

Efficient *in vitro* regeneration system from seed derived callus of perennial ryegrass (*Lolium perenne*) and annual ryegrass (*Lolium multiflorum*)

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Introduction The commercially important ryegrasses in cool temperate climates throughout the world are annual ryegrass (*Lolium multiflorum* L.) and perennial ryegrass (*Lolium perenne* L.). Improvements through conventional breeding have been slow as they are usually heterozygous and highly self-infertile. Hence, there is a need to use modern biotechnological tools to the development of improved rye grass cultivars for incorporating value added traits. Successful transformation of rye grasses has been done using suspension cells, which is time consuming and laborious (Spangenberg *et al.*, 1995, 1998). We report here a rapid and highly efficient *in vitro* plant regeneration system from seed derived callus in annual and perennial rye grasses.

Materials and methods Mature seeds of one variety of annual ryegrass (RGANN) and one variety of perennial ryegrass (RGPEN), were surface sterilized in 70% ethanol for 1 min followed by various treatments with H₂SO₄ and Bleach, bleach alone or HgCl₂ with intermittent shaking. Surface sterilized seeds were germinated on MS medium (Murashige and Skoog, 1962) solidified with agar (0.8%) and supplemented with varying concentrations of 2,4-D (2 mg/l, 4mg/l and 6 mg/l), and petri dishes were kept in dark at 24± 2 °C. Ten seeds were placed on each petri dish (100 x 15 mm) containing 20 ml of solidified MS medium and 100 seed per treatment for each genotype. After one week of seed germination, the emerging shoot and root were chopped to suppress germination and stimulate callus formation. After 6 weeks on fresh medium, the resulting callus was maintained by sub-culturing every 4 weeks. To evaluate the regeneration potential, the calli were transferred to MS augmented with different combinations of plant growth regulators. For regeneration, cultures were incubated at 24 ± 2 °C under a 16/8-hour dark photoperiod.

Results Among various surface sterilization procedures, the treatment with 0.1% HgCl₂ for 10 minutes was found to be optimum for seed germination. The treatment with 4.0 mg/l 2,4-D was found to be the best for callus induction for both RGANN and RGPEN and with further increase in 2,4- D concentration, the callus induction frequency decreased. Highest frequency of callus induction was observed in RGANN (94%) followed by RGPEN (72%) on MS medium supplemented with 4.0 mg/l 2,4-D. Callus initiation and growth took about 6 weeks. Callus was maintained every 4 weeks on MS medium supplemented with 4.0 mg/l 2,4 D and 0.1 mg BAP. Shoot regeneration was observed, after 4 weeks of transferring the callus to regeneration medium, containing various combinations of BAP and 2,4.D. Highest frequency of shoots was regenerated per callus clump on MS +0.5 mg/l BAP in RGPEN (13.67). Whereas in RGANN, highest frequency of shoots (11.13) was observed in MS medium with 0.1mg/l BAP. In both the varieties, a decrease in shoot regeneration frequency was observed in the combination treatments of BAP (0.1 mg/l, 0.5 mg/l) and 2, 4-D (0.1 mg/l 1.0 mg/l). Hundred percent rooting was observed when regenerated plants were transferred to MS media supplemented with 0.2 mg/l NAA alone or in combination with 0.5 mg/l GA₃. Rooted plantlets were transferred to soil and acclimatized in the greenhouse.

Conclusions We report here an efficient and rapid (four months) tissue culture regeneration protocol for both annual and perennial ryegrasses.

References

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