Available online www.jsaer.com

***Journal of Scientific and Engineering Research*, 2018, 5(11):228-236**

Research Article

ISSN: 2394-2630
CODEN(USA): JSERBR

Does Glutamine Promote the Development of Pepper (*Capsicum annuum* L.) Anthers *in vitro*?

Tugce OZSAN*, A. Naci ONUS

Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Turkey
*tugceozsan@akdeniz.edu.tr

Abstract Pepper (*Capsicum annuum* L.) plant has a very important place both across the world and in Turkey in terms of economic sense. Although many researchers working in the field of plant tissue culture, pepper plants have a nature that is 'recalcitrant' in terms of their genetic characteristics that they exhibit, plant biotechnology has a potential that is quite common in its work. Within these, biotechnological studies that pepper has been most prominent are in the framework of haploid techniques. However, as the embryo formation and the frequency of transformation of these embryos to plants are low, researchers are concentrating on different applications. One of these applications is adding various additives. Glutamine is known as an organic nitrogen source to supply energy for cells that cannot use energy sources effectively. This study was carried out by using four different pepper types (Erciyes, Filinta, Ergenekon and Bellisa F_1) as plant materials and 12 different nutrient media combinations. To enhance embryo formation we evaluated the effects of different combinations of MS and Gamborg B5 nutrient media with or without glutamine. When the results of the study were evaluated, it was observed that the glutamine concentration used in the study effected pepper androgenesis in the positive direction. Obtained results showed that while Bellisa and Ergenekon varieties were remarkable over others, media X (Gamborg B5 + 4.0 mg/l NAA + 0.1 mg/l BAP + 0.25% AC + 15.0 mg/l $AgNO_3$ + 1.0 g/l glutamine) and XII (Gamborg B5 + 4.0 mg/l NAA + 1.0 mg/l BAP + 0.25% AC + 15.0 mg/l $AgNO_3$ + 1.0 g/l glutamine) seem to be successful in relation to the varieties.

Keywords *Capsicum annuum* L., pepper, haploids, anther culture, glutamine

Introduction

Pepper (*Capsicum annuum* L.) is a plant which is quite high economically important plant that in terms of cultivating and breeding in the world and in our country. In the case of vegetable breeding, conventional breeding programs require both time and labor. Tissue culture techniques are not only used on their own but also as a means of helping traditional breeding methods. Among these techniques, classical breeding is seen as the technical haploid technique which can be used as quickly and effectively as possible. In this context, thanks to this unique ability to shorten the breeding period, this technique has an undeniable prominence in traditional plant breeding. Obtaining homozygous dihaploid lines is one of the most important applications in haploid plant production. In breeding programs prepared using conventional breeding methods, it takes quite a long time to obtain homozygous lines in both self-pollinated and foreign pollinated plants. At this point, haploidy techniques are used to shorten the breeding period in reaching the desired target, and this process, which lasts for 6-7 years at best in studies conducted by traditional methods, can be shortened to 1-2 years. Nowadays, thanks to the developed effective protocols, haploid plant and homozygous dihaploid plants can be obtained in as short a period as several months by using androgenesis techniques such as anther and microspore cultures. These homozygous plants are very important to develop high quality new varieties by creating homozygous lines and therefore genetic diversity.

To form the fundamental molecules (nucleic acids, amino acids, vitamins) for plant cells' life, need nitrogen. When needed, the plant cells use the molecules as energy sources that are most readily available. One of the most preferred organic nitrogen sources is glutamine [1] which can be used alternatively [2] and it serves the purpose of alternative energy for developing plant cells that cannot use carbohydrate sources efficiently [3, 4]. Even though it prevents developmental stages at high concentrations [5] the addition of various doses glutamine has been mostly used *in vitro* cultures [4]. Glutamine allows maintaining and promoting functions of plant cells better growth for a longer term [6-8]. Exogenous glutamine has many positive improvements such as enhancing the organogenesis competence, plantlet formation, micropropagation, embryo regeneration, embryogenic callus formation in many species in tissue culture studies [9-16].

As reported to date, due to the fact that it described as the 'recalcitrant' genetic structure, pepper androgenesis studies are challenging. For the reason that haploidy studies show less embryo formation and plant conversion, it is tried different modulations. In this study, we tried to enhance embryo formation and plant conversion based on this approach. The effects of different combinations of MS and Gamborg B5 nutrient media with or without glutamine were investigated.

Material and Method

The study was carried out in the Tissue Culture Laboratory of the Department of Horticulture, Faculty of Agriculture, Akdeniz University and was carried out using the buds of pepper plants grown in the greenhouses of same department.

In the present study, we used different pepper types such as capia, green and bell pepper which have strong, good and high yielding in terms of plant structure and fruit quality properties. Erciyes, Filinta, Ergenekon and Bellisa F_1 varieties were used as plant materials. April, May and June months were seen as suitable for growing and flower bud formation periods. The flower buds that came to the most appropriate stage for anther culture were collected early in the day. Based on previous androgenesis studies it has been observed that the most favorable bud stage is the beginning of the first mitotic division where microspores have a late uninucleate or early binucleate phase [17]. Morphologically, suitable buds for anther culture were found to have an equal length of sepals and petals, or have slightly longer petiole leaves than the petals, and in the anthers of these buds an anthocyanin color pigment was observed.

Following the classification of the buds, surface sterilization was carried out for 15 minutes in 10% sodium hypochlorite solution with 1-2 drops of Tween-20, followed by 1 minute in 70% alcohol. The buds were washed 3-4 times with sterile distilled pure water after these procedures. Then the anthers were isolated from the buds which had been sterilized and separated from their filaments. At first, isolated anthers were cultured in the dark and pretreated at high temperature (35 °C) for the first two days. At the end of the pre-treatment period, the cultures were transferred to the growth chamber, which had 16 hours of light at 28 °C, 8 hours of dark photoperiod and 3000 lux of illumination.

Twelve different nutrient media combinations have been tried in the study and the media 1, 2 and 3 by [18] and their modified concentrations have been used in the study (Table 1). Among the modifications, glutamine, one of the amino acids that can be found quite easily, as well as used it as an energy source. Besides MS basal medium [19], Gamborg B5 basal medium [20] have been used (Table 1).

In the first two days of cultivation, the anthers subjected to preheating at 35 °C were transferred to the growth chamber, which had 16 hours of light, 8 hours of dark photoperiod and 3000 lux illumination at 28 °C to continue their development. According to this, the development status of the anthers in cultures (differentiation, swelling, etc.), embryo and vegetative state were observed and recorded.

Table 1: Contents of culture media used in the study

Media Code	Basic Composition	NAA*Ratio (mg/l)	BAP**Ratio (mg/l)	AC*** Ratio (%)	$AgNO_3$****Ratio(mg/l)	GlutamineRatio (g/l)
I.	MS	4.0	0.1	0.25	15.0	-
II.	MS	4.0	0.5	0.25	15.0	-
III.	MS	4.0	1.0	0.25	15.0	-
IV.	MS	4.0	0.1	0.25	15.0	1.0

V.	MS	4.0	0.5	0.25	15.0	1.0
VI.	MS	4.0	1.0	0.25	15.0	1.0
VII.	GamborgB5	4.0	0.1	0.25	15.0	-
VIII.	GamborgB5	4.0	0.5	0.25	15.0	-
IX.	GamborgB5	4.0	1.0	0.25	15.0	-
X.	GamborgB5	4.0	0.1	0.25	15.0	1.0
XI.	GamborgB5	4.0	0.5	0.25	15.0	1.0
XII.	GamborgB5	4.0	1.0	0.25	15.0	1.0

* NAA= naphthalene acetic acid; **BAP=benzylaminopurine; ***AC= Activated Carbon; ****$AgNO_3$=Silver Nitrate

The experiment was carried out in three replications, for the different genotypes and media variants. The data obtained in the trial were subjected to a two-way ANOVA with interaction using the SAS 9.4 software. The differences between the averages were compared with the Duncan test.

Results and Discussion

After determining the optimum bud sizes and anther characteristics for the cultured anthers, the anthers from these buds were cultured in a combination of 12 different media and the changes were observed. Consistent with previous studies, the most suitable bud stage was determined by looking at the morphology of the buds, which can be regarded as suitable for a successful anther culture. According to this, the length of the sepals and petals are equal or the petals are slightly longer than the sepals. The color differences are observed from the end part to almost half of the anthers, and accordingly, the accumulation of the anthocyanin is realized. Also, microspores in these anthers are at the first mitotic phase [17, 21 - 27]. The determined buds were also found suitable for this study. It has also been reported that in the previous studies, exposed to high temperatures are ideal for pepper anther and microspore cultures. Embryogenesis of pepper anthers are affected positively [25, 28 - 30] and the results obtained from this study are consistent with previous studies. In the present study, it has been demonstrated once again that it is very important of exposing to high temperatures under dark conditions in the first few days after the beginning of culture for triggering the pepper androgenesis.

Genotype is also one of the key considerations to achieve success in androgenesis studies. Previous studies put the emphasis on genotype variety to get haploid embryo and plants [31 - 34]. The present study consistent with previous studies. When anther developments, embryo formations, and plant conversion rates were examined, there were seen statistically differences between genotypes. In this sense, amongst varieties Bellisa is the best responding, followed by Ergenekon (Figures 1-4). In Erciyes and Filinta varieties, however, no embryo or plant formation has been observed, although anthers developed (Table 2-8).

MS medium was developed for tobacco cultures and has relatively high salt levels, especially of K and N, while Gamborg B5 medium was developed for soybean callus cultures. In addition to MS basal medium, Gamborg B5 basal medium was also used in this study. On account of the high concentration of macro- and microelements, it is thought that MS medium is very salty for pepper varieties that are used in this study. To avoid this problem the Gamborg B5 basal medium was tested in order to reduce the salinity resulting from the macro elements in MS. In addition, according to the previous studies, the addition of thiamine at different concentrations was found to increase development of in some plant species that generally showed slow growth [35]. Therefore, since the thiamine ratio in the Gamborg B5 medium is higher than in the MS medium, it was preferred the Gamborg B5 medium in this study. In this sense there are statistically differences between media combinations used in the study. According to evaluated parameters, when media consist of Gamborg B5 basal nutritions, results were better. As seen from Table 2-8, medium number X (Gamborg B5 + 4.0 mg/l NAA + 0.1 mg/l BAP +0.25% AC + 15.0 mg/l $AgNO_3$ + 1.0 g/l glutamine) and XII (Gamborg B5 + 4.0 mg/l NAA + 1.0 mg/l BAP + 0.25% AC + 15.0 mg/l $AgNO_3$ + 1.0 g/l glutamine) with regards to embryo formation and plant conversion showed much better performances (Figures 1-4).

Glutamine, an alternative energy source, supports the rapid growth of cells while providing large quantities of protein and nucleic acid synthesis. It is an energy source that cells *in vitro* can easily access to meet their amino acid needs in order to provide energy where the glucose level is low but the energy demand is high. Glutamine

has been used in this study due to this special condition of cells *in vitro*. Based on the results obtained, glutamine was found to be more effective in the Gamborg B5 medium compared to the MS medium depending on the genotypes (Table 2-8). Glutamine helps promoting the development of embryoids from microspores of cultivated pepper anthers. These results are supported by previous studies used as nitrogen sources at different concentrations *in vitro* nicotiana cultures, carrot suspension cultures, wheat anther cultures and barley microspore cultures [36].

Table 2: Results obtained from anther cultures in Erciyes pepper variety

Media	Number of anthers taken in culture (number)	Number of developing anthers		Number of embryos formed		Number of plants	
		number	%	number	%	number	%
I.	222	119	53.60	-	-	-	-
II.	208	119	57.21	-	-	-	-
III.	225	133	59.11	-	-	-	-
IV.	121	81	66.94	-	-	-	-
V.	117	71	60.68	-	-	-	-
VI.	112	76	67.85	-	-	-	-
VII.	56	22	39.28	-	-	-	-
VIII.	57	20	35.08	-	-	-	-
IX.	60	30	50.00	-	-	-	-
X.	62	28	45.16	-	-	-	-
XI.	57	40	70.17	-	-	-	-
XII.	45	36	80.00	-	-	-	-

Table 3: Results obtained from anther cultures in Bellisa pepper variety

Media	Number of anthers taken in culture (number)	Number of developing anthers		Number of embryos formed		Number of plants	
		number	%	number	%	number	%
I.	151	92	60.92	23	25.00	2	2.17
II.	127	86	67.71	30	34.88	2	2.32
III.	143	78	54.54	-	-	-	-
IV.	103	48	46.60	-	-	-	-
V.	110	55	50.00	-	-	-	-
VI.	121	74	61.15	-	-	-	-
VII.	63	19	30.15	-	-	-	-
VIII.	56	23	41.07	-	-	-	-
IX.	65	6	9.23	-	-	-	-
X.	65	25	38.46	-	-	-	-
XI.	68	1	1.47	-	-	-	-
XII.	67	35	52.23	123	351.4	1	2.85

Table 4: Results obtained from anther cultures in Filinta pepper variety

Media	Number of anthers taken in culture (number)	Number of developing anthers		Number of embryos formed		Number of plants	
		number	%	number	%	number	%
I.	116	84	72.41	-	-	-	-
II.	124	95	76.61	-	-	-	-

III.	121	97	80.16	-	-	-	-
IV.	98	60	61.22	-	-	-	-
V.	80	70	87.5	-	-	-	-
VI.	88	88	100.0	-	-	-	-
VII.	102	54	52.94	-	-	-	-
VIII.	102	72	70.58	-	-	-	-
IX.	109	75	68.80	-	-	-	-
X.	106	86	81.13	-	-	-	-
XI.	130	87	66.92	-	-	-	-
XII.	75	23	30.66	-	-	-	-

Table 5: Results obtained from anther cultures in Ergenekon pepper variety

Media	Number of anthers taken in culture (number)	Number of developing anthers		Number of embryos formed		Number of plants	
		number	%	number	%	number	%
I.	123	90	73.17	-	-	-	-
II.	131	83	63.35	-	-	-	-
III.	117	90	76.92	-	-	-	-
IV.	103	48	46.60	-	-	-	-
V.	93	19	20.43	-	-	-	-
VI.	103	45	43.68	-	-	-	-
VII.	24	24	100.0	-	-	-	-
VIII.	45	24	53.33	-	-	-	-
IX.	72	42	58.33	-	-	-	-
X.	58	41	70.68	147	358.5	3	7.31
XI.	99	56	56.56	-	-	-	-
XII.	87	36	41.37	-	-	-	-

Table 6: Response of developingantherratio (%)

Media	Genotypes				Averages of media
	Erciyes	**Bellisa**	**Filinta**	**Ergenekon**	
I	$53,60^{L-O}$	$60,92^{I-L}$	$72,35^{D-G}$	$73,16^{D-G}$	$65,01^{A}$
II	$57,48^{K-N}$	$67,71^{GHI}$	$76,63^{C-F}$	$63,33^{H-K}$	$66,29^{A}$
III	$59,10^{J-M}$	$54,53^{L-O}$	$80,14^{BCD}$	$76,91^{CDE}$	$67,67^{A}$
IV	$66,89^{G-J}$	$46,57^{O-R}$	$61,26^{I-L}$	$46,63^{O-R}$	$55,34^{BC}$
V	$60,67^{I-L}$	$49,99^{N-Q}$	$87,55^{B}$	$20,42^{V}$	$54,66^{C}$
VI	$67,87^{GHI}$	$61,13^{I-L}$	$100,00^{A}$	$43,69^{QRS}$	$68,17^{A}$
VII	$39,27^{RST}$	$30,15^{U}$	$52,94^{L-P}$	$100,00^{A}$	$55,59^{BC}$
VIII	$35,08^{TU}$	$41,12^{RST}$	$70,58^{E-H}$	$53,32^{L-O}$	$50,02^{DE}$
IX	$50,00^{N-Q}$	$9,23^{W}$	$68,76^{F-I}$	$58,32^{KLM}$	$46,58^{E}$
X	$45,15^{P-S}$	$38,52^{ST}$	$81,10^{BC}$	$70,61^{E-H}$	$58,84^{B}$
XI	$70,17^{E-H}$	$1,44^{X}$	$66,98^{G-J}$	$56,56^{K-N}$	$48,79^{DE}$
XII	$79,99^{BCD}$	$52,17^{M-P}$	$30,66^{U}$	$41,37^{RST}$	$51,05^{D}$
Averages of genotypes	$57,10^{B}$	$42,79^{C}$	$70,74^{A}$	$58,69^{B}$	

Different letters in the same column and rows indicate a statistically significant difference at *** $P \leq 0.001$; ** $P \leq 0.01$; and * $P \leq 0.05$, respectively. NS, not significant

Table 7: Response of embryoformationrates (%)

Media	Genotypes				Averages of media
	Erciyes	Bellisa	Filinta	Ergenekon	
I	0.00^{C}	24.94^{B}	0.00^{C}	0.00^{C}	6.23^{B}
II	0.00^{C}	34.80^{B}	0.00^{C}	0.00^{C}	8.70^{B}
III	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}
IV	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}
V	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}
VI	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}
VII	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}
VIII	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}
IX	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}
X	0.00^{C}	0.00^{C}	0.00^{C}	360.07^{A}	90.01^{A}
XI	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}
XII	0.00^{C}	353.80^{A}	0.00^{C}	0.00^{C}	88.45^{A}
Averages of genotypes	0.00^{C}	34.46^{A}	0.00^{C}	30.00^{B}	

Different letters in the same column and rows indicate a statistically significant difference at *** $P \leq 0.001$; ** $P \leq 0.01$; and * $P \leq 0.05$, respectively. NS, not significant

Table 8: Response of plantconversion rate (%)

Media	Genotypes				Averages of media
	Erciyes	Bellisa	Filinta	Ergenekon	
I	0.00^{C}	2.14^{B}	0.00^{C}	0.00^{C}	0.53^{B}
II	0.00^{C}	2.29^{B}	0.00^{C}	0.00^{C}	0.57^{B}
III	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{B}
IV	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{B}
V	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{B}
VI	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{B}
VII	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{B}
VIII	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{B}
IX	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{B}
X	0.00^{C}	0.00^{C}	0.00^{C}	7.37^{A}	1.84^{A}
XI	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{B}
XII	0.00^{C}	2.56^{B}	0.00^{C}	0.00^{C}	0.64^{B}
Averages of genotypes	0.00^{B}	0.58^{A}	0.00^{B}	0.61^{A}	

Different letters in the same column and rows indicate a statistically significant difference at *** $P \leq 0.001$; ** $P \leq 0.01$; and * $P \leq 0.05$, respectively. NS, not significant

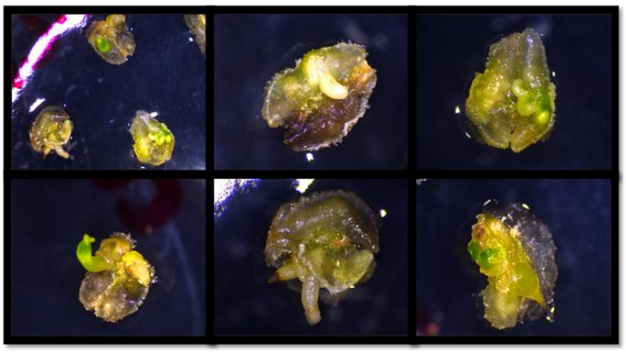

Figure 1: Changes in Bellisa F_1 pepper cultivar cultured in the medium X. at the end of 2 months

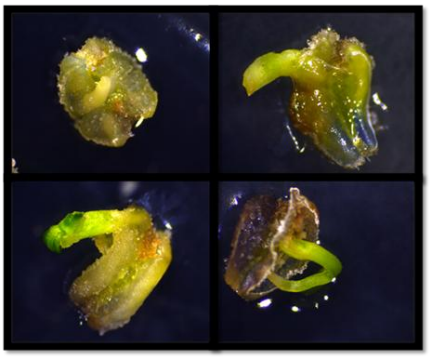

Figure 2: Changes in Bellisa F_1 pepper cultivar cultured in the medium II. at the end of 2 months

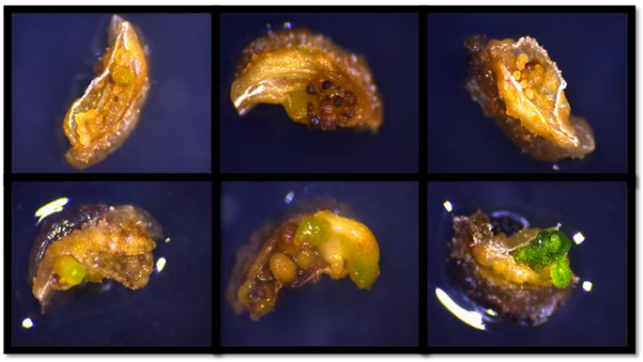

Figure 3: Changes in Bellisa F_1 pepper cultivar cultured in the medium XII. at the end of 2 months

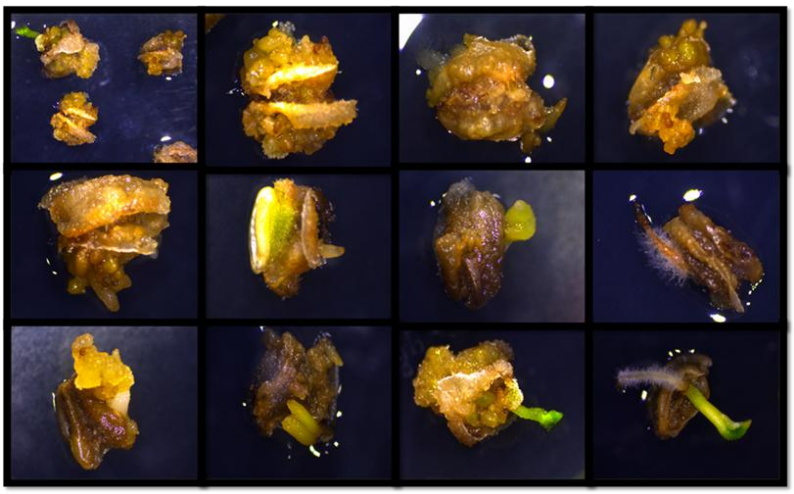

Figure 4: Changes in Ergenekon F_1 pepper cultivar cultured in the medium X. at the end of 2 months

Conclusion

In this study, it was concluded that the responses of Bellisa and Ergenekon varieties were remarkable compared to Erciyes and Filinta varieties. In addition, media X (Gamborg B5 + 4.0 mg/l NAA + 0.1 mg/l BAP + 0.25% AC + 15.0 mg/l $AgNO_3$ + 1.0 g/l glutamine) and XII (Gamborg B5 + 4.0 mg/l NAA + 1.0 mg/l BAP + 0.25% AC + 15.0 mg/l $AgNO_3$ + 1.0 g/l glutamine) seem to be successful in relation to the varieties, in terms of combinations of tested media. The glutamine concentration used in the study was effected pepper anther's embryogenesis positively. When glutamine was added to the Gamborg B5 medium combinations, it was observed that getting better accomplished rather than the MS medium. In the present study which covers April, May, and June it is also observed that May is the most productive period in terms of pepper embryogenesis. As a result of this study we believe that trying different concentrations of glutamine in future anther culture studies may shed more light onimproving the progress in peppers.

References

[1]. Thom, M., Maretzki, A., Komor, E. & Sakai, W. (1981). Nutrient uptake and accumulation bysugarcane cell cultures in relation to the growth cycle. *Plant Cell Tissue and Organ Culture*, 1: 3–14.

[2]. Al-Zuhairi, A.E.M. & Ghanm, N.S. (2017). Effect of Jasmonic Acid (JA) and Glutamine on Callus Induction of Madagascar Periwinkle Plant *(Catharanthus roseus* L. cv. Nirvana Pink Blush) by *in vitro* Culture.*Int. J. Curr. Microbiol. App. Sci.* 6(5):1415-1422. doi: https://doi.org/10.20546/ijcmas.2017.605.154

[3]. Ageel, S., Elmeer, P. (2011). Effects of casein hydrolysates and glutamine on callus and somaticembryogenesis of date palm (*Phoenix dactylifera*). *New York Science Journal*, 4:121–125.

[4]. Daniel, M.A., David R.H.A., Caesar, S.A., Ramakrishnan, M., Duraipandiyan, V., Ignacimuthu, S. & Al-Dhabi, N.A. (2018). Effect of L-glutamine and casein hydrolysate in the development of somatic embryos from cotyledonary leaf explants in okra (*Abelmoschus esculentus* L. monech). *South African Journal of Botany*, 114:223–231.

[5]. Haroun, S.A., Shukry, W.M. & El-Sawy, O. (2010). Effect of asparagine or glutamine on growth and metabolic changes in phaseolus vulgaris under *in vitro* conditions. *Bioscience Research*, 7(1): 01-21.

[6]. Newsholme, P., Lima, M.M.R., Procopio, J., Pithon-Curi, T.C., Doi, S.Q., Bazotte, R. B. & Curi, R. (2003). Glutamine and glutamate as vital metabolites. *Braz J Med Biol Res*, 36:153–163.

[7]. Mazri, M.A., Meziani, R., El Fadile, J. & Ezzinbi, A. (2016). Optimization of medium composition for *in vitro* shoot proliferation and growth of date palm cv. Mejhoul. *3 Biotech*, 6:111.DOI 10.1007/s13205-016-0430-x

[8]. Borpuzari, P.P. & Kachari, J. (2018). Effect of glutamine for high frequency *in vitro* regeneration of *Aquilaria malaccensis* Lam. through nodal culture. *Journal of Medicinal Plants Studies*, 6(2): 09-16.

[9]. Franklin, C.I., Trieu, T.N., Gonzales, R.A. & Dixon, R.A.(1991). Plant regeneration from seedling explants of green bean (*Phaseolus vulgaris* L.) via organogenesis. *Plant Cell Tissue Organ Cult*., 24:199-206.

[10]. Shetty, K., Asano, Y. & Oosawa, K. (1992).Stimulation of *in vitro* shoot organogenesis in *Glycine max* (Merrill) byallantoin and amides. *J. Plant Sci. Limerick*, 81:245–251.

[11]. Ogita, S., Sasamoto, H., Yeung, E.C. & Thorpe, T.A. (2001). The effects of glutamine on the maintenance of embryogenic cultures of *Cryptomeria japonica*. *In Vitro Cell. Dev. Biol. – Plant*, 37:268-273.

[12]. Sudarsana Rao, G.V., Chandra, R. & Polisetty, R. (2001). Role of amino acids in evolution of ethylene and methane, and development of microshoots in *Cajanus cajan*. *Biol. Plant*, 44(1):13-18.

[13]. Vasudevan, A., Selvaraj, N., Ganapathi, A., Kasthurirengan, S., Ramesh Anbazhagan, V. & Manickavasagam, M. (2004). Glutamine: a suitable nitrogen source for enhanced shoot multiplication in *Cucumis sativus* L. *Biol. Plant*, 48:125-128.

[14]. Hamasaki, R.M., Purgatto, E. & Mercier, H. (2005). Glutamine enhances competence for organogenesis in pineapple leaves cultivated *in vitro*. *Braz. J. Plant Physiol*., 17(4):383-389.

[15]. Mariani, T.S., Purnaning, A.S. & Latif, D.S. (2014). Effect of glutamine addition in maturation stage on the germination and plantlet conversion of oil palm (*Elaeis guineensis* Jacq.)Somatic Embryo. *Asian J. Appl. Sci*., 2(5):663-667.

[16]. Samarina, L., Kolomiets, T., Malyarovskaya, V., Gubaz, S. & Platonova, N. (2016). Effect of Glutamine, Biotin and ADP on Micropropagation and Growth of *Chrysanthemum hybridum, Gerbera jamesonii* and *Cordyline fruticosa In vitro. Plant Tissue Cult. & Biotech.*, 26(1):97-104.

[17]. Chambonnet, D. (1988). Production of haploid pepper plants. Bulletin Interne de la Station d'Amélioration des PlantesMaraîchéres, d'Avignon-Montfavet, France 1–10.

[18]. Taşkın, H., Büyükalaca, S., Keleş, D. & Ekbiç, E. (2011). Induction of microspore-derived embryos by anther culture in selected pepper genotypes. *African Journal of Biotechnology*, 10:17116-17121.

[19]. Murashige, T. & Skoog, F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant*, 15:473.

[20]. Gamborg, O.L., Miller, R. A. & Ojima, K. (1968). Nutrient requirement of suspensions cultures of soybean root cells. *Exp. Cell Res.*, 50:151.
[21]. Çağlar, G., Aras, V., & Bayram, A. (2004). Kurutmalık Kırmızı Biberlerde Androgenesis Yoluyla In Vıtro Haploid Embriyo Uyartımı. *Mediterranean Agricultural Sciences*, *17*(1), 87-94.
[22]. Özkum Çiner, D. & Tıpırdamaz, R. (2002). The effects of cold treatment and charcoal on the *in vitro* androgenesis of pepper (*Capsicum annuum* L.). *Turk J Bot,* 26:131-139.
[23]. Kim, M., Kim, J., Yoon, M., Choi, D. I. & Lee, K. M. (2004). Origin of multicellular pollen and pollen embryos in cultured anthers of pepper (*Capsicum annuum* L.). *Plant Cell Tissue Organ Culture*, 77:63–72.
[24]. Kim, M., Jang, I. C., Kim, J. A., Park, E. J., Yoon, M. & Lee, Y. (2008). Embriyogenesis and plant regeneration of hot pepper (*Capsicum annuum* L.) through isolated microspore culture. *Plant Cell Rep*, 27:425-434.
[25]. Lantos, C., Juhász, A. G., Somogyi, G., Ötvös, K., Vági, P., Mihály, R., Kristóf, Z., Somogyi, N. & Pauk, J. (2009). Improvement of isolated microspore culture of pepper (*Capsicum annuum* L.) via co-culture with ovary tissues of pepper or wheat. *Plant Cell Tiss Organ Cult*, 97:285-293.
[26]. Parra-Vega, V., González-García, B. & Seguí-Simarro, J.M. (2013). Morphological markers to correlate bud and anther development with microsporogenesis and microgametogenesis in pepper (*Capsicum annuum* L.). *Acta Physiol Plant*, 35:627–633.
[27]. Terzioğlu, Ş., Ellialtıoğlu, Ş., & Abak, K. (2000). İnkübasyon koşullarının biber anter kültüründe embriyo oluşumu üzerine etkisi. III. *Sebze Tarımı Sempozyumu*, 11-13.
[28]. Dumas de Vaulx, R., Chambonnet, D. & Pochard, E. (1981). Culture *in vitro* d'anthères de piment (*Capsicum annuum* L.): Amè1ioration des tauxd'obtention de plantes chez different génotypes par des trâtitement a + 35 ° C. *Agronomie*, 1:859-864.
[29]. Lantos, C., Juhász, A. G., Vági, P., Mihály, R., Kristóf, Z. &Pauk, J. (2012). Androgenesis induction in microspore culture of sweet pepper (*Capsicum annuum* L.).*Plant Biotechnol Rep*, 6:123-132.
[30]. Parra-Vega, V., Renau-Morata, B., Sifres, A. &Seguí-Simarro, J.M. (2013b). Stress treatments and *in vitro* culture conditions influence microspore embryogenesis and growth of callus from anther walls of sweet pepper (*Capsicum annuum* L.). *Plant Cell Tiss Organ Cult*, 112:353-360.
[31]. Kristiansen, K. & Andersen, S.B. (1993). Effects of donor plant temperature, photoperiod, andage on anther culture response of *Capsicum annuum* L. *Euphytica*, 67:105-109.
[32]. Mityko, J., Andrasfalvy, A., Csillery, G. & Fári, M. (1995). Anther-culture response in different genotypes and F_1 hybrids of pepper (*Capsicum annuum* L.). *Plant Breeding*, 114:78-80.
[33]. Rodeva, V., Irikova, T. & Todorova, V. (2004). Antherculture of pepper (*Capsicum annuum* L.): Comparative study on effect of the genotype. *Biotechnol Biotech Eq.*, 3:34-38.
[34]. Rodeva, V., Grozeva, S. & Todorova, V. (2006). *In vitro* answer of Bulgarian pepper (*Capsicum annuum* L.) varieties. *Genetica (Serbia)*, 38: 129-136.
[35]. Bonner, J. & Greene, J. (1938). Vitamin B1 and the Growth of Green Plants. *Botan. Gaz.* 100:226-237.
[36]. Olsen, F. L. (1987). Induction of Microspore Embryogenesis in Cultured Anthers of *Hordeum vulgare*. The Effects of Ammonium Nitrate, Glutamine and Asparagine as Nitrogen Sources. *Carlsberg Res. Commun*. 52:393-404.