Short Communication

Propagation of *Oenothera erythrosepala* BORBAS by Shoot Apex Culture

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Shoot apices of *Oenothera erythrosepala* were cultured on MS medium supplemented with various concentrations and combinations of naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA). Effect of hormonal concentration on number of newly induced shoots per shoot apex placed was evident. The media containing 0.2mg/l NAA 0.2mg/l BA and/or 0.2mg/l NAA 2.0mg/l BA were suitable for shoot multiplication and 2.0mg/l NAA for rooting. There was a little difference in root formation and root hair development according to the gelling agents used, but the survival rate was not affected by them.

KEY WORDS: *Oenothera erythrosepala* BORBAS, shoot apex culture, gelling agent.

Introduction

Oenothetin B, ellagitannin extracted from *Oenothera erythrosepala* BORBAS, has been known to have antitumor activity (MOTOYAMA *et al.* 1988). In our preliminary experiment, oenothetin B content of *Oenothera erythrosepala* plant varied 2.24% to 6.67%. WOLFSON and SEARS (1989) cultured shoot apex of *Oenothera hookeri* on agar medium but no reports focusing on multiple shoot induction of *Oenothera* plant have been published. For the purpose of rapid propagation of plant containing a high percentage of oenothetin B, shoot apex culture was attempted.

Materials and Methods

Portion of stem bearing numerous lateral buds were taken from two-year-old *Oenothera erythrosepala* plants, washed with running water to remove soil debris. Four plants were used. After removing leaves, lateral buds cut in 5mm, sterilised by soaking in 70% ethanol for 1 minute and 1% sodium hypochlorite solution for 10 minutes and were finally rinsed three times with sterile distilled water. The lateral buds were dissected under a binocular microscope and shoot apices measuring 0.5mm in length were removed for culture. MS mineral salt medium (MURASHIGE and SKOOG 1962) with organic constituents added at the following concentrations; 30g/l sucrose; 10mg/l thiamine hydrochloride; 5mg/l nicotinic acid; 10mg/l pyridoxine hydrochloride; 100mg/l myo-inositol was used as basal medium in all experiments. Naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA) were added to the basal medium in different concentrations and combinations as indicated in the results (Fig. 1 and Table 1). The medium was solidified with 8.0g/l agar. All culture tubes (25mm × 100mm) contained 10ml of medium adjusted to pH5.6 before autoclaving and was sealed with aluminium foil. One shoot apex was placed in a culture tube. Four tubes were used for each combination of NAA and BA.

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Newly induced shoots of 1.0–1.5 cm length in the medium containing 0.2 mg/l NAA and 0.2 mg/l BA were excised and transferred to rooting media with 2.0 mg/l NAA. Gelling agents (8.0 g/l agar and 2.0 g/l gelrite) were used for the solidification of rooting media. When liquid medium was used the shoots were supported by a filter paper bridge. Cultures were grown in the growth room at $27 \pm 1^\circ\text{C}$ under continuous illumination using white fluorescent lamps (2500 lux). Rooted plants were transferred to pots filled with vermiculite and maintained under high humid condition for the initial 14 days in the glasshouse. After a hardening period of 2 to 3 weeks in the glasshouse, the young plants were transplanted to the field.

Results and Discussion

Freshly excised shoot apices were white in colour, but turned green within 4 to 5 days when placed on the media containing NAA at relatively low concentrations (0–0.2 mg/l NAA, 0–4.0 mg/l BA). On the other hand, at higher NAA concentrations (2.0–4.0 mg/l NAA, 0–4.0 mg/l BA) callus developed at the bottom end of a shoot apex. These calli rapidly grew and covered shoot apex within 20 days at the combinations of higher NAA and BA levels (2.0–4.0 mg/l NAA and 2.0–4.0 mg/l BA). After 20 days most cultures had produced new shoots (Table 1). NAA at relatively low concentration (0.2 mg/l) with high concentration of BA (0.2–4.0 mg/l) stimulated shoot proliferation. Average number of shoots per shoot apex placed were 9.8, 10.8, 8.0 on the medium containing 0.2 mg/l NAA 0.2 mg/l BA, 0.2 mg/l NAA 2.0 mg/l BA, 0.2 mg/l NAA 4.0 mg/l BA, respectively. A comparison of the new shoots induced on these media showed growth to be slightly better at the lower BA level. After

Table 1. Effect of NAA and BA concentrations on multiple shooting, rooting and callus formation after 20 days, culture

BA (mg/l)	NAA (mg/l)				
	0	0.02	0.2	2.0	4.0
0	$1.0 \pm 0^{1)}$	1.0 ± 0	1.0 ± 0	2.3 ± 1.5 (R-4)	1.5 ± 1.0 (C-2) (R-2)
0.02	2.5 ± 1.3	4.0 ± 0.8	5.3 ± 1.0	2.3 ± 1.5 (C-4) (R-1)	0.3 ± 0.5 (C-4) (R-2)
0.2	5.3 ± 1.0	5.0 ± 0.8	9.8 ± 1.7	7.5 ± 2.4 (C-4)	0 ± 0 (C-4)
2.0	4.5 ± 1.3	5.3 ± 1.3	10.8 ± 2.2	3.3 ± 2.6 (C-4)	0 ± 0 (C-4)
4.0	5.8 ± 1.0	5.5 ± 1.0	8.0 ± 1.4	0 ± 0 (C-4)	0 ± 0 (C-4)

Four shoot apices were used for each combination of NAA and BA.

1) Mean $\pm$ standard deviation of newly induced shoots per culture

(C-): No. of cultures formed callus

(R-): No. of cultures rooted

20 to 40 days at the combinations of higher NAA and BA (2.0, 4.0mg/l NAA and 0.2–4.0mg/l BA), new shoots were induced from the central part of the callus. It is reasonable to consider that newly formed axillary buds elongated after callus formation. They grew rapidly, but morphology of the newly formed leaves were slightly abnormal, slender and semitransparent (Fig. 1). Rooting was observed at the combinations of 2.0, 4.0mg/l NAA and 0.02mg/l BA within 20 days (Table 1). On the 2.0mg/l NAA 0mg/l BA medium, growth of the shoots and newly induced roots were better than another three. From these results, we concluded that the media containing 0.2mg/l NAA 0.2mg/l BA and/or 0.2mg/l NAA 2.0mg/l BA were suitable for shoot multiplication and 2.0mg/l NAA for rooting.

Fig. 1. Effect of NAA and BA concentrations on multiple shooting, rooting and callus formation after 40 days culture.

Fig. 2. Roots induced on the medium containing 2.0mg/l NAA. (a: 2.0g/l gelrite, b: 8.0g/l agar, c: paper bridge in liquid medium)

Fig. 3. Plantlets transferred to a pot filled with vermiculite.

Table 2. Effect of gelling agent on root formation after 30 days culture

Gelling agent	No. of shoots used	No. of shoots rooted
Agar (8.0 g/l)	10	9
Gelrite (2.0 g/l)	10	10
Paper bridge (No gelling agent)	10	8

Roots were formed within 1 to 2 weeks on all rooting media containing 8.0 g/l agar, 2.0 g/l gelrite and filter paper bridge in liquid medium (Table 2). In early phase of culture, number of roots formed per shoot was more on the medium containing gelrite and paper bridge than on agar. Elongation of the roots formed was slightly superior on gelrite. In latter phase, 30 days culture, there was no difference in the rate of root development on these media, but root hair development was better on paper bridge (Fig. 2). When the plantlets were transplanted to the pots filled with vermiculite, all of them survived (Fig. 3). After a hardening period of 2 to 3 weeks in the glasshouse, the young plants were successfully established and transplanted to the field. From these results we concluded that both gelling agents tested and paper bridge method were suitable for rooting of this species.

Literature Cited

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茎頂培養によるオオマツヨイグサ (*Oenothera erythrosepala* BORBAS) の増殖

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オオマツヨイグサの葉に含まれる Oenotherin B は、抗腫瘍活性を有することが知られている。高 Oenotherin B 系統育成の一環として、茎頂培養による大量増殖法について検討した。2 年生個体の側芽を滅菌 (70%エタノール 1 分間, 1%次亜塩素酸ナトリウム 10 分間, 滅菌水で 3 回洗浄) し、長さ約 0.5 mm の茎頂を摘出置床した。MS 培地 (MURASHIGE and SKOOG 1962) に 0, 0.02, 0.2, 2.0, 4.0 mg/l のナフタレン酢酸 (NAA) と、6-ベンジルアミノプリン (BA) を添加した 25 種類の培地を用い、 $27 \pm 1^\circ\text{C}$ 明条件下 (2500 lux) で培養した。

培養 20 日目には、ほとんどの区においてシュートが形成されたが、NAA 濃度の高い区ではカルス化するものも見られた (Table 1)。新しく形成されたシュートの数と発根に対する NAA と BA の濃度の影響が顕著に認められた。カルス化した NAA 高濃度区においても、培養 40 日目までにはカルス中央部よりシュートが形成された (Fig. 1)。新しく形成されたシュートの数および葉の形態から、シュートの増殖には 0.2 mg/l の NAA と 0.2 mg/l の BA の組み合わせ、あるいは 0.2 mg/l の NAA と 2.0 mg/l の BA の組み合わせが適当と考えられる。発根については、シュート基部のカルス化の少ない 2.0 mg/l NAA のみの区が適当と考えられる。

培養の初期にはゲル化剤 (8.0 g/l 寒天, 2.0 g/l gelrite, paper bridge) による発根速度、根毛の発生程度などに多少の差が見られるが、活着率に差は認められなかった (Fig. 2, 3 Table 2)。