J Indian bot Soc Vol 71 (1992) 47-49

MICROPROPAGATION OF WHEAT (*TRITICUM DURUM* DESF) THROUGH MICROTILLERING

ANJU RAO AND S.L.KOTHARI
Department of Botany, University of Rajasthan, Jaipur-302 004, India
(Accepted January 1992)

Shoot multiplication through microtillering was observed from cultured mature embryos of wheat on MS medium supplemented with 2,4-D. More than 20 shoots developed on medium with 0.2 and 1.0 mg/1 2,4-D in 8-10 weeks. Number of shoots increased in ageing cultures and after serial transfers on media with lower levels of 2,4-D. Media with 2,4-D in combination with Kn induced more than fifty shoots in second subculture. Only callusing was favoured in media with 2,4-D concentrations greater than 2.0 mg/1. Shoots were rooted on half MS medium with or without 0.5 mg/1 IAA.

Key words: Mature embryo, micropropagation, microtillering, *Triticum aestivum.*

Wheat is one of the most important food crops of the world. Multiplication of plants from cultured tissues of this species is required for the successful application of biotechnology in its improvement. Many attempts have been made for plant regeneration via organogenesis or embryogenesis from several genotypes of wheat in tissue culture (Eeapen and Rao, 1982; Mackinnon *et al.,* 1986; Ozias-Akins and Vasil, 1983; He *et al.,* 1989 and Redway *et al.,* 1990a). However, regeneration potential has been reported to be differing depending on genotypes, explants and culture media (Carman *et al.,* 1988 and Redway *et al.,* 1990b). In this paper we report a simple protocol for micropropagation through microtillering from mature embryos of one of the genotypes of wheat. Microtillering as a method of shoot multiplication has been described in other grasses also viz., *Sorghum* (Dunstan *et al.,* 1978, 1979) and *Lolium* (Dalton and Dale, 1985).

MATERIALS AND METHODS

Seeds of genotype Raj. 155 of Wheat (*Triticum durum* Desf.) were collected from Agriculture Research Station, Durgapura, Jaipur (India). Seeds were surface sterilized in 0.1% $HgCl_2$ (w/v) solution for 3-5 minutes, rinsed 3-4 times in sterile distilled water and then soaked in sterile distilled water for 24-36 hours. Mature embryo explants were dissected out and cultured on MS medium (Murashige and Skoog, 1962) supplemented with different concentrations of 2, 4-dichlorophenoxy acetic acid (2, 4-D) contained in Erlenmeyer flasks (100 ml) as well as culture tubes (25x150 mm). Sucrose (3%) was added as carbon source, agar (0.8%) for solidification and pH of the media was adjusted to 5.8 before autoclaving at 1.06 kg/cm^2 pressure for 15-20 minutes. All the cultures were incubated at 26±2°C with 16 h. photoperiod (1200 lux) provided with fluorescent tubes and incandescent bulbs in growth chamber. The cultures were transferred to fresh medium at regular 25-30 days intervals after 40-45 days of initial culture period. Some replicates of each culture were incubated for 10-12 weeks. Shoots were transferred to MS medium containing lower levels of 2,4-D added singly or in combination with Kinetin (Kn) for their multiplication. Elongated shoots were cultured on half strength MS medium with or without 0.5 mg/l IAA for rooting. Six replicates of each treatment were taken and all the manipulations were aseptically performed. Each experimental set was repeated two to three times.

RESULTS AND DISCUSSION

After 7-10 days of culture, mature embryos induced 2-6 shoots with very little callusing on media containing 0.2 and 1.0 mg/12, 4-D (Fig. 1a). Within 40-45 days cultured embroys formed 10-15 shoots and more than 20 shoots developed in 60-70 days through microtillering (Fig. 1b). However, germination of cultured mature embroys was observed in some cultures. Callus and shoots were separated from each other and cultured on the same medium where the number of shoots increased. The amount of callus increase was lesser on subculturing on the same medium. At higher concentration of 2,4-D (1.0 mg/l) the number of shoots developed was reduced (Fig. 2) and at still higher levels of 2, 4-D (2,3,4 and 8 mg/

Received August, 1991.

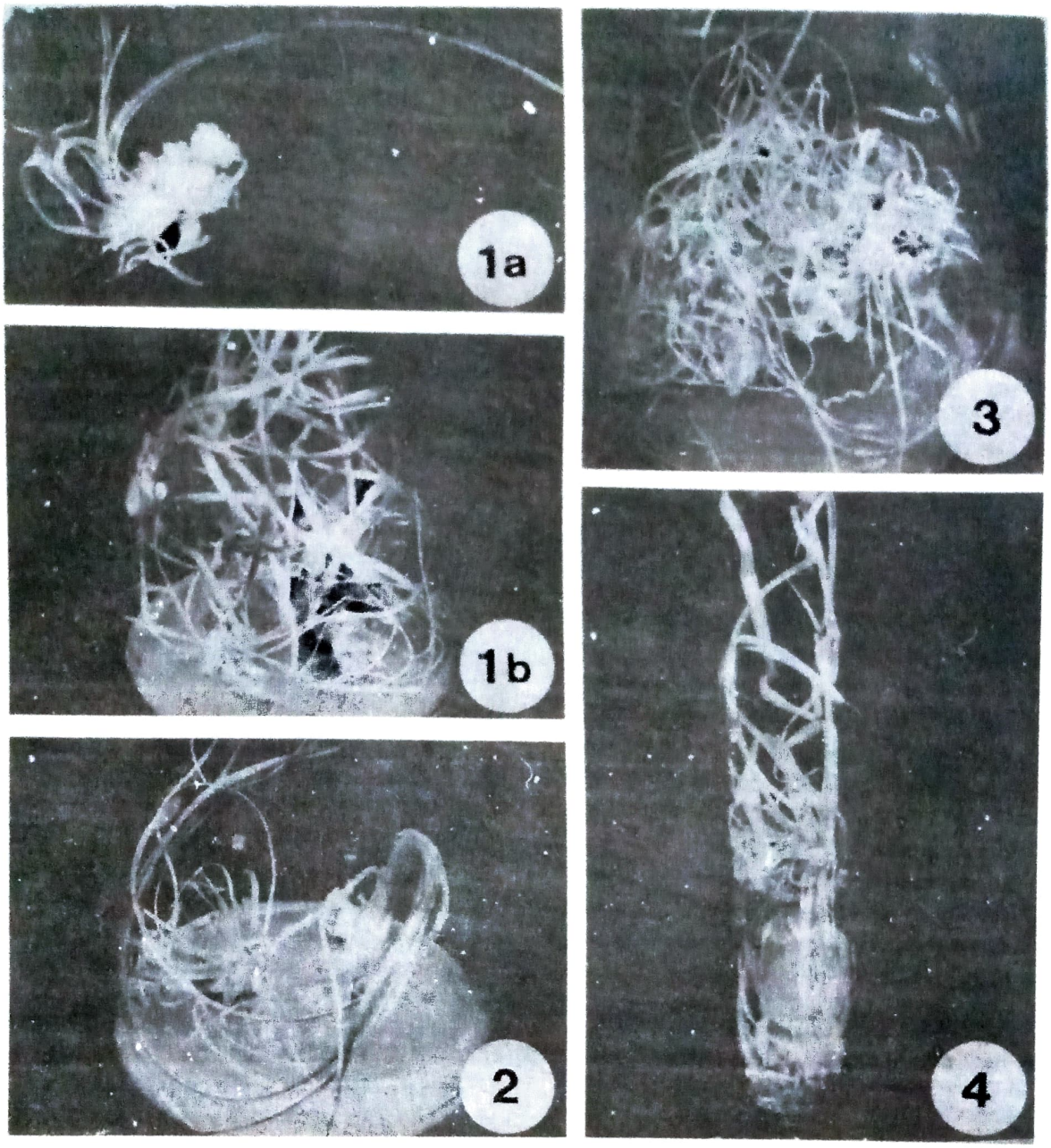

Figure 1-4: Microtillering from mature embryos of wheat on MS medium. Fig. 1a: Multiple shoots (2-6 in no.) from a 25-30 day old mature embryo cultured on medium with 0.2 mg/l 2,4-D. Fig. 1b: Same after 60-70 days. Fig. 2: Multiple shoots after 40-50 days old culture on media with 1.0 mg/l 2,4-D. Fig. 3: Best multiplication of shoots (> 50) on medium with 2,4-D (1.0 mg/l) + Kn (0.5 mg/l) after 25-30 days in second passage. Fig. 4: Rooted shoots on 1/2 MS with IAA (0.5 mg/l).

1) the cultured embroys callused from the entire surface. Callus formed was creamish, friable and water soaked. More than 50 shoots were formed after 25-30 days when differentiating shoots with callus were cultured on media containing 1.0 mg/l 2,4-D in combination with 0.5 mg/1 kn in the second passage (Fig. 3).

Elongation of shoots occurred in old cultures and in serial transfers on 2, 4-D (0.2 and 1.0 mg/1). Elongated shoots (4-5 cm long) were rooted on MS as well as half strength MS medium with or without 0.5 mg/1 IAA (Fig. 4).

In the present ivestigation multiplication of shoots through microtillering was observed. Microtillering has also been observed in *Sorghum* (Dunstan *et al.*, 1978, 1979) and *Lolium* (Dalton and Dale, 1985). the process was influenced by 2,4-D levels and serial transfers. Only lower levels of 2,4-D induced multiple

shoots from cultured mature embryos. Higher 2, 4-D levels supported callusing only. In second passage more than fifty shoots were recorded on medium containing 2,4-D in combination with lower levels of kn. The number of shoots formed in 8-10 week old cultures along with profuse callusing increased on medium with 0.2 mg/l 2, 4-D. In serial transfers callus turned light brown, wbhitish in colour and compact and nodular in texture.

We thank Professor Dalbir Singh and Professor N. Chandra for help and encouragement. This work was supported by CSIR grant [No. 38 (747)/88-89/ EMR-II] to SLK.

REFERENCES

Carman J G, N E Jefferson & W F Campbell 1988 Induction of embryogenic *Triticum aestivum* L. Calli. I. Quantification of genotype and culture medium effects. *Plant Cell Tissue Organ Cult* **12** 83-95.

Dalton S J & P J Dale 1985 The application of in vitro tiller induction in *Lolium multiflorum*. *Euphytica* **34** 897-904.

Dunstan D I, K C Short & E Thomas 1978 The anatomy of secondary morphogenesis in cultured scutellum tissue of *Sorghum bicolour*. *Protoplasma* **101** 355-361.

Eapen S & P S Rao 1982 Organogenesis and plantlet formation from callus cultures of different cultivars of bread wheat (*Triticum aestivum*) *Proc Indian Nat Sci Acad* B **48** No. 3, 371-377.

He D G, Y M Yang & K J Scott 1989 The effect of macro-element in the induction of embryogenic callus from immature embryos of wheat (*Triticum aestivum* L.) *Plant Sci* **64** 251-258.

Mackinnon C, G Gunderson & M W Nabors 1986 Plant regeneration by somatic embryogenesis from callus cultures of sweet sorghum. *Plant Cell Rep* **5** 349-351.

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

Ozias-Akins P & I K Vasil 1983 Improved efficiency and normalization of somatic embryogenesis in *Triticum aestivum* (wheat) *Protoplasma* **117** 40-44.

Redway F A, V Vasil, D Lu & I K Vasil 1990a Characterisation and regeneration of wheat (*Triticum aestivum* L.) embryogenic cell suspension cultures *Plant Cell Rep* **8** 714-717.

Redway F A, V Vasil & I K Vasil 1990b Identification of callus types for long-term maintenance and regeneration from commercial cultivars of wheat (*Triticum aestivum* L.) *Theor Appl Genet* **79** 609-617.