A novel genotype independent protocol for in vitro plant regeneration from mature seed derived callus of tall fescue (*Festuca arundinacea* Schreb.)

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Introduction Tall fescues (*Festuca arundinacea* Schreb.) are cool season forage and turf grasses of significant agricultural importance in different grassland countries. Genetic improvement of tall fescues by conventional selection procedures is slow, since these are predominantly, cross-pollinated, hexaploid and generally infertile (Jauhar, 1993). Genetic Engineering approaches for incorporation of agronomically useful traits may contribute to the development of improved tall fescue cultivars (Spangenberg *et al*., 1998). However for any genetic engineering studies, it is essential to develop a genotype-independent, reproducible and efficient *in vitro* plant regeneration protocol. In the present study, we analyzed the effects of different sterilization procedures for *in vitro* seed germination and studied the effects of different concentrations and combinations of 2,4-D and BAP on callus induction, growth and regeneration potential of two cultivars of tall fescue.

Materials and methods Seeds of two varieties of tall fescue, EnviroBlend (FESEVB) and EnviroSHADE (FESEVS) were surface sterilized in 70% ethanol for 1 min followed by various treatments with H_2SO_4 with Bleach and bleach alone or $HgCl_2$ and germinated in petriplates with 20 ml of MS medium (with 0.8% Agar) (Murashige and Skoog, 1962) supplemented with varying concentrations of 2,4-D (2 mg/l, 4mg/l and 6 mg/l). Ten seeds were placed on each petri dish, with 100 seeds per treatment for each cultivar. After one week of seed germination at 24± 2 °C, the emerging shoot and root were chopped to suppress germination and stimulate callus formation and sub cultured onto fresh medium and kept in dark. To evaluate the regeneration potential, the calli were transferred to MS medium with different combinations of hormones and incubated at 24 ± 2 °C under a 16/8-hour dark photoperiod provided by cool-white fluorescent lights at a quantum flux density of 30µmol $s^{-1}m^{-2}$.

Results Surface sterilization of seed with 0.1% $HgCl_2$ 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 the varieties and further increase in 2,4- D concentration decreased the callus induction frequency. Highest frequency of callus induction was observed in FESEVS (88%) followed by FESEVB (86%) on MS medium supplemented with 4.0 mg/l 2, 4-D. Callus was maintained every 4 weeks on MS medium containing 4.0 mg/l 2,4-D and 0.1 mg BAP. Callus turned greenish and shoots appeared after 4 weeks on regeneration medium. Higher frequency of shoots were regenerated per callus clump on MS +0.5 mg/l BAP alone compared to other hormonal combinations in FESEVB (13.07) followed by FESEVS (11.13). When 2, 4, D was present (either 0.1 mg/l or 1.0 mg/l) in regeneration medium a drastic reduction in shoot regeneration frequency was observed. One hundred percent rooting was observed in both the varieties on MS media supplemented with 0.2 mg/l NAA in two weeks. In the combination treatments of NAA and GA_3 all the plants were rooted in FESEVS while only 80% of the plants rooted in FESEVB. When BAP (1.0 mg/l) was added to the rooting medium, either with NAA alone or in combination with NAA and GA_3, a drastic reduction in rooting frequency was observed. No significant differences were observed for both callus induction and shoot regeneration in both varieties. All rooted plants were transferred to the soil and acclimatized in the greenhouse.

Conclusions Previously, *in vitro* regeneration and the production of transgenic plants of tall fescues were done by using suspension cells, which is time consuming and often produces more somaclonal variation. For the first time, we developed a genotype independent, rapid and efficient *in vitro* plant regeneration system in four months from mature seed derived callus of tall fescue making this grass more amenable for genetic engineering studies.

References

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