Callus initiation and plant regeneration in ragi (*Eleusine coracana* Gaertn.)

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**Abstract.** Callus was successfully initiated on root, mesocotyl and leaf base segments of 3- to 4-day-old seedlings of ragi (*Eleusine coracana* Gaertn.). 2,4-D along with casein hydrolysate for Murashige and Skoog's basal medium was found to be most effective for callus initiation and maintenance. Mesocotyl and leaf base tissue derived calli gave shoot buds in medium in which the 2,4-D concentration was lowered.

**Introduction**

Regeneration of plantlets from cultured tissues of recalcitrant monocot species like cereals and millets has been recently achieved. Although a few millets like *Panicum* and *Pennisetum* have been grown successfully in culture [5, 9], the knowledge with respect to *Eleusine coracana* a tropical minor millet is meagre [7, 8]. Earlier work [8] on *Eleusine* had shown that regeneration could be achieved using mesocotyl callus tissues. A suitable procedure for initiation of callus and plantlet regeneration from various explants of *Eleusine* is yet to be developed.

**Materials and methods**

Seeds of *Eleusine coracana* var. AKP 7 and var. Dibysinha were surface sterilized in 0.1% mercuric chloride solution for 5 min, washed 3 times with sterilized distilled water and germinated aseptically on moist filter paper. Root, mesocotyl and basal portion of leaf segments of 3- to 4-day-old seedlings were placed on Murashinge and Skoog's (MS) [6] and Gamborg's B5 [1] basal media supplemented with different growth hormones such as 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichloroacetic acid (2,4,5-T), Naphthaleneacetic acid (NAA) or Indole-3-acetic acid (IAA), at various concentrations. All, media (pH 5–8) were solidified with 0.8% agar and autoclaved at 121 °C for 15 min. IAA was filter sterilized and added to media after autoclaving.

For callus initiation three explants were inoculated per tube and there
were fifteen tubes in each treatment and each treatment was repeated three times. Cultures were placed under 16 h daily photoperiod at 22 ± 1 °C and 25 μE m⁻² s⁻¹ Photon Flux Density (PFD) provided by Phillips fluorescent tubes. For shoot initiation, calli were subcultured into media with 0, 0.25 and 0.5 mg/l of 2,4-D and exposed to continuous illumination in growth chamber with 260 μE m⁻² s⁻¹ PFD of both combined Phillips fluorescent-incandescent light.

For histological analysis calli were fixed with FAA (90 ml 70% ethanol, 5 ml formaldehyde, 5 ml glacial acetic acid) and dehydrated in a tertiary-butyl-alcohol series [3]. After dehydration, materials were passed through butyl alcohol:chloroform series (3:1, 2:1 and 1:1) and finally chloroform. The chloroform was changed and paraffin chips were added gradually. Tubes containing materials were placed at 58 °C for 48 h for paraffin infiltration. Paraffin blocks were prepared and sections were cut at 7 to 10 μ thickness using a spencer's microtome. Double staining procedures of Johanson [3] was followed. Slides were made permanent with Canada balsam.

Results and discussion

More than 90% of the explants form each variety formed callus when grown on MS or B₅ medium supplemented with 1–4 mg/l of 2,4-D. The optimal concentration was found to vary for each type of explant (4 mg/l of 2,4-D for leaf base segments and 2 mg/l for root and mesocotyl explants) but calli turned brownish to black within 8 to 10 days. The addition of casein hydrolysate (500 mg/l) along with 2,4-D yielded white friable callus from both the varieties, irrespective of explant sources. Callus formation was not improved with NAA, 2,4,5-T or IAA. NAA (1–4 mg/l) caused initial swelling of explants followed by blackening. Inclusion of casein hydrolysate with NAA prevented the blackening but did not give better callus induction than 2,4-D. 2,4,5-T and IAA were ineffective and coconut water (cw), (5%, 10% and 15%) with 2,4-D did not show any significant effect on callusing. Previous work on cereals and millets has shown 2,4-D to be effective in callus induction [10]. However, the superiority of 2,4-D is probably due to its stable nature [4]. In the present investigation, 2,4-D along with casein hydrolysate was found to be most effective for initiation of ragi culture. Thiru and Mohan Ram [7] found NAA to be suitable for tissue culture of ragi in var. HPB 7–6 and var. ROH 2 whereas, here with varieties AKP 7 and Dibysinha it was not suitable.

Regeneration of Shoots occurred either on omission of 2,4-D from the medium or with low concentrations of 2,4-D. The frequency of shoot regeneration is presented in Table 1. The highest frequency of shoot formation was achieved at 0.25 mg/l of 2,4-D on MS basal medium. The mesocotyl and leaf base calli of var. AKP 7 showed 33 and 29%, shoot differentiation, respectively. The shoot differentiation from mesocotyl and leaf base calli of Dibysinha