eJBio

Electronic Journal of Biology, 2011, Vol. 7(1): 1-5

Some Physiological Characteristics of Pepper (*Capsicum annuum* L.) Produced *in vitro*

Liljana Koleva Gudeva*, Snezana Stavreva Veselinovska

Goce Delcev University – Stip, Krste Misirkov b.b, PO. box 201, 2000 Stip, R. of Macedonia.

* Corresponding author. Tel: +389 32 550 627; Fax: +389 32 390 700; E-mail: liljana.gudeva@ugd.edu.mk

Abstract

Apical buds were isolated from aseptically grown seedlings of pepper (*Capsicum annuum* L.), then they were cultivated on MS medium (Murashige and Skoog), with different concentrations and combinations of hormones. After four weeks, pepper shoots were obtained from apical buds in culture tissue *in vitro* conditions. After eight weeks the plantlets were transferred from sterile conditions in the laboratory to the outside conditions where the regenerate plants became adapted to the environment. Growth regulators affect the translocation of mineral substances in the vegetative organs of plant species. The aim of this research was to determine the content of certain biogenic elements and some photosynthesis pigments in pepper produced *in vivo* and *in vitro* conditions. The results showed that the plants obtained of conventional production produced *in vivo*, which were used as a control, compared with *in vitro* produced plants were significant differences in only a few of the examined features. Differences occurred primarily due to different hormonal treatment in the stages of preparation of the seedling. *In vitro* obtained plants have tended to preserve the juvenility characteristics i.e. rejuvenating, which characteristic is proved with the obtained results from examinee parameters.

Keywords: Apical buds culture; Micro propagation; Morphogenesis; Growth regulators.

1. Introduction

In vitro regeneration of *Capsicum* species has been achieved in many different tissues and organs, such as hypocotils [1,2], cotyledons [3-6], apical buds explained [7-9], stem segments [10-12] and mature zygotic embryos [13].

The impact of individual factors in *in vitro* conditions on organogenesis and differentiation of established cultures can be followed. From all factors the most examined are growth regulators and their impact in different combinations and concentrations on organogenesis and regeneration on the initial explantant in *in vitro* culture [9,14].

With using of meristem culture successful production of free of viruses could be obtained as well as elimination of some bacteria and fungi. The most important race of bacteria such as *Erwinia, Pseudomonas, Xanthomonas* and *Bacillus* as well as the most important races of fungy *Fusarium, Verticillium, Phytophthora* and *Rhizoctonia* are eliminated only with the tissue culture of meristem. There are literary data, which indicate that regulators of growth have also an impact on translocation of mineral substances in vegetative organs in different plant species [15].

Therefore, one of the main aims of this research was to determine the content of certain biogenic elements and some photosynthetic pigments in pepper produced *in vivo* and *in vitro* conditions.

Regeneration of pepper in *in vitro* conditions and their adaptation in external conditions has not been studied before in the Republic of Macedonia. Our approach to this problem consist in monitoring of the development of regenerate plants in external conditions, compared with a control group of plants (control) made with the traditional way of pepper production in external conditions. More morphological, physiological and biological characteristics of plants developed *in vitro* and *in vivo* external conditions were investigated comparatively.

2. Methods

In our analysis, the culture of apical buds was set in order to introduce the feature of the tissue in *in vitro* conditions, in particular its potential for organogenesis and regeneration in a whole plant. The fact that different growth regulators, used in different concentrations and combinations act differently to the regeneration of plantlets was confirmed.

2.1 Plant material and growth conditions

Apical buds were isolated from seeds that are germinated in aseptic conditions (Figure 1A). The seeds were washed 15 seconds in 70% ethanol, kept 10 minutes in 1% Izosan G, than stirred 15 minutes in Na-hypochlorite and rinse three times in sterile distilled water.

From the aseptically grown seedlings, apical buds were isolated and then they were cultivated on MS medium [16], mineral solution with 3% sucrose, 0.7% agar, 100 mg·l^{-1} inositol and 200 mg·l^{-1} casein hydrolysis. In the MS media plant growth regulators IAA, IBA, GA_3, NAA, BAP and KIN were used and

were added in different concentrations and combinations in the medium (Figure 1B, 1C).

The pH of the medium before autoclaving was 5.8. Cultures were kept in a climate chamber in controlled conditions:

- Relative humidity 80%;
- Photoperiod of 16 / 8, light / dark;
- Temperature 25±1˚C and;
- Illumination with the intensity of 50 µmol·m^{2}·s^{-1}.

Well-rooted plants were transferred to plastic plate filled with sterile mixture of sand, peat and perlite (1:1:1), and their acclimatization took place in two phases: climate chamber (Figure 1D), and then transferred to greenhouse.

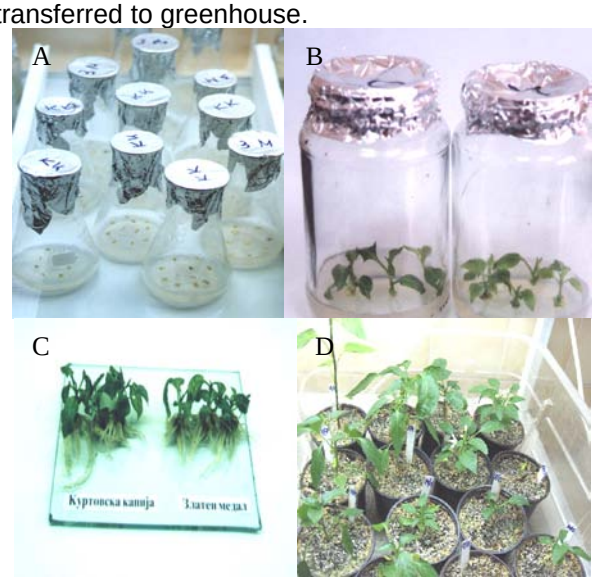

Figure 1. (**A**) Pepper seed placed in initial MS medium for aseptic germination; (**B**) Development of apical buds on MS hormonal medium; (**C**) Rooting of pepper shoots on MS medium supplemented with auxins; (**D**) Acclimatization of pepper in vitro regenerated plants in climate chamber.

2.2 Determination of some biogenic elements and photosynthetic pigments

The material for analysis was taken twice in the vegetative period in the flowering phase and in the fruitful phase. The plant material (roots, steams, leafs, flowers and fruits) collected from *in vitro* regenerated plant and from the *in vivo*, conventionally grown plant, was dried until it was dry mass at the temperature of 60-80°C.

The dray plant material is used for determination of examine physiologic characteristic. The percentage of water and dry matter was calculated as the difference of the measured mass of fresh and dry condition of the plant material.

The contents of biogenic elements K, Ca, Mg, and Fe was determined in parent solution with atomic absorption spectrophotometer Perkin Elmar 5000, and the content of the P with ammoniumvandat molybdate method.

The content of photosynthetic pigments was determined in the third leaf, numerous top-down, by the method of Röbellen [17], in *in vivo* and *in vitro* conditions. The calculations of the photosynthetic pigments in the 96% ethanol extracts were done according the Wintermans J.F.G.M. and Mots De A, 1965, formulas [18]:

$$Chl\ a = 13.70 \times A_{665} - 5.76 \times A_{649}$$
$$Chl\ b = 25.80 \times A_{649} - 7.60 \times A_{665}$$
$$Chl\ a+b = 6.10 \times A_{665} + 20.04 \times A_{649}$$

3. Results and Discussion

3.1 The effect of growth regulators on in vitro pepper morphogenesis

Pepper explants cultivated in the MS medium, enriched with BAP and IAA, have had a faster recovery, than those cultivated in the same medium with hormones KIN, GA_3 and IAA. GA_3 showed inhibitory effect on formation of plant shoots, influencing on the proliferation of callus in the medium. Root-formation of well-shaped shoots was performed on MS medium with low concentration of auxin (0.1 – 0.04 mg·l^{-1} IAA + 0.1 mg·l^{-1} IBA), which were essential for the root-formation of the plantlets. The results of the influence of different combinations and concentrations of growth regulators on callus formation, leaf rosettes and roots formation are given in Table 1.

Table 1. The effect of growth regulators combination and concentration in MS medium on morphogenesis of pepper apical buds.

MS medium + growth regulators mg·l^{-1}						Rooting %	callus formation %	shoot formation %
IAA	IBA	GA_3	KIN	BAP	NAA			
0.05	-	0.05	0.10	-	-	-	100.00	-
0.10	-	0.10	0.10	-	-	-	92.24	-
0.10	-	-	0.10	-	-	-	84.00	2.27
0.10	-	0.20	2.00	-	-	-	80.00	7.27
0.20	-	-	2.00	-	-	-	42.22	-
0.10	-	0.10	5.00	-	-	-	52.50	-
0.10	-	-	5.00	-	-	-	2.40	2.40
0.10	-	0.1	-	0.1	-	-	3.70	3.70
1.00	-	-	-	2.0	-	-	16.13	9.70
-	3.00	-	-	1.5	-	-	51.06	57.44
-	-	-	-	-	1.0	-	54.34	26.08
0.10	1.00	-	-	-	-	29.76	-	-
0.05	0.10	-	-	-	-	79.78	-	-
0.04	0.10	-	-	-	-	83.95	-	-

After eight weeks, the plantlets were moved from sterile to non-sterile conditions where they adapted to the external environment. All further analyses of *in vitro* obtained plants, for the content of biogenic elements and some other physiological characteristics, were comparatively analyzed with the control group of *in vivo* plants. Significant differences showed only a few of the investigated characteristics.

3.2 Content of mineral elements

The results of the content of analyzed biogenic elements (P, K, Ca, Mg and Fe) indicate that there is a difference in the presence of analyzed elements in separate organs, in *in vitro* obtained and control plants obtained by conventional procedure in the *in vivo* conditions (Table 2).

Table 2. The content of mineral elements of pepper (Capsicum annuum L.) obtained in vitro and in outside conditions in vivo (mg·g-1 dry matter).

phase	group	organs	content				
			P	K	Ca	Mg	Fe
flowering phase	*in vitro*	root	1.61	28.60	21.20	19.59	0.34
	in vivo		1.50	14.00	24.00	15.50	1.04
	in vitro	stem	0.86	62.20	20.90	18.40	0.16
	in vivo		2.34	47.10	26.40	30.70	0.13
	in vitro	leaf	1.57	33.80	35.80	22.80	0.21
	in vivo		2.36	39.00	40.30	33.70	0.15
	in vitro	flower	5.34	28.80	47.00	23.80	0.24
	in vivo		7.54	27.20	29.10	22.10	0.14
fruitful phase	*in vitro*	root	0.77	7.90	23.00	13.20	0.49
	in vivo		0.07	8.00	16.20	7.00	0.08
	in vitro	stem	0.82	14.70	29.80	27.50	0.05
	in vivo		1.19	16.20	36.70	37.90	0.05
	in vitro	leaf	2.17	32.90	42.20	32.20	0.13
	in vivo		1.26	18.20	48.20	39.02	0.07
	in vitro	fruit	3.18	23.60	14.20	6.10	0.07
	in vivo		3.28	26.00	14.00	9.32	0.07

The dynamic of phosphorus has a completely normal stream both in *in vitro* obtained plants and control plants obtained in *in vivo* conditions. The content of phosphorus is greater in phase of flowering unlike phase of fruiting and its accumulation in flower and fruit is evident. These element translocations in generative organs in a larger extend then in the vegetative organs, which are shown by our results too.

The content of potassium has greater values in *in vitro* regenerated plants (with the exception of the leaf in the phase of flowering and in a fruit and stem in phase of fruiting), suggesting that regenerated plants have a high capability for preserving its juvenility. A certain accumulation of calcium is observed in the root of plants obtained *in vitro* phase of fruiting.

The content of calcium in both examined phases is the lowest in the roots and has better value in the leaf and stem. Translocation of this ion goes from the roots to the elevated parts of the plant. Affinity for calcium to the roots is less than the affinity towards other ions although its concentration in soil solution is usually eight to ten times greater than that of potassium.

The largest presence of magnesium is observed in leaves in both groups of plants. It is known that photosynthetic activity in *in vitro* obtained plants is started later, behind the control that is shown also with the content of exanimate chloroplast pigments in our experiment. Therefore the lower content of magnesium in leaves of regenerated plants in phase of blooming is quite logical.

In phase of flowering the value of iron in the over ground organs of regenerated plants is higher, and the content of plant pigments in the leaves have larger values in the control group plants. In phase of fruiting there is a positive correlation of these two physiological parameters, where regenerated plants have a greater value of these two parameters compare with the control.

With the exception of leaves in the phase of flowering, all other organs have greater water content in *in vitro* obtained plants. Regenerated plants thus containing a greater percentage of water throughout the vegetation in terms of control tend to preserve its juvenilities (Table 3). The content of the dry matter is in inverse correlation with the water content.

According to Swartz [19] and many other authors, a feature of plants obtained in *in vitro* conditions are often described as a "rejuvenation" where blooming is delayed (occurs later). According the same author growth regulators and their synthetic derivatives cause rejuvenation of plants obtained in *in vitro* conditions, and in particular gibberellins and cytokines who have the ability to extend the juvenile stages.

Table 3. Some physiological characteristics of pepper (Capsicum annuum L.) obtained in vitro and in vivo conditions.

		flowering phase				fruitful phase			
content	group	root	stem	leaf	flower	root	stem	leaf	fruit
water %	*in vitro*	86.05	87.39	81.07	94.01	86.49	79.07	78.69	93.25
	in vivo	74.77	85.30	82.41	87.76	68.18	78.89	73.44	92.75
dry matter %	*in vitro*	13.95	42.61	18.93	5.99	13.51	20.93	21.31	6.75
	in vivo	25.13	14.70	17.59	12.21	31.82	12.11	26.56	7.20
chl. a in leaf	*in vitro*	103.74 $mg \cdot g^{-1}$ fresh weight				105.59 $mg \cdot g^{-1}$ fresh weight			
	in vivo	138.95 $mg \cdot g^{-1}$ fresh weight				89.00 $mg \cdot g^{-1}$ fresh weight			
chl. b in leaf	*in vitro*	36.10 $mg \cdot g^{-1}$ fresh weigh				37.62 $mg \cdot g^{-1}$ fresh weight			
	in vivo	45.53 $mg \cdot g^{-1}$ fresh weight				33.37 $mg \cdot g^{-1}$ fresh weight			
chl. a+b	*in vitro*	139.84 $mg \cdot g^{-1}$ fresh weight				143.21 $mg \cdot g^{-1}$ fresh weight			
	in vivo	184.48 $mg \cdot g^{-1}$ fresh weight				122.37 $mg \cdot g^{-1}$ fresh weight			
caroten-oids in leaf	*in vitro*	58.60 $mg \cdot g^{-1}$ fresh weight				65.08 $mg \cdot g^{-1}$ fresh weight			
	in vivo	81.24 $mg \cdot g^{-1}$ fresh weight				56.60 $mg \cdot g^{-1}$ fresh weight			

3.3 Content of the photosynthetic pigments

Photosynthetic activity is followed by examine pigments in the chloroplast, chlorophyll a, chlorophyll b, chlorophyll a+b and total carotenoids. Carotenoids found in chloroplasts are helping photosynthesis, while those present in chromoplasts are the main ingredients of the colour of flowers and fruits of many plant species. Photosynthetic activity delay in *in vitro* obtained plants behind *in vivo* obtained plants, showed by lower content of all chloroplast pigments tested in the phase of blooming in *in vitro* obtained plants. Toward the end of vegetation, these differences in the content of photosynthetic pigments show inverse dynamics, and larger values are obtained in *in vitro* plants in phase of fruiting. This is result of the change of the way of nutrition in *in vitro* obtained plants and their acclimatization and adaptation to totally autotrophic way of nutrition. In the phase of fruiting plant pigments have greater value in *in vitro* obtained plants (Table 3), although the content of magnesium in the leaf of *in vitro* obtained plants is behind the control. Positive correlation is known between the content of iron and chlorophyll. This fact is confirmed in our research in the phase of fruiting, after completely adaptation of *in vitro* regenerated plant on external conditions.

4. Conclusions

According to the results concerning the area of researching in the *in vitro* pepper production form meristem tissue, and comparing with the conventional obtained plant, the following conclusions can be made:

The development of shoots in pepper apical bud culture has shown that cytokines, in the presence of auxins in smaller concentrations, effected stimulating on the organogenesis. In this regard BAP overrides the kinetin and BAP sows the best effect on shot formation.

The best rooting of well-shaped shoots was performed on MS medium with low concentration of auxins (0.04 $mg \cdot l^{-1}$ IAA + 0.1 mg·l IBA) were 83.95 % of shoots were rooted.

In vitro regenerated plants have all sort characteristics of pepper.

Biogenic elements P, K, Ca, Mg and Fe have a normal dynamic in regenerate group of plants and in the control group of plants. The content of potassium is higher in *in vitro* obtained plants throughout the vegetation (with the exception of the leaf in flowering phase and fruit and stem in fruiting phase), indicating that regenerated plants have the capacity for prolongation the juvenility.

From the results for the content of plant pigments can be concluded that *in vitro* obtained plants begin with their photosynthetic activity immediately after their full adaptation, which confirm why their content of plant pigments is less in phase of flowering than in phase of fruiting. After the fully established enzyme biosynthesis and the start of photosynthetic processes, *in vitro* regenerated plants increase the content of photosynthetic pigments. This result in greater value of photosynthetic pigments in *in vitro* obtained plants, compared to the control in phase of fruiting.

Regarding the fact that the region is mainly agricultural oriented the interest for *in vitro* culture of some vegetable crops could be used for mass production as well as for further researches concerning tissue culture methods.

References

[1] Gunai L. and Rao P.S. (1978) *In vitro* plant regeneration from hypocotyls and cotyledon explants of red pepper (*Capsicum*). *Plant Science Letters*, **11**: 365-372.

[2] Fari M., and Czako M. (1981) Relationship between position and morphogenetic response of pepper hypocotyls explants cultured in vitro. *Scientia Horticulturae*, **5**: 205-213.

[3] Kisaburo H., Zhiqing Y., Kenij K. (1988) The effect of cotyledon explants and culture condition on *in vitro* formation of advention buds in red pepper (*Capsicum annuum* L.). *Agricultural Science*, **37**: 153-159.

[4] Husain S., Jain A., Kothari S.I. (1999) Phenolatic acid improves bud elongation and *in vitro* plant regeneration efficient of *Capsicum annuum* L., *Plant Cell Reports*, **19**: 64-68.

[5] Dabuaza M., and Pena L. (2001) High efficiency organogenesis in sweet pepper *Capsicum annuum* L. tissues from different seedlings explants. *Plant Growth Regulations*, **33**: 221-229.

[6] Joshy A. and Kothari S.L. (2007) Height copper levels in the medium improved shoot bud differentiation and elongation from the cultured cotyledons of *Capsicum annuum* L. *Plant Cell Tissue and Organ Culture*, **88**: 127-133.

[7] Ficher M. (1990) Establishment of *Pepper nigrum* in vitro. *Acta Horticulturae*, **275**: 285-291.

[8] Mathewa H, Rao P.S. (1984) *In vitro* response of black pepper (*Pepper nigrum*). *Current Science*, **53**(4): 183-185.

[9] Koleva Gudeva L., Mitrev S., Spasenoski M. (2001) Possibilities of uses of some new methods for virus-free plant production. *Yearbook of Institute of Southern Crops, Strumica*, **1**: 37-45.

[10] Garcia R.A. (1990) Tissue and cell culture of pepper (*Capsicum annuum* L. c.v. Pico and Piqulio). *APHF/SECH*, pp. 249-254.

[11] Sim S.L. (1986) Propagation of single - node pepper *Piper nigrum* auttings. *Journal Announcment*, **1037**: 105-113.

[12] Hosain A., Konisho K., Minami M., Nemoto K. (2003) Somaclonal variation of regenerated plants in chili pepper *Capsicum annuum* L. *Euphitica*, **130**: 233-239.

[13] Arous S., Boussaid M., Marrach M. (2001) Plant regeneration from zygote hypocotyls embryos of Tunisian chilly *Capsicum annuum* L. *Journal of Applied Horticulture*, **3**(1): 17-22.

[14] Koleva Gudeva L., Spasenoski M. (2001) The effect of some cytokinines on pepper organogenesis (*Capsicum annuum* L.). *Yearbook of Institute of Southern Crops, Strumica*, **1**: 31-35.

[15] Spasenoski M. (1993) Vegetative propagation of some plant species in vitro and possibilities for obtaining health plant material. *Proceeding of XVIII plant protection meeting, Ohrid R. of Mecedonia*, **5**: 145-148.

[16] Murashige T., Skoog F. (1962) A revised medium for rapid grouth and bio assay with tobacco tissue cultures. *Phisiologia Planatarum*, **15**: 473-497.

[17] Röbellen G. (1968) Desynapsis als Fehlerquelle bei der Aufstellung von Monosomen-Sortimenten des Weizens. *Z Pflanzenzüchtg*, **60**: 1-18.

[18] Wintermans J.F.G.M., Mots De A. (1965) Spectrophotometric characteristics of chlorophylls *a* and *b* and their phenophytins in ethanol. *Biochemica et Biophysica Acta (BBA) – Biophysics including Photosintesis*, **109**(2): 448-453.

[19] Swart H.J. (1993) Genetic and epigenetic effects and related problems. *Post culture behavior*, pp. 95-121.