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Callus Induction and Plant Regeneration from Mature Embryos in Oat

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ABSTRACT : Mature embryos of five oat genotypes were cultured to develop an efficient method of callus induction and plant regeneration. Murashige and Skoog (MS) and N6 media supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin were used for callus induction. Percentage of callus induction showed significant among the combinations of plant growth regulators. Callus induction showed high efficiency in medium containing 3 mg/l of 2,4-D. The high frequency of callus induction was obtained in Gwiri37. For plant regeneration, calli induced from mature embryos were transferred onto MS and N6 media supplemented with combinations of 6-benzyladenine (BA) and naphthaleneacetic acid (NAA) for 5 weeks. Percentage of plant regeneration showed high in MS medium containing 0.2 mg/l of NAA and 1 mg/l of BA. The callus initiation medium affected the subsequent plant regeneration. Treatment with 3 mg/l of 2,4-D, and 3 mg/l of 2,4-D and 3 mg/l of kinetin in callus induction media showed high frequency for plant regeneration. Plant regeneration frequency among the genotypes showed significant. Especially, Gwiri37 showed high regeneration frequency. Regenerated shoots were treated with 200, 350 and 500 mg/l of indole-3-butyric acid (IBA) transferred onto half-strength MS medium without plant growth regulators. Treatment of shoots with IBA induced root formation rapidly.

Keywords **:** *Avena sativa*, mature embryo, callus induction, regeneration

Oats are nutritious food that gently restores vigour after debilitating illnesses, helps lower cholesterol levels in the blood. Another benefits are that the dietary fiber and protein in oats make feel full fast, keep away from more fattening foods and help control weight.

An efficient procedure for plant regeneration from various tissues is a prerequisite for the application of gene transfer methods in crop improvement. The efficiency of callus induction and plant regeneration in tissue culture of oat is commonly influenced by the explant sources (Gless *et al.*, 1998a; Chen *et al.*, 1995a, Chen *et al.*, 1995b) and genotypes (Kiviharju *et al.*, 2000; Kiviharju *et al.*, 1998; Rines, 1983; Torbert *et al.*, 1998b).

Various explant sources such as immature embryos (Bregitzer *et al.*, 1989; Bregitzer *et al.*, 1991; Cummings *et al.*, 1976; Lörz *et al.*, 1976; Rines and McCoy, 1981; Rines and Luke, 1985; Torbert *et al.*, 1998a), mature embryos (Birsin *et al.*, 2001; Cater *et al.*, 1967), seedling mesocotyls (Heyser and Nabors, 1982), apical meristems (Zhang *et al.*, 1996), axillary tiller buds (Shewkhawat *et al.*, 1984), leaf-base segments (Chen *et al.*, 1995b; Gless *et al.*, 1998), young leaves (Chen *et al.*, 1995a), and anther culture (Kiviharju *et al.*, 1998; Kiviharju and Tauriainen, 1999; Rines, 1983) have been used for callus induction and plant regeneration in oat.

The most frequently and successfully used explants in oat cultures are immature embryos. However, immature embryos which are only available at a very limited time in a growing season, generally requires growth of oat plants in climate-controlled rooms (Torbert *et al.*, 1998, Özgen *et al.*, 1998). Mature embryos are readily available at all times. An efficient regeneration method from mature embryos provides enough material for plant transformation studies. Therefore, mature embryos can be used as an effective and convenient explant source in plant transformation.

The purpose of this study was to develop an efficient method of callus induction and plant regeneration from mature embryo and to evaluate the Korean oat genotypes with high regeneration efficiency to apply for gene transfer method.

MATERIALS AND METHODS

Plant materials

Four Korean genotypes and one foreign genotype, Gwiri37, Malgwiri, Megwiri, Samgeolgwiri, and Swan, were used. Mature seeds were sterilized for 2 min in 70% ethanol. And then seeds were sterilized with 70% chlorox containing 3 drops of Tween® 20 per 100 ml for 40 min with gentle shaking and rinsed three times with sterile distilled water and placed on media for callus induction.

Callus induction

To induce callus formation, mature seeds were cultured on

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Table 1. Combination of plant growth regulators treated in this experiment.

Callus induction	Plant regeneration
1 mg/l 2,4-D	0.2 mg/l NAA, 1 mg/l BA
3 mg/l 2,4-D	1 mg/l NAA, 2 mg/l BA
1 mg/l 2,4-D, 1 mg/l kinetin	
3 mg/l 2,4-D, 1 mg/l kinetin	
1 mg/l 2,4-D, 3 mg/l kinetin	
3 mg/l 2,4-D, 3 mg/l kinetin	

MS (Murashige and skoog, 1962) and N6 (Chu *et al.*, 1975) media in the dark at 25°C for 4 weeks (Table 1). Both media contained 3% sucrose and 0.6% agar, and were adjusted to pH 5.8. The media were supplemented with the combinations of 1 and 3 mg/l of 2,4-D and 1 and 3 mg/l of kinetin to investigate the effect of plant growth regulators on efficiency of callus induction. Callus was subcultured onto the same medium after 2 weeks, and cultured for a further two weeks at 25°C in the dark. Each treatment contained two replications with at least 40 embryos per replication. Percentage of callus induction was calculated as the number of embryos inducing callus out of the total number of embryos placed on the callus induction medium.

Plant regeneration

After 4 weeks, callus was cultured on regeneration medium at 25°C in 16 hr light and 8 hr dark condition. MS and N6 media were supplemented with the combinations of 0.2 and 1 mg/l of NAA and 1 and 2 mg/l of BA (Table 1). Callus was subsequently subcultured onto the same medium after two weeks. When plantlets developed three to five leaves, they were transferred onto half-strength MS media without plant growth regulators to induce root formation. In addition, shoots were immersed in 200, 350 and 500 mg/l of IBA for five seconds and transferred onto half-strength MS medium without plant growth regulators. Percentage of plant regeneration was calculated as the number of embryos showing plant regeneration out of the total number of embryos placed on the initial medium.

RESULTS AND DISCUSSION

Callus induction

All oat genotypes produced callus from mature embryos on the six callus induction media. Calli were creamish and friable on the 7th days (Fig. 1). The effects of 2,4-D and kinetin concentration on callus induction from mature embryos was examined (Table 2). The percentage of callus induction did not showed significant difference between MS and N6 media and showed significant among the combinations of plant growth regulators. Callus induction showed high efficiency in medium containing 3 mg/l of 2,4-D. Though the callus induction among genotypes did not showed significant, Gwiri37 showed high percentage of callus induction compared with other genotypes. In addition, callus induction showed high efficiency in medium containing 3 mg/l of 2,4-D and 3 mg/l of kinetin.

Fig. 1. Callus induction and plant regeneration from mature embryos in oat. A: Seven-day-old calli on 3 mg/l 2,4-D, B: Shoot regeneration on MS medium containing 0.2 mg/l of NAA and 1 mg/l of BA, C: Regeneration plant with root.

Table 2. Percentage of callus induction from mature embryos in oat.

Genotype	MS						N6					
	C1†	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
Gwiri37	85.0	90.0	75.0	75.0	72.5	90.0	77.5	87.5	72.5	82.5	77.5	90.0
Malgwiri	77.5	82.5	72.5	77.5	67.5	82.5	75.0	82.5	70.0	75.0	67.5	80.0
Megwiri	77.5	85.0	70.0	77.5	70.0	87.5	80.0	85.0	67.5	72.5	70.0	77.5
Samgeolgwiri	77.5	87.5	72.5	72.5	70.0	82.5	72.5	80.0	65.0	72.5	62.5	75.0
Swan	72.5	85.0	65.0	70.0	65.0	80.0	65.0	75.0	72.5	72.5	70.0	75.0

†C1: 1 mg/l 2,4-D, C2: 3 mg/l 2,4-D, C3: 1 mg/l 2,4-D, 1 mg/l kinetin, C4: 3 mg/l 2,4-D, 1 mg/l kinetin, C5: 1 mg/l 2,4-D, 3 mg/l kinetin, C6: 3 mg/l 2,4-D, 3 mg/l kinetin.

‡Percentage of callus induction was calculated as the number of embryos inducing callus out of the total number of embryos placed on the initial medium. The analysis of variance showed significant differences among the hormone composition ($P=0.05$) $LSD_{0.05}=11.83\%$. Differences among the genotypes were not significant.

Table 3. Percentage of plant regeneration from mature embryos in oat. [§]

Genotype	R1[†]						R2					
	C1[‡]	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
						MS medium						
Gwiri37	17.5	22.5	12.5	27.5	27.5	27.5	12.5	17.5	7.5	20.0	7.5	20.0
Malgwiri	15.0	17.5	12.5	20.0	12.5	20.0	10.0	15.0	12.5	12.5	15.0	20.0
Megwiri	15.0	27.5	7.5	17.5	7.5	20.0	15.0	20.0	7.5	12.5	10.0	12.5
Samgeolgwiri	20.0	20.0	15.0	10.0	10.0	25.0	10.0	7.5	7.5	7.5	7.5	25.0
Swan	10.0	12.5	5.0	10.0	7.5	17.5	5.0	12.5	5.0	10.0	5.0	12.5
						N6 medium						
Gwiri37	12.5	27.5	7.5	20.0	12.5	20.0	7.5	22.5	10.0	17.5	10.0	20.0
Malgwiri	17.5	17.5	7.5	17.5	7.5	17.5	10.0	15.0	5.0	7.5	12.5	15.0
Megwiri	15.0	17.5	7.5	12.5	10.0	17.5	10.0	12.5	5.0	12.5	7.5	20.0
Samgeolgwiri	15.0	15.0	5.0	12.5	12.5	17.5	12.5	10.0	10.0	10.0	12.5	17.5
Swan	15.0	12.5	12.5	10.0	5.0	15.0	7.5	10.0	7.5	12.5	10.0	15.0

[†]R1: 0.2 mg/l NAA, 1 mg/l BA, R2: 1 mg/l NAA, 2 mg/l BA.
[‡]C1: 1 mg/l 2,4-D, C2: 3 mg/l 2,4-D, C3: 1 mg/l 2,4-D, 1 mg/l kinetin, C4: 3 mg/l 2,4-D, 1 mg/l kinetin, C5: 1 mg/l 2,4-D, 3 mg/l kinetin, C6: 3 mg/l 2,4-D, 3 mg/l kinetin.
[§] Percentage of plant regeneration was calculated as the number of embryos showing plant regeneration out of the total number of e mbryos placed on the initial medium. The analysis of variance showed significant differences for regeneration among the genotypes ($P<0.01$) $LSD_{0.01}$=2.15%, as well as the hormone composition $LSD_{0.01}$=4.71%.

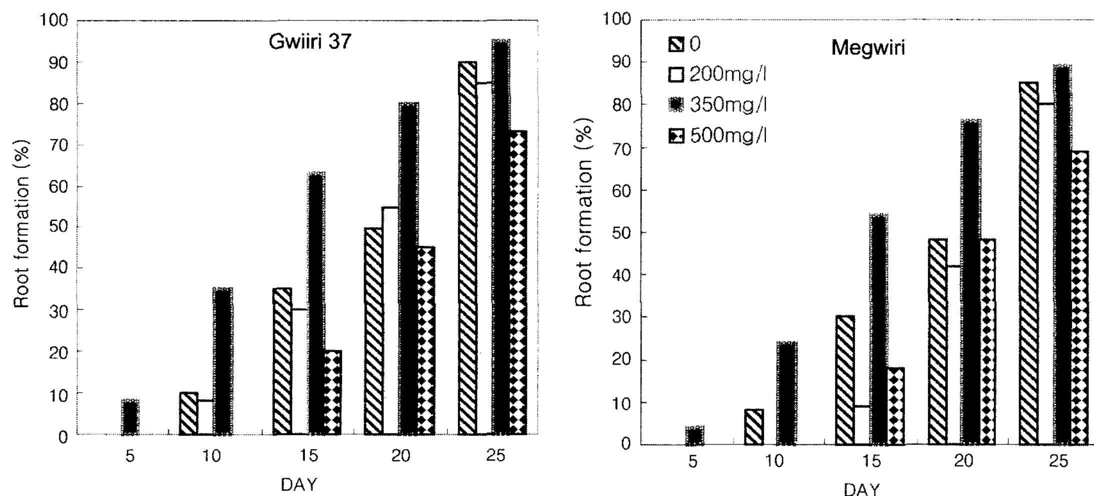

Fig. 2. Root formation with IBA treatment in Gwiri37 and Megwiri. Shoots were immersed in 200, 350 and 500 mg/l of IBA for five seconds and transferred onto half-strength MS medium without growth regulators.

Plant regeneration

Plant regeneration efficiency was examined in MS and N6 media containing 0.2 mg/l of NAA and 1 mg/l of BA, and 1 mg/l of NAA and 2 mg/l of BA (Table 3). Calli with green spots rapidly developed shoots and leaves in regeneration medium (Fig. 1). Percentage of plant regeneration showed high in MS medium containing 0.2 mg/l of NAA and 1 mg/l of BA. The callus initiation medium affected the subsequent plant regeneration. Treatment with 3 mg/l of 2,4-D, and 3 mg/l of 2,4-D and 3 mg/l of kinetin in callus induction media showed high frequency for plant regeneration. It appears that the callus initiation medium may be an important factor for subsequent plant regeneration. Kiviharju and Tauriainen (1999) reported that high 2,4-D and low kinetin in anther culture of oat were beneficial for plant regeneration. Seong and Sohn (1990) reported that 2 mg/l of kinetin was optimal concentration for plant regeneration in rice. Plant regeneration frequency among the genotypes showed significant. Especially, Gwiri37 showed high plant regeneration frequency. This result supported that plant regeneration was influenced by the genotypes (Kiviharju *et al.*, 1998; Torbert *et al.*,

1998b; Özgen *et al.* 1999; Sears and Deckard, 1982).

Root formation

The effect of MS half-strength medium containing 200, 350 and 500 mg/l of IBA on root induction was examined (Fig. 2). Treatment with 350 mg/l of IBA showed high root induction at 20 days compared with control. However, root formation of control showed similar with those of 350 mg/l of IBA treatment at 25 days. This appears that IBA induces root formation rapidly. All IBA treated shoots exhibited a superior rooting capacity when compared to the control. Barrueto Cid *et al.* (1999) also reported that an IBA pre-treatment was important to increase rooting efficiency.

In conclusion, the present study demonstrates that the composition of the callus initiation medium is an important factor for subsequent plant regeneration. It also shows the influence of genotype on plant regeneration from mature embryos of oat. An oat regeneration system from mature embryos may provide an effective and convenient explant for plant transformation studies.

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