

Phytohormonal and Genotypic Factors Affecting Shoot Regeneration from Cotyledonary Explant of Radish (*Raphanus sativus* L.)

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The success of *Agrobacterium*-mediated transformation in plants depends on the establishment of a plant regeneration system through organogenesis or embryogenesis. Radish (*Raphanus sativus* L.) which is classified in Cruciferae is an important vegetable in the Far East Asia. Though a few attempts at tissue culture have been reported in radish¹⁻³, this plant is considered to be one of most recalcitrant of Cruciferous species. On the other hand, in tissue culture and transformation studies of some *Brassica* species, cotyledonary petiole has been used as an explant having high regeneration competence⁴⁻⁸. The aim of this study is to investigate the phytohormone condition and genotypic variation for shoot regeneration from cotyledonary explant of radish.

Protocol of cotyledonary explant culture was according to Ono *et al.*⁹ with minor modification. To determine the optimum conditions of phytohormones for shoot regeneration, 12 combinations of 6-benzyladenine (BA) and 1-naphthaleneacetic acid (NAA) were tested using 4 cultivars of 'Aishikuru', 'Aonaga', 'Sushirazu-shogoin' and 'Taibyo-sohbutori'. Cotyledons including 1-2 mm petioles were excised from 4 day-old seedlings which were aseptically germinated in hormone-free MS medium (germination medium). Eight explants were cultured in 15×90 mm petri dish containing regeneration medium. The regeneration medium was composed of MS basal medium supplemented with 3% sucrose, 0.7% agar, and a combination of BA (0-8 mg/l) and NAA (0-2 mg/l) (Fig. 1). After 4 weeks of culture, the number of explants forming adventitious shoots were counted. After determination of phytohormone conditions, 32 cultivars of radish, which are listed in Fig. 2, were used to examine shoot-regeneration capacity. Regeneration frequency was averaged for at least 3 replications. All cultures were incubated at 25°C under 16h photoperiod.

Though shoots were regenerated in various media, the frequency was low (Fig. 1). Of 4 cultivars used, 'Taibyo-sohbutori' which was responsive in various combinations of BA and NAA, showed maximum frequency of shoot regeneration (10.3%) in the presence of 4 mg/l BA. On the other hand, no induction of shoot regeneration was observed in 'Sushirazu-shogoin'. The response of 'Aishikuru' and 'Aonaga' was intermediate between them. Of the 12 kinds of media tested, 4 phytohormone combinations induced shoot regeneration from two cultivars. Of these, 4 mg/l BA and 2 mg/l BA + 1 mg/l NAA was reported to be optimum in *B. napus*^{5,8} and *B. campestris*⁶, respectively. Though root formation was observed on all media, the presence of BA in the medium was inhibitory for root induction. On the other hand, the elongation of petiole was promoted by BA, but was inhibited by NAA (data not shown).

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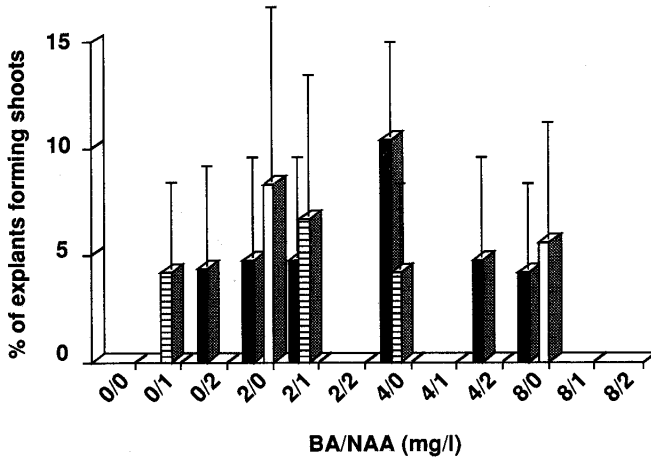


Fig. 1 Regeneration of shoots from 4 day-old cotyledonary explants of 4 cultivars of *R. sativus* on various concentrations of BA and NAA.

Data consist of at least 3 replicates and for each replicate 8 cotyledonary explants were used. Bar represents S. E. Of 4 cultivars, 'Sushirazu-shogoin' could not produce any adventitious shoots on any media.

□: 'Aishikuru', ▨: 'Taibysohbutori', ▩: 'Aonaga'.

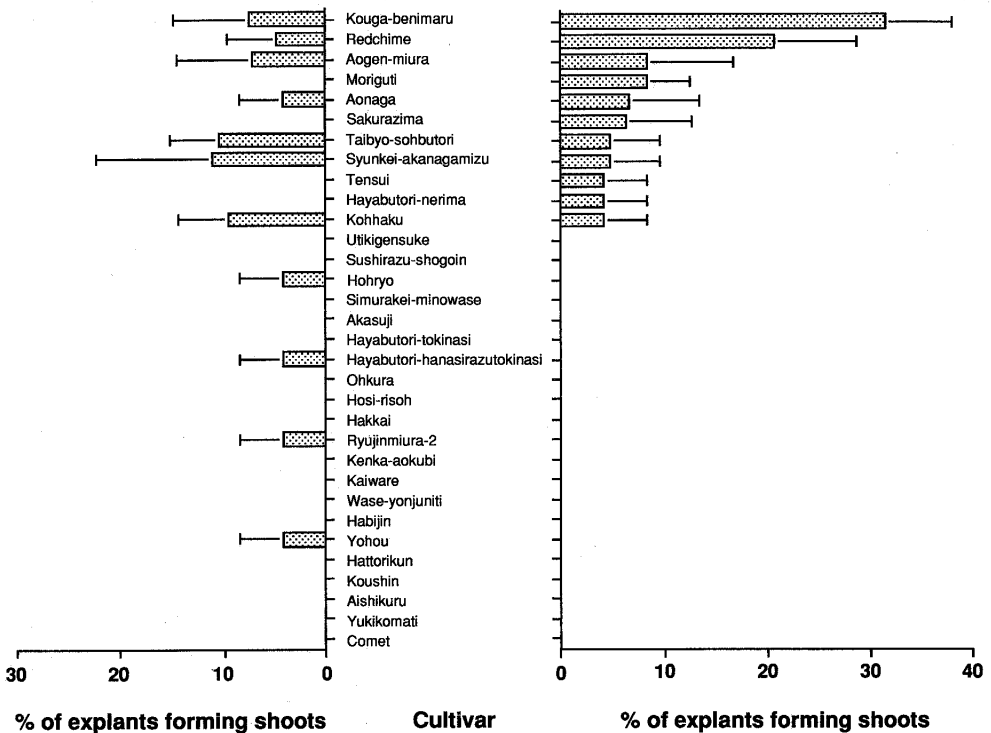


Fig. 2 Effect of genotypes on shoot regeneration from cotyledonary explants of *R. sativus*.

Four day-old cotyledonary explants of 32 cultivars were cultured on MS medium supplemented with 2 mg/l BA and 1 mg/l NAA (right) or with 4.0 mg/l BA (left). Data consist of at least 3 replicates and for each replicate 8 cotyledonary explants were used. Bar represents S. E.

To increase the frequency of shoot regeneration, the effect of the addition of BA (2 mg/l) in the germination medium and ethylene inhibitor AgNO₃ (2 mg/l) in the regeneration media was investigated, because these additives were reported to be effective in *B. campestris*⁹⁾. However, they did

not enhance shoot regeneration frequency in radish(data not shown). The use of thidiazuron (TDZ) instead of BA could not increase shoot regeneration response, either.

Using two kinds of phytohormone condition (2 mg/l BA + 1 mg/l NAA and 4 mg/l BA) which was decided by the results of this study and the previous reports of *Brassica*^{5,6,8}, shoot regeneration of 32 cultivars of radish were examined. As shown in **Fig. 2**, genotypic variations for shoot regeneration were observed. Fifteen of the 32 cultivars responded at 31.5-4.2% frequency, but 17 cultivars did not exhibit any shoot regeneration on both media. The high frequency of shoot regeneration of 31.5% and 20.1% was obtained in 'Kouga-benimaru' and 'Redchime', respectively, when cultured on the medium containing 2 mg/l BA and 1 mg/l NAA. The response of the other cultivars was about 10% or less. Of 15 cultivars responded, 7 regenerated shoots on both media, 4 responded to 2 mg/l BA + 1 mg/l NAA, and 4 responded to 4 mg/l BA. Especially, all Chinese radish of 'Kouga-benimaru', 'Aonaga' and 'Syunkei-akanagamizu' produced shoots on both media with the exception of 'Koushin'. These results indicated that the shoot regeneration ability of radish is under genetic control, which is reported in *B. napus*¹⁰.

In the present study, we examined the shoot regeneration potential of radish cotyledonary explants in various cultivars, but the frequency of regeneration was still low. A more comprehensive study will be required to establish an efficient regeneration system for radish.

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《和文要約》

ダイコン (*Raphanus sativus* L.) 子葉片からの不定芽誘導に及ぼす植物ホルモンと遺伝子型の影響

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ダイコン 4 品種を用いて子葉片からの不定芽誘導に及ぼす植物ホルモンの影響について検討した。BA と NAA の種々の組み合わせで不定芽が形成されたが、形成率は最高で 10% であった。次に、32 品種について 2 種類の培地を用いて遺伝子型の影響を調査したところ、15 系統で不定芽形成が見られ、最高で 30% 程度の形成率を持つ品種が見出された。