Note

Plant Regeneration from the Callus Derived from Mature Embryos of Hiproly Barley, *Hordeum distichum* L.

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Plant regeneration from barley callus has been reported in several varieties of *Hordeum vulgare*. For example, plantlets have been regenerated from calluses derived from apical meristems of young seedlings,1) from immature ovaries,2) from mature embryos3) and from immature haploid barley embryos.4)

A high auxin concentration is required for both initiation and maintenance of callus, and then transferring the callus onto an auxin-free medium results in organogenesis or plant regeneration. Some of the reports have shown that supplementation of cytokinin encouraged shoot formation in the callus.5,6)

Hiproly barley *Hordeum distichum* L. is a producer of exceptionally high protein and lysine yields. Nevertheless, plant regeneration has not been reported from the callus of this barley. In this paper we report plant regeneration from the callus derived from mature embryos of Hiproly barley.

The mature embryos of Hiproly barley (*Hordeum distichum* L. variety Hiproly) were sterilized by soaking in an ethanol–hydrogen peroxide mixture (1:1, v/v) for 10 min, washing twice in sterile water, and then aerating for 24 hr. For callus initiation they were placed on agar-solidified White’s medium containing 20 mg/liter of 2,4-D and incubated in the dark at 23°C for callus induction. Callus was induced from embryos and root segments. After 30 days in the dark at 23°C, induced calluses were transferred to fresh White’s medium containing 2 mg/liter of 2,4-D. Calluses were then subcultured every 8 weeks.

Calluses were kept for at least one passage on White’s medium containing 2 mg/liter of 2,4-D. Roots and shoots were formed on Murashige and Skoog’s medium without 2,4-D. Complete plants were regenerated on B5 medium7) without 2,4-D, under light (about 3000–8000 lux).

Chromosomes of the regenerated plantlets were counted as described by Orton2) except that 55 min of hydrolysis in 5 N HCl at room temperature was used. Root tips were fixed and softened for one min in 1 N HCl at 60°C before squashing and stained by Feulgen reagent.

Up to 80% of the 20 calluses which had been subcultured twice showed root formation. Roots were easily produced in both the calluses derived from embryos and from root explants. Figure 1A shows excessive root formation from the callus under light (about 3000 to 8000 lux). Callus derived from root segments did not form roots, but some of the callus derived from embryos did form shoots. About 30% of the total calluses formed shoots after two subcultures (number of calluses tested was 40).

Under the high light intensity (about 3000 to 8000 lux) shoots became green and continued steady growth on B5 medium. Five plantlets were regenerated and transferred into soil (Fig. 1C). Figure 1D shows chromosomes in a root tip cell of a regenerated plantlet. All plantlets maintained the diploid chromosome number (2n = 14). No aberrant phenotypes were detected among the regenerated plants.

REFERENCES

Fig. 1. A) Excessive Root Formation from Callus of Hiproly Barley under High Light Intensity on Murashige and Skoog's Medium without 2,4-d. B) Shoot Formation from Callus of Hiproly Barley on B5 Medium without 2,4-D. Note Root Formation Directly from Shoot Base. C) Plantlet after Transfer into Pot in Soil. D) Chromosomes in Root Tip Cell of Regenerated Plantlet.