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Gezira j. agric. sci. 1 (2): 128-134 (2003)

SHORT NOTE

Embryoid induction and plantlet regeneration from leaf segments of sugarcane (Saccharum officinarum L.)

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Sugarcane (Saccharum officinarum L.) is an economically important crop in Sudan for domestic consumption and export. It is the first and essential source for production of high purity sugar. Tissue culture techniques can be used for in vitro conservation of sugarcane (Taylor and Dukic, 1993) and mass propagation of elite cultivars of crop species. Moreover, it was used for production of pathogen-free planting material from infected mother plants. Variability induced in vitro can furnish a base for improvement of vegetatively propagated crops including sugarcane. Different tissue culture techniques were applied successfully to sugarcane propagation and plant regeneration through organogenesis of shoot meristem (Nadar and Heinz, 1977; Ho and Vasil, 1983a), cell suspension cultures (Ho and Vasil, 1983b, Aftab et al., 1996) and protoplast cultures (Liu, 1994). Cell suspen - Sion cultures were also used for cytological, pathological (Peros and Lombard, 1992), biochemical and physiological investigations of sugarcane (Heinz et al., 1977).

This study was initiated, during 1998-2000, to investigate the effect of 2,4-D on induction of embryogenic callus from leaf explants of sugarcane and regeneration of somatic embryos on different concen-trations of Murashige and Skoog (1962) medium (MS).

Leaf explants were obtained from shoot tips of sugarcane plants of the cultivar Co-6806 grown in the nursery of the Tissue Culture Laboratory, Agricultural Research Corporation, Wad Medani, Sudan. The cultivar is commercially cultivated in the fields of Kenana Sugar Company, Sudan. All the outer mature leaves were removed and shoot tip explants were sterilized by dipping in 75% ethanol for) few seconds, followed by 50% v/v Chlorox (commercial bleach) with few

drops of Tween 20, as a wetting agent, for 30 minutes on a shaker. plant material was then washed with three changes of sterilized distilled water. The third and fourth innermost leaves from the sterilized shoot tips were used in this study and they were sequentially cut into 5-mm long segments. The leaf segments were then placed each in a test tube (25 x 150 mm) containing approximately 1205 mls of a nutrient medium. The basal medium used was MS with 20 g/l sucrose as a carbon source. The medium was jelled with 0.8% agar and the pH was adjusted to 5.8 ± 0.1 before autoclaving.

Induction of embryogenic callus from leaf explants was tested on MS medium supplemented with different concentrations of 2,4-D (0, 1, 2, 4 and 6 mg/l) with 10 replications per treatment in a completely randomized desiB1 (CRD). The number of explants with callus, callus size and colour were recorded every two weeks.

Regeneration of somatic embryos from callus was tested on different dilutions of MS medium (Full MS, h MS and 1/4 MS) without addition of growth regulators in CRD with 20 replications. Data on the number of explants with shoot and root morphogenesis, number of shoots and roots per explant were recorded weekly. Growth of embryogenic callus was tested on MS medium with and without activated charcoal at 3 g/l in CRD with 20 replications.

Callus tissue became visible at the cut ends of almost all leaf explants cultured on MS medium supplemented with 1 to 6 mg/12,4-D after 4 to 5 weeks, while no callus was formed on 2,4-D free medium. Two types of callus tissues were obtained: a) Compact and smooth looking callus which was white in colour and embryogenic in nature and b) Mucilaginous, shiny, non-embryogenic callus. Embryogenic callus was induced on MS medium supplemented with 2,4-D from 1 to 4 mg/l. The highest 2,4-D concentration (6 mg/l) induced non-embryogenic callus. The best growth of the embryogenic callus was induced on 4 mg/1 2,4-D.

Embryogenic callus induced on 4 mg/1 2,4-D was transferred to different dilutions of MS basal medium (Full MS, l, : BIS and 1/4 MS) without any growth substances to promote embryoid development and to induce plantlet regeneration, Significantly higher percentage of embryoids were induced from embryogenic callus cultured on full MS medium compared to quarter and half MS medium after four weeks (Table 1).

Table 1. Morphogenesis of embryogenic callus on different strengths of Murashige and Skoog basal medium (MS) after 4 weeks.

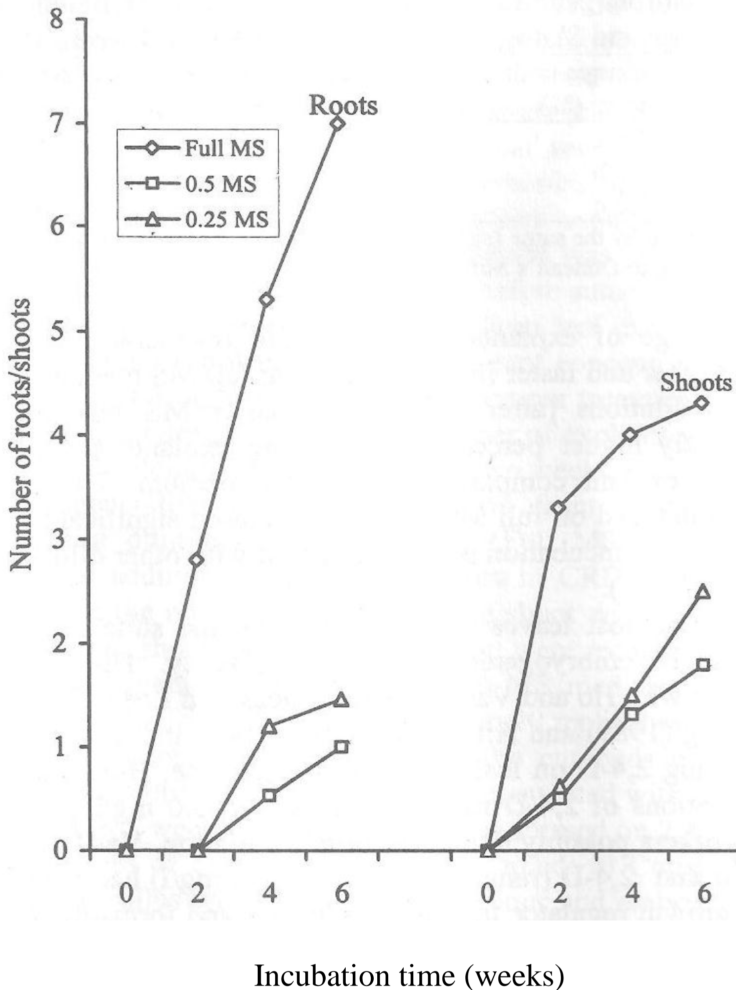
MS strength	Cultures with shoots (%)	Cultures with roots(%)	No. of shoots per callus	No. of roots per plantlet
Full MS	87.5 a	83.3 a	4.3 a	6.6 a
1/2 MS	45.8 b	16.7 c	1.7 c	0.9 b
1/4 MS	29.2 c	37.5 b	2.5 b	1.4 b

Means followed by the same letter(s) are not significantly different at 5% probability level according to Duncan's Multiple Range Test.

Percentage of explants with embryoid regeneration was significantly higher and faster (after 2 weeks) on full MS medium compared to other dilutions (after 4 weeks). Quarter MS medium showed significantly higher percentage of rooting explants and number of roots per explant compared to half MS medium. The number of embryos induced on full MS medium increased significantly with the increase in the incubation period compared with other dilutions of MS medium (Fig. 1).

The innermost leaves were found to be the suitable explant for induction of embryogenic callus in this study. These results are consistant with Ho and Vasil (1983b), Sheng and Lee (1987), Grisham and Bourg (1989) and Aftab et al. (1996), who induced embryogenic callus using 2,4-D on leaf explants of sugarcane. However, different concentrations of 2,4-D ranging from 0.5 to 5.0 mg/I were used by these workers possibly due to genotypic variation. Vasil et al. (1982) reported that 2,4-D (ranging from 0.5 to 3.0 mg/l) had been the most potent growth regulator for callus induction and formation of somatic embryos in cell and tissues of all gasses including sugarcane. Ho and Vasil (1983b) reported that the frequency and vigour of embryogenic callus formation depended on leaf age. They found that the leaf sheath and the midrib were more suitable for production of embryogenic callus than the leaf blade and that the most suitable was the fourth or fifth leaf-

Somatic embryos were regenerated on MS medium without any plant growth regulators. These results were consistent with Peros et al. (1990) and Aftab et al. (1996), who were able to regenerate plantlets from sugarcane callus cultured on MS medium without 2,4-D. Contrary to these results, Ho and Vasil (1983b), Sheng and Lee (1987) and Grisham and Bourg (1989) reported that 1/2MS medium was



Incubation time (weeks)

Fig. 1. Effect of MS medium concentrations and incubation time on number of roots and shoots regenerated from embryogenic callus of sugarcane cultivar Co6806.

better for plantlet establishment. The 1/2MS medium was supplemented with 6% sucrose, but only 3% sucrose was used in this study. This high carbon source might have a positive effect on the regeneration of plantlets. Murashige and Skoog's medium was consistantly better in the frequency of plant regeneration and all morphogenetic parameters as compared to MS medium dilutions. However, 1/4MS

medium was better in percentage of rooting and number of shoots per explant compared to 1/2MS medium. Although the number of regenerated shoots per explant was significantly lower on 1/2MS than on 1/4MS medium, the total number of shoots (percentage x number of shoots per explant) was comparable on both treatrnents. These results indicated that all plants regenerated on full MS medium were somatic embryos (with shoot and root), while on MS medium dilutions a few somatic embryos were induced first and the rest were adventitious shoots which started to develop their root system two weeks later. The low nutrient concentration and the lack of auxin in the dilutions of MS medium might be another reason for the inconsistency of rooting. Taylor and Dukic (1990) found that NAA improved the rate of root formation on somatic embryos of sugar-cane. In conclusion embryogenic callus can be induced on the leaf explants of the sugarcane cultivar C06808 on 4 mg/1 2,4-D and somatic embryos were regenerated on fill MS mediun without plant growth regulators.

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