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## Plantlet regeneration from the mature embryo of *Bothriochloa ischaemum*

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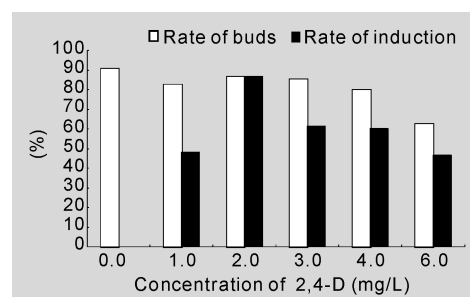
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**Key words:** *Bothriochloa ischaemum*, mature embryo, embryogenic callus, plant regeneration

**Introduction** *Bothriochloa ischaemum* is known to have a strong vitality, produce high yields, is tolerant to grazing and has a high feeding value. The regeneration from mature embryos was studied using the mature embryos of *Bothriochloa ischaemum* as explants. The effect of various hormone compositions on callus induction and growth state was studied.

**Materials and Methods** The explants were first immersed for 10 min in distilled water, followed by immersion in 75% ethanol for 30 s, then in 0.1% (w/v) HgCl<sub>2</sub> solution for 15 min with constant agitation, lastly extensive washing (four-five times) with sterile distilled water was undertaken. Murashige and Skoog's (MS) medium containing 3% sucrose and 0.5% agar was used as the basal medium. The MS medium supplemented with 2,4-D (0, 1.0, 2.0, 3.0, 4.0, 6.0 mg/L) was tried in a single factorial treatment. Each treatment consisted of three replications with 15 explants. Callus was maintained at 25 ± 2°C, 60% relative humidity for 27 d. Rapidly growing callus and embryogenesis were maintained in the callus subculture medium plus 1.0 mg/L 2,4-D for 5-6 d. The embryogenic callus (EC) were subsequently subcultured on MS media supplemented with NAA (0.04, 0.05, 0.06, 0.07 mg/L) in combination with 0.1 mg/L of 6-BA for inducing callus differentiation.

**Results** The frequency of callus induction reached 86.7% on the callus induction medium supplemented with 2.0 mg/L 2,4-D (Figure 1). The rate of differentiation frequency from callus sub-cultured was 60.9% on the differentiation medium supplemented with 0.1 mg/L 6-BA and 0.05 mg/L NAA. Differentiation frequency from combinations of 0.1 mg/L 6-BA with 0.04 or 0.06 mg/L NAA was 43.6% and 34.6%, respectively (Table 1).



**Figure 1** The growth of seeds in different concentrations of 2,4-D.

**Table 1** Callus Differentiation in different combined of hormones.

Hormone composition(mg/L)		Number of inoculation	Number of EC	Induction frequency of EC (%)	Number of differentiation	Differentiation frequency (%)
6-BA	NAA					
0.1	0.04	86	71	82.6	31	43.6
0.1	0.05	87	46	52.9	28	60.9
0.1	0.06	88	81	92.0	28	34.6
0.1	0.07	86	73	84.8	21	28.7

**Conclusions** The callus induction Frequency reached 46.6%-86.7% on most of the media combinations during 20-27 days. It has been shown that the callusing in differentiation required a low auxin and comparatively high cytokinin level.

### Reference

Parrott, W. A., 1991. Auxin-stimulated somatic embryogenesis from immature cotyledons of white clover. *Plant Cell Report*, 10, 17-21.