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

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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 graviolens* 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 decease free planting material and germplasm conversation. 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 this problems, there are e large number of species being micropropagated on a commercial scale throw 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 form *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 Delcev 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, than with distilled water, followed by 15-20 seconds in 70% C_2H_5OH, 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 $mg \cdot l^{-1}$ inositol, 200 $mg \cdot l^{-1}$ casein hydrolysate, 0,1 $mg \cdot l^{-1}$ B1, 1,0 $mg \cdot l^{-1}$ B6 and 0,5 $mg \cdot l^{-1}$ nicotinic acid. Different phytohormones were used in a medium such as: IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), NAA (α-naphtaleneacetic acid), BAP (6-benzylaminopurine), BA (N6-benzyladenine), KIN kinetin (6-furfuryl aminopurine), ZEA zeatin (N^6-4 hydroxyl-3-methyl-trans-2butenyl anunopurine), 2iP (N^6-2-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 form *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 an new medium supplemented by same or different plant hormone were cultivated in climate chamber with controlled conditions and temperature of 25±2ºC, photoperiodic of 16/8 light/dark, 50% relative humidity, and 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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 form *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 micropropagatein. 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 graviolens* L.

Micropropagation of some species form *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. *Apiaceae*	Explant	Medium + mg·L^{-1} Growth Regulators	Results	Efficiency
Apium graviolens 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	+++
Daucus carota subsp. *sativus* 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	+++
Petroselinum crispum 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. *Brassicaceae*	Explant	Medium + mg·L^{-1} Growth Regulators	Results	Efficiency
Brassica oleracea var. *italica*	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	+++
Brassica oleracea var. *capitata* 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	+++
Raphanus sativus var. *radicola*	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 *Cucurbitacea* family

The *Cucurbitacea* 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. *Cucurbitaceae*	Explant	Medium + mg·L^{-1} Growth Regulators	Results	Efficiency
Cucumis sativus 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	+++
Cucurbita pepo var. *cylindrical*	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. *Solanaceae*	Explant	Medium + mg·L^{-1} Growth Regulators	Results	Efficiency
Capsicum annuum 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	+++
Lycopersicon esculentum 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	+++
Lycopersicon esculentum Mill. var. *cerasiforme* (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	+++
Solanum	sprouts	MS + 0.5 BAP + 1.0 NAA	shoots	++

tuberosum 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 consider 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 form 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.

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