal of Mountain Agriculture on the Balkans, 2017, 20 (1), 251-258*
*Research Institute of Mountain Stockbreeding and Agriculture, Troyan*
ISSN 1311-0489 (Print)
ISSN 2367-8364 (Online)

# Усвояване на минерални елементи и натрупване на сухо вещество при микоризирани разсадникови растения Гизела 6/Ван

Иван Цветков[1]*, Евлоги Марков[2], Теодора Джамбазова[1], Диян Георгиев[3]

*[1]Агробиоинститут, бул. Драган Цанков 8, 1164 София, България*
*[2]Институт по почвознание, агротехнологии и защита на растенията "Н. Пушкаров", ул. Шосе Банкя 7, 1331 София, България*
*[3]Институт по планинско животновъдство и земеделие, ул. "Васил Левски" 281, 5600 Троян, България*
**E-mail: ivantsvetkov@abi.bg*

# Mineral elements uptake and dry matter accumulation in mycorrhizated nursery plants Gisela 6/Van

Ivan Tsvetkov[1]*, Evlogi Markov[2], Teodora Dzhambazova[1], Diyan Georgiev[3]

*[1]Agrobioinstitute, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria*
*[2]Institute of Soil Science, Agrotechnologies and Plant Protection "N. Pushkarov", 7 Shausee Bankya Str., 1331 Sofia, Bulgaria*
*[3]Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Str., 5600 Troyan, Bulgaria*

## РЕЗЮМЕ

Целта на изследването е да се проучи влиянието на различни микоризни гъби върху усвояването на минерали и натрупването на сухо вещество при разсадникови овощни растения Гизела 6/Ван. Натрупването на сухо вещество е анализирано два пъти през вегетационния период (през май и октомври). Паралелно са проведени анализи по отношение усвояването и разпределението на шест основни макро и микроелементи (N, P, K, Ca, Mg, Fe) от експерименталните растения. Получените резултати показват, че по отношение на свежо тегло Glomus варианта изпреварва контрола 1 с 14,47% и контрола 2 с 22,60%. По

## SUMMARY

The aim of the study is to investigate the influence of different mycorrhizal fungi on the absorption of minerals and accumulation of dry matter in nursery fruit plants Gisela 6 / Van. The accumulation of dry matter was analyzed twice during the vegetation period (May and October). Parallel analyzes were conducted regarding the mineral uptake and distribution of six major macro- and microelements (N, P, K, Ca, Mg, Fe) from the experimental plants. Obtained results shows that concerning fresh weight *Glomus* variant overrun Control 1 with 14,47% and Control 2 with 22,60%.

In case of the dry weight the same

*Journal of Mountain Agriculture on the Balkans, 2017, 20 (1), 259-266*
*Research Institute of Mountain Stockbreeding and Agriculture, Troyan*
ISSN 1311-0489 (Print)
ISSN 2367-8364 (Online)

# Афинитет и динамика на растеж на селектирана форма дива лоза (*Vitis vinifera* ssp. *sylvestris*) присадена върху различни подложки

Иван Цветков[1*], Мария Георгиева[2], Теодора Джамбазова[1]

[1]*Агробиоинститут, бул. Драган Цанков 8, 1164 София, България*
[2]*Институт по планинско животновъдство и земеделие, ул. "Васил Левски" 281, 5600 Троян, България*
**E-mail: ivantsvetkov@abi.bg*

# Affinity and growth dynamic of selected wild grape form (*Vitis vinifera* ssp. *sylvestris*) grafted on different rootstocks

Ivan Tsvetkov[1], Maria Georgieva[2], Teodora Dzhambazova[1]

[1]*Agrobioinstitute, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria*
[2]*Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Str., 5600 Troyan, Bulgaria*

## РЕЗЮМЕ

Загубата на биологично разнообразие е основен проблем на лозарството в света. Последният доклад на Осмата сесия на работната група на Междуправителствената Техническа Работна Група за Растителни Генетични Ресурси показва, че само 8-9 процента от наличните световни сортове се използват за производство грозде и вино (FAO, 2016). Основният път за решението на този нарастващ проблем е подобряването на генетичните и селекционните програми при лозата, чрез ефективно използване на генетичния потенциал на непроученото местно биоразнообразие (EC, 2013). В случая на дивата лоза, друг съществен проблем е загубването на вече маркирани индивидуални форми поради климатични промени, урбанизация или промяна на статуса на естествените им местообитания. Това предполага търсене на

## SUMMARY

Biodiversity loss is a major problem in viticulture worldwide. The last report of the Eighth Session of the Intergovernmental Technical Working Group on Plant Genetic Resources showed that only 8-9 percent of the available world varieties are used for grape and wine production (FAO, 2016).

Principal way to the resolution of this growing problem is the improvement of the grapevine genetic and breeding programs by effective use of the genetic potential of unexplored local biodiversity (EC, 2013).

In the case of wild grape, another significant problem is the loss of already tagged individual forms due to climate change, urbanization or change the status of their natural habitats. This assumes finding ways for initial preservation and

*GMOs in Integrated Plant Production*
*IOBC-WPRS Bulletin Vol. 114, 2016*
*pp. 37-38*

# Impact of *Bt* crops on non-target organisms – 3 systematic reviews

**Michael Meissle[1], Jörg Romeis[1], Judith Riedel[1], Steve E. Naranjo[2], Kaloyan Kostov[3], Petya Christova[3], Borislav Assenov[3], Ivan Tsvetkov[3], Slavtcho Slavov[3], Christian Frølund Damgaard[4], Paul Henning Krogh[4], Niels Bohse Hendriksen[5], Jeremy Sweet[6]**
*[1]Agroscope, Institute for Sustainability Sciences, Zurich, Switzerland; [2]USDA-ARS, Arid-Land and Agricultural Research Center, Maricopa, AZ, USA; [3]AgroBioInstitute, Sofia, Bulgaria; [4]Department of Bioscience, Aarhus University, Silkeborg, Denmark; [5]Department of Environmental Science, Aarhus University, Roskilde, Denmark; [6]Sweet Environmental Consultants, Cambridge, UK*
*E-mail: michael.meissle@agroscope.admin.ch; kkostov@abi.bg*

**Key words:** *Bacillus thuringiensis*, evidence synthesis, field studies, arthropods, soil invertebrates, microorganisms, microbial endpoints.

The cultivation of genetically modified (GM) crops producing Cry toxins, originating from the bacterium *Bacillus thuringiensis* (*Bt*), has raised environmental concerns over their sustainable use and consequences for biodiversity and ecosystem services in agricultural land. During the last two decades, a range of biosafety research programmes in Europe and elsewhere conducted experimental field studies to investigate potential effects of GM crops on non-target organisms. In the EU project GRACE (GMO Risk Assessment and Communication of Evidence) available evidence from those field studies was reviewed following a predefined systematic review process (Kohl *et al*., 2013).

The first systematic review covered *Bt* maize and non-target animals (mainly invertebrates) including field margin populations. The review question was "Does the growing of *Bt* maize change abundance or ecological function of non-target animals compared to the growing of non-GM maize?" Details of the methods used in this review are published in a peer-reviewed protocol (Meissle *et al.*, 2014).

The second review considered field studies that compared the abundance and diversity of soil inhabiting invertebrates, such as Protozoa, Nematoda, Collembola, mites, enchytraeids and earthworms. The systematic review protocol was published by Kostov *et al*. (2014a) and focuses on the question "Are population abundances and biomasses of soil invertebrates changed by *Bt* crops compared with conventional crops?"

The third systematic review examined abundance, activity and functional and genetic diversity of microorganisms, including bacteria and fungi, in *Bt* and conventional crops. The review question was "Are soil microbial endpoints changed by *Bt* crops compared with conventional crops?" and the protocol was published by Kostov *et al.* (2014b).

Stakeholder involvement was an important element in the GRACE project. During two workshops and several online consultations, input from stakeholders from science, regulatory authorities, and civil society was obtained at different stages of the review process: 1) in the prioritisation and formulation of the review questions; 2) before the draft review protocols were submitted; and 3) when results were available.

The systematic reviews were performed in transparent and reproducible manners following a uniform approach (Kohl *et al*., 2013). Literature was searched through a range of bibliographic databases, with full text search engines, and on relevant webpages. Identified

# 10

# Modern Biotechnologies and Phytonutritional Improvement of Grape and Wine

*Atanas Atanassov[1], Teodora Dzhambazova[2], Ivanka Kamenova[2], Ivan Tsvetkov[2], Vasil Georgiev[3], Ivayla Dincheva[2], Ilian Badjakov[2], Dasha Mihaylova[4], Miroslava Kakalova[4], Atanas Pavlov[4] and Plamen Mollov[4]*

[1] *JGC, Sofia, Bulgaria*
[2] *Agrobioinstitute, Sofia, Bulgaria*
[3] *Center for Viticulture and Small Fruit Research, College of Agriculture and Food Sciences, Florida A & M University, Tallahassee, FL, USA*
[4] *University of Food Technologies, Plovdiv, Bulgaria*

## 10.1 Grape Genomics

Grapevine (*Vitis vinifera*) is the one of the most important fruit species both globally and economically, linked to agricultural and religious activities in the earliest writings and chronicles (Vivier and Pretorius 2000, 2002). Worldwide, 7.6 million hectares were reported to be used for grape-growing providing the yearly production of approximately 70 million tons of grapes for food, beverages, cosmetics, pharmaceutical and nutraceutical sectors (FAO 2012). Because of its nutritional and health promoting properties, grape was amongst the first fruits domesticated and cultivated by mankind.

Agricultural practise, developed for commercial grape varieties, is used to improve grape quality and yield. However, the achieved advances are at the expense of higher costs and unsustainable viticulture, that involves increased use of pesticides, fertilisers and mechanisation, contributing to the degradation of natural environment.

In the era of global climate change as a result of continuous globalisation and industrialisation, the environment is highly endangered and the scientists have focused their attention to sustainable agriculture directed to preserving the nature. To accomplish this, the main step is obtaining of deep knowledge of grape biology, which can be used to achieve the desired viticultural improvements. The main idea is that studying the grape genome will reveal the role of those genes, crucial for viticulture, that could be used for improvement of grape individual characteristics and the quality of wine. Knowing the genes responsible for the ripening, sugar accumulation , aroma and flavour compounds synthesis will help the growers to balance these quality factors and to produce more consistent wines of excellent quality. Till now, the knowledge about genetic background of important agronomical traits still remains poor.

However, the recent sequencing of grape genome offers a good base for exploring the genes that determine certain traits.

*Phytonutritional Improvement of Crops*, First Edition. Edited by Noureddine Benkeblia.
© 2017 John Wiley & Sons Ltd. Published 2017 by John Wiley & Sons Ltd.

BIOTECHNOLOGY & BIOTECHNOLOGICAL EQUIPMENT, 2018
https://doi.org/10.1080/13102818.2018.1427509

Taylor & Francis
Taylor & Francis Group

REVIEW; AGRICULTURE AND ENVIRONMENTAL BIOTECHNOLOGY

OPEN ACCESS Check for updates

# Plant organic farming research – current status and opportunities for future development

Ivan Tsvetkov[a], Atanas Atanassov[b], Mariana Vlahova[b], Lucien Carlier[c], Nikolai Christov[a], Francois Lefort[d], Krasimir Rusanov[a], Ilian Badjakov[a], Ivayla Dincheva[a], Mark Tchamitchian[e], Goritsa Rakleova[b], Liliya Georgieva[a], Lucius Tamm[f], Anelia Iantcheva[a], Joelle Herforth-Rahmé[f], Epaminondas Paplomatas[g] and Ivan Atanassov[a]

[a]AgroBioInstitute, Agricultural Academy, Sofia, Bulgaria; [b]Joint Genomic Center, Sofia University 'St. Kliment Ohridski', Sofia, Bulgaria; [c]Plant Sciences Department, Institute for Agricultural and Fisheries Research, Merelbeke, Belgium; [d]University of Applied Sciences and Arts Western Switzerland, Delemont, Switzerland; [e]INRA French National Institute for Agricultural Research, Avignon, France; [f]FiBL Forschungsinstitut für biologischen Landbau, Frick, Switzerland; [g]Faculty of Crop Sciences, Agricultural University of Athens, Athens, Greece

**ABSTRACT**

This paper reviews the recent development of the scientific, legislative, economic and environmental aspects of plant organic farming. The impact of organic farming on biodiversity and soil fertility is discussed in comparison with conventional systems. A significant barrier for wide application and future development of organic farming is the existing diversity of national and international policy instruments in this sector. Special attention is paid to up-to-date research techniques that could help solve a number of the problems typically faced in plant organic farming. It is argued that organic farming is still not productive enough to be considered fully sustainable. This underlines the necessity of strong support for more effective implementation of scientific research innovations and improvement of the networking between all stakeholders – organic producers, scientists and corresponding policy makers at the national and international level.

**ARTICLE HISTORY**
Received 12 October 2017
Accepted 10 January 2018

**KEYWORDS**
Organic farming; conventional farming; biodiversity; biocontrol; soil fertility; scientific innovations; sustainable agriculture

## Introduction

The need of continuously raising the yield, relevant to the continuously increasing market demand, has led to inordinate utilization of the natural and non-renewable resources and energy. Conventional farming in the face of the Green-revolution system allows the agricultural production capacity to be significantly increased, leading to the highest possible yield per hectare. The improvement of soil fertility through additional application of fertilizers and plant protection chemicals, as well as the utilization of modern agricultural machineries for tilling, irrigation, sowing and crops harvesting, could reach the most remarkable economic benefits on the farm level and thus to provide food production necessary for the fast growing human population [1–4]. The high yield per hectare and good quality products for the market have given farmers the guarantee of a secure income and consumers the availability of a large spectrum and choice of agricultural products at a reasonable price, independent of the season. The overproduction of different foodstuffs, e.g. sugar, wheat, milk, meat etc., is the luxury problem of the consumerist society. Most of the agricultural foodstuffs on the market in developed countries are mainly produced by such intensive agriculture systems. The excessive use of synthetic chemicals, which vastly contaminate the environment, as well as the mechanical soil disturbance and irrigation, have led to a generation of resistant insects, fungi, weeds, etc., accumulation of chemicals in crops and soil, pollution of water and air and consequently contribute to some extent to the greenhouse effect and global warming [5].

To set boundaries to this method of production, the European Commission initiated a quota regulation system with a guaranteed price for a limited production of milk and sugar beet per farm [6]. In the former Common Agricultural Policy (CAP) this quota system was coupled with the set-aside of arable land [7]. The European Commission enacted several regulations and directives to oblige governmental authorities of the member countries and also to motivate farmers in changing their attitude and to pay more attention in respect to the quality of life and preservation of environment. Council Directive 75/440/EEC determined the maximum allowed nitrate concentration in drinking water, while Directive 91/676/EEC refers to the water protection against the pollution with nitrates from agriculture). The regulation

CONTACT Ivan Tsvetkov ivantsvetkov@abi.bg

© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.