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BOOK OF PROCEEDINGS

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FOREWORD

A Word from the Editor-in-Chief

Dear colleagues,

In your hands is the Book of Proceedings of the X International Scientific Agricultural Symposium "AGROSYM 2019", which I hope you will find useful in your work. As many as 900 contributions, from 82 countries, have been accepted for oral or poster presentations. Symposium themes cover all branches of agriculture and are divided into 7 sessions: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environmental protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, 7) Forestry and agroforestry. Papers dealing with agricultural engineering and technology were included into one of the seven sessions depending on their focus.

In the plenary lectures were addressed interesting topics; one keynote was on biotechnology and two others dealt with organic farming in Australia and Europe. This confirms the role of AGROSYM as a forum for open discussions and exchanges on agriculture, food, the environment and rural development in the Balkans and beyond. Many of the papers identify a number of approaches and market-based incentives to encourage producers to achieve higher levels of performance (from both economic and environmental points of view) and as a result to meet the expectations of governments and consumers.

The successful management of agricultural resources to satisfy changing human needs, while maintaining or enhancing the quality of the environment and conserving natural resources, indicate a long-term agricultural development imperative. Advances in productivity, profitability and stability of modern cropping, animal and forestry systems will have to be achieved globally on an ecologically sustainable basis. Today, it is obvious that conventional methods of agricultural production, while providing sufficient food and various products to humanity, have led to a number of negative impacts, including the transgression of many planetary boundaries. These negative impacts raise serious questions about the long-term sustainability of high-input agriculture and call for a genuine transition towards sustainable agro-food systems, which achieve food and nutrition security for present and future generations within the safe operating space for humanity.

Full texts of the submitted communications will be available on the website of AGROSYM (http://agrosym.ues.rs.ba). Each paper included in the present Book of Proceedings was positively reviewed.

Much appreciation is due to the authors of all papers submitted and presented at the symposium as well as to all symposium participants whose ideas and contributions allowed rich and lively discussions during the various sessions. Many thanks to all reviewers, session moderators and colleagues for their help in editing the Book of Proceedings. Special thanks go to all co-organizers, partners and sponsors for their unselfish collaboration and comprehensive support.

Editor-in-Chief

Dusan Kovacevic

Dusan Kovacevic, PhD

East Sarajevo, 12 October 2019

EVALUATION OF ANDROGENIC COMPETENCE OF DIFFERENT PEPPER, TOMATO AND EGGPLANT GENOTYPES

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Abstract

The methods of biotechnology such as androgenesis introduce new possibilities for faster creation of new varieties or at least faster development of improved genotypes with desirable traits that can give an answer to new abiotic and biotic challenges in agricultural production. Androgenesis is a method that opens possibilities for development of haploids and spontaneous dihaploids plants via anther culture. *In vitro* anther culture utilized for gaining haploid/dihaploid plants serves as a tool for improvement of some solanaceous crops such as tomato, eggplant and pepper, but it always faces obstacles for high productivity of regenerants in those crops. In the present study, androgenic competence of 3 pepper genotypes (Edita, Bela Duga and Homera), 3 tomato genotypes (Bellfort, Rally and Policarpo) and 1 eggplant genotype (Domaci srednje dugi) were evaluated. The pepper anthers were cultured according to the method developed by Dumas de Valux *et al.* (1981), tomato anthers were cultivated according to Corral-Martínez *et al.* (2011), while eggplant anthers were cultivated according to Dumas de Valux and Chambonnet (1982). The experiment showed that androgenesis was successfully implemented only in pepper genotype Edita, whereas the eggplant genotype did not show any response to anther induction media. Cultivation of anthers from all tomato genotypes resulted only in callus formation. Our results are one more confirmation that androgenesis applied on pepper, tomato and eggplant has its limitations and the successfulness of androgenesis depends on many factors as growing conditions and donor plant age, donor plant genotype, microspore developmental stage, culture media and cultivation conditions.

Keywords: *Androgenesis, Anther culture, Capsicum annuum, Lycopersicon esculentum, Solanum melongena*

Introduction

Androgenesis is one of the successful biotechnological methods that is included in the group of novel techniques for improving agricultural crops and it can be combined with other biotechnological methods for achieving new selection goals (Koleva Gudeva *et al.*, 2008; Irikova *et al.*, 2016). It is considered as the fastest way to achieve homozygosity and to get homozygotic lines, where dihaploids have many advantages for their involvement in fundamental and breeding research (Koleva Gudeva *et al.*, 2007a; Seguí-Simarro, 2016). Nevertheless, obtaining regenerated and fertile androgenetic plants in the Solanaceae horticulture crops is still low (Seguí-Simarro, 2016).

Generally, there are a small number of scientific publications related to the full regeneration of the androgenetic solanaceous plants, their characterization and evaluation and direct participation in selection programs for scientific and commercial purposes. According to Seguí-Simarro *et al.* (2011), pepper (*Capsicum* sp.) is characterized as a third-class crop by fam. Solanaceae which is "resistant" to methods of androgenesis, after tomato (*Lycopersicon esculentum* Mill.) and eggplant (*Solanum melangena* L.). The aim of our research was to test and establish effective androgenesis protocols for pepper, tomato and eggplant.

Material and Methods

Pepper anther culture

Three pepper (*Capsicum annuum* L.) varieties were used as anther donors in the experiment: Edita (sweet, long type), Bela Duga (sweet, long type), Homera (long, hot type). The flower buds were harvested when the corolla was of the same length as the calyx or slightly longer. Flower buds were surface sterilized in 70% ethanol for 2 minutes, then in 5% Isozan G for 10 min, and rinsed three times in sterile distilled water. After the removal of the filaments, anthers from three flower buds were placed in Petri dish, with the concave face down, touching the culture medium. The media employed for anther culture was: Cp (Dumas de Valux *et al.*, 1981) supplemented with kinetin (0.01 $mg \cdot l^{-1}$) and 2.4-D (0.01 $mg \cdot l^{-1}$). The anthers cultivated on Cp medium with the supplementary hormones were incubated for 8 days at 35±2 ^oC in the dark, and then transferred to 25 ± 2 ^oC with 12 h photoperiod. After 12 days of induction on Cp medium, the anthers were transferred each month onto fresh R_1 medium supplemented with 0.01 $mg \cdot l^{-1}$ kinetin and simultaneously the deteriorated or infected anthers were removed. The cultures were observed regularly, and the data were recorded every week. The frequency of callus formation and the number of emerged embryoids were recorded. Young shoots emerging from the anthers were transferred onto hormone free V3 media in order roots to be formed.

Tomato anther culture

Three tomato (*Lycopersicon esculentum* Mill.) varieties were used as anther donors in the experiment: Bellfort (rounded shape), Rally (rounded shape) and Policarpo (plum-shaped tomato). Buds varying in length were collected from flowering plants and they were surface sterilized in 70% ethanol for 2 minutes, then in 5% Isozan G for 10 min, and rinsed three times in sterile distilled water. The anthers were dissected and plated on the MS induction medium prepared according to Corral-Martínez *et al.* (2011). It consisted of MS basal medium + vitamins (Murashige and Skoog 1962), supplemented with 2.5 $g \cdot l^{-1}$ Phytagel, 20 g/l sucrose, 1 $mg \cdot l^{-1}$2ip and 2 $mg \cdot l^{-1}$ IAA, pH 5.7. Petri dishes were kept in a growth cabinet at 25 ^oC, in darkness for 1 month, and then under a 16/8 photoperiod. Anthers and developing calli were transferred to fresh medium on a monthly basis. Green or partially green, proliferating calli were transferred to regeneration medium composed of 4.4 g/l MS medium plus vitamins, 2.5 $g \cdot l^{-1}$ Phytagel, 20 $g \cdot l^{-1}$ sucrose and 0.25 $mg \cdot l^{-1}$ zeatin, pH 5.7.

Eggplant anther culture

One eggplant (*Solanum melanogena* L.) genotype, Domaci srednje dugi, was used as anther donor genotype. Flower buds of the certain size were harvested from anther donor plants and surface sterilized in 70% ethanol for 2 minutes, then in 5% Isozan G for 10 min, and rinsed three times in sterile distilled water and immediately dissected. Anthers were cultured according to Dumas de Vaulx and Chambonnet (1982) on Ct inductive medium. The Ct medium was supplemented with 0.01 $mg \cdot l^{-1}$ 2,4-D and 0.01 $mg \cdot l^{-1}$ kinetin. Anthers were inoculated in petri dishes on Ct inductive medium and cultured for 8 days in darkness at 35^oC. Then, they were transferred to light (12 h light/12 h darkness photoperiod) and 25 ^oC for 4 more days. At day 12, anthers were transferred to R_1 medium supplemented with 0.01 mg/l kinetin, where they were cultured indefinitely at 25 ^oC, with medium refreshing every 20 days.

Results and Discussion

The results of androgenic response of different pepper genotypes are presented in Table 1 and Figure 1. On the inductive Cp medium were incubated 555 anthers from Edita, 619 anthers from Bela Duga and 640 from Homera with mean anther length of 3.3; 3.3 and 3.2 mm, respectively. The cultivated anthers of three varieties responded with callus formation with different percentage as Edita (12.7%), Bela Duga (11.6%) and Homera (17.2%). The

percentage of embryogenic anthers varied from 0.22% for Bela Duga and 2.3% for Edita. Only for the variety Edita there was successful regeneration of 5 embryo into plants. As expected, the anthers of the hot variety Homera did not responded with embryo formation.

Table 1. Androgenic response of different pepper genotypes (*Capsicum annuum* L.) incubated on Cp medium + 0.01 mg·l^{-1} kinetin and 0.01 mg·l^{-1} 2.4-D (+35 ^{o}C, 8 days in darkness).

Species / Variety	Total cultured anthers	Mean length of anthers (mm)	Callusogenic anthers (%)	Embryogenic anthers (%)	Regenerated androgenic plants
Edita	555	3.3	12.7	2.3	5
Bela Duga	619	3.3	11.6	0.22	/
Homera	640	3.2	17.2	/	/

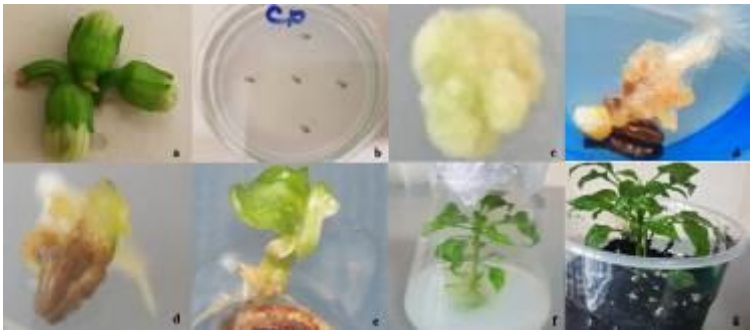

Figure 1. a) pepper buds collected for anther isolation b) anthers inoculated on R_1 medium developing c) callus developed from anther d) emerging androgenic embryo e) fully developed androgenic embryo f) regenerated androgenic plant on V_3 medium g) acclimatized androgenic plant.

According to Koleva-Gudeva *et al.* (2007a,b), the process of embryo formation on different media under different thermal conditions showed that the formation of haploid embryos occurred only in the CP medium exposed to heat thermal stress (+35 ^{o}C) and hot pepper genotypes are low in androgenic response which is in agreement with our results findings (Koleva Gudeva *et al.*, 2007a). Even with difficulties and low efficiency of pepper, there are several laboratories that successfully applied pepper androgenesis and fully regenerated androgenic plants which are characterized either phenological or/and molecular as the Hungarian group (Mitykó and Gémes Juhász, 2006); the Macedonian group (Trajkova, 2013; Trajkova and Koleva Gudeva, 2017); the Bulgarian group (Todorova *et al.*, 2013); the Korean group (Luitel *et al.*, 2012) and the Polish group (Olszewska *et al.*, 2015; Nowaczyk *et al.*, 2015).

The results of androgenic response of different tomato genotypes are presented in Table 2 and Figure 2. On inductive MS medium were incubated 80 anthers from Bellfort, 49 anthers from Rally and 64 from Policarpo with mean anther length of 2.4; 3.0 and 3.5 mm, respectively. The anthers of these three varieties responded with callus formation with different percentage as Bellfort (63.8%), Rally (28.6%) and Policarpo (28.1%). There was no embryo formation from tomato anthers from all three varieties, consequently no regenerated androgenic plants.

Table 2. Androgenic response of different tomato genotypes (*Lycopersion esculentum* Mill.) incubated on MS medium + 1 mg·l^{-1} 2ip and 2 mg·l^{-1} IAA (+25 ºC, 1 month in darkness).

Variety	Total cultured anthers	Mean length of anthers (mm)	Callusogenic anthers (%)	Embryogenic anthers (%)	Regenerated androgenic plants
Bellfort	80	2.4	63.8	/	/
Rally	49	3.0	28.6	/	/
Policarpo	64	3.5	28.1	/	/

Figure 2. a) tomato buds collected for anther isolation b) inoculated anthers on MS medium c-d) calli developing from anthers.

In tomato, anther culture has also been demonstrated possible, but for very few genotypes. Seguí-Simarro (2016) highlight that mostly two key bottlenecks impose strong limitations to the efficiency of double haploids technique in recalcitrant solanaceous crops as induction efficiency and embryo development, where it is widely known that the genotype is the most influential factor. Seguí-Simarro (2016) discussed that only two laboratories have published the complete regeneration of entire tomato plants with a demonstrated haploid or DH origin (Shtereva *et al.*, 1998; Zagorska *et al.*, 2004; Seguí-Simarro and Nuez, 2005, 2007; Corral-Martínez *et al.*, 2011).

The results of androgenic response of one eggplant variety are presented in Table 3. On inductive Ct medium were incubated 144 anthers with mean anther length of 2.4 from eggplant genotype Domaci srednje dugi. The utilized medium and incubation conditions did not induce any response of cultivated anthers.

Salas *et al.* (2011) published successful protocol for eggplant androgenesis where responding anthers produced both calli derived from anther tissue and embryos derived from microspores, suggesting that the protocol established by Dumas de Vaulx and Chambonnet (1982) it's not always a source of androgenic competence among different eggplant genotypes.

Table 3. Androgenic response of eggplant (*Solanum melongena* L.) incubated on Ct medium + 0,01 mg·l^{-1} 2,4-D and 0,01 mg·l^{-1} kinetin (+35 ºC, 8 days in darkness).

Genotype	Total cultured anthers	Mean length of anthers (mm)	Callusogenic anthers (%)	Embryogenic anthers (%)	Regenerated androgenic plants
Domaci srednje dugi	144	2.4	No response	No response	/

However, our results showed that the same protocol did not worked for the eggplant genotype Domaci srednje dugi and no anthers response was reached. On the other hand, Rivas-Sendra *et al.* (2017) evaluated androgenic capacity through microspore culture of the eggplant commercial F1 hybrid Bandera and its first and second generation of DHs and obtained a population of 80 DH individuals via microspore cultures. These findings show that beside the genotype, cultivation medium has the main role in positive androgenic response in eggplant, as well as thickness of eggplant anther walls and heterostyly, which might delay the access of inductive factors to the anther locule, thus reducing their effect over inducible microspores (Salas *et al.*, 2012).

Conclusions

The low androgenic rate in pepper and no androgenic response in tomato and eggplant, as shown in our experimental results, are one more confirmation that these solanaceous crops are still far from an efficient and reliable technology to be applied on a routine basis in breeding programs. Even with the incredible importance of this family for agriculture, double haploid technology is not yet competently applied in these crops and they appear to respond to androgenic induction very differently.

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