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In vitro culture of bell pepper (*Capsicum annuum* cv. Bryza) and its greenhouse performance

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Abstract. Two methods of pepper regeneration regarding the rate of obtaining microcuttings were compared. In the first one, halves of imbibed seeds were cultured on Murashige and Skoog (MS) medium without growth regulators (GR). In the second one, seedling explants, cotyledon segments and shoot tips, which were cultured on MS medium with GR, were used. The induction of adventitious buds occurred on the cut surface of the explant and depended upon the type of the explants, the presence and concentration of GR. The microcuttings which were fit for the ex vitro use, developed mainly from adventitious buds formed on the explants derived from the seeds. Adventitious buds on the explants derived from the seedlings developed mainly into leaflike structures while the axillary buds – into shoots. Rooted shoots were grown successfully and potted out in a greenhouse where they grew into normal fruit bearing plants.

Key words: *Capsicum annuum*, micropropagation, organogenesis.

Introduction

There are reports on regeneration of pepper plants from adventitious buds on cotyledons, hypocotyls and shoot tips induced by growth regulators (GR) (FARI, CZAKO 1981, PHILLIPS, HUBSTENBERGER 1985, AGRAWAL et al. 1989, OCHOA-ALEJO, IRETA-MORENO 1990, ARROYO, REVILLA 1991, EBIDA, HU 1993). The regeneration system depends on the genotype (ROGOZIŃSKA, TOBOLEWSKA 1992, EZURA et al. 1993, SZASZ et al. 1995), physiological condition of the donor material (GATZ et al. 1995), explant source (FARI, CZAKO 1981), culture medium composition and incubation conditions (light quality

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and intensity, day length, temperature) (PHILLIPS, HUBSTENBERGER 1985). Poor development of adventitious buds on pepper explants limits the procurement of improved breeding material with the use of molecular biology methods. With the aim of obtaining a more efficient regeneration system of pepper, the effect of age, the kind of explants and the GR were determined in this study. The preliminary results of greenhouse performance of the plants obtained through in vitro culture are also reported.

Material and methods

In vitro culture

Based on the researches of EBIDA and HU (1993) and EZURA et al. (1993) two methods of in vitro regeneration of the Polish pepper cultivar Bryza were compared. In the first method, halves of imbibed seeds (0-6 days preculture) containing the proximal part of hypocotyl and the radicle of embryos were used as explants; they were cultured on MURASHIGE and SKOOG (1962) (MS) medium without GR. In the second method, the explants constituting cotyledon segments and shoot tips isolated from 2-week-old seedlings were cultured on MS medium with combinations of GR: 1-naphthalene acetic acid (NAA) and 6-benzylamino purine (BAP). The concentrations of auxin and cytokinin varied depending on the type of explant (Table 2).

The procedure of explant isolation and the conditions of culture were described previously (ROGOZIŃSKA, TOBOLEWSKA 1992, GATZ, ROGOZIŃSKA 1994). The results are presented as the mean of three replicates.

Greenhouse culture

The pepper microcuttings obtained using both methods of regeneration and propagation were transplanted to the pots with peat bed and placed in the greenhouse. After two-three weeks of growth, forty microcuttings with similar developmental stages and good vigour were selected and established in the soil. 28 microcuttings derived from adventitious buds and 12 from axillary buds. Fertilisers and chemical plant protectors were applied in the course of the culture. After the development of six to eight sets of fruits, the plants were detopped (NOWACZYK, NOWACZYK 1995). Observations on plant development were conducted throughout the period of plant growth in the greenhouse till the time of harvest. The effect of seed preculture on fruit bearing was determined. The fruiting of the plants developed from adventitious

buds (3-5 day of preculture) was compared to that of the plants obtained from axillary buds. Control plants were obtained by a traditional way.

The results obtained were evaluated by the analysis of variance. Additionally the methods of regression and correlation were used to confirm the relationship between some useful features of pepper fruits and the time of seeds preculture.

Results

In vitro culture

Seed explants. Explants derived from the precultured seeds formed roots during the first two weeks of culture. Simultaneously the hypocotyls elongated and adventitious buds were induced around the cut surface of the hypocotyls. Shoots were formed after the next two weeks of culture (Fig. 1A). The number of explants forming buds increased with the length of seed preculture (Table 1). Also the number of explants forming shoots was positively correlated with the time of seed preculture. The correlation coefficient (0.457) was significant

Table 1. Effect of the time of pepper seed preculture on the formation of adventitious buds and further development after successive passages*

Duration of preculture (days)	Explants (%)		Mean number of shoots per explant after passages		
	with buds	with shoots	I	II	III
0	62.2	14.2	0.6	1.4	0.4
1	66.4	23.5	0.5	1.5	1.7
2	68.6	32.8	0.5	1.2	0.7
3	70.2	33.4	0.8	2.0	1.2
4	70.1	33.8	0.6	1.9	1.0
5	67.1	35.6	0.3	0.0	0.6
6	70.5	25.0	0.6	1.6	3.0

* means from three replicates, each replicate comprised twenty four explants

at level $\alpha = 0.01$. This relationship was linear (regression equation: $y = 19.68 + 5.04x$) and the coefficient of determination in 33% explains this variability. However, the shoots were formed only from some buds (Fig. 1A). The per-

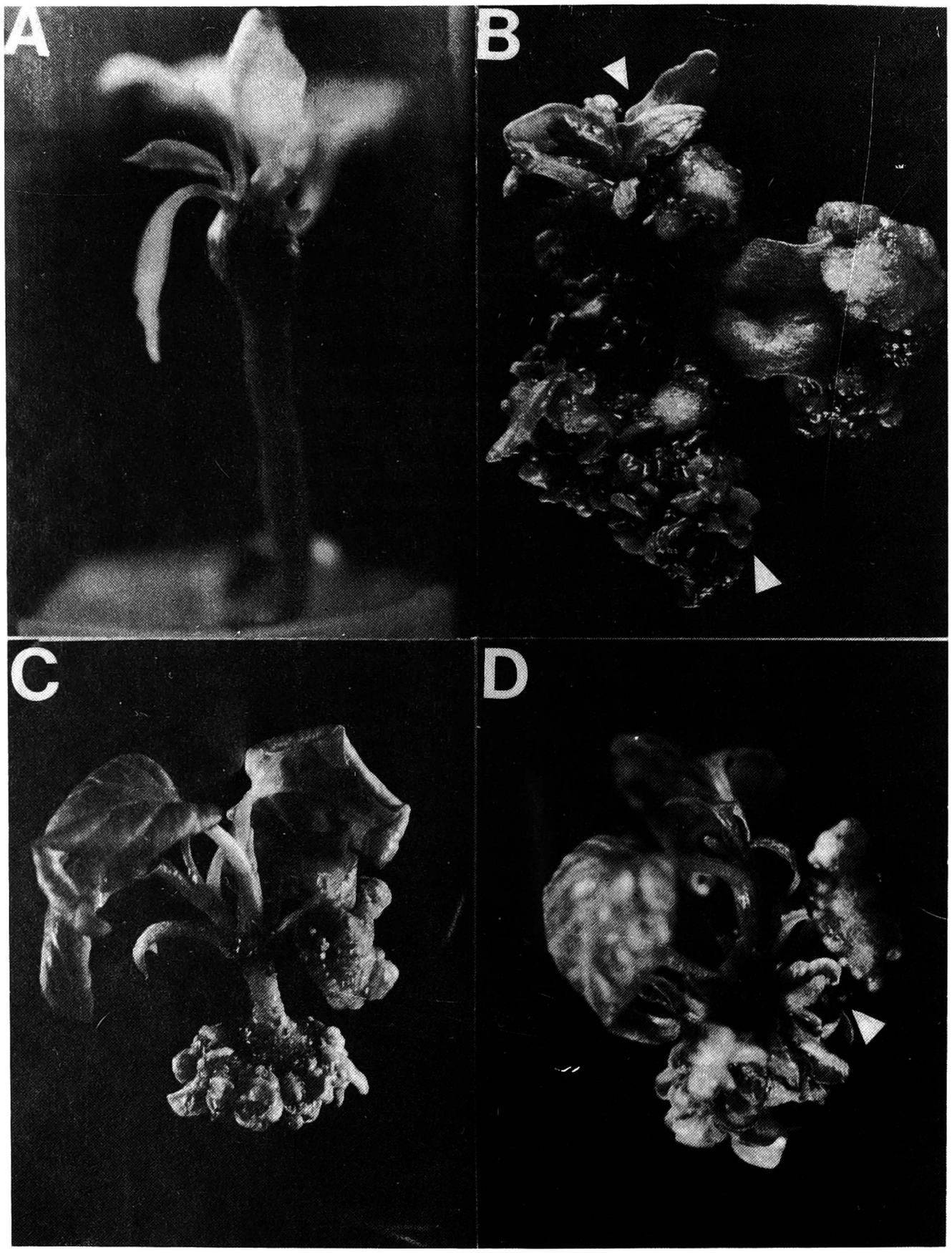

Fig. 1. In vitro differentiation of pepper explants derived from precultured seeds (A) and seedlings (B, C, D) after four weeks of incubation on MS medium

A – elongated shoots developed from adventitious buds formed at the cut surface of hypocotyl (0 GR), B – multiple adventitious shoot buds and leaflike structures proliferated at the cut surface of cotyledon (0.1 mg/l NAA + 10 mg/l BAP), C – multiple adventitious shoot buds proliferated at the cut surface of shoot tip (5 mg/l BAP), D – differentiation of axillary buds at the shoot tip (5 mg/l BAP)

centage of explants, on which adventitious shoots were produced was the highest after 3-5 days of seed preculture (Table 1). After about four weeks, 2 mm hypocotyl segments with adventitious buds were transferred to a fresh medium, where further bud development, shoot elongation and root initiation occurred. After another four weeks the rooted adventitious shoots were transplanted to the *ex vitro* conditions, while the buds were subcultured to be multiplied. The details concerning the successive subcultures and the effectiveness of the method are shown in Table 1. As results from the given data (Table 1), the maximum number of adventitious shoots obtained from one explant was two in the second passage and three in the third one, respectively.

Seedling explants. The explant sources used consisted of cotyledons and seedling shoot tips. The MS medium was supplemented with GR. Callus and numerous adventitious buds were formed on the cut surface of cotyledons during the culture period. The induction of adventitious buds occurred also at the base of shoot tips. However, the number of buds per explant was smaller than that obtained from cotyledon explants. During four week of culture, shoots were formed also from the axillary buds of the shoot tip explants (Fig. 1B-D, Table 2).

Table 2. Induction of adventitious buds and development of axillary shoots on seedling explants of pepper after four weeks of incubation on MS medium with growth regulators

Explant source	Concentration of growth regulators (mg/l)	Explants forming (%)	
		adventitious buds[1]	axillary shoots[1]
Cotyledons	0.1 NAA + 10 BAP	99.0	0
Shoot tips	5 BAP	28.3	43.4

[1] means from three replicates, each replicate comprised forty eight explants

After four weeks of culture, adventitious buds and the developed axillary shoots were transferred into MS medium without GR or with the addition of auxin. The subcultured adventitious buds appeared on cotyledon explants usually developed into leaflike structures. Shoots were formed only from some buds after another passage. Leaflike structures developed also from buds at the base of shoot tips, whereas shoots developed from axillary buds, elongated and produced roots. The efficiency of shoot formation after four weeks amounted to 2.7 plantlets per explant (the mean of three replicates).

Greenhouse culture

The microcuttings obtained by in vitro method were potted out in the greenhouse where they grew into self-sufficient plantlets (Fig. 2). The transition of plantlets from in vitro conditions and the hardening-off process were successful. The survival rate approached 100%. After about two weeks of growth the plants began to produce flowers and set normally shaped fruits with seeds.

Fig. 2. Ex vitro development and fruit set of pepper cv. Bryza

Characters of pepper fruits obtained by in vitro culture are presented in Tables 3 and 4.

As shown earlier, the time of seed preculture affected adventitious bud and shoot production, as well as some features of fruits. The percentage of explants,

Table 3. The effect of seed preculture from which the explants derived on fruit bearing of pepper

Character	Duration of seeds preculture (days)						
	0	1	2	3	4	5	6
Number of fruits per plant	7	6	6	5	6	4	7
Fresh weight of one fruit (g)	57.79	52.30	58.40	61.00	70.88*	73.08*	60.07
Dry weight of one fruit (%)	6.78	6.89	8.18*	6.77	7.20	6.31	6.10
Fresh weight of one pericarp (g)	48.93	44.58	50.04	52.06	61.33*	62.06*	51.90
Coefficient of fruit shape (length/width)	1.24	1.19	1.19	1.14	1.21	1.28	1.20
Mean thickness of pericarp (cm)	0.41	0.38	0.40	0.39	0.42	0.41	0.30

* $P \leq 0.05$.

on which adventitious shoots were produced, was the highest after 3-5 days of preculture. Also the plants which were developed from these shoots formed fruits with the highest fresh weight. The time of seed preculture influenced the pericarp weight in a similar way. These relationships were linear and their regression equations are: $y_{FWF} = 55.29 + 2.25\,x$, $y_{FWP} = 47.07 + 1.98\,x$ (FWF – fresh weight of fruit, FWP – fresh weihgt of pericarp, x – time of seed preculture). The coefficient of determination in 19% explains the dependency of the both features from the time of seed preculture.

Table 4. The comparison of pepper fruiting depending on the way of obtaining micro-cuttings

Characters	Control – intact seeds	Microcuttings obtained from	
		adventitious buds (seed explants)	axillary buds (seedling explants)
Number of fruits per plant	7	6	6
Fresh weight of one fruit (g)	68.42	69.02	68.21
Dry weight of one fruit (%)	5.99	7.15	7.28
Fresh weight of one pericarp (g)	57.29	64.24	58.46
Coefficient of fruit shape (length/width)	1.27	1.24	1.22
Mean thickness of pericarp (cm)	0.36	0.42	0.37

The investigations carried out have shown that morphological and useful features of fruits of cv. Bryza grown in our greenhouse conditions and derived from in vitro culture were similar to those of fruits obtained by conventional way. A comparison of the ways of obtaining microcuttings (adventitious and axillary buds) showed no essential differences in the characters of the analysed fruits.

Discussion

There are various ways in which plants can be propagated through tissue culture. The simplest type of in vitro plant propagation is the stimulation of axillary bud development, the other one-adventitious shoot proliferation. In the experiments on pepper reported here, vegetative buds were produced through two different organogenetic pathways depending on the explant cultured: axillary buds from seedling explants (shoot tips) and adventitious buds from seed explants and seedling explants (cotyledons). Culture media and growth conditions were optimised for highest rates of multiplication.

Micropropagation depends, among others, on culture medium composition and interaction of endogenous hormones with exogenously supplied growth regulators. EZURA et al. (1993) have reported on bud induction in fourteen cultivars of pepper without GR. The explants used consisted of proximal part of hypocotyl and radicle. There were considerable differences among the investigated cultivars. The number of buds per explant ranged 1.1-3.9. The method of EZURA et al. (1993) applied to the pepper cv. Bryza gave similar results. In the first stage of culture, the bud induction mainly occurred and this process depended on the time of seed preculture which influenced embryo development. Successive passages affected the efficiency of the bud induction and shoot development.

The ability to organogenesis of various explants of pepper seedlings in the presence of GR was investigated, among others, by PHILLIPS and HUBSTENBERGER (1985). The authors have shown essential interactions between GR and the explant source. The explants of meristematic origin (shoot tips, cotyledon nodes) as compared to nonmeristematic explants (hypocotyls, distal part of cotyledon) had a 2-10-fold greater capability of forming shoots. The results described by EBIDA and HU (1993) show much smaller differences in the ability to bud formation on cotyledon explants and shoot tips. However, they used much younger seedlings and a medium with a lower agar concentration (0.4%). Similar conditions of explant isolation and culture applied to

the cv. Bryza showed that adventitious buds were formed most numerously on the cotyledons grown on a medium with GR. However, the number of shoots developed from these buds was not high because the majority of them developed into leaflike structures. The application of gibberellin to these structures to obtain shoots caused a very strong enlargement instead of the expected elongation and did not increase the number of formed shoots (unpublished data). Like in the case of lily of the valley (VERRON et al. 1995), it appeared very difficult to modify this type of organogenesis. Moreover, shoot and root organogenesis in pepper tissue cultures decreased with the subculture and it was impossible to maintain the organogenetic callus in continuous culture (PHILLIPS, HUBSTENBERGER 1985). A new promising way was found by SZASZ et al. (1995) in which the recalcitrant pepper genotypes not inducible by conventional methods employing BAP and IAA as plant hormones could regenerate shoots in cotyledon explants after thidiazuron treatment.

From this study, it appears that in the case of the cv. Bryza, in vitro culture of seed explants and their further development into plantlets seem to be more promising for the production of adventitious shoots than the use of seedlings as explant source. In the case of seedling explants further investigations should focus on establishing optimal conditions for stimulating the development of leaflike structures into shoots.

The development of microcuttings of the cv. Bryza obtained with the use of the both procedures (EZURA et al. 1993, EBIDA, HU 1993) was compared in the preliminary greenhouse investigations. The studies included 40 plants. There is a lack of similar data in the literature for comparison. Up to date, no papers (including those of EZURA et al. 1993 and EBIDA, HU 1993, whose methods were used in the present investigations) have given data on the yield and analysis of pepper fruits obtained through in vitro culture. The presented paper is an attempt in this direction.

Investigations on the regeneration and propagation system in pepper are of current interest. It was possible to introduce into and express in bell pepper tissues foreign genes, however, no success has been obtained until now in regeneration of whole plants from transformed shoot buds (LIU et al. 1990).

The results obtained in the presented paper could find application in the breeding program of pepper using tissue culture methods, namely for propagation of improved genotypes.

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