Efficient *In Vitro* Regeneration System From Seed Derived Callus of Perennial Ryegrass (*Lolium Perenne*) And Annual Ryegrass (*Lolium Multiflorum*)

S. Chennareddy
University of Toledo

R. V. Sairam
University of Toledo

S. L. Goldman
University of Toledo

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

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

S. Chennareddy, R.V. Sairam and S.L. Goldman*Plant Science Research Center, University of Toledo, Toledo, Ohio-43606, USA, Email: srudrab@utnet.utoledo.edu*

Keywords callus, regeneration, ryegrass

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 (RGPER), were surface sterilized in 70% ethanol for 1 min followed by various treatments with H_2SO_4 and Bleach, bleach alone or $HgCl_2$ 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% $HgCl_2$ 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 RGANN and RGPER 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 RGPER (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 RGPER (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 GA_3. 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

Murashige T and Skoog F. (1962) A revised medium for rapid growth and bioassays with tobacco cultures. Physiol Plant. 15: 473-497.

Spangenberg G, Wang Z Y., Nagel, J., Iglesias, V.A., Potrykus, I. (1995). Transgenic tall fescue (*Festuca arundinaceae*) and red fescue (*Festuca rubra*) plants from microprojectile bombardment of embryogenic suspension cells. J. Plant Physiol. 145:693-701

Spangenberg G, Wang Z Y, Potrykus I, (1998). Biotechnology in Forage and Turf grass improvement. In: Frankel R., Grossman M, Linskens HF, Maliga P, Riley, R (eds) Monographs on theoretical and applied genetics, Vol. 23, Springer, Berlin Heidelberg New York, 200 pp.