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High frequency plant-regeneration through direct shoot development and somatic embryogenesis from immature inflorescence cultures of finger millet (*Eleusine coracana* Gaertn)

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Summary

Plant regeneration from cultured immature inflorescence segments of *Eleusine coracana* was obtained by direct shoot development and somatic embryogenesis. Direct development of shoots from cultured inflorescence segments occurred on MS medium supplemented with 2,4-D in combination with zeatin. Inflorescences with well developed spikelets differentiated at a low frequency (< 5%) from callus cultures initiated on media supplemented with 2,4-D in combination with zeatin or coconut water or picloram + kinetin. Somatic embryogenesis was also induced in callus cultures growing on MS + picloram + kinetin at the end of four passages. Supplementation of the media with different concentrations of sucrose showed 3% sucrose as the best concentration for plant differentiation from somatic embryos. The majority of the regenerated plants showed the diploid chromosome constitution in their root tips. The regenerants were in general shorter with an increased number of tillers compared to the control.

Abbreviations: CW – Coconut water; 2,4-D – 2,4-dichloro phenoxyacetic acid; Kn – Kinetin; Z – Zeatin.

Introduction

Immature inflorescences have been recognized as an important source of totipotent cultures in many cereals, millets and grasses (Vasil, 1982). The main routes of plant differentiation from cultured inflorescences in Gramineae have been reported to be through direct shoot differentiation by transformation of floret primordia (Heinz & Mee, 1969; Dale et al., 1981; Dale & Dalton, 1983), organogenesis (Dudits et al., 1975) and somatic embryogenesis (Brettell et al., 1980).

Finger millet (*Eleusine coracana* Gaertn) is an important millet of the tropics and is generally grown in areas where rainfall is scanty. It is desirable to have tissue cultures capable of high frequency plant differentiation so as to use the system for genetic improvement of the species. The previous reports on *E. coracana* dealt with organogenesis from mesocotyl callus (Rangan, 1976) and multi-shoot differentiation from apical domes (Wakizuka & Yamaguchi, 1987). The present communication deals with high frequency plant regeneration through somatic embryogenesis and direct shoot development from cultured immature inflorescence segments of *E. coracana.*

Material and methods

Field-grown plants of Finger millet *(Eleusine coracana)* were used as the source material. Immature inflorescences (10–60 mm in length) along with the leaf sheaths collected from the plants were surface-sterilized by dipping in 70% alcohol (V/V) for two minutes, the inflorescences were taken out of the leaf sheath, cut into 2–3 mm segments and cultured on Murashige and Skoog's basal medium (Murashige & Skoog, 1962) supplemented with different phytohormones. The pH of the medium was adjusted to 5.8 prior to autoclaving the medium and was solidified with 0.7% agar (Bipinchandra & Co., Bombay). The cultures were incubated under continuous illumination of 950 lux at 25 ± 2° C.

The media used for initiation of callus were: (1) MS + 2,4-D (2 mgl^{-1}) + Z (0.1 mgl^{-1}), (2) MS + 2,4-D (5 mgl^{-1}) + Z (0.1 mgl^{-1}), (3) MS + 2,4-D (2.5 mgl^{-1}) + CW (5%) and (4) MS + picloram (2 mgl^{-1}) + Kn (0.1 mg^{-1}). The cultures which showed direct induction of shoots were first transferred to MS + Z (1 mgl^{-1}) + picloram (0.1 mgl^{-1}) and later to MS medium devoid of growth supplements. For induction of somatic embryogenesis, callus tissues initiated on MS + picloram (2 mgl^{-1}) + Kn (0.1 mgl^{-1}) were subcultured on the same medium for three more passages. The embryogenic cultures which developed on this medium were transferred to MS medium devoid of growth regulators for development of somatic embryos into plantlets.

The effect of different concentrations of sucrose on germination of somatic embryos was studied by incorporating it into the basal medium at 3, 6, 9 and 12%. The cultures were scored at the end of 45 d and all experiments were repeated at least twice.

For histological studies, inflorescence segments after different period of culture were fixed in formalin-acetic alcohol (FAA), processed in an alcohol xylol series and embedded in paraffin. Sections were cut at 12 μm and stained with safranine.

For cytological analysis, root tips of 23 plants obtained by direct shoot differentiation and 150 plants obtained through somatic embryogenesis were pretreated with a saturated solution of α-bromonaphthalene for 2 hrs, fixed in acetic acid-alcohol (1: 3) for a minimum period of 24 hrs, hydrolysed with IN HCl and squashed in a drop of acetocarmine.

The regenerated plants with well developed roots were transferred to the field and reared to maturity. The height and number of tillers of 20 plants each obtained through direct shoot differentiation and somatic embryogenesis were studied.

Results

Direct shoot and inflorescence differentiation

When inflorescence segments were cultured on MS medium supplemented with 2,4-D (2,5 mgl^{-1}) + Z (0.1 mgl^{-1}), the pedicels of florets elongated and shoot buds were seen to originate from the region of florets at the end of 2 weeks of culture. These shoot regenerants remained in clusters of 10–15 and developed further at the end of 3 weeks (Fig. 1). Histological examination of the cultured inflorescence segments showed that the shoot buds arose from the region of florets directly without any intervening stage (Fig. 2). The development of direct shoot buds was dependent on the length of the inflorescence used for culture. Direct shoot development occurred when the inflorescence length ranged from 10–40 mm, while older inflorescences (> 40 mm) showed no induction of shoot buds. When clusters of shoot buds were transferred to MS medium + Z (1 mgl^{-1}) + picloram (0.1 mg^{-1}), the shoots developed further and on MS medium devoid of phytohormones, plants with well developed roots were obtained.

An average of 25–30 plants were obtained by direct shoot development from inflorescence segments of 2–3 mm length. Along with the differentiation of shoot buds, initiation of callus was also seen from the rachis. Callus cultures initiated on the four different media gave rise to inflorescences at a low frequency (< 5%) (Fig. 3). These *in vitro* induced inflorescences obtained directly from callus cultures had well developed spikelets and some of them even set seeds in the test tube. Plants obtained through direct shoot development also flowered and set seeds *in vitro* (Fig. 4).

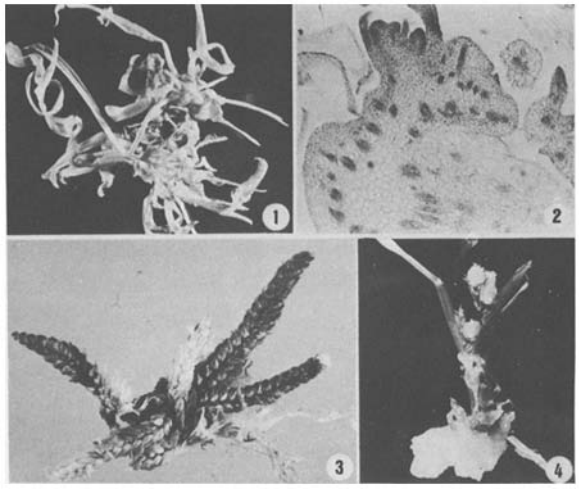

Fig. 1. Differentiation of clusters of shoot buds from cultured inflorescences of *E. coracana; Fig. 2.* Differentiation of a shoot bud from the region of floret primordium; *Fig. 3.* Inflorescences differentiated directly from callus culture; *Fig. 4.* A regenerant with well developed seeds *in vitro*.

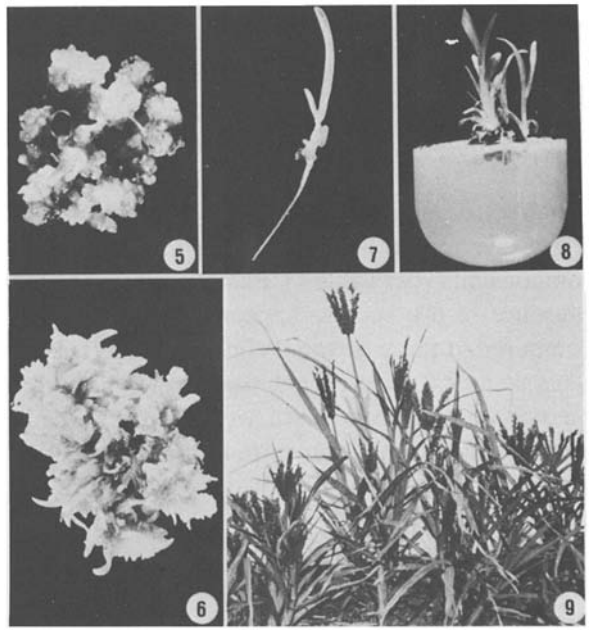

Fig. 5. Embryogenic tissue showing development of numerous somatic embryos on MS + picloram ($2\,mg\,l^{-1}$) + Kn ($0.1\,mg\,l^{-1}$); *Fig. 6.* Clusters of well developed somatic embryos developing into plants; *Fig. 7.* A germinated somatic embryo with shoot and root meristems at opposite pole; *Fig. 8.* A group of germinated somatic embryos on MS basal medium; *Fig. 9.* Regenerants growing in the field at the seed setting stage.

Plant differentiation through somatic embryogenesis

Initiation of callus and subsequent somatic embryogenesis were observed only when inflorescences of 10–40 mm were used for culture. Callus cultures initiated on picloram ($2\,mgl^{-1}$) + Kn ($0.1\,mgl^{-1}$) were creamish white in colour and somatic embryos were not observed during the first two passages. However, at the end of third passage, three distinct types of calli were observed: (1) extremely white friable calli with root like structures inside and composed of small, isodiametric, protoplasmic rich cells, (2) friable pale yellow to brown callus which did not show any sign of organogenesis, (3) nodular white callus which developed into embryogenic cultures in the subsequent passage. Only 20% of the cultures showed the presence of nodular embryogenic callus. On further selection and subculture, the embryogenic tissue grew rapidly and over a period of 18 months of study, produced only embryogenic cultures. Large number of somatic embryos were seen to differentiate from embryogenic tissues (Fig. 5). Some of the embryos were creamish white while others were dark green in colour. The somatic embryos were seen in aggregates and separated out only on germination on MS medium devoid of growth regulators. An average of 180 germinating somatic embryos were obtained from 50 mg of tissue in the first passage. There was a sharp increase in the number of somatic embryos produced at the end of one year and an average of 450 germinating somatic embryos were obtained from 50 mg of tissue. On MS medium, the somatic embryos grew vigorously (Fig. 6). Each embryo while germinating was an independent structure with shoot-root meristems at opposite poles and closely resembled germinating zygotic seedlings (Figs. 7, 8). Albino plants were not observed even after one year of culture. Over 25,000 plants differentiated from the cultures initiated from a dozen immature inflorescences. About 5000 plants were transplanted to the soil and

reared to maturity (Fig. 9). The survival rate of the regenerants was more than 95%.

Effect of different sucrose concentrations

When tested at different concentrations, sucrose at 3% produced the highest number of germinating somatic embryos (Table 1). Plants produced in the presence of 6% sucrose appeared more vigorous compared to those on other concentrations tested. Sucrose at 9% reduced plantlet development frequency and they appeared weaker. No somatic embryogenesis and plant differentiation was observed on medium devoid of sucrose and at 12% sucrose.

Cytological analysis

Cytological analysis of regenerated plants showed that while all the 150 plants obtained through somatic embryogenesis were normal diploids ($2n = 4x = 36$), one out of 23 plants obtained through direct shoot differentiation was an aneuploid ($2n = 54$) and the rest were diploid. Regenerants of *Panicum maximum* (Hanna et al., 1984) and *Pennisetum americanum* (Swedlund & Vasil, 1985) obtained through somatic embryogenesis were also found to have the diploid chromosome constitution.

Preliminary analysis of the regenerated plants obtained through direct shoot regeneration and somatic embryogenesis showed that they had reduced height and increased number of tillers compared to control plants. Detailed agronomic evaluation of these regenerated plants is being carried out and will be published elsewhere.

Table 1. Effect of different sucrose concentrations on somatic embryogenesis and plant development in *Eleusine coracana* cultured on MS medium*

Sucrose %	Average number of plants/50 mg of tissue ± S.E.	Nature of plant development
Nil	Nil	−
3	183.2 ± 31.3	++
6	98.2 ± 7.9	+++
9	51.3 ± 6.4	+
12	Nil	−

− No plant growth.
\+ Plantlets very weak.
++ Plantlets normal.
+++ Plantlets very vigorous.
* Average of 12 replicates.

Discussion

In the present study using *E. coracana,* plant regeneration from cultured immature inflorescences was obtained through two routes; (1) direct shoot development from regions of floret primordia, (2) somatic embryogenesis. Direct development of shoots from floral primordia has been reported from cultured inflorescence of *Lolium, Festuca, Phleum* and *Dactylis* (Dale & Dalton 1983, Dale et al., 1981).

In nature, inflorescences of many grasses are known to become transformed into shoots instead of producing seeds (Aber, 1934). This phenomenon, called as vivipary, is a mode of reproduction in these plants. Infection with pathogenic organisms, which disturb the hormonal balance can also transform inflorescences into shoot buds (Semeniuk & Mankin, 1964). In the present study, the transformation of floral primordia into vegetative shoots may be triggered by phytohormones such as 2,4-D and zeatin in the medium. Multiple shoot production from cultured inflorescences has been suggested as a means of clonal propagation (Lo et al., 1980) and may find use in propagation of *E. coracana* genotypes.

Another interesting aspect of the present study was the induction of high frequency somatic embryogenesis from inflorescence callus cultures. Somatic embryogenesis has been reported from inflorescence cultures of many cereals and millets such as *Triticum aestivum* (Ozias-Akins & Vasil, 1982), *Oryza sativa* (Chen et al., 1985), *Sorghum bicolor* (Brettell et al., 1980) *S. arundinaceum* (Boyes & Vasil, 1984), *S. almum* (George & Eapen, 1988), *Hordeum vulgare* (Thomas & Scott,

1985), *Paspalum species* (Bovo & Mroginski, 1986) and *Panicum miliare* (Rangan & Vasil, 1983). However, in an earlier investigation on *E. coracana* (Rangan, 1976) only shoot morphogenesis was reported from mesocotyl callus culture. Multiple bud production was also reported from the apical dome (Wakizuka & Yamaguchi, 1987) in this species. In our observation, embryogenesis in *E. coracana* was unique in the fact that there was an increase in the number of germinating somatic embryos produced with subculture, while normally in tissue cultures of cereals, the embryogenic potential decreases with age (Lorz et al., 1988).

The present observation that the age of the inflorescence is critical for the production of shoot buds and somatic embryos in *E. coracana* is in conformity with the report in *S. bicolor* (Brettell et al., 1980).

The callus cultures initiated from inflorescence segments of finger millet directly gave rise to whole inflorescences which later set seeds *in vitro*. Although the induction of pistil like structures has been reported in callus cultures of wheat (Hunsinger & Schauz, 1987), in our knowledge, this is the first report in cereals where whole inflorescences with well developed spikelets were initiated directly from callus established from immature inflorescences. The capability of callus to produce inflorescences directly deserves further studies.

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