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Plant Regeneration from Hypocotyl Section and Callus in Buckwheat (*Fagopyrum esculentum* Moench.)

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Abstract

A tissue culture experiment on buckwheat hypocotyl was carried out by using B5 and MS media supplemented with various concentrations of 2,4-D and 6-BA. In the presence of 1.0-2.0 mg/l 2,4-D, callus induction was frequent and it was enhanced by the addition of 6-BA. Callus was also induced on the B5 media that contained only 6-BA (0.5-2.0 mg/l). Adventitious shoots were obtained from hypocotyl directly when they were cultured on the MS medium with 2.0 mg/l 2,4-D and 2.0 mg/l 6-BA. The calli induced in the media with 2.0 mg/l 2,4-D and 1.0-2.0 mg/l 6-BA and successively transferred to the media without 2,4-D regenerated plantlets, but those induced with lower concentrations of 6-BA did not. This suggests the effect of 6-BA to the regenerating ability of callus. Majority of the regenerated plants were diploids, but a few tetraploids and mixoploids were observed.

Introduction

In vitro tissue and cell culture have recently been introduced to the programe of plant breeding. In buckwheat, Yamane (1974) and Srejović and Nešković (1981) succeeded in plant regeneration from the calli of seedlings and cotyledons, respectively. However, more information will be needed for practical application of this technique to plant breeding.

In the present paper, callus culture was studied on the hypocotyl explant of buckwheat and regenerated plants were obtained from callus as well as from hypocotyl explant directly.

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Materials and Methods

A local variety 'Iwate-zairai Akisoba' of *Fagopyrum esculentum* Moench. was used. Seeds were germinated on filter paper in petri dishes at 25°C in darkness. Five day-old seedlings were sterilized by dipping them in 70% ethanol for 30 sec and 5% calcium hypochlorite solution for 10 min successively. About 5 mm long pieces were dissected from a hypocotyl and used as the explants.

The basal media were MS (Murashige and Skoog 1962) and B5 (Gamborg et al. 1968) solidified with 0.8% agar. They were supplemented with various concentrations of 2,4-dichlorophenoxy-acetic acid (2,4-D), indole-3-acetic acid (IAA), 1-naphthalene acetic acid (NAA), indole-3-butyric acid (IBA) and 6-benzyladenine (6-BA), solely or in combinations, as will be described in each experiment. Callus culture was made at 25°C under continuous light.

Chromosome numbers of the regenerated plants were determined on the root tips by means of Feulgen's squash method.

Results and Discussion

Callus formation from explants was tested on the B5 media supplemented with various concentrations of 2,4-D and 6-BA (Table 1). Callus was well formed when 1.0

Table 1. Effect of 2,4-D and 6-BA on callus and root formation induced from buckwheat hypocotyl section after 30 day culture on B5 medium

(a) Callus

Concentration of 2,4-D (mg/l)	Concentration of 6-BA (mg/l)					
	0	0.05	0.1	0.5	1.0	2.0
0	−	+	+	++	+++	+++
0.1	−	+	+	+++	+++	+++
1.0	++	+++	+++	+++	+++	+++
2.0	+++	++	++	+++	+++	+++
5.0	+	+	++	+++	+++	+++
(b) Root						
0	+++	+++	+++	+	−	−
0.1	+++	+++	+	+	−	−
1.0	+	−	−	−	−	−
2.0	−	−	−	−	−	−
5.0	−	−	−	−	−	−

Percentage of callus and root formation is indicated as follow, −: no callus and no root, +:<35%, ++: 35-70%, +++:>70%.

and 2.0 mg/l 2,4-D was given as well as when more than 0.5 mg/l 6-BA was supplied. It was noticed that callus could be induced by only 6-BA without 2,4-D. The callus under the combination of 1.0-2.0 mg/l 2,4-D and 0.5-2.0 mg/l 6-BA became somewhat greenish in color. By giving the hormonal combination of 2.0 mg/l 2,4-D and 0.1-2.0 mg/l 6-BA to the MS medium, callus formation was tested again (Table 2). The callus formation was enhanced by high concentration of 6-BA, and they became greenish under these conditions.

Table 2. Percentage of callus, shoot and root formation induced from buckwheat hypocotyl section after 30 day culture on the MS medium supplemented with 2,4-D and 6-BA

Concentration of		Callus		Shoot	Root
2,4-D (mg/l)	6-BA (mg/l)	Large	Small		
2.0	0.1	74	26	0	0
2.0	0.2	88	4	0	4
2.0	0.5	88	4	0	0
2.0	1.0	92	0	0	0
2.0	2.0	91	0	4	0

One explant on the MS with 2.0 mg/l 2,4-D and 2.0 mg/l 6-BA induced buds directly (Table 2 and Fig. 1). When these buds were transferred to a hormone free MS medium, they produced numerous buds and some of them elongated shoots. Well developed shoots were again transplanted to the MS with 1.0 mg/l IBA and without hormones to induce roots according to the method of Srejović and Nešković (1981) (Fig. 2). The rooted plants were grown in pots outside (Fig. 3). The adventitious shoot formation from hypocotyl explant was also observed on a different cultivar 'Hashikami-wase' (unpublished).

After 30 day culture in the MS with 2.0 mg/l and 0.1-2.0 mg/l 6-BA (Table 2),

Table 3. Percentage of shoot formation from hypocotyl callus after transfer from callus inducing media to regenerating ones

Regenerating medium		Callus inducing medium*				
Concentration of		6-BA concentration (mg/l)				
IAA (mg/l)	6-BA (mg/l)	0.1	0.2	0.5	1.0	2.0
0	0	0	0	0	0	40
0	0.5	0	0	0	20	20
0	1.0	0	0	0	20	0
0	2.0	0	0	0	20	20
0.2	2.0	0	0	0	20	20

* Callus inducing media contain MS, 2.0 mg/l 2,4-D and 6-BA.

each callus was cut into pieces and transferred to shoot formation media that were composed of MS and combinations of IAA and 6-BA in various concentrations as shown in Table 3. Adventitious buds were induced from the calli in all media, though the calli that developed buds were limited to those initially cultured on the medium with 2.0 mg/l 2,4-D and 1.0-2.0 mg/l 6-BA (Fig. 4). Effect of callus inducing medium to the shoot differentiation was pointed out.

When the adventitious buds were transferred to the MS medium supplemented with NAA (0.1-0.2 mg/l) and 6-BA (0.1-1.0 mg/l), or without hormones, numerous buds and a number of shoots were obtained. Rooting was better on the hormone free medium and its frequency decreased with the increasing concentration of hormones. After an incubation with the medium with 1.0 mg/l IBA or without hormones for obtaining more roots, a number of regenerated plantlets were grown in pots.

Finally 26 regenerated plants in total were obtained and subjected for chromosome observation. The chromosome numbers of these plants are shown in Table 4. The majority of regenerated plants had diploid chromosome number (2n=16), though some were tetraploids or mixoploids that consisted of diploid and tetraploid cells (Figs. 5 and 6). Since there was no difference in the frequency of diploid between the plants regenerated from hypocotyl and from callus tissue, the plant from hypocotyl might have passed once the callus status on the hypocotyl. One mixoploid involved 63% diploid and 37% tetraploid cells in it. Mixoploids in the regenerated plants from callus have been reported in many plants, e.g. *Brassica oleracea* (Horák 1972), *Hordium vulgare* (Mix et al. 1978), *Lycopersicum pervianum* (Ancora and Sree Ramula 1980) and *Solanum melongena* (Matsuoka and Hinata 1983).

Table 4. Chromosome numbers of the plants regenerated from hypocotyl and callus

Origin of regenerated plants	Number of plants with somatic chromosome number of			Total
	16	32	Mixoploid	
Plants regenerated from hypocotyl	11	2	2	15
Plants regenerated from callus	8	3	0	11

The present results showed a little difference from the data of Yamane (1974) on the optimum hormone concentrations for the callus formation and plant regeneration. Such discrepancy may be due to the difference of the used media, material cultivars and the organs from which explants were obtained.

For the successful incorporation of tissue culture to plant breeding, high regeneration ability is required for the material plants. Buckwheat may be one of good materials because of its high regenerating ability from cullus.

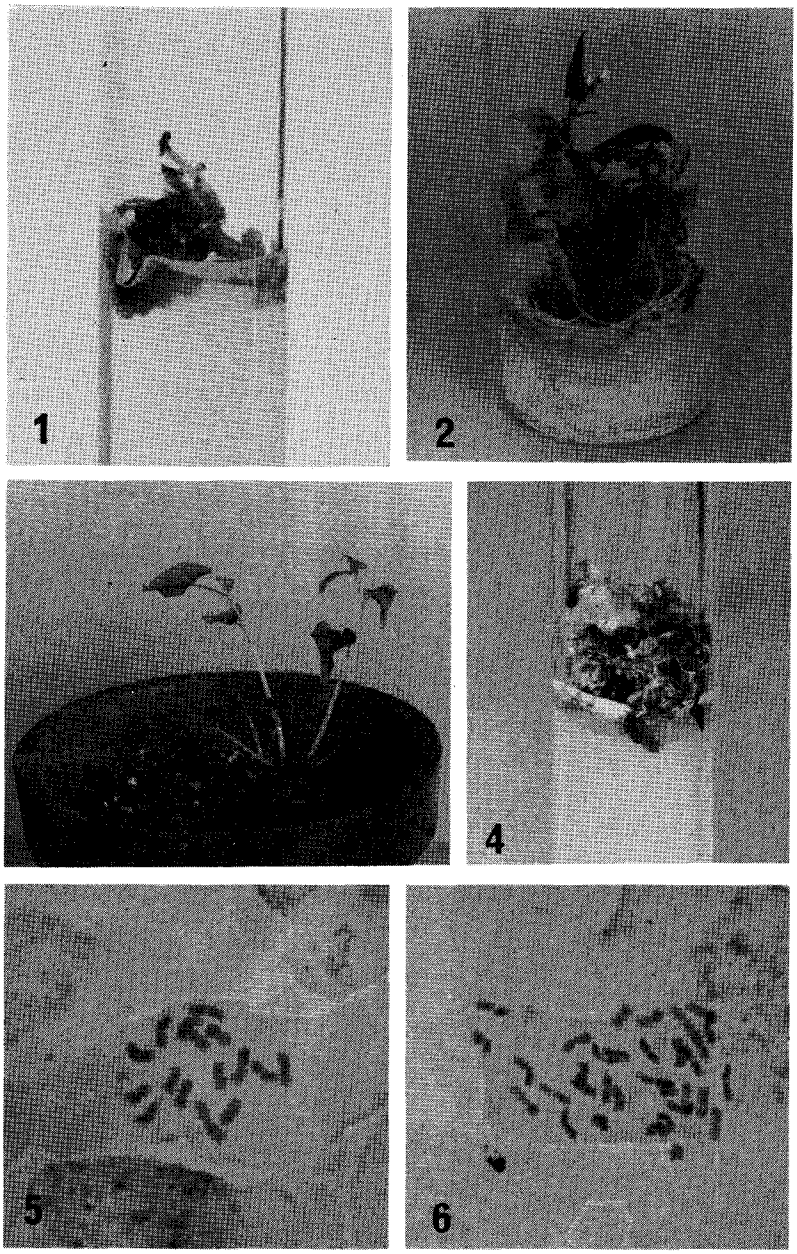

Fig. 1. Bud formation from hypocotyl section on the MS medium supplemented with 2.0 mg/l 2,4-D and 2.0 mg/l 6-BA

Fig. 2. Root formation from differentiated shoots on the MS medium supplemented with 1.0 mg/l IBA

Fig. 3. A regenerated plant in a pot

Fig. 4. Differentiated buds from the callus on the MS medium without hormones

Fig. 5. Chromosomes at metaphase in a root tip cell of a regenerated plant that determined to be diploid (2n=16)

Fig. 6. Same as Fig. 5 but of a tetraploid plant (2n=32)

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