

Hormonal effect on shoot multiplication in Sugarcane genotypes

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ABSTRACT

Studies were carried out for rapid micro propagation of five sugarcane genotypes i.e. HSF-242, SPF-213 HSF-240, CP-77-400 and CP-43-33. The explants were surface sterilized with 40% clorex for 45 minutes. Multiplication of the cultures was obtained by using BAP and Kinitin in various combinations and concentrations in MS medium. The optimum multiplication for genotype HSF-240 was obtained at 1.5 mg/l BAP, 0.5 mg/l Kin with 12.6 cm shoot, 11 tillers and 8 leaves per plant. Similarly optimum multiplication for variety CP-77-400 was obtained at 0.5 mg/l BAP and 1.0 mg/l Kinitin, with a maximum of 9 cm shoot length, 1 tiller and 16 leaves. The variety SPF-213 was obtained at 1.5 mg/l BAP and 0.1 mg/l Kin, with a maximum of 9.6 cm shoot length, 8 tillers and 11 leaves. Best multiplication rate for variety HSF-242 and CP-43-33 was observed at 1.5 mg/l BAP and 0.1 mg/l Kin with a maximum of 13 cm shoot, 8 tillers and 10 leaves per plant and 1.0 mg/l BAP and 0.1 mg/l Kin with a maximum of 10 cm shoot, 11 tillers and 12 leaves per plant. Rooting of the plantlets was obtained on half strength MS medium containing 6% sucrose and various concentrations of IBA. The experiment will be help for further selection and multiplication of desirable sugarcane varieties.

Keywords: Sugarcane, kinetin, Multiplication, BAP, IBA

1 INTRODUCTION

Sugarcane crop is a major raw material source for the production of white sugar, brown sugar and gur and is also a cash crop. Its share in value addition in agriculture and GDP is 3.6 and 0.8 percent, respectively. Sugarcane was cultivated on an area of 988 thousand hectares during the year 2010-11, 4.8 percent higher than last year's level of 943 thousand hectares production for the year 2010-11 is estimated at 55.3 million tons as against actual production of 49.3 million for the year 2009-10 (MINFA, 2011). Commercially, sugarcane is propagated from stem cuttings with each cutting or set having two or three buds. After the establishment of variety, major bottleneck in spreading of the variety is slow propagation rate through conventional method, which takes years (Cheema & Hussain, 2004). Modern commercial sugarcane varieties are obtained through breeding and a multi-stage selection scheme over a period of 10-15 years. Tissue culture techniques have been widely used for large-scale micro propagation and can effectively reduces the time period between selection and commercial release of new sugarcane varieties (Lorenzo et al., 2001; Taylor, 1997). Micro propagation is currently the only realistic means of achieving rapid, large-scale production of disease-free seed canes of newly

developed varieties in order to speed up the breeding and commercialization process in sugarcane (Feldmann et al., 1994; Lal & Krishna, 1994). In contrast to conventional method where one bud produces 4-5 shoots, tissue culture, if estimated conservatively can produce around 10,000 identical plants from a single bud in about 3-4 months (Lee, 1987). Micropropagation can also be used for obtaining disease free plants. Many scientists have described methods for micropropagation (Hendre et al., 1983, Gosal et al., 1998, Jadhav et al., 2001). Gosal et al., (1998) reported rapid multiplication in liquid MS medium on BAP (0.5 mg/l) and Kin (0.5 mg/l) and rooting on NAA (0.5 mg/l) and sucrose 70%. In the present study we was to developed protocol for fast and efficient micro propagation of five sugarcane genotypes viz., HSF-242, SPF-213, HSF-240, CP-77-400 and CP-43-33 by using BAP and Kinetin in various concentrations and combinations, which will help to cope with the increasing demand for sugarcane production on large scale to meet the future challenges for sugar production.

2 MATERIALS AND METHODS

2.1 Plant material

The plant materials were collected form glasshouse of faculty of agriculture Rawalakot and all the experimental work was carried out at Plant tissue culture lab Department of Plant Breeding and Molecular Genetics. Five sugarcane varieties HSF-242, HSF-240, CP-77-400, SPF-213 and CP-43-33 were used in this study. The explants materials were taken from six months old sugarcane plants.

2.2 Explants preparation and surface sterilization

Size of the shoot tip taken was 4-6 mm. For surface sterilization of explants 40% Clorox (commercial bleach containing 5.25% v/v Sodium hypochlorite) for 45 minutes was used but before clorex treatment the explants were first thoroughly washed with tap water for several times, then put in 60% ethanol for 30 minutes and finally in antioxidants solution for 45 minutes. The explants were washed with autoclaved distilled water thrice time for 5 minutes.

2.3 Hormonal concentrations

Solid MS (Murashige & Skoog's medium, 1962) supplemented with 0.1 mg/l GA3 and 1.0 mg/l Kin for initiation of cultures, various concentrations and combinations of BAP and Kin in liquid MS medium for the multiplication of cultures and with 0.5 mg/l-1.5 mg/l of IBA with 6% sucrose for the rooting of cultures. Data for shoot initiation were recorded 30 days after culturing, for shoot multiplication 30 days and 15 days for rooting after culturing. Data were analyzed using a computer package MSTAT.

3 RESULTS AND DISCUSSION

Multiplication of shoots was observed at different concentrations of BAP in combination with Kin. The varieties indicated variable towards the different concentrations used, however BAP (0.5, 1.5-1.0 mg/l) with Kin (1.0, 0.1-0.5 mg/l) were found best for multiple shoots formation. At maximum of 13 cm long shoot produced 11 tillers and 16 leaves per plant on different combinations in 30 days.

The average shoot length found from varieties HSF-242, HSF-240, SPF-213, CP-43-33 and CP-77-40 were highly significantly different. The shoot length ranged from 9 cm for variety CP-77-400 to 12.6 cm for variety HSF-242 (Fig-3). The impact of different hormones combinations on average shoot length was also highly significantly different.

The impact of tillers between genotypes showed less difference. However the number of tillers ranged from 8 in variety HSF-240 and SPF-213 to 12 in CP-77-400 (Fig-2). The different hormones combinations showed highly significant impact on average number of tillers. The highest value of tillers was observed at T3 (BAP-1.5, Kin.-0.5) while the lowest one was for that of control T2 (Fig: 2). Rapid shoot multiplication was achieved in liquid MS medium supplemented with cytokinins, Kin (0.5 mg/l) and BAP (0.5 mg/l) with 20 shoots per plant (Gosal et al., 1998). The interaction effect between varieties and different hormones combinations was non-significantly different on tillers number (Fig-2). The average number of leaves for different varieties (HSF-242, HSF-240, CP-77-400, CP-43-33 and SPF-213) were highly significantly different. The means for number of leaves

ranged from 10 for variety HSF-242 to 16 for that of variety CP-77-400 (Fig: 1). Chattha et al., (2001) reported that multiple shoots formation was excellent at 1.5 mg/l BAP in combination with 1.5 mg/l GA3 and recorded a maximum of 4 tillers and 16 leaves per plant. The impact of different hormones combinations was also highly significant against leaves number. However the leaves number ranged from 10 as the lowest value for T2 to 16 as the highest value for T4 (Fig: 1). Patel et al., (2001) recorded highest multiplication on 1.5 mg/l Kin. The combinations T1, T3 and T4 were highly significantly different while the combinations T2, T3 and T5 were non-significantly different (Fig: 1).

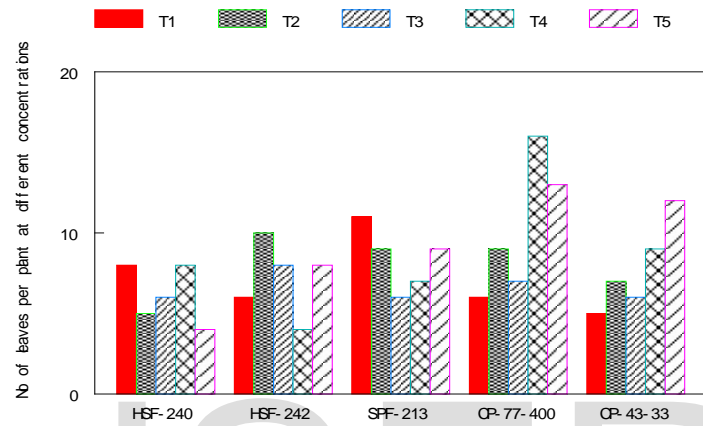


Fig: 1 comparison between no of leaves at five hormonal treatment

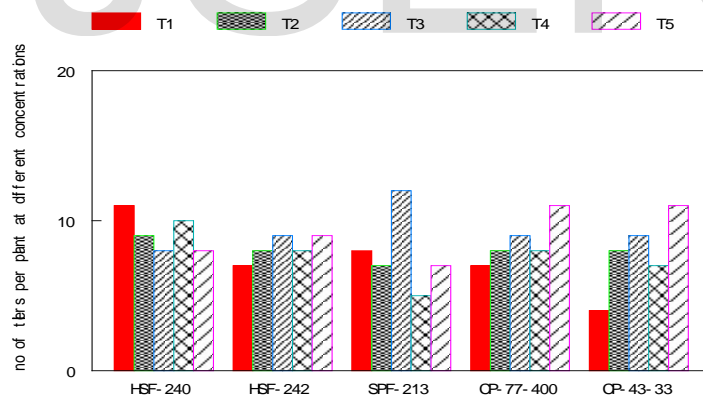


Fig: 2 comparison between no of tillers at five hormonal treatments

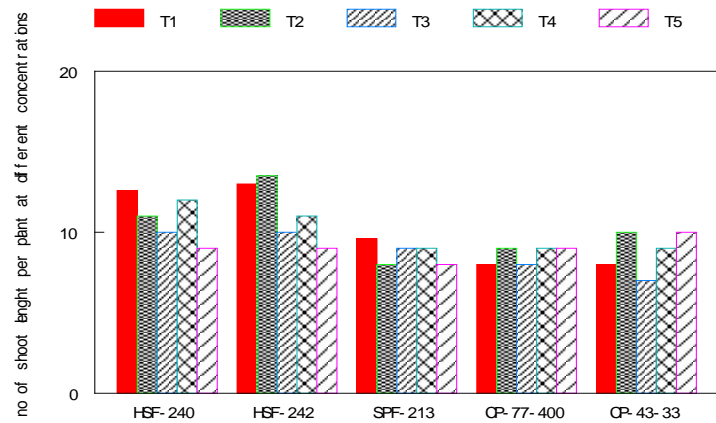


Fig: 3 comparison between shoot length at five hormonal concentrations

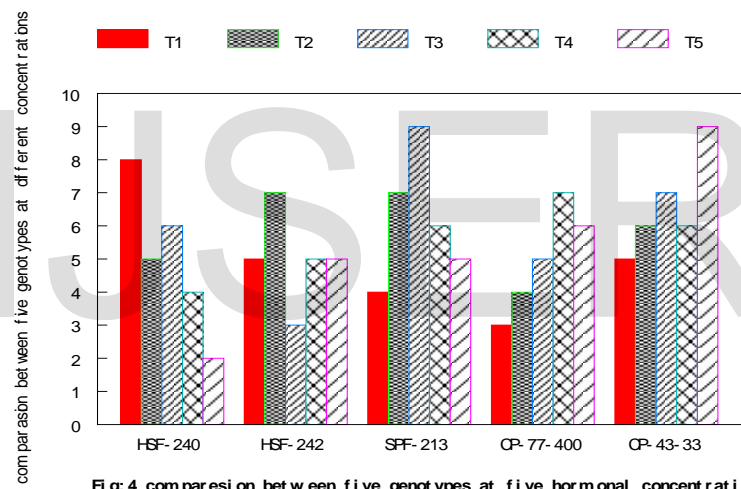


Fig: 4 comparison between five genotypes at five hormonal concentrations



HSF-242



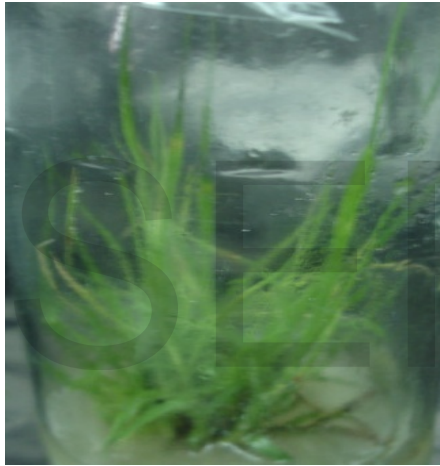
HSF-240



CP-77-400



SPF-213



CP-43-33

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