Short Communication

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SOMATIC EMBRYOGENESIS AND PLANTLETS REGENERATION IN *HETEROPOGON CONTORTUS* (L.) P. BEAUV.

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For maturation and maintenance of embryogenic cultures 0.1 mgl^{-1} 2, 4-D and 100 mgl^{-1} ascorbic acid were sufficient. Out of 1500 embryos transferred to hormone free MS medium 1088 (ca 70 per cent) produced complete plantlets. They were transplanted to pots containing Soil-Soilrite ™ (1:1) mixture where they were grown to maturity.

Key Words : *Heteropogon contortus*, somatic embryogenesis, plnat regeneration, mature caryopses.

Success in the application of *in vitro* techniques, plant genetic manipulation and plant cell molecular biology depends on the particular techniques of tissue, cell and protoplast culture developed for each species. One desirable attribute would be the ability to establish cultures from graminaceous species (Shekhawat *et al.*, 1984; Kackar and Shekhawat, 1989; George *et al.*, 1991; Kackar and Shekhawat, 1991). However, regenerative embryogenic cultures from mature seeds have been obtained in few cases (Smith and Bhaskaran, 1986; Kothari and Chandra, 1988; Purohit *et al.*, 1992). The present paper deals with establishment of callus cultures, induction of somatic embryogenesis and plantlet regeneration in *Heteropogon contortus* from mature caryopses.

Seeds of *Heteropogon contortus* were collected from the forest areas of Jhadol, Udaipur. After thorough washing with running tap water, seeds were surface sterilized with 0.2 per cent mercuric chloride for 10 minutes, followed by washing with sterile distilled water. Seeds were put axenically on Murashige and Skoog's medium (1962) containing 0.5-5.0 mgl^{-1} 2, 4-D. The pH of the medium was adjusted to 5.8 prior to autoclaving. Cultures were maintained by subculturing every three weeks on fresh medium containing 0.1 mgl^{-1} 2, 4-D and 100 mgl^{-1} of ascorbic acid. Callus cultures were kept in dark at 28±2°C and 40-50 per cent relative humidity. For plantlet regeneration, competent embryogenic cultures were transferred to auxin-free MS medium and kept under 16-18 hr light (3000 lux) and dark periods. Regenerated plantlets were carefully removed from culture vessels, washed with distilled water and transferred to pots containing Soil - Soilrite™ (1:1) mixture.

The callus initiation was observed on 8th day after inoculation of seeds on MS medium containing 2,4-D (0.5-5.0 mgl^{-1}). The best callus was obtained on MS medium containing 0.5 mgl^{-1} 2,4-D. Out of 50 seeds transferred, 28 seeds produced white friable calli. On subculture, two distinct types of calli were observed: One was soft, fluffy, translucent and non-morphogenic (non-embryogenic callus) while the other was hard, nodular, white and regenerative callus (embryogenic type Fig. 1). The former non-embryogenic cultures produced roots when they were transferred to hormone free MS medium. Later embryogenic cultures were highly regenerative and sensitive to mechanical injury and disturbances which turned brown if kept on the same medium for longer times. The regenerative cultures were required to be isolated carefully to maintain the purity of cultures, otherwise non-regenerative tissues grew rapidly and the regenerative cultures disappeared with time.

Competent embryogenic cultures could be developed into well organized globular embryos when they were transferred to MS medium containing 0.1 mgl^{-1} 2,4-D and kept under dark conditions. For maintenance and healthy growth of these embryos, it was necessary to incorporate 100 mgl^{-1} ascorbic acid in the medium. It was also necessary to subculture these embryos on fresh medium every 21 days to maintain their regenerative potential. For obtaining complete plantlets such embryos when transferred to hormone free MS medium with full strength salt and kept under light. About 70 per cent embryos (1088 out of 1500) germinated showing emergence of leaves from coleoptile and roots from coleorhiza (Figs. 2, 3). There was however, no phenotypic variation observed in such regenerated plantlets.

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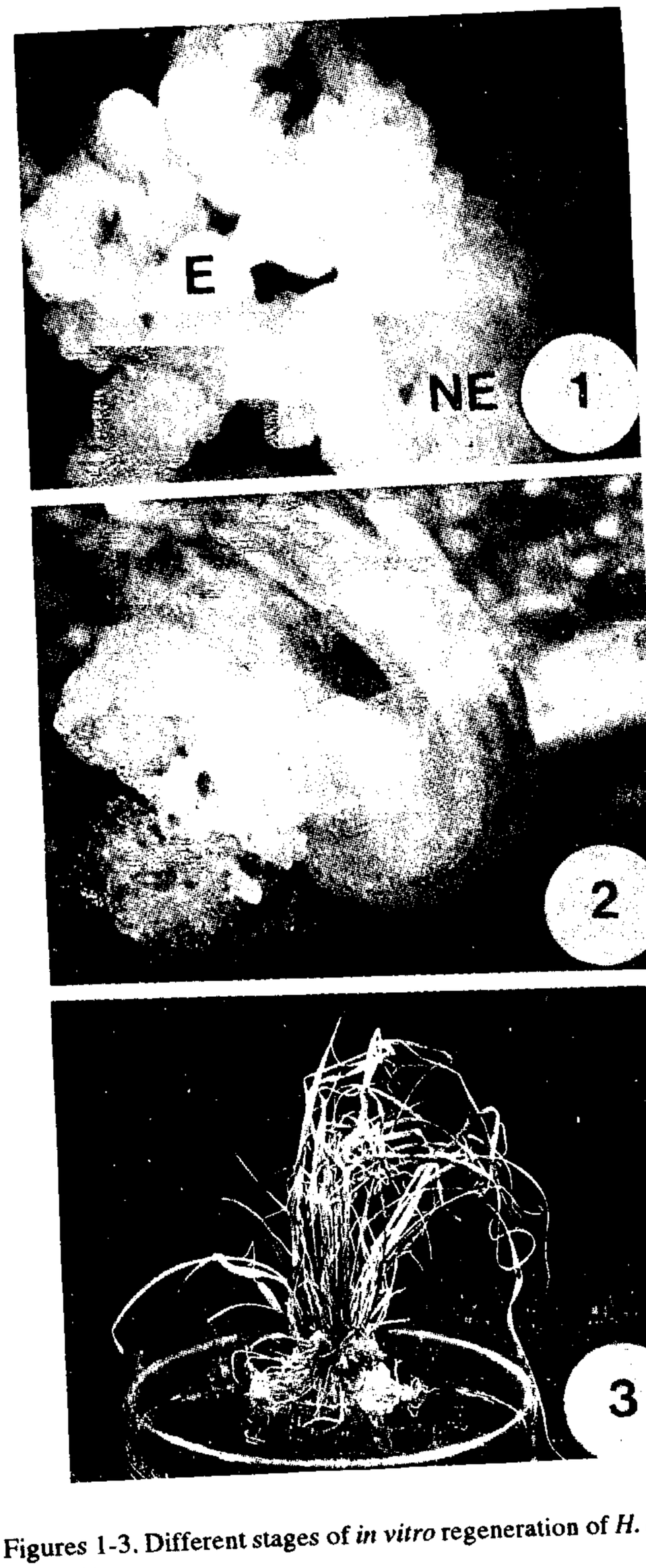

Figures 1-3. Different stages of *in vitro* regeneration of *H. contortus* (L.) P. Beauv.
Fig. 1. Embryogenic (E) and non-embryogenic (NE) callus cultures on MS medium containing 0.5 mgl^{-1} 2,4-D.
Fig. 2 Germinated somatic embryo on hormone free MS medium.
Fig. 3. Complete plantlet with tillers on hormone free MS medium.

Ten to fifteen days old plantlets removed from culture vessels were transferred to pots containing Soil-Soilrite™ (1:1) mixture with 70-80 per cent transplantation success. Under greenhouse condition, out of 1088 plants transferred to pots, 864 survived till maturity.

In graminaceous species embryogenic cultures from mature seeds have been obtained in few cases (Smith & Bhaskaran, 1986; Kackar & Shekhawat, 1989; Purohit *et al.*, 1992). In present investigation mature caryopses have been used to obtain competent embryogenic cultures for plantlet regeneration. Use of 2,4-D to induce embryogenesis and its removal for embryo germination observed in present investigation is consistent with those reported for so many other cases (Marousky & West, 1990; Kackar & Shekhawat, 1991).

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REFERENCES

George L, S Eapen & P S Rao 1991 Plant regeneration by somatic embryogenesis from inflorescence cultures of *Sorghum halepense* L. *Pers. Ind J Expt Biol* **29** 16-18.

Kackar A & N S Shekhawat 1989 Regeneration of *Lasiurus scindicus* from tissue culture. *Annals of Botany* **64** 455-458.

Kackar A & N S Shekhawat 1991 Plant regeneration from cultured immature inflorescence of *Cenchrus setigerus* and *C. ciliaris. Ind J Expt Biol* **29** 62-64.

Kothari S L & Chandra N 1988 Somatic embryogenesis and plant regeneration from seed callus of barley (*Hordeum vulgare* L.). *Current Science* **57** 1351-52.

Marousky F J & S H West 1990 Somatic embryogenesis and plant regeneration from cultured mature caryopses of bahia grass (*Paspalum notatum* Flugge). *Plant Cell Tissue and Organ Culture* **20** 125-129.

Murashige T & F Skoog 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plantrum* **15** 473-497.

Purohit S D, G Kukda & R Sukhwal 1992 Plant regeneration in *Apluda mutica* L. through somatic embryogenesis. *In J Expt Biol* **30** 1154-1157.

Shekhawat N S, P N Gordon & A W Galston 1984 Highly regenerative tissue cultures from axillary tiller buds of sexually mature oat plants (*Avena sativa* L.). *In Vitro* **20(9)** 707-711.

Smith R H & S Bhaskaran 1986 Sorghum. In *Biotechnology in Agriculture & Forestry* (YPS Bajaj ed), Springer-Verlag, Berlin p 220-233.