Notes Plant Tissue Culture Letters, 7(3), 202-204 (1990)

Callus Induction and Plant Regeneration from Barley Coleoptile Tissues

Hideyoshi TOYODA, Yuji KOURA, Yoshinori MATSUDA, Kouji HORIKOSHI, Takayuki TAMAI, Takako YAMAGA, Seiji IKEDA, Masato SHIMABAYASHI, Masahiro MORITA and Seiji OUCHI

Laboratory of Plant Pathology, Faculty of Agriculture, Kinki University, Nakamachi 3327-204, Nara 631, Japan

(Accepted May 29, 1990)

Genetic transformation of graminaceous plants has been mainly conducted by directly introducing foreign genes into their protoplasts by means of electroporation[7] or polyethylene glycol treatment.[10] In these methods, plant regeneration from protoplasts is a prerequisite for producing transgenic plants. However, the inability of protoplasts of many monocotyledonous plants to regenerate into plants makes it difficult to generalize these methods in a broad range of monocotyledonous species. Therefore, the substitutive method such as a microprojectile bombardment[4] has been developed and applied to direct delivery of foreign genes into monocotyledonous plant materials.[2,5] Recently the authors reported the transient expression of foreign gene introduced into barley coleoptile cells by microinjection and discussed the possible application of coleoptile tissues to a production of transgenic barley.[8] In the present paper, therefore, callus induction and plant regeneration from coleoptile tissues are described in order to complete the coleoptile system of barley. The progress of these methods would make it possible to produce transgenic plants in a broad range of monocotyledonous species in which protoplast regeneration is difficult.

Seeds of barley (*Hordeum vulgare* L. cv. Kobinkatagi) were soaked in 70% ethanol for 30 sec, followed by 2% sodium hypochlorite for 2 min with continuous shaking, and rinsed several times with sterile distilled water. Surface-sterilized seeds were germinated at 20°C on water-moistened filter paper in a Petri dish. Germinating seedlings were transferred to agar-solidified plates supplied with sterile chemical fertilizer (1000-fold diluted Hyponex) and incubated at 20°C under a continuous illumination of 5,000 lux. After 10 days of incubation, coleoptiles were excised from primary leaves and transferred to Murashige-Skoog[6] (MS) medium supplemented with various concentrations of growth regulators, and adjusted to be pH 5.8 with 0.1 N NaCl before autoclaving. The regulators used in the present study were 2,4-dichlorophenoxyacetic acid (2,4-D) and α-naphthaleneacetic acid (NAA) as auxin, and 6-benzylaminopurine (BA) and kinetin (K) as cytokinin. To evaluate the role of auxin and cytokinin on callus induction and plant regeneration, an experiment was designed with all 54 possible combinations of nine different concentrations (0, 0.1, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, and 10.0 μg/ml) of auxin and six concentrations (0, 0.01, 0.05, 0.1, 0.5, and 1.0 μg/ml) of cytokinin.

It has been generally recognized that synthetic auxin such as 2,4-D or NAA is effective for inducing callus tissues from different explants of graminaceous species.[1] In the present study, therefore, the effects of mainly 2,4-D and NAA were examined. Callus induction from coleoptile tissue was observed in a broad range of 2,4-D concentrations (1.0-10.0 μg/ml), with no relation to concentrations of K or BA combined, and even in the absence of cytokinin. Vigorous growth of callus tissues was observed especially when they were cultured with 1.0-0.05, 1.0-0.1, 1.5-0.5, 2.0-0.1, 3.0-0.1, 3.0-0.5, 5.0-0.05, and 10.0-0.05 μg/ml of 2,4-D and K. Callus tissues were not induced, however, when coleoptile explants were cultured with the medium in which 2,4-D was replaced with NAA.

Jelaska *et al.*[3] and Rengel and Jelaska[9] examined the regeneration of callus tissues derived from

mesocotyl and apical meristem of barley, and reported that a transfer of 2, 4-D-induced callus tissues to a regeneration medium containing 2, 3, 5-triiodobenzoic acid was necessary to initiate the shoot formation. In the present study, however, the transfer of callus tissues to a regeneration medium was not an essential step. Induced callus tissues were cut into small pieces (5 mm diameter), transferred to the fresh same medium at an interval of 20 days, and subcultured till some morphological changes were observed. Greening spots were frequently formed when callus tissues were subcultured for 3–4 passages with 3.0 μg/ml 2, 4-D and 0.1 μg/ml K. Under this condition, 2–3 greening spots were formed in 80–85% of callus tissues excised. Greening spots differentiated shoots after incubation of a further 10–15 days (**Fig. 1 A**). The regenerative capability of coleoptile-derived callus tissues was stably maintained during successive subcultures for at least 3 months.

Root initiation was observed by transferring leaf-developing shoots to growth-regulator-free MS medium (**Fig. 1 B**). Regenerated plants were transplanted to soil and acclimated in a moist chamber for a few days. **Figure 1 C** shows regenerants forming ears under the field condition. These regenerants produced and maturated viable seeds 2 months after planting.

The present study demonstrated that the combination of 2, 4-D and K is effective for plant regeneration of coleoptile-derived callus tissue of barley. In addition, the authors evaluated the effect of these

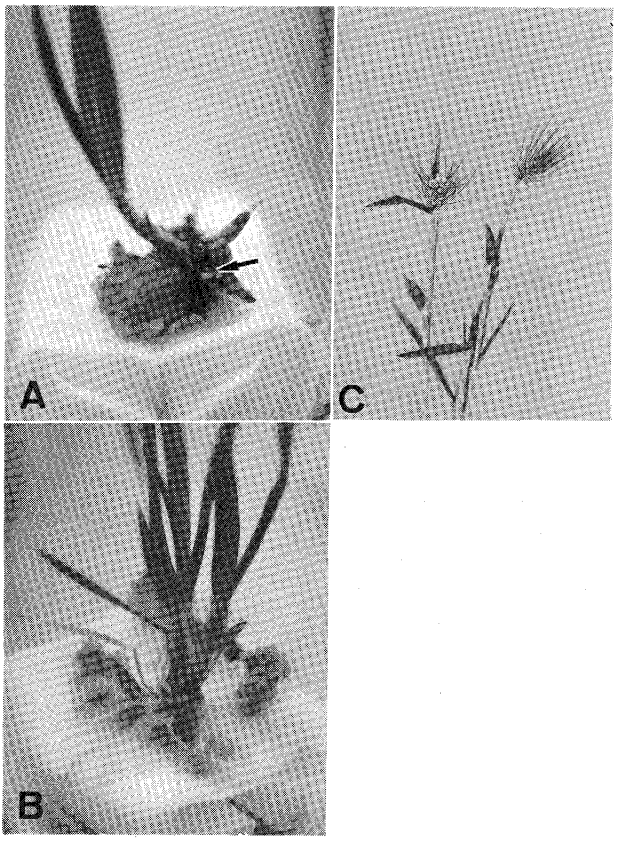

Fig. 1. Plant regeneration of callus tissues induced from barley (*H. vulgare* L. cv. Kobinkatagi) coleoptile.
A : Shoot differentiation from greening spot formed in callus tissues (with MS medium containing 3.0 μg/ml 2, 4-D and 0.1 μg/ml K). Arrow indicates greening spot which does not differentiate shoot. B : Root formation of shoot in growth-regulator-free MS medium. C : Regenerated plant forming ear under the field condition.

growth regulators on the callus induction and plant regeneration from explants obtained from different tissues. With the present combination of both growth regulators, callus tissues were similarly induced from mature embryo and germinating seeds, and regenerated into shoots. These results suggest that different tissues of Kobinkatagi would be commonly depend on 2, 4-D and K to produce callus tissues and redifferentiate shoots.

References

1) Flick, C. E., D. A. Evans, W. R. Sharp, 1983. In "Handbook of Plant Cell Culture" (ed. by Evans, D. A., W. R. Sharp, P. V. Ammirato, Y. Yamada), p. 13-81, Macmillan Publishing, New York.
2) Kartha, K. K., R. N. Chibbar, F. Georges, N. Leung, K. Caswell, E. Kendall, J. Qureshi, 1989. Plant Cell Rept., **8**: 429-432.
3) Jelaska, S., Z. Rengel, V. Cesar, 1984. Plant Cell Rep., **3**: 125-129.
4) Klein, T. M., E. D. Wolf, R. Wu, J. C. Sanford, 1987. Nature, **327**: 70-73.
5) Mendel, R. R., B. Muller, J. Schulze, V. Kolesnikov, A. Zelenin, 1989. Theor. Appl. Genet., **78**: 31-34.
6) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
7) Shimamoto, K., R. Terada, T. Izawa, H. Fujimoto, 1989. Nature, **338**: 274-276.
8) Toyoda, H., T. Yamaga, Y. Matsuda, S. Ouchi, 1990. Plant Cell Rep., **9**: 299-302
9) Rengel, Z., S. Jelaska, 1986. J. Plant Physiol., **124**: 385-392.
10) Zhang, W., R. Wu, 1988. Theor. Appl. Genet., **76**: 835-840.

≪和文要約≫

オオムギ子葉鞘カルスからの植物体再生

豊田秀吉，小浦勇次，松田克礼，堀越浩二，玉井隆行，
山家高子，池田成志，島林正人，守田昌弘，大内成志

近畿大学農学部

オオムギの子葉鞘を外植片とし，それらを種々の植物生長調節物質を添加した MS 培地で培養して，カルス誘導や個体再生のための培養条件を検討した．その結果，子葉鞘からのカルス誘導は，2, 4-D とカイネチンを種々の濃度で添加した実験区で観察されたが，カルス組織からの緑色小斑形成ならびに緑色小斑からの shoot 形成には，3.0 μg/ml の 2, 4-D と 0.1 μg/ml のカイネチンを加えた区がもっとも効果的であった．カルス組織の再分化能は数世代継代したあとも安定して保持され，発根は生長調節物質を添加しない培地に移植することで容易に誘導された．本条件で得た再分化個体は，馴化後通常の土壌栽培条件下で正常に生育し，それらから自殖種子を得ることができた．