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Ru Yang

Chinese Academy of Agricultural Sciences, China

Qinghua Yuan

Chinese Academy of Agricultural Sciences, China

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Effect of different explants on callus induction and differentiation of perennial ryegrass

Yang Ru, Yuan Qing-hua*

Institute of Animal Sciences, CAAS, Beijing 100094, China

* Corresponding author. E-mail address: yuanqinghua@hotmail.com

Key words perennial ryegrass, explant, mature seeds, hypocotyl, callus induction, differentiation

Introduction Perennial ryegrass, in north China, is the most important forage and turf grass that plays an extensive role and is used in many fields. At present, cultivated area of perennial ryegrass is expanded faster than other grass cultivars due to its great quality, such as longer green period, heat resistant, high yield etc. Transgene as a utility tool provides a new means to improve culture quality. Accordingly, it is of importance to establish a high efficiency system of callus induction and differentiation.

Materials and methods The sterilized seeds of *Lolium perenne* L. varieties were sown in MS medium. Keeping them in culture room for 4-6 days, we picked a part of germination seeds and cut shoot tip, root tip and hypocotyledonary axis as explant which had an inoculation at induction medium with different concentration 2,4-D, 6-BA. About 30 days later, the callus has a well growth that was transferred a new induction medium to continue growth. When we had enough callus, we transferred some of them to differentiation medium without 2,4-D and with different concentration 6-BA.

Results Experiment results showed that MS medium without 2,4-D was ineffective in induction callus (Saalbach, 1978). Different explants had different induction rate and differentiation rate. Shoot tip and root tip were ineffective in callus induction. MS medium with 7mg/L 2,4-D and 0.1 mg/L 6-BA was propitious to callus induction from mature seeds, with 5 mg/L 2,4-D and 0.05 mg/L 6-BA was propitious to callus induction from hypocotyledonary axis (Table 2). Time of callus subculture had markedly effect on differentiation rate (McDonnell, 1984) (Figure 1). Callus subculture for 60 days was propitious to callus differentiation from mature seeds, for 80 days was propitious to callus differentiation from hypocotyledonary axis.

Conclusions The callus induction medium supplemented with two auxins (2,4-D and 6-BA) was much better than that with 2,4-D individuals. Mature seeds and hypocotyledonary axis are most propitious to callus induction. It is not advantaged for subculture callus to induce differentiation. We established a good system for callus induction and differentiation that was a base stage for us to improve quality of perennial ryegrass utilizing transgene tool.

Table 2 Effect of different degrees of 6-BA, 2,4-D concentration on the callus formation frequency of *Lolium perenne*.

treatment	concentration		Rate of callus(%)	
	2,4-D	6-BA	Mature seeds	Hypocotyl
1	5	0.05	53.3	25.3
2	5	0.1	60.0	21.3
3	5	0.5	50.6	18.0
4	7	0.05	68.0	16.6
5	7	0.1	73.3	24.0
6	7	0.5	57.3	14.6

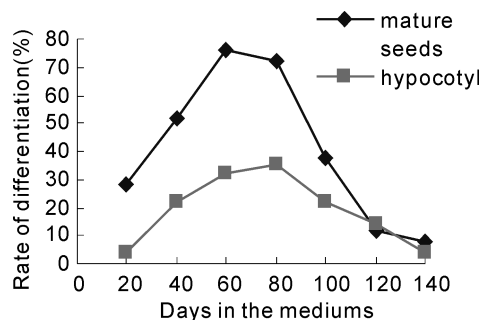


Fig 1 Comparison of *Lolium perenne* callus differentiation with different culturing days.

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