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IN VITRO REGENERATION EFFICIENCY OF SOME TURKISH PEPPER GENOTYPES

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Abstract
Pepper is one of the important vegetables consumed in different ways in the world Pepper cultivation is done in many regions of our country. Recently, tissue culture methods have been used to increase yield and quality and to obtain plants resistant to diseases and pests. The basic system used in plant tissue culture processes and genetic improvements is plant regeneration. Tobacco and petunia became a model for the study of certain aspects of modern biotechnology and molecular biology about thirty years ago. Additionally, transgenic tomato and eggplant varieties have been introduced to the market and have reached the stage of different field trials. However, pepper (Capsicum annuum L.) is a little behind the age of advanced biotechnology and transgenic breeding. In this study, it was aimed to determine the regeneration efficiency using some local Turkish pepper genotypes. In the study, 15 pepper genotypes grown as standard in Turkey were used and callus formation from explants of the genotypes and transformation from callus to plant were examined. According to the obtained results, callus formation among pepper genotypes was between 0-60%, while the transformation from callus to plant was between 0-25%. Results of present study may contribute to gene transfer studies and micropropagation studies in pepper.

Keywords: Pepper, in vitro, regeneration

1. INTRODUCTION

Pepper (*Capsicum annuum* L.) is a member of the Solanaceae family and is native to the Americas. There are about 30 species in the genus *Capsicum* and the most common and cultivated species are *C. annuum* L., *C. pubescens* Ruiz & Pav., *C. chinense* Jacq., and *C. baccatum* L., *C. frutescens* L., (Greenleaf, 1986; Eken and Mavi, 2014). Pepper is a cultivated plant that can be grown mostly as an annual plant but also as a perennial plant (Şalk et al., 2008). The countries with the highest pepper production worldwide are Vietnam, Brazil, Indonesia, Burkina Faso and India (FAO, 2021).
In addition to being consumed as a fresh vegetable, pepper is utilized in many ways such as spices, pepper paste, sauces, pickles, pepper gas, pesticides such as insecticides and bactericides, and also as a folk remedy in the treatment of digestive system, rheumatism, muscle pains, hypertension and cancer diseases (Oğuzkan et al., 2018; Li et al., 2020).
Biotechnological breeding of pepper has been started in recent years due to some reasons such as long time, costly, labor-intensive and labor-intensive traditional breeding studies. Especially in the last four decades, intensive studies have been carried out on the production of pepper by tissue culture (Ammirato, 1983; Christopher and Rajam, 1994; Çömlekçioğlu et al., 2001; Çiner and Tıpırdamaz, 2002; Otroshy et al., 2011). As in other cultures, it is very important to determine the

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effect of genotype in the propagation of pepper by tissue culture. A great many efforts have been made to achieve successful regeneration of Capsicum. The main method of regeneration has been via organogenesis(Dabauza, and Peña, 2001; Bora et al. 2014; Sanatombi et al. 2008). The researchers demonstrate that the principal factors influencing the development of an effective protocol for plant regeneration in Capsicum genotypes are the selection of an appropriate explant and the formulation of a medium containing specific growth regulators.

2. MATERIALS AND METHODS

A total of 21 pepper genotypes were used in this study, and 15 of these genotypes consist of genotypes grown locally in Turkey and 6 of them consist of commercial hybrid varieties that are widely grown in Turkey. For seed germination, 25 seeds of each genotype were planted on 60 mm petri dishes in the medium containing MS (Murashige and Skoog, 1962) supplemented with 3% sucrose, 0.7% plant agar. Depending on the genotype, just before the true leaf stage (12th-16th day), the cotyledonous leaves are cut in half and explants were cultured on MS (Murashige and Skoog, 1962) the media was supplemented with 3% sucrose, 0.7% plant agar, 5 mg/L BAP (6-benzylaminopurine), 0.5 mg/L IAA (indole-3-acetic acid) and 0.47 mg/L $CuSO_4$, followed by a subculture in the same medium, which was further supplemented with 10 mg/L $AgNO_3$(Martínez-López et al., 2021) on 60 mm petri dishes. For rooting, the medium containing half strength MS (Murashige and Skoog, 1962) medium supplemented with 1 mg/L indole-3-butyric acid (IBA) were used. The pH of the medias were adjusted to 5.9 before adding agar and autoclaving, and then these media were sterilized in the autoclave at 121 ºC for 20 minutes. After the autoclaving process, the sterile nutrient medium and culture containers were taken into the sterile cabinet and 10 ml of nutrient medium was distributed to each plate. The explants were cultured with a photoperiod of 16 h light/8 h dark at a temperature of 25 ◦C at plant growth chamber. After one month of culture, the presence of callus in the explants was evaluated and callus were transfered to fresh same media. Gelişen sürgünler kök ortamına transfer edilmiş ve köklenen bitkicikler acclimatizated under a photoperiod of 16 h light/8 h dark at a temperature of 25 ◦C at plant growth chamber. Number of explants to callus, callus formation ratio(%), number of explants with elongated shoot, shoot elongation ratio(%), number of shoots to root, rooting ratio(%), number of acclimatizated plants and acclimatization ratio(%) were determined.

3. RESULTS AND DISCUSSIONS

In this study, 2100 explants belonging to 21 genotypes were cultured and number of explants to callus, callus formation ratio(%), number of explants with elongated shoot, shoot elongation ratio(%), number of shoots to root, rooting ratio(%), number of acclimatized plants and acclimatization ratio(%) were determined(Figure 1 and Figure 2). According to the results, the highest number of explants transform to callus was obtained in Kahramanmaraş pepper (78 explants), while the lowest number of explants transform to callus was obtained in Ege Sivri Acı genotype (22 explants).Callus formation ratio(%) was highest to Kahramanmaras pepper. While highest shoot elongation ratio(%) was observed at Yalova Yaglik genotype lowest shoot elongation ratio(%) recorded at Ege Aci Sivri, Sari Sivri genotypes. Rooting ratio(%)was highest at F1 Hybrid-3 and there were no rooting at 10 genotypes(Cırgalan, Hatay pepper, Bağci Çarliston, Karaisali, Urfa pepper, Demre Sivri, F1 Hybrid-1, Ege Aci Sivri, Sari Sivri). Acclimatization ratio(%) varied between 18,2%(Çetiner Sivri)- 62,5%(Yalova Çarliston)(Table 1).

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Table 1. Number of explants to callus, callus formation ratio(%), number of explants with elongated shoot, shoot elongation ratio(%), number of shoots to root, rooting ratio(%), number of acclimatizated plants, acclimatization ratio(%) of pepper genotypes

Genotypes	Total cultured explant	Number of explants to callus	Callus formation ratio(%)	Number of Explants with elongated shoot	Shoot elongation ratio(%)	Number of shoots to root	Rooting ratio(%)	Number of acclimatizated plants	Acclimatization ratio(%)
Cırgalan	100	55	55	21	21	0	0.0	0	0.0
Kahramanmaras	100	78	78	36	36	11	30.6	6	54.5
Hayat pepper	100	46	46	33	33	0	0.0	0	0.0
Bağci Çarliston	100	52	52	36	36	0	0.0	0	0.0
Karaisali	100	56	56	18	18	0	0.0	0	0.0
Urfa pepper	100	39	39	11	11	0	0.0	0	0.0
Demre Sivri	100	33	33	14	14	0	0.0	0	0,0
F1 Hybrid-1	100	54	54	18	18	0	0.0	0	0,0
F1 Hybrid-2	100	48	48	39	39	13	33.3	8	61.5
F1 Hybrid-3	100	36	36	36	36	15	41.7	5	33.3
F1 Hybrid-4	100	59	59	25	25	8	32.0	2	25.0
F1 Hybrid-4	100	52	52	33	33	5	15.2	2	40.0
F1 Hybrid-6	100	63	63	39	39	12	30.8	7	58.3
Yalova Yaglik	100	66	66	43	43	6	14.0	3	50.0
Kandil Dolma	100	66	66	41	41	5	12.2	2	40.0
Ornamental pepper	100	53	53	15	15	0	0.0	0	0.0
Antep Dolma	100	48	48	10	10	0	0.0	0	0.0
Çetiner Sivri	100	64	64	36	36	11	30.6	2	18.2
Yalova Çarliston	100	66	66	39	39	8	20.5	5	62.5
Ege Aci Sivri	100	22	22	0	0	0	0,0	0	0.0
Sari Sivri	100	31	31	0	0	0	0,0	0	0.0
Max		78	78	43	43	15	41.7	8	62.5
Min		22	22	0	0	0	0	0	0
Mean		51.6	51.6	25.5	25.5	4.7	13.2	2.2	22.0

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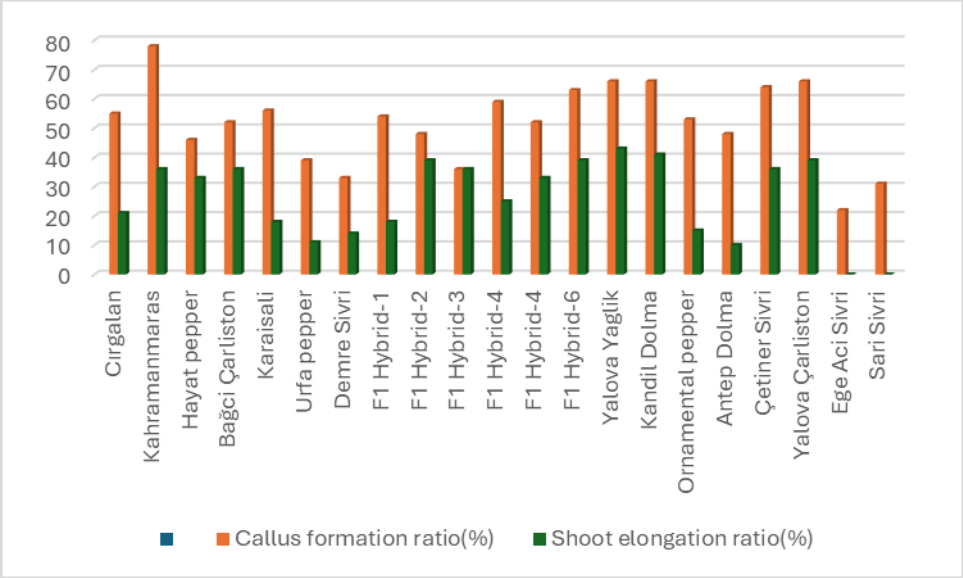

Figure 1. Callus formation and Shoot elongation ratio(%) of pepper genotypes

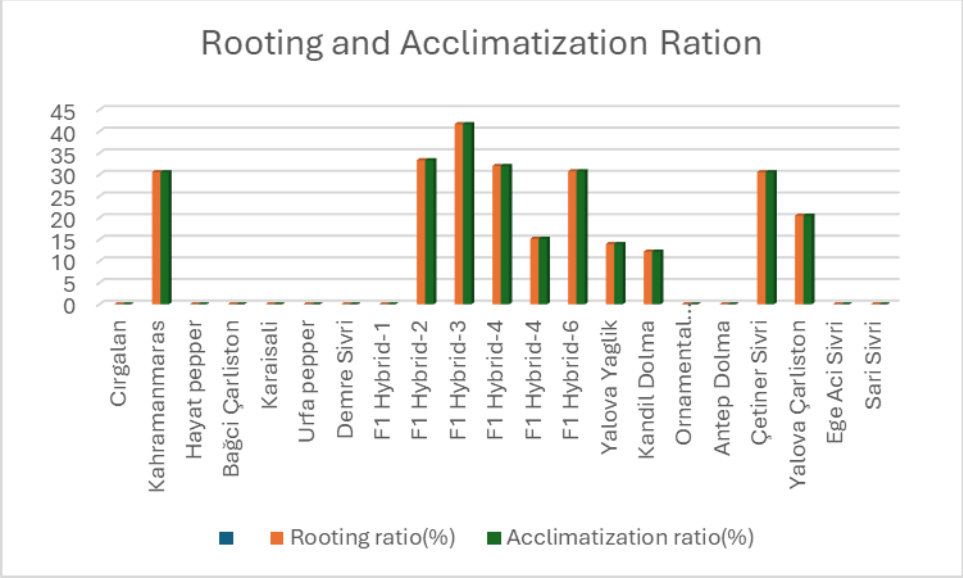

Figure 2. Rooting and acclimatization ratio(%) of pepper genotypes

The in vitro regeneration ability of the 21 pepper genotypes which 15 of them were Turkish local varieties genotypes investigated showed that genotypes had a similar genetic background for shoot formation and that there were varietal differences in the regeneration ability among all pepper genotypes grown in Turkey. This results was not unexpected, as previous studies had documented genotypic differences in plant regeneration from seedling explants (Ochoa-Alejo and Ireta Moreno, 1990; Rogozinska and Tobolewska 1992; Ezura et al., 1993; Ramirez-Malagon and Ochoaalejo, 1996) The findings of the present study substantiate the hypothesis that *Capsicum* peppers exhibit recalcitrance to direct organogenesis regeneration. However, it is noteworthy that variation was observed among pepper genotypes in their responses to in vitro culture(Sanatombi and Sharma,

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2008; Valadez-Bustos et al. 2009). Plant regeneration through organogenesis in *Capsicum* was reported from cotyledon, hypocotyl, leaf, shoot tip, embryo, root and seed explants (Kothari et al. 2010; Gammoudi et al. 2018; Bora et al. 2019). But we used cotyledon of pepper genotypes. Because and Martínez-Lópe et al. (2021) reported that, In general, the most effective explant for the regeneration of shoots via organogenesis in C. annuum genotypes was the cotyledon, which exhibited the highest number of shoots per explant. As was observed in previous studies, regeneration was more pronounced in Capsicum cotyledons than in hypocotyls. In this regard, Gunay and Rao (1978) observed enhanced regeneration in cotyledons when a medium containing IAA and BAP was used with a California Wonder (C. annuum) material. The survival rate in the acclimatisation stage was found to be lower than expected (less than 25%), in contrast to the generally higher rates (of over 80%) reported in the literature (Sanatombi and Sharma, 2008; Kumar et al., 2012). This discrepancy is attributed to the insufficient length of the explants transferred to the substrate. This discrepancy may be attributed to the environmental and climatic conditions employed in the experimental setup.

4. CONCLUSIONS

In the present study, the in vitro regeneration efficiency of 15 local Turkish peppers and 6 commercial hybrid pepper varieties was determined. According to the results of present study, significant variation was determined between genotypes. It is recommended to planing to studies, especially by taking into account the results obtained in clonal propagation and gene transfer studies, and to develop genotype-specific protocols for genotypes with low regeneration efficiency or unresponsive genotypes.

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