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In vitro Plant Regeneration from Pepper (*Capsicum annuum* L. cv. 'Soroksari') Seedling Explants

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S u m m a r y

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In vitro plant regeneration from pepper (*Capsicum annuum* L. cv. 'Soroksari') explants were investigated. Shoot-tip, cotyledon and hypocotyl explants excised from 2 weeks old seedlings were cultured on MURASHIGE & SKOOG medium supplemented with B5 or L2 vitamins, and different content of growth regulators: 2,4-dichlorophenoxyacetic acid, benzyladenine, indole-3-acetic acid, and zeatin. Abundant callus have been developed on explants cultured on initial media with 2,4-dichlorophenoxyacetic acid. The best response of regenerative ability showed calli induced on cotyledon explants after transfer on MS-B5 medium supplemented with benzyladenine and gibberellic acid. Direct shoot regeneration have been obtained only from basal part of shoot-tip explants cultured on media with benzyladenine or zeatin alone, and with benzyladenine and indole-3-acetic acid. Regenerated shoots, rooted on MS-B5 medium with indole-3-acetic acid, were successfully transferred in the glasshouse conditions. Plants regenerated from callus cultures grown ex vitro showed differences in their morphological and physiological traits.

Introduction

Pepper (*Capsicum annuum* L.) is an important vegetable crop grown worldwide (AGRAWAL & al. 1989), and there is the strong intention to improve existing cultivars using a new biotechnological methods as somaclonal variability and/or plant transformation (ARROYO & REVILLA 1991, EBIDA & HU 1993, CHRISTOPHER & RAJAM 1996, SZAS & al. 1997).

Somaclonal variability - variation originating in cell and tissue culture (LARKIN & SCOWCROFT 1981) - may be of value in crop improvement in two ways: 1) to augment that component of the variation which may not exist in the natural

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gene pools, and 2) to change one or more characters in an existing cultivar which may lead to its improved performance (AHLOOWALIA 1986).

In our breeding programmes we are interested to use, along with traditional breeding methods, also plant tissue culture as one of the possibilities to obtain pepper plants with changes in qualitative and quantitative characters. This requires an efficient, reliable and reproducible procedure for plant regeneration from callus cultures. Many studies on plant regeneration from different explant types confirm that pepper is recalcitrant species for work in plant tissue culture, and also highly genotype dependent.

The results reported here are the initial work on in vitro plant regeneration from explants of pepper cultivar 'Soroksari'.

Abbrevations: BA=benzyladenine; B5=Gamborgs B5 medium (GAMBORG & al. 1968); 2,4-D=2,4-dichlorophenoxyacetic acid; GA_3=gibberellic acid; IAA=indole-3-acetic acid; L2=Phillips medium (PHILLIPS & COLLINS 1979); MS=Murashige and Skoog medium (MURASHIGE & SKOOG 1962); MSB5=medium consists of MS macrosalts and B5 vitamins; MSL2=medium consists of MS macrosalts and L2 vitamins; Z=zeatin.

Material and Methods

Seeds of *Capsicum annuum* L. cv. 'Soroksari' obtained from Breeding Center Semenarna Ptuj, were surface sterilized in 10% (v/v) commercial bleach for 10 min and rinsed in several changes of sterile distilled water. One sterile seed was placed in culture tube on wet cotton and germinated aseptically at temperature +25°C under a 16h-photoperiode of dim light. Excised shoot-tips, cotyledons and hypocotyls of two weeks old seedlings were used as explants.

Initial media consisted of MS salts (MURASHIGE & SKOOG 1962), and B5 vitamins (GAMBORG & al. 1968) supplemented with growth regulators: 1) 4.5 µmol/l 2,4-D; 2) 4.5 µmol/l 2,4-D+2.2 µmol/l BA; 3) 8.9 µmol/l BA+2.9 µmol/l IAA; 4) 13.3 µmol/l BA+5.7 µmol/l IAA, and MS salts with L2 vitamins (PHILLIPS & COLLINS 1979) supplemented with growth regulators: 5) 4.4 µmol/l BA, and 6) 9.1 µmol/l Z. Cultures were transferred from initial media after two weeks on MS-B5 medium supplemented with 17.8 µmol/l BA+5.8 µmol/l GA_3 (R1 medium) for shoot regeneration during next two weeks. Explants with regenerated shoots were transferred on MS-B5 medium with 8.9 µmol/l BA+2.9 µmol/l GA_3 (R2 medium) for shoot elongation. Elongated shoots were individually rooted on MS-B5 medium supplemented with 2.9 µmol/l IAA, and successfully transferred ex vitro under high air humidity.

Results and Discussion

Abundant, white and friable callus have been developed in the first weeks of culture on all explants (shoot-tip, cotyledon and hypocotyl) cultured on media with 2,4-D.

Shoot-bud regeneration and leafy structures have been obtained only on calli transferred on medium with BA (17.8 µmol/l) and GA_3 (5.8µmol/l).

No organic response have been observed from calli grown on media with 2,4-D more than two weeks, and similar results reported ARROYO & REVILLA 1991 for pepper cultivars Pico and Piquillo.

It was found that very important step for shoot development in pepper callus is transfer them after two weeks on the regenerative medium with lower concentration of BA (8.9 μmol/l) and GA_3 (2.9 μmol/l). Using this procedure it was possible to obtain shoot regeneration in 60% of explants. The best response was obtain in cotyledon explants inoculated with upper part on medium surface (100%).

Table 1. Effect of initial media and growth regulators on organogenesis in different explants of pepper cv. 'Soroksari'; percentage of explants with organogenesis.

Explant type	Media 1	2	3	4	5	6
ST	20	0	100	100	100	100
H	0	0	40	20	20	80
C	40	20	40	20	0	40
CD	60	40	100	40	60	40
C*	60	80	40	20	20	0
CD*	100	60	60	40	60	60

Explant type: ST-shoot type; H-hypocotyl; C-cotyledon; CD-distal part of cotyledon; *-explant with upper part on medium surface.
Note: Five explant types were inoculated on each medium.

Table 2. Shoot regeneration in different explants of pepper cv. "Soroksari' after transfer from initial media (1-6) on medium for regeneration (MSB5+17.8 μmol/l BA+5.8 μmol/l IAA) and elongation (MSB5+8.9 μmol/l BA+2.9 μmol/l IAA).

Explant	Media 1		2		3		4		5		6	
type	a	b	a	b	a	b	a	b	a	b	a	b
ST	1	0.2	0	0	31	6.2	10	2.5	81	11.6	78	9.8
H	0	0	0	0	3	1.0	1	1.0	7	3.5	11	1.0
C	2	0.4	1	0.2	13	4.3	1	1.0	0	0	0	0
CD	12	2.4	3	0.6	21	4.2	4	2.0	0	0	0	0
C*	5	1.0	21	4.2	28	14.0	1	1.0	8	4.0	0	0
CD*	29	5.8	18	3.6	71	2.3	10	5.0	7	2.3	27	9.0

Explant type: ST-shoot type; H-hypocotyl; C-cotyledon; CD-distal part of cotyledon; *-explant with upper part on medium surface.
a) total number of regenerated shoots; b) average number of regenerated shoots per explant
Note: Five explant types were inoculated on each medium.

The average number of regenerated shoots was 5.8 per explant. Only a few shoots developed roots spontaneously, and efficient root induction have been achieved after four weeks on medium with 2.9 μmol/l IAA.

Direct shoot regeneration have been obtained in tissue of basal part of shoot-tips (100% of explants) on initial media with cytokinins BA (4.4 μmol/l) or Z (9.1 μmol/l), and also when BA (8.9 μmol/l) and IAA (2.9 μmol/l) were applied

together. No callus was observed on the explants that expressed shoot-bud organogenesis. The average number of regenerated shoots was 11.6 per explant. EBIDA & HU 1993 also obtained the highest shoots number from shoot-tip explants of pepper cv. Early California Wonder, when exogenous cytokinin BA with no auxin added in medium. Higher concentrations of BA (13.3 μmol/l) and IAA (5.7 μmol/l) supressed ability for shoot regeneration on initial medium, and shoots (2.5 shoot/explant) were observed when shoot-tip explants were transferred on regenerative medium.

Conclusion

Presented results of our initial work on in vitro plant regeneration from different explants of pepper cultivar 'Soroksari' showed the possibilities to obtain plants also from callus tissue induced by auxin 2,4-D, which could be used in our further breeding programmes of this cultivar.

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