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Plant Regeneration from Cultured Immature Inflorescence of Common Buckwheat (*Fagopyrum esculentum* MOENCH) and Perennial Buckwheat (*F. cymosum* MEISN.)

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Maintenance of valuable genotypes of common buckwheat is difficult owing to heterostylic self-incompatibility. To establish vegetative propagation system, a procedure for plant regeneration from immature inflorescence culture was developed. Immature inflorescences of common buckwheat and perennial buckwheat were cultured on B5 media supplemented with NAA, 2,4-D and BA at various concentrations. Direct shoot production from the inflorescence of common buckwheat was promoted by the addition of 0-2.0 mg/*l* NAA+0-2.0 mg/*l* BA (optimum, 0.2 mg/*l* NAA+1.0 mg/*l* BA). In perennial buckwheat, direct shoot was induced only on the medium containing 1.0 mg/*l* NAA and 1.0 mg/*l* BA with lower frequency than common buckwheat. In the presence of more than 1.0 mg/*l* 2,4-D or more than 2.0 mg/*l* NAA, callus was well formed in both species. Shoots were induced from common buckwheat callus on B5 medium containing 0.2 mg/*l* NAA and 1.0 mg/*l* BA and lacking plant growth regulators, but not from perennial buckwheat. The shoots obtained by the two different pathways developed a root system on MS medium containing 1.0 mg/*l* IBA. Chromosomal analysis of the directly induced regenerants showed that they had diploid chromosome numbers (2n=16).

KEY WORDS : *Fagopyrum esculentum*, *F. cymosum*, inflorescence, plant regeneration, tissue culture.

Introduction

Common buckwheat (*Fagopyrum esculentum* MOENCH) is one of the plants in which valuable genotypes cannot be easily maintained and propagated owing to heterostylic self-incompatibility. Recently, *in vitro* techniques have been used for clonal propagation and plant breeding in many species. In common buckwheat, plant regeneration has been achieved from calli of seedlings, cotyledons and hypocotyles (YAMANE 1974, SREJOVIĆ and NEŠKOVIĆ 1981, TAKAHATA and JUMONJI 1985). However, these organs cannot be used as culture sources for clonal propagation, when valuable and rare characteristics are identified in developing plants. Currently, immature inflorescences have been used as a suitable source for the maintenance of desirable genotypes and clonal propagation in Graminae (BRETTELL *et al.* 1980, DALE *et al.* 1981, VASIL and VASIL 1982, NAKAMURA and KELLER 1982, CHEN *et al.* 1982, EAPEN and RAO 1985).

In the present study, the effect of plant growth regulators on the culture of immature inflorescences of common buckwheat and perennial buckwheat (*F. cymosum* MEISN.) was investigated, and plantlets were regenerated from immature inflorescences directly as well as from calli.

Materials and Methods

Two common buckwheat cultivars 'Iwatezairai-Akisoba' and 'Hashikamiwase' and one strain S-1 of perennial buckwheat were used as materials. Immature inflorescences,

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collected from plants grown in a vinyl house, were sterilized with 70% ethanol for 30 sec followed by a sodium hypochlorite solution containing 1.0% active chlorite for 10 min and washed three times with sterile distilled water. Inflorescences about 3 mm in size were cut from the tip and cultured on B 5 medium (GAMBORG *et al.* 1968) supplemented with 1-naphthalene acetic acid (NAA), 2, 4-dichlorophenoxy acetic acid (2, 4-D) and 6-benzyladenine (BA) at various concentrations, as indicated in Tables 1 and 2. At least ten explants were cultured on each medium. For the regeneration of shoots, the calli were transferred to B 5 media containing 0.2 mg/*l* NAA and 1.0 mg/*l* BA and lacking plant growth regulators. Shoots induced from immature inflorescence directly and from callus were transplanted on MS medium (MURASHIGE and SKOOG 1962) supplemented with 1.0 mg/*l* indole-3-butyric acid (IBA) for the development of the root system. All the cultures were incubated at 25°C under a 16 hour photoperiod regime.

The chromosome numbers of the regenerated plants were counted in the root tip cells by using the Feulgen squash method.

Results and Discussion

Shoots were directly induced from common buckwheat immature inflorescences (Fig. 1 A). They initiated in the region between flower buds 2-3 weeks after culturing. The shoot initiation signaled with the appearance of leaf. As shown in Table 1, the frequency of shoot induction varied with the medium used. The media containing 0-2.0 mg/*l* NAA and 0-2.0 mg/*l* BA promoted shoot induction, unlike NAA at high concentration or 2, 4-D. The highest shoot production was recorded on the medium containing 0.2 mg/*l* NAA and 1.0 mg/*l* BA in two cultivars (40.0% in 'Iwatezairai-Akisoba' and 47.6% in 'Hashikamiwase'). In the inflorescences inducing shoots, one to five shoots were formed from

Table 1. Effect of NAA, 2, 4-D and BA on shoot, root and callus formation from common buckwheat immature inflorescences after 30 days of culture on B 5 medium.

Concentration of			shoot formation	root formation	callus formation
NAA	2, 4-D	BA (mg/*l*)			
a) 'Iwatezairai-Akisoba'					
0	0	0	+	−	−
0.2	0	1.0	++	+	++
2.0	0	0	+	+++	++
2.0	0	2.0	+	++	+++
5.0	0	2.0	−	+++	+++
0	1.0	1.0	−	−	+++
0	2.0	0	−	−	+++
0	2.0	2.0	−	−	+++
0	5.0	2.0	−	−	+++
b) 'Hashikamiwase'					
0.2	0	1.0	++	+	++
2.0	0	2.0	+	++	+++

Percentage of shoot, root and callus formation is indicated as follows, − : 0%, + : <35%, ++ : 35~70%, +++ : >70%.

Table 2. Effect of NAA, 2,4-D and BA on shoot, root and callus formation from perennial buckwheat immature inflorescences after 30 days of culture on B5 medium.

Concentration of			shoot formation	root formation	callus formation
NAA	2,4-D	BA (mg/*l*)			
0	0	0	−	−	−
0.2	0	1.0	−	−	+
1.0	0	1.0	+	+	##
2.0	0	0	−	+	##
2.0	0	2.0	−	+	##
0	1.0	1.0	−	−	##
0	2.0	0	−	−	##
0	2.0	2.0	−	−	##
0	5.0	2.0	−	−	##

Symbols, as in Table 1.

Table 3. Percentage of shoot differentiation from immature inflorescence callus on two regeneration media.

Concentration of		*F. esculentum*		*F. cymosum*
NAA	BA (mg/*l*)	Iwatezairai-Akisoba	Hashikamiwase	
0	0	18.8	20.0	0
0.2	1.0	17.5	28.6	0

a single inflorescence, and in some cases concomitant callus initiation was observed. The phenomenon of the direct shoot initiation is similar to that in Graminae, where direct shoot production was considered to originate from undifferentiated spikelet primordia (Dale *et al.* 1981), axillary buds (Chen *et al.* 1982) or epidermal cells or primordia of floral organs (Ling *et al.* 1983). Chen *et al.* (1982) speculated that exogenous hormones in the medium or wounding hormones associated with the excision may trigger axillary vegetative budding from reproductive organs. However, I cannot conclude the origin of the direct shoot from immature inflorescence of common buckwheat. Further investigations are required to determine the origin and the mechanisms of this phenomenon.

Calli were initiated at the cut-end of the inflorescence and on the surface of the raceme, when hormones were supplied. In contrast to shoot production, NAA at a high concentration or 2,4-D was most effective in inducing callus formation (Table 1).

The results obtained in perennial buckwheat are indicated in Table 2. The response for shoot and root induction was weaker than that in common buckwheat. Direct shoot induction occurred only on the medium containing 1.0 mg/*l* NAA and 1.0 mg/*l* BA with a frequency of 14.3%. On the other hand, callus formation was similar to that in common buckwheat. Calli were well formed on all the media containing 2,4-D or more than 1.0 mg/*l* NAA.

The calli obtained from common buckwheat and perennial buckwheat were transferred to two regeneration media (Table 3). Differences in shoot induction between species were observed. Adventitious shoots were induced from the calli of the two common buckwheat cultivars with a frequency of 17.5–28.6% (Fig. 1 B). The two regeneration media had a

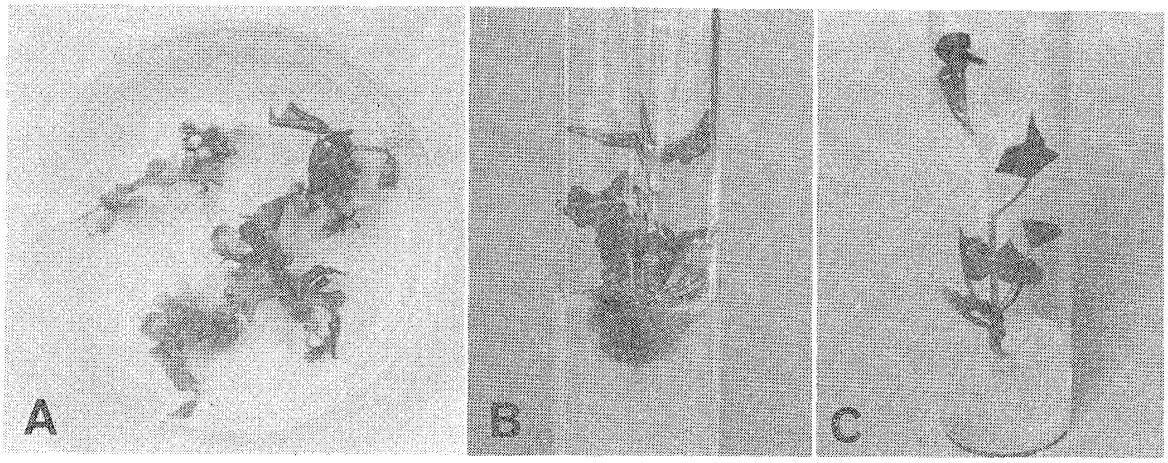

Fig. 1. Plant regeneration from immature inflorescence culture of common buckwheat. (A) Direct shoot production from inflorescence ; (B) Shoot differentiation from inflorescence callus ; (C) Adventitious root formation from shoot.

similar effect in shoot induction. The callus of perennial buckwheat failed to produce shoots on the regeneration media and such a weak response was identical with that of direct shoot production. No shoot induction from leaf and stem callus of perennial buckwheat has been observed (unpublished).

When the shoots induced directly from immature inflorescences and from callus tissues were transferred to MS medium containing 1.0 mg/*l* IBA, they developed roots and plantlet regeneration was achieved (Fig. 1 C). For chromosomal analysis, 25 plantlets for common buckwheat and one for perennial buckwheat were randomly selected from the regenerants that directly initiated. All the plants had diploid chromosome numbers ($2n=16$) and no variations in the chromosomal number were observed. The direct regeneration system from immature inflorescence may have potential in the clonal propagation of common buckwheat because of the high ability of shoot induction and the chromosomal stability.

Common buckwheat is one of the plants in which valuable genotypes cannot be readily maintained owing to heterostylic self-incompatibility. The present study demonstrated that plant regeneration could be achieved from cultured immature inflorescences of common buckwheat. The somatic chromosomes were stable in the inflorescence-derived regenerants. SONGSTAD *et al.* (1986) indicated that callus cells from immature inflorescences may be used in mutation studies since many desirable characteristics of source plants have been manifested when source plants were selected for culture initiation. The culture scheme using immature inflorescences appears to be a useful means for maintaining a desirable genotype as well as for clonal propagation and mutation studies in common buckwheat.

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普通ソバ（*Fagopyrum esculentum* MOENCH）および宿根ソバ（*F. cymosum* MEISN.）の幼花序からの植物体再生

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普通ソバは異型花柱性の自家不和合性で，有用な遺伝子型を効率的に維持，増殖することが困難である．そこで組織培養による増殖法の確立を目的として，幼花序からの植物体再生について検討した．普通ソバおよび宿根ソバの幼花序を NAA，2,4-D および BA を含んだ B5 培地で培養した．普通ソバでは NAA 0～2.0＋BA 0～2.0 mg/*l* 添加で幼花序から直接不定芽が生じ，特に NAA 0.2＋BA 1.0 mg/*l* 添加区で最高の分化率を示し，岩手在来秋ソバで 40.0%，階上早生で 47.6% の不定芽形成がみられた（Table 1, Fig. 1A）．宿根ソバは NAA 1.0＋BA 1.0 mg/*l* 区でのみ不定芽が生じ，その頻度も低かった（Table 2）．一方，両種とも NAA 2.0 mg/*l* 以上あるいは 2,4-D 1.0 mg/*l* 以上の添加により高いカルス形成率を示した．これらのカルスをホルモン無添加あるいは NAA 0.2＋BA 1.0 mg/*l* 添加の再分化培地に継代したところ，普通ソバでは 17.5～28.6% の頻度で芽を分化したが，宿根ソバではまったく分化しなかった（Table 3, Fig. 1B）．幼花序から直接分化した場合も，カルス経由の場合も，芽を IBA 1.0 mg/*l* 添加の MS 培地に移植し発根させた（Fig. 1C）．幼花序から直接再生した植物体のうち，普通ソバ 25 個体，宿根ソバ 1 個体について染色体数を調査したところ，すべて 2n＝16 の 2 倍体であった．