

BOOK OF PROCEEDINGS

**Sixth International Scientific Agricultural Symposium
“Agrosym 2015”**

AGROSYM 2015



Jahorina, October 15 - 18, 2015

Impressum

Sixth International Scientific Agricultural Symposium „Agrosym 2015“

Book of Proceedings

Published by

University of East Sarajevo, Faculty of Agriculture, Republic of Srpska, Bosnia
University of Belgrade, Faculty of Agriculture, Serbia
Mediterranean Agronomic Institute of Bari (CIHEAM - IAMB) Italy
International Society of Environment and Rural Development, Japan
Balkan Environmental Association, B.EN.A, Greece
University of Applied Sciences Osnabrück, Germany
Selçuk University, Turkey
Perm State Agricultural Academy, Russia
Biotechnical Faculty, University of Montenegro, Montenegro
Institute for Science Application in Agriculture, Serbia
Institute of Lowland Forestry and Environment, Serbia
Institute of Forestry, Podgorica, Montenegro
Academy of Engineering Sciences of Serbia, Serbia
Agricultural Institute of Republic of Srpska - Banja Luka, Bosnia and Herzegovina
Maize Research Institute „Zemun Polje“ Serbia
Balkan Scientific Association of Agricultural Economics, Serbia
Institute of Agricultural Economics, Serbia

Editor in Chief

Dusan Kovacevic

Technical editors

Sinisa Berjan
Milan Jugovic
Velibor Spalevic
Noureddin Driouech
Rosanna Quagliariello

Website:

<http://www.agrosym.rs.ba>

CIP - Каталогизација у публикацији

Народна и универзитетска библиотека
Републике Српске, Бања Лука

631(082)(0.034.2)

INTERNATIONAL Scientific Agricultural Symposium "Agrosym
2015" (6 ; Jahorina)

Book of proceedings [Elektronski izvor] / Sixth International
Scientific Agricultural Symposium "Agrosym 2015", Jahorina,
October 15 - 18, 2015 ; [editor in chief Dušan Kovačević]. - East
Sarajevo =Istočno Sarajevo : Faculty of Agriculture =Poljoprivredni
fakultet, 2015. - 1 elektronski optički disk (CD-ROM) : tekst, slika ;
12 cm

CD ROM čitač. - Nasl. sa nasl. ekrana. - Bibliografija uz svaki rad. -
Registar.

ISBN 978-99976-632-2-1

COBISS.RS-ID 5461016

**Sixth International Scientific Agricultural Symposium “Agrosym 2015”
Jahorina, October 15-18, 2015, Bosnia and Herzegovina**

HONORARY COMMITTEE

STEVO MIRJANIC, Minister of Agriculture, Water Management and Forestry of Republic of Srpska, Bosnia; JASMIN KOMIC, Minister of Science and Technology of Republic of Srpska, Bosnia; DANE MALESEVIC, Minister of Education and Culture of Republic of Srpska, Bosnia; RADOSLAV GRUJIC, Rector of the University of East Sarajevo, Bosnia; MILICA PETROVIC, Dean of the Faculty of Agriculture, University of Belgrade, Serbia; COSIMO LACIRIGNOLA, Director of the Mediterranean Agronomic Institute of Bari, (Italy) and Secretary General of the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM), Italy; MARIO T. TABUCANON, President of the International Society of Environment and Rural Development, Japan; FOKION K. VOSNIAKOS, President of the Balkan Environmental Association (B.EN.A), Greece; BERND LEHMANN, Vice-President of the University of Applied Sciences Osnabruck, Germany; HAKKI GOKBEL, Rector of the Selcuk University, Turkey; IURII ZUBAREV, Rector of the Perm State Agricultural Academy, Russia; MIOMIR JOVANOVIC, Dean of the Biotechnical Faculty, University of Podgorica, Montenegro; SNEZANA JANKOVIC, Director of the Institute for Science Application in Agriculture, Serbia; SASA ORLOVIC, Director of the Institute of Lowland Forestry and Environment, Serbia; BRANKO KOVACEVIC, President of the Academy of Engineering Sciences of Serbia, Serbia; VOJISLAV TRKULJA, Director of Agricultural Institute of Republic of Srpska - Banja Luka, Bosnia and Herzegovina; BRANKA KRESOVIC, Director of the Maize Research Institute “Zemun Polje”, Serbia; JONEL SUBIC, Director of the Institute of Agricultural Economics, Serbia

SCIENTIFIC COMMITTEE

DUSAN KOVACEVIC, Faculty of Agriculture, University of Belgrade, Serbia; WILLIAM MEYERS, Howard Cowden Professor of Agricultural and Applied Economics, University of Missouri, USA; JOHN BRAYDEN, Norwegian Agricultural Economics Research Institute (NILF), Norway; STEVE QUARIE, Visiting Professor, School of Biology, Newcastle University, United Kingdom; ATEF HAMDY, Emeritus Professor, Land and Water Resources Department; IAMB, Italy; DANI SHTIENBERG, full professor, Department of Plant pathology and Weed Research, ARO, the Volcani Center, Bet Dagan, Israel; THOMAS G. JOHNSON, University of Missouri – Columbia, USA; DIETER TRAUTZ, University of Applied Science, Germany; MACHITO MIHARA, Tokyo University of Agriculture, Japan; MARKUS SCHERMER, Department of Sociology, University of Innsbruck, Austria; SERGEI ELISEEV, Vice-Rector for Research and Innovations, Perm State Agricultural Academy, Russia; NOVO PRZULJ, Faculty of Agriculture, University of Banjaluka, Bosnia and Herzegovina; FOKION VOSNIAKOS, Balkan Environmental Association (B.EN.A), Greece; ADRIANO CIANI, Department of Agricultural, Foods and Environmental Sciences, Perugia University, Italy; MATTEO VITTUARI, Faculty of Agriculture, University of Bologna, Italy; VELIBOR SPALEVIC, Institute of Forestry, Montenegro; REGUCIVILLA A. POBAR, Bohol Island State University, Philippines; SUDHEER KUNDUKULANGARA PULISSERY, Kerala Agricultural University, India; EPN UDAYAKUMARA, Faculty of Applied Sciences, Sabaragamuwa University, Sri Lanka; VLADIMIR SMUTNÝ, full professor, Mendel University, Faculty of agronomy, Czech Republic; FRANC BAVEC, full professor, Faculty of Agriculture and Life Sciences, Maribor, Slovenia; NICOLAE ISTUDOR, full professor, Academy of Economic Studies, Bucharest, Romania; JAN MOUDRÝ, full professor, Faculty of Agriculture, South Bohemia University, Czech Republic; STEFAN TYR, full professor, Faculty of Agro-biology and Food Resources, Slovakia; NATALIJA BOGDANOV, Faculty of Agriculture, University of Belgrade, Serbia; SABAHUDIN BAJRAMOVIC, Faculty of Agriculture and Food Sciences, University of Sarajevo, Bosnia; FRANCESCO PORCELLI, University of Bari Aldo Moro, Italy; VASILJE ISAJEV, Faculty of Forestry, University of Belgrade, Serbia; ELAZAR FALLIK, Agricultural Research Organization (ARO), Volcani, Israel; JUNAID ALAM MEMON, Pakistan Institute of Development Economics, Pakistan; HIROMU OKAZAWA, Faculty of Regional Environment Science, Tokyo University of Agriculture, Japan; MLADEN TODOROVIC, Land and Water Resources Department; IAMB, Italy; HAMID EL BILALI, Sustainable Agriculture, Food and Rural Development Department. IAMB, Italy; NOUREDDIN DRIOUECH, Environmental Sciences and Organic Agriculture, IAMB, Italy; LALITA SIRIWATTANANON, Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi (RMUTT), Thailand; ABID HUSSAIN, International Centre for Integrated Mountain Development (ICIMOD), Nepal; AMRITA GHATAK, Gujarat Institute of Development Research (GIDR), India; NASER SABAGHNI, University of Maragheh, Iran; MÁRTA BIRKÁS, full professor, St. Istvan University, Godollo – Hungary; UDAI PRATAP SINGH, Department of Mycology and Plant Pathology, Banaras Hindu University, India; ANDRZEJ KOWALSKI, Director of the Institute for Agricultural and Food Economy, Warszawa-Poland; YALCIN KAYA, The Director of the Plant Breeding Research Center, University of Trakya, Turkey; SANJA RADONJIC, Biotechnical Faculty, University of Montenegro, Montenegro; KOSANA KONSTATINOV, Academy of Engineering Sciences of Serbia, Serbia; SNEZANA MLADENOVIC-

DRINIC, Maize Research Institute “Zemun Polje”, Serbia; NEBOJSA MOMIROVIC, Faculty of Agriculture, University of Belgrade, Serbia; ZORAN JOVOVIC, Biotechnical Faculty, University of Montenegro, Montenegro; VLADIMIR VUKADINOVIC, full professor, Faculty of Agriculture, University of Osijek, Croatia; DANIJEL JUG, associate professor, Faculty of Agriculture, University of Osijek, Croatia; VLADO KOVACEVIC, full professor, Faculty of Agriculture, University of Osijek, Croatia; MILAN MARKOVIC, Department for Animal husbandry, Biotechnical Faculty, University of Montenegro, Montenegro.

ORGANIZATION COMMITTEE

VESNA MILIC, Dean of the Faculty of Agriculture, University of East Sarajevo, Bosnia; STEVAN TRBOJEVIC, Vice rector of the University of East Sarajevo, Bosnia; DEJAN BOKONJIC, Vice rector of the University of East Sarajevo, Bosnia; ZELJKO DOLJANOVIC, Faculty of Agriculture, University of Belgrade, Serbia; ROBERTO CAPONE, Mediterranean Agronomic Institute of Bari, Italy; ROSANNA QUAGLIARIELLO, Mediterranean Agronomic Institute of Bari, Italy; NOUREDDIN DRIOUECH, Coordinator of MAIB Alumni Network (FTN), Mediterranean Agronomic Institute of Bari, Italy; ALEKSANDRA DESPOTOVIC, Biotechnical Faculty Podgorica, University of Montenegro, Montenegro; MILIC CUROVIC, The journal “Agriculture and Forestry”, Biotechnical Faculty Podgorica, University of Montenegro, Montenegro; SLADJAN STANKOVIC, Institute for Science Application in Agriculture, Serbia; SRDJAN STOJNIC, Institute of Lowland Forestry and Environment, Serbia; OKSANA FOTINA, International Relations Center, Perm State Agricultural Academy, Russia; MORTEZA BEHZADFAR, Tarbiat Modares University, Tehran, Iran; ULRIKE SCHLIEPHAKE, Dipl. agr.oec., University of Applied Science, Germany; BILJANA GRUJIC, Institute of Agriculture Economics, Serbia; GORAN PERKOVIC, Faculty of Agriculture, University of East Sarajevo, Bosnia; MIRJANA RADOVIC, Faculty of Agriculture, University of East Sarajevo, Bosnia; MILAN JUGOVIC, Faculty of Agriculture, University of East Sarajevo, Bosnia; SINISA BERJAN, Faculty of Agriculture, University of East Sarajevo, Bosnia, secretary

Original scientific paper

10.7251/AGSY1505238K

THE EFFECT OF PLANT GROWTH REGULATORS ON MORPHOGENESIS IN TISSUE CULTURE OF SOME AGRICULTURE SPECIES

Liljana KOLEVA GUDEVA, Fidanka TRAJKOVA

Goce Delcev University, Faculty of Agriculture, Department of Plant Biotechnology,
Krste Misirkov, No.10-A, P.O. Box 201, Stip 2000, Republic of Macedonia

*Corresponding author: liljana.gudeva@ugd.edu.mk

Abstract

The vegetative propagation of the plants in *in vitro* conditions enables to abbreviate the process of selection, enhance the genetic stability of plants and improve the production of healthy plants free of viruses.

In this paper the results of our experimental work for determination of the potential of *in vitro* morphogenesis and micropropagation of some agricultural species are presented (*Apium graveolens* L., *Daucus carota* subsp. *sativus* L., *Cucumis sativus* L., *Cucurbita pepo* var. *cylindrica*, *Raphanus sativus* var. *radicola*, and important agricultural species from *Apiaceae*, *Brassicaceae*, *Cucurbitaceae* and *Solanaceae* family). Different initial explants were cultivated *in vitro* on various media supplied by different combinations and concentration of plant growth regulators. The main objective of this research was to set up meristem tissue culture and non-meristem explants, to explore the properties of the tissues *in vitro*, and to observe their possibilities for morphogenesis and micropropagation.

Keywords: *in vitro*, vegetative propagation, phytohormones, regeneration, micropropagation.

Introduction

Biotechnological approaches like micropropagation, somaclonal variation, *in vitro* conservation, protoplast fusion, and development of novel transgenic plants have great potential in conservation, utilization and increasing the production of spices. Efficient micropropagation systems are available for many spices which are being used for propagation, conservation, safe movement and exchange of germplasm, crop improvement through somaclonal variation and transgenic pathways (Babu et al., 2015).

At the peak of the plant tissue culture era in the 1980s, in a relatively short time, many commercial laboratories were established around the world to capitalize on the potential of micropropagation for mass production of clonal plants for the horticulture industry. Today plant tissue culture applications encompass much more than clonal propagation (Akin-Idowu et al., 2009).

Micropropagation has been employed a large scale production of disease free planting material and germplasm conservation. High rate of multiplication coupled with the additional advantage of obtaining diseases free planting material makes micropropagation a viable alternative to a conventional propagation.

Morphogenesis in plants is a complex phenomenon and is being regulated by numerous factors and in-between relationships of plant organs, tissues and cells also. The correlation between cells tissues and organs of a plant plays important role in the growth and development in *in vivo* and in *in vitro* conditions. The study of such complex system can be simplified with cell, tissue or organ isolation and their cultivation *in vitro*. In such conditions, the influence of certain factors on the organogenesis and differentiation of the plant tissue can be traced (Koleva Gudeva and Trajkova, 2012). Even today much more is not fully understood about micropropagation, and morphogenesis in tissue culture, and although general guidelines for micropropagation have been established, each plant species is unique.

Despite these problems, there are a large number of species being micropropagated on a commercial scale throughout the world (Kitto, 1997).

This paper surveys the effect of plant growth regulators as an important role in the plant morphogenesis of some important agricultural species from *Apiaceae*, *Brassicaceae*, *Cucurbitaceae* and *Solanaceae*, as a main factor for successful commercial micropropagation.

Material and methods

This study was conducted in the Laboratory for Plant Biotechnology at Faculty of Agriculture, Goce Delchev University-Stip, Republic of Macedonia during the period 2010-2015. The plant material used for isolation of the initial explants, was sterilized in the following manner: rinsing with tap water, then with distilled water, followed by 15-20 seconds in 70% C₂H₅OH, Tween 20 enriched, 10-15 minutes in 5% Ca(ClO)₂ enriched with Tween 80, and on the end the explants were rinsed few times with sterile water. Sterilized initial explants were cultivated on MS medium in which various concentrations and combinations of plant hormones were added.

Plant growth medium ingredients

In the following tables is shown that all plant explants except pepper anthers were cultivated on either on MS (Murashige and Skoog, 1962) or LS (Linsmaer and Skoog, 1965) media containing 3% sucrose, 0,7% agar, 100 mg·l⁻¹ inositol, 200 mg·l⁻¹ casein hydrolysate, 0,1 mg·l⁻¹ B1, 1,0 mg·l⁻¹ B6 and 0,5 mg·l⁻¹ nicotinic acid. Different phytohormones were used in a medium such as: IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), NAA (α -naphthaleneacetic acid), BAP (6-benzylaminopurine), BA (N⁶-benzyladenine), KIN kinetin (6-furfuryl aminopurine), ZEA zeatin (N⁶-4 hydroxyl-3-methyl-trans-2butenyl ananopurine), 2iP (N⁶-2-isopentyl adenine) and 2,4 D (2,4-dichlorophenoxy acetic acid).

In order to favorite the microtuberisation of potatoes the medium for cultivation the nodal explant from *Solanum tuberosum* L. was supplemented also with different concentrations of sucrose (30, 60 and 90g/l) (Anoop et al., 2009; Dieme et al., 2013)

The androgenetic potential of the pepper was examined on medium developed by Dumas de Valux et al. (1981) method for pepper anther culture.

Growth conditions

Apical buds, cotyledons and hypocotyls from the species under research originated from plant seeds germinated on basal media. All the explants and their subculture to a new medium supplemented by same or different plant hormone were cultivated in climate chamber with controlled conditions and temperature of 25±2°C, photoperiodic of 16/8 light/dark, 50% relative humidity, and 50 μ mol·m⁻²·s⁻¹ light intensity. The growth conditions for examination of morphogenetic potential in pepper anther culture and the ability for androgenesis and/or somatic embryogenesis was set up according the method of Dumas de Valux et al. (1981).

Results and discussion

Micropropagation of some species from *Apiaceae* family

The species from family *Apiaceae* are a well-known source of many important herbal products. A number of research studies focus on the biosynthesis of secondary metabolites in *in vitro* plant cultures. There are also numerous reports concerning the methods of plant micropropagation, especially using somatic embryogenesis (Ekiert, 2000). The results from experiment shown in Table 1, confirmed that these species have an excellent potential for micropropagation. Two types of initial explants, apical buds and shoots, were used to set up a tissue culture from celery, carrot and parsley on six different growth mediums. The best potential for morphogenesis of all mediums have the cultures from *Apium graveolens* L.

Micropropagation of some species from *Brassicaceae* family

This family includes many economically important edible and industrial oilseed, vegetable, condiment, and fodder crop. It also includes the molecular plant model, such as *Arabidopsis thaliana* and a rich source of agronomic and economic traits in its highly diverse wild germplasm (Warwick, 2011). Therefore the knowledge about the ability of micropropagation of these economically important species is of great importance for plant biotechnology. In Table 2 are presented the results of morphogenetic potential research of *in vitro* culture of broccoli, cabbage and radish. The best potential for morphogenesis of all examined mediums have the cultures from *Brassica oleracea* var. *italica*.

Table 1. The effect of plant growth regulators on morphogenesis in tissue culture of some species from family *Apiaceae*

Species form fam. <i>Apiaceae</i>	Explant	Medium + mg·L ⁻¹ Growth Regulators	Results	Efficiency
<i>Apium graveolens</i> L.	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+++
		MS + 3 KIN + 3 BAP	shoots	+++
		LS + 3 KIN+2 IAA+2 IBA	shoots	+++
		LS + 5 KIN	shoots	+++
	shots	MS + 1 NAA	roots	+++
		MS + 1 IAA	roots	+++
<i>Daucus carota</i> subsp. <i>sativus</i> L.	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+
		MS + 3 KIN + 3 BAP	shoots	++
		LS + 3 KIN+2 IAA+2 IBA	shoots	++
		LS + 5 KIN	shoots	+++
	shots	MS + 1 NAA	roots	+++
<i>Petroselinum crispum</i> Mill.	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+++
		MS + 3 KIN + 3 BAP	shoots	++
		LS + 3 KIN+2 IAA+2 IBA	shoots	+
		LS + 5 KIN	shoots	+++
	shots	MS + 1 NAA	roots	+++

Table 2. The effect of plant growth regulators on morphogenesis in tissue culture of some species from family *Brassicaceae*

Species form fam. <i>Brassicaceae</i>	Explant	Medium + mg·L ⁻¹ Growth Regulators	Results	Efficiency
<i>Brassica oleracea</i> var. <i>italica</i>	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+++
		MS + 3 KIN + 3 BAP	shoots	+++
		LS + 3 KIN+2 IAA+2 IBA	shoots	+++
		LS + 5 KIN	shoots	+++
	shots	MS + 1 IAA	roots	+++
<i>Brassica oleracea</i> var. <i>capitata</i> L.	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+++
		MS + 3 KIN + 3 BAP	shoots	++
		LS + 3 KIN+2 IAA+2 IBA	shoots	+
		LS + 5 KIN	shoots	+++
	shots	MS + 1 NAA	roots	+++
<i>Raphanus sativus</i> var. <i>radicola</i>	apical buds	MS + 2 KIN + 0.4 NAA	shoots	+++
		MS + 3 KIN + 3 BAP	shoots	++
		LS + 3 KIN+2 IAA+2 IBA	shoots	+++
		LS + 5 KIN	shoots	++

	shots	MS + 1 NAA	roots	++
--	-------	------------	-------	----

Micropropagation of some species form *Cucurbitaceae* family

The *Cucurbitaceae* is a remarkable family with great and proven economic, aesthetic, cultural, medicinal and botanical significance.

Table 3. The effect of plant growth regulators on morphogenesis in tissue culture of some species from family *Cucurbitaceae*

Species form fam. <i>Cucurbitaceae</i>	Explant	Medium + mg·L ⁻¹ Growth Regulators	Results	Efficiency
<i>Cucumis sativus</i> L.	apical buds	MS + 11.0 KIN + 3.5 IBA	shoots	+++
	hypocotyls	MS + 2.0 KIN	callus	+++
	1/3 cotyledons	MS + 6.5 BA+10.0 2,4 D	callus	+++
<i>Cucurbita pepo</i> var. <i>cylindrical</i>	apical buds	MS + 2 KIN + 0,4 NAA	shoots	++
		MS + 3 KIN + 3 BAP	shoots	++
		LS + 3KIN+2 IAA+2 IBA	shoots	+
		LS + 5KIN	shoots	+++

Table 4. The effect of plant growth regulators on morphogenesis in tissue culture of some species from family *Solanaceae*

Species form fam. <i>Solanaceae</i>	Explant	Medium + mg·L ⁻¹ Growth Regulators	Results	Efficiency
<i>Capsicum annuum</i> L.	apical buds	MS + 5.0 BAP + 0.5 NAA	callus	++
		MS + 10.0 BAP + 0.5 IAA	shoots	++
		MS +1.0ZEA	shoots	+
	anthers	CP + 0,01 KIN + 0,01 2,4D	incubation	+
		R ₁ + 0,01 KIN	embryos	++
	hypocotyls 1/3 cotyledons	MS + 30.0 BAP + 1.0 IAA	callus	+
		MS + 10.0 BAP + 0.5 NAA	callus	++
		MS + 5.0 ZEA	callus	+++
		MS + 2.5 2iP	callus	+++
	<i>Lycopersicon esculentum</i> Mill.	apical buds	MS + 6.0 BAP + 0.4 IBA	shoots
MS + 4.5 BAP + 0.3 IBAA			shoots	++
MS + 4.5 KIN + 0.3 IAA			shoots	++
hypocotyls 1/3 cotyledons		MS + 6.0 BAP + 0.4 IBA	Callus	+
		MS + 3.0 KIN + 0.1 IAA	Callus	++
		MS + 1.5 BAP + 0.1 IBA	Callus	+++
<i>Lycopersicon esculentum</i> Mill. var. <i>cerasiforme</i> (Dunal)	apical buds	MS + 2.5 BAP + 1.5 NAA	shoots	++
		MS + 2.0 2iP + 0.5 IAA	shoots	+
		MS + 2.0 BAP + 2.5 2,4 D	shoots	++
		MS + 0.5 KIN + 1.0 IAA	shoots	+++
	hypocotyls	MS + 2.0 2iP + 0.5 IAA	shoots	+
		MS + 2.0 BAP + 2.5 2,4 D	shoots	+
		MS + 2.5 BAP + 1.5 NAA	shoots	+
	1/3 cotyledons	MS + 0.5 KIN + 1.0 IAA	callus	++
		MS + 2.0 2iP + 0.5 IAA	callus	++
		MS + 2.0 BAP + 2.5 2,4 D	callus	+++
MS + 2.5 BAP + 1.5 NAA		callus	+++	
<i>Solanum</i>	sprouts	MS + 0.5 BAP + 1.0 NAA	shoots	++

<i>tuberosum</i> L.		MS + 2.0 BAP	shoots	++
		MS + 4.0 KIN	shoots	+++
	nodule	1BAP+0.5NAA+30g/L sucrose	tubers	++
		4BAP+2 NAA + 60g/L sucrose	tubers	+++
		6BAP+2 NAA + 90g/L sucrose	tubers	+++

The family *Cucurbitacea* along with the other two *Brassicaceae* and *Asteraceae* can be considered as a family of extraordinary importance to humans, as they follow cereals and legumes in their economic significance to human kind (Lebeda et al., 2006). A good micropropagation protocol could reduce the cost of hybrid seed production, which can account for 30% of the total seedling cost. The commercial application of *in vitro* techniques in *Cucurbitaceous* taxa has been well demonstrated and the regeneration of plants has been reported from different types of tissue cultures (Ahmad and Anis, 2005). Our experimental work involved meristem and non-meristem tissues cultivated on MS and LS media. The results show that *Cucumis sativus* L. possess bigger potential for morphogenesis as compared to *Cucurbita pepo* var. *cylindrical* (Table 3).

Micropropagation of some species from family *Solanaceae* family

The *Solanaceae* family provides some of the world's most popular vegetables. The family ranges from annual and perennial herbs to vines, lianas, epiphytes, shrubs, and trees, and includes a number of important agricultural crops, medicinal plants, spices, weeds, and ornamentals. Many members of the family contain potent alkaloids, and some are highly toxic.

In the current research the meristematic apical buds and non-meristematic cotyledonary and hypocotyledonary segments were used as initial explants for research of regenerative potential and further morphogenesis of pepper in *in vitro* culture (Table 4). The androgenetic potential of the pepper and the ability for embryos induction in pepper anther culture were tested as well (Table 4). From all tested media and treatments, haploid embryo production was accomplished only by the method of Dumas de Valux et al. (1981).

As experimental material for tomato micropropagation, apical buds, cotyledons and hypocotyls were used (Table 4). Tomato possesses excellent potential for *in vitro* morphogenesis which was proven by our and in many other experiments (Bhatia et al., 2004; Chandra, 2013; Koleva Gudeva et al., 2006)

The results from micropropagation of *Solanum tuberosum* L. presented in the Table 4 are in line with results of Dieme et al. (2013) who found that media enriched with BAP, KIN and sucrose give better microtuber formation. Different researchers agreed that higher percent of sucrose in the medium has positive results on microtuberisation process and increases the number and quality of microtubers (Farran and Mingo-Castel, 2006; Motallebi-Azar and Kazemiani, 2012; Ahmed et al., 2013; Iqbal et al., 2014; Koleva Gudeva et al., 2014) which confirm our findings during this research.

Conclusion

Today, without the use of *in vitro* plant methodology, many sophisticated and complex processes at the molecular level cannot be imagined and implemented, which is one of the most important challenges of the XXI century. The mass production of many species is based on the use of tissue culture and micropropagation. Furthermore, the ability to produce disease free plant material transformed plant propagation to an emerging industry for commercial micropropagation. This industry will continue to expand, but expansion will not be completed without introduction of new improved protocols for successful morphogenesis in plant tissue culture for economically important species, as they were presented in these paper.

References

- Ahmad, N., Anis, M., 2005. In vitro mass propagation of *Cucumis sativus* L. from Nodal Segments. *Turk J Bot*, 29 (2005) 237-240.
- Ahmed, M., Saha, S., Islam, M.M. and Ali, M.R. (2013) Effect of different levels of sucrose on microtuberization and different substrates on minituber production resulted from potato meristem culture. *Journal of Agriculture and Veterinary Science*, Vol.4(6)58-62.
- Akin-Idowu, P.E., Ibitoye, D.O. and Ademoyegun, O.T. (2009) Tissue culture as a plant production technique for horticultural crops. *Afri. J. of Biotechnology* Vol. 8(16): 3782-3788.
- Anoop, B. and Chauhan J.S. (2009) Effect of Growth Regulators on Meristem-tip Development and in vitro Multiplication of Potato cul. kufri himalini. *Nature and Science* 7(9):31-34.
- Babu, N., Divakaran, M., Raj, R., Anupama, K., Sarma P. (2015) Biotechnological Approaches in Improvement of Spices: A Review, *Plant Biology and Biotechnology* 2015:487-516.
- Bhatia, P., Nanjappa Ashwath, Senaratna, T., Midmore. D (2004) Tissue Culture Studies of Tomato (*L. esculentum*). *Plant Cell, Tissue and Organ Culture*, Vol.78 (1): 1-21.
- Chandra, I., Singh, P., Bhattacharya, A., Singh, P., Javed, S. and Singhamahapatra, A. (2013) In vitro callus induction, regeneration and micropropagation of *Solanum lycopersicum*. *Int. J. Curr. Microbiol. App. Sci* (2013) 2(12): 192-197.
- Dieme, A., Sambe, M., Agbangba, E.C. and Sy, M.O. (2013) Residual effects of sucrose and hormonal treatments of the tuberization medium on in vitro germination of potato (*Solanum tuberosum* L.) Microtubers. *American Journal of Plant sciences*, 2013, 4, 1872-1878.
- Dumas de Valux, R., Chambonnet, D. and Pochard. E., (1981) In vitro culture of pepper (*Capsicum annum* L.) Anthers: high rate plant production from different genotypes by + 35°C treatments. *Agronomie*, 1(10):859-864.
- Ekiert, H. (2000) Medicinal plant biotechnology: The Apiaceae family as the example of rapid development. *Pharmazie*. Aug. 55(8):561-7.
- Iqbal, M., Jaskani, M., Rafique, R., Hasan, S., Iqbal, M., Rasheed, M., and Mushtaq, S. (2014) Effect of Plant Growth Regulators on Callus Formation in Potato. *AgriFood App Sci*.2:77-81.
- Farran, I., Mingo-Castel, A.M. (2006) Potato minituber production using aeroponics: Effect of plant density and harvesting intervals. *Am. J. of Potato Research*, Vol. 83(1): 47-53.
- Koleva Gudeva, L., Trajkova F. and Stojkova, I. (2014) Microtuberization of potato (*Solanum tuberosum* L.). *Yearbook of Faculty of Agriculture, Goce Delcev University, Stip, R. Macedonia*, Vol.12: 19-37.
- Koleva Gudeva, L. and Trajkova F. (2012) In vitro response from different explants at some vegetable species. *Scientific conference of UFT Food science engineering and technology Proceedings*, 548-552.
- Kitto, S.L., (1997) Commercial micropropagation. *HortScience* Vol. 32(6): 1012-1014.
- Lebeda, A., Widrlechner, MP., Staub, J.Zalapa, J., Kristkova E. (2006) Genetic resource, chromosome engineering and crop improvement: Cucurbits (*Cucurbitaceae*; *Cucumis* spp; *Cucurbita* spp; *Citrulus* spp). *CRC Press, Francis & Taylor Group*. Vol.3 272-344.
- Linsmaer, E.M., Skoog, F., (1965) Organic growth factor requirements of tobacco tissue culture. *Physiol. Plant* 18, 100–127.
- Motallebi-Azar, A. and Kazemiani, S. (2011) A new concept about carbon source roles on in vitro microtuberization of potato (*S.tuberosum* L.). *AAB Bioflux*. 3(3), 160-167.

- Murashige, T., Skoog, F., (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant* 15, 473–497.
- Warwick S.I. (2011) Genetics and Genomics of the Brassicaceae, *Plant Genetics and Genomics: Crops and Models*: 34-50.