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SOME MORPHOLOGICAL AND BIOLOGICAL CHARACTERISTICS OF PEPPER PRODUCED UNDER *IN VITRO* CONDITIONS

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Abstract

Apical buds were isolated from pepper (*Capsicum annuum* L. sort Kurtovska kapija), from aseptically grown seedlings; then they were cultivated on MS medium (Murashige and Skoog) with hormones IAA and KINETIN. After 4 weeks, pepper shoots were obtained from the apical buds. Rooting of pepper shoots was stimulated on MS medium with a low level of auxins, which were essential for rooting. After 6 weeks the plantlets were adapted to the environment.
Compared with the regularly cultivated plants, which were used as a control, the *in vitro* procedure yielded plants with significant differences in some morphological characteristics. In the next vegetation, the seeds from *in vitro* obtained plants were planted in the field. These plants were less variable in some morphological characteristics than the control group. Phenological phases appeared at the same time in both *in vitro* and control plants.

1. Introduction

In vitro regeneration of *Capsicum* species has been achieved from many tissues and organs - hypokotil explants (Gunai, *et al.*, 1978; Fari, M. and Czako, M. 1981), cotiledon explants (Kisaburo, *et al.*, 1988), apical buds (Fitcher, M. 1990), stem segments (Garcia, R.A. 1990; Sim, S.L. 1986).

The purpose of this work was to extend cultured tissue of apical buds taken from red pepper (*Capsicum annuum* L. c.v. Kurtovska kapija), to observe the tissue in distinctively "*in vitro*" conditions, to note their responsibility for organogenesis and regeneration, and to produce plants from plantlets established from tissue culture.

This article describes some morphological and biological characteristics of pepper produced under *in vitro* conditions and normal cultivated plants.

2. Material and methods

Apical buds of red pepper were isolated from aseptically grown seedlings. The seeds were washed 15 sec in 70% alcohol, stirred 10 min in 1% Izosan, then stirred 15 min in Na - hypochloride and rinsed twice in sterilized distilled water.

Apical buds were trimmed to 0,3 - 0,5 cm in length and were cultivated in a modified Murashige-Skoog medium containing benzyladenine BA 1,0-2,0 mg/l; 6furfurylaminopurine KIN 1,0-5,0 mg/l; indole3acetic acid IAA 0,1 mg/l and gibberellic acid GA3 0,1-0,2 mg/l, in different combinations and concentrations. The medium also contained 200 mg/l casein hydrolysate, 100 mg/l inositol and 7g/l agar (pH 5,8).

Cultures were held in the primary growth room under illumination of 2 000 - 3 000 Lux, photo-period 16/8 light/dark, 25±1°C temperature and relative humidity of 80%.

Proc. First Balkan Symp.
Vegetables and Potatoes
Eds. S. Jevtić, B. Lasić
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After 4 weeks, pepper shoots were obtained from apical buds. Rooting of plantlets was stimulated also on MS containing low level of auxins (IAA and IBA indole3buteric acid).

The plants were adapted to the environment within 6 weeks, and they grew into regular normal plants. The seeds from the plants obtained *in vitro* were picked and planted in the next vegetation. These plants had better morphological characteristics than the control group.

3. Results and Discussion

Pepper shoots were obtained from initial plantlets after 4 weeks. The effect of auxin-cytokinin combinations on shoot formation was observed (Table 1).

Table 1 - The effect of different hormone combinations in MS medium on pepper shoot formation.

MS + hormones (mg/l)				pepper shoot	
BA	IAA	KIN	GA_3	formation	callus
1,0	0,1	-	-	++	-
2,0	0,1	-	-	+++	-
-	0,1	1,0	0,1	+	+++
-	0,1	5,0	-	+++	+
-	-	1,0	0,2	-	+++

Plantlets had faster regeneration when they were cultivated on MS medium with BA and IAA than others which were cultivated on the same medium with KIN, GA3 and IAA. GA3 had an inhibitory influence on shoot formation because this hormone caused a large callus formation, especially on MS + 0,2mg/l GA3 + 1,0 mg/l KIN.

After the elongation on the above mentioned mediums (Table 1), rooting was stimulated on MS + 0,04 mg/l IAA + 0,1 mg/l IBA. Low levels of auxins were essential for rooting.

The rooted plants were transferred into plastic pots in a sterile mixture of pert, sand and perlit (1: 1: 1) and were kept in the primary growth room. Their acclimatization went through 3 stages: the first in a primary growth room, the second in greenhouse conditions, and the third in outside climate conditions.

After 6 weeks the plants produced from plantlets, established from tissue culture, became adapted to the environment and they grew into regular normal plants. In the outside climate conditions, normal cultivated plants were also transferred to be used as a control group, and as a check for some biological and morphological characteristics of the plants.

In vitro obtained plants showed significant differences in the length of the roots and the height of the entire plants, during all phenological phases. The morphological and production characteristics of the fruits were less variable and without significant differences.

In the next vegetation, seeds from *in vitro* obtained plants were planted and these plants, during vegetation, were less variable than the control group (Table 2, 3 and 4).

Table 2 - Morphological characteristics of plants in seedling phase.

	height of plant cm	length of internodes cm	thickness of stem cm	number of leafs	length of leaf cm	width of leaf cm	length of root cm
in vitro	10,36	4,84	0,33	7,50	4,17	2,27	5,23
control	11,40	5,87	0,23	6,30	3,94	2,37	5,84
LSD	0,31	0,73	1,11	0,49	0,32	0,33	0,07

Table 3 - Morphological characteristics of plants in flowering phase.

	height plant cm	height branch cm	thickness of stem cm	length interno-des cm	No of leafs	length of leaf cm	width of leaf cm	number flowers cm	length of root cm
in vitro	25,75	14,35	0,94	2,93	37,20	7,10	3,50	23,80	22,00
control	26,30	14,85	0,88	2,80	40,90	6,70	3,80	25,60	20,00
LSD	0,01	0,02	0,53	0,03	0,06	0,03	0,62	0,18	0,66

Table - 4 Morphological characteristics of plants in fruitful phase.

	height plant cm	height of branching I cm	II cm	III cm	length intero-des cm	width of stem cm	No of leafs	length of leaf cm	width leaf cm	length leaf cm
in vitro	73,10	23,20	21,40	30,30	7,18	0,83	110,90	6,55	3,69	24,59
control	73,60	13,18	21,10	29,50	6,60	0,82	86,00	7,00	3,60	26,13
LSD	0,004	0,17	0,01	0,16	0,29	0,03	0,41	0,35	0,13	0,54

A large number of the "*in vitro*" plants had better morphological characteristics, especially in the fruit phase (Table 4).

The results obtained from the morphological and production measures of fruits show that they were better from "*in vitro*" plants (Table 5 and 6).

Table 5 - Morphological characteristics of fruits.

	length of fruit cm	width of fruit cm	index of fruit cm	mass of fruit g	mass fruit without seeds g	thickness of pericarp cm	number of chambers	dry matter %
in vitro	14,86	6,36	2,27	132,51	94,59	0,49	2,50	6,85
control	13,96	5,67	2,46	114,76	85,74	0,47	2,50	7,17
LSD	0,39	1,01	0,05	0,49	0,33	0,05	0,00	0,27

Table 6 - Production characteristics of fruits.

	number of seeds per fruit	mass of seeds per fruit g	mass of one seed g	number of fruit up to III branch	number of fruit per plant	mass of fruit up to III branch g	mass of fruit per plant g	yield kg/h
in vitro	368,20	3,00	0,0089	4,20	8,30	467,84	321,84	33414,00
control	271,60	2,46	0,0078	2,40	6,90	351,81	248,88	25128,00
LSD	1,16	0,98	0,02	0,78	0,26	0,48	0,32	1,34

The production characteristics of fruits from "*in vitro*" plants showed higher values such as number of seeds per plant, mass of seeds per fruit, mass of fruit up to the III branch, muss of fruit per plant, therefore the yield from "*in vitro*" plants was higher.

Table 7 - Biological characteristics - phenological phases.

	planting	germi-nation	flowering	ripening	technological ripening	botanical ripening	vegetation days
in vitro	27.3.'95	12.4.'95	03.7.'95	12.7.'95	21.8.'95	11.9.'95	148
control	27.3.'95	12.4.'95	03.7.'95	12.7.'95	21.8.'95	11.9.'95	148

The results from table 7 show that there was no differences in biological characteristics between the two groups of pepper. All phenological phases appeared at the same time, and the duration of the vegetation period was 148 days for both groups.

4. Concluding remarks

The results obtained from the experiments proved that the most effective combination of growth hormones in induction shoot formation and plant regeneration were BA+IAA and KIN+IAA. It was obvious that higher concentrations of BA and KIN stimulated the regeneration of explants. Rooting was stimulated only with auxins.

In vitro obtained plants showed significant differences in some morphological characteristics of the plants. In the next vegetation, they had less variables and were without any significant differences, compared with the control, for all examined values, and they showed higher yield.

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