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Influence of Genotype and Phytohormones on Somatic Embryogenesis and Plant Regeneration in Finger Millet

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Mature seeds of three finger millet (*Eleusine coracana*) genotypes were cultured on media containing picloram, 2, 4-D or dicamba (1-10 mg l^{-1}) either alone or in combination with Kn, BA or 2, i-P (0.5 mg l^{-1}). Small green, nodular structures developed from the seedling shoot apices 4-6 weeks from culture. On transfer to MS medium supplemented with picloram (2 mg l^{-1}) and Kn (0.1 mg l^{-1}), the nodular masses produced a highly embryogenic tissue which was maintained on the same medium, without loss of the embryogenic potential. Plantlets were obtained by transferring the embryogenic callus to MS medium devoid of phytohormones. The genotypes differed in their ability to produce callus from shoot apices and plantlets from somatic embryos. Among the auxins tested for callus initiation, 2, 4-D was the most effective either alone or in combination with Kn, and among the cytokinins, Kn was more effective than BA or 2, i-P in combination with 2, 4-D or picloram. The usefulness of this system for improvement of fingermillet is discussed.

Key Words: ***Eleusine coracana*, Finger millet, Somatic embryogenesis, Plant regeneration, Phytohormones**

Introduction

The development of cell and tissue culture techniques is an essential prerequisite for the *in vitro* genetic manipulation of crop plants and this has been attempted for most of the major cereals. Finger millet (*Eleusine coracana* Gaertn) is a major crop in the arid and semi-arid regions of developing countries, and *in vitro* techniques are yet to be standardised for the genetic improvement of this species. Regeneration of plants through organogenesis (Rangan 1976), multiple shoot production (Wakizuka & Yamaguchi 1987), and somatic embryogenesis (Eapen & George 1989, Sivadas et al. 1990) has been reported earlier. Because precise information on *in vitro* response of different genotypes and effects of phytohormones on organogenesis and somatic embryogenesis in finger millet is unavailable, the present communication fills up this lacuna.

Materials and Methods

Plant Materials

Three cultivars of finger millet Co-9 (white seeded), Co-12 and Co-13 (brown seeded, blast tolerant, supplied by Tamil Nadu Agricultural University, Coimbatore, India) were used in this study.

Callus Initiation

After prescribed methods of surface-sterilization (Eapen & George 1989) the seeds were cultured on Murashige & Skoog (1962) basal medium (BM). BM was supplemented with picloram (4, amino-3, 5,

6-trichloro-picolinic acid), 2, 4-D (2, 4-dichlorophenoxy acetic acid) or dicamba (3, 6-dichloro-O-anisic acid) (1, 2, 4, 6, 8 and 10 mg l^{-1}) in factorial combination with Kn (Kinetin), BA (6-benzyl amino purine) or 2, i-P (6-γ, γ, dimethyl allylaminopurine) 0.5 mg l^{-1}. The cultures were incubated under continuous illumination (900 Lux) at 25 ± 2°C. For somatic embryogenesis, callus cultures that developed from the shoot apices were transferred to MS + picloram (2 mg l^{-1}) + Kn (0.1 mg l^{-1}), and were maintained on this medium through monthly subcultures.

Germination of Somatic Embryos

About 500 mg of embryogenic tissue was transferred to the BM lacking phytohormones for the germination of the somatic embryos. The number of plantlets formed 6 weeks from transfer was recorded.

Results

The three cultivars showed 100% seed germination on MS + picloram, 2, 4-D or dicamba either alone or in combination with Kn, BA or 2, i-P in 4-5 days from culture. Swelling of the mesocotyl and leaf bases followed by slight callusing was observed within 1 week. The callus which developed from mesocotyl and the leaf base was white and transluscent, but turned brown and necrosed eventually. However, when the cultures were maintained on the same medium for 4-6 weeks, dark-green nodular calli developed from the seedling shoot apices.

The effects of phytohormones on callus initiation was studied in cv. Co-12.

At low concentrations picloram was effective for callus induction; at high levels its effect diminished. Either alone or in combination with Kn, 2, 4-D was more effective in inducing callus from seedling shoot apices (figure 1). Along with BA or 2, i-P, 2, 4-D was less effective except in the combination BA + 2, 4-D each at 1 mg l^{-1}. At low levels dicamba was ineffective, whereas at 8 mg l^{-1} it elicited callus growth in 43% of the cultures. In combination with Kn, all the concentrations of dicamba tested induced callus although the frequency was higher at lower concentrations.

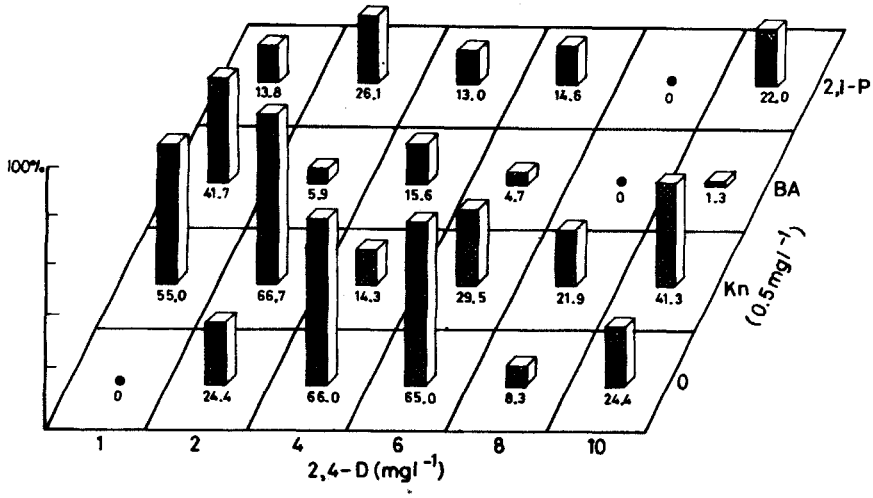

Figure 1 Effects of 2, 4-D and cytokinins on callus induction from seeds of *Eleusine coracana* cv Co-12. The number at the bottom of each bar denotes % callus induction

Differences in Genotype Responses

Table 1 gives the frequency of callus initiation in cultures of shoot apices, mesocotyl and leaf base of the 3 cultivars on MS + picloram (4 mg l^{-1}) + Kn (0.5 mg l^{-1}). The growth of the calli from shoot apices was rather slow on the callus initiation medium. On transfer to medium supplemented with picloram (2 mg l^{-1}) and Kn (0.1 mg l^{-1}), the calli grew rapidly and became embryogenic. The highly embryogenic callus tissue was maintained in this medium through subcultures every month.

Table 1 *Callus initiation in cultures of shoot apices, mesocotyl and leaf base on MS + picloram (4 mg l^{-1}) + Kn (0.5 mg l^{-1}) and plantlet regeneration in seed-derived callus on BM of Eleusine coracana*

Cultivar	Percent cultures which showed callus formation			Mean No. of plantlets/ culture* ±S E
	Shoot apices	Meso cotyl	Leaf base	
Co-9	27.6	Zero	Zero	597.9±46.5**
Co-12	71.6	9.8	14.8	895.8±49.5
Co-13	26.3	19.7	23.0	615.6±67.2

** t values not significantly different at p=0.05
S E = standard error; * Mean of 24 cultures

Upon transfer to MS devoid of phytohormones the embryogenic cultures of the 3 cultivars readily produced plantlets resembling seed-derived plants with well-developed roots. There were significant differences in the plantlet regeneration potential in two of the three cultivars (table 1).

Discussion

Of the 3 cultivars studied, cv. Co-12 was the most responsive for callus initiation, somatic embryogenesis and plantlet regeneration from somatic embryos, and cv Co-9 was the least responsive. The genotype of the experimental material is known to influence *in vitro* morphogenesis in other cereals also (Bhaskaran & Smith 1990).

That for callus induction, 2, 4-D is superior to dicamba and that picloram is also effective have been reported earlier for finger millet (Sivadas et al. 1990, Eapen & George 1989). Of the 3 cytokinins tested for induction of callus and somatic embryogenesis, Kn used in combination with 2, 4-D or picloram was superior to BA and 2, i-P. The promotive effect of Kn on somatic embryogenesis is comparable to that already reported for finger millet (Wakizuka & Yamaguchi 1987, Rangan 1976, Sivadas et al. 1990).

The green nodular callus obtained from the cultured seeds of finger millet originated from the shoot apices as in *Poa pratensis* (Vander Valk et al. 1989). Contrary to the earlier report (Rangan 1976), the callus developed from the mesocotyl was non-morphogenic and necrosed on subculture.

In contrast to several other cereal tissue cultures in which organogenic and embryogenic potential declined during prolonged subcultures (Nabors et al. 1983), in finger millet, tissue cultures older than 2 years exhibited embryogenic potential comparable to that of the fresh cultures. The availability of such a culture system could be exploited for improvement of finger millet.

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