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Development of Sugarcane Mutants Through *in vitro* Mutagenesis

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Abstract: The present study was carried out to develop mutants in sugarcane using *in vitro* mutagenesis technology. The calli were exposed to four radiation doses. The plants did not develop from calli exposed to radiation dose of 6.0 Kr, whereas plants developed well with other radiation doses. Among the other doses, 0.5 Kr showed good effect on some agronomic characters i.e. plant height, number of tillers/plant, cane thickness and number of green leaves/plant. These characters are directly linked with yield and sugar contents of cane. it will be worth to study the stability of improved characters in developed mutant plants in the succeeding generations.

Key words: Sugarcane, mutants, mutagenesis

Introduction

Sugarcane is one of the important cash crop of the area. A sugar-mill has been installed in the area. It is expected that sugarcane will be cultivated extensively in this area. However, none of the cultivated variety is promising enough to give good yield. As a result average yield in the area is about 40 metric t ha^{-1} which is much lower than the average yield of any area of the country. There is a great need to start breeding of high yielding cultivars of sugarcane. There is much evidence to prove the usefulness of *in vitro* culture combined with induced mutation for bringing about the desirable characters of high yield in the crop plants (Novak *et al*., 1990; Novak, 1991; Klu, 1993). *In vitro* culture technique offer unique opportunity for the creation of genetic variability and rapid isolation of clones with desired characters. This technique has been employed in sugarcane in other parts of the country. The regenerated plants showed variability in quantitative and qualitative characteristics. The potential usefulness of this technique for plant improvement became apparent in sugarcane with the work of Nickell (1977) and Shahid *et al*. (1994).

Mutation of plant cell culture is carried out through use of either physical or chemical mutagen. Physical mutagen has advantage over chemical as it needs no washing/manipulation to remove the mutagen. After mutagenesis it is important to incubate cells/callus in the dark so as to reduce photo-inducible DNA repair. Mutagen cause a modification in the DNA in one strand. It is essential that the DNA be replicated before selection for a phenotype so that at least both DNA strands carry the mutation. In case the modified strand do not replicate, the mutation may not be transcribed and expressed (Flick, 1983). The use of *in vitro* culture combined with induced mutation can speed up breeding program in vegetatively propagated crops like sugarcane. This is very effective method for plant improvement (Novak *et al*., 1990; Novak, 1991; Sonnino *et al*., 1986; Ahloowalia, 1990; Klu, 1993). The large size of propagule allow mutagenic treatment of large number of cells. With the *in vitro* culture of such propagules a relatively uniform and large populations of cells and tissues in a disease-free situation is obtained for radiation and it is possible to separate the desired mutated sectors from the others in a short time. Induction of mutations *in vitro* cultured material and subsequently *in vitro* multiplication for 2-3 cycles is also helpful in separating mutated sectors from chimeric tissue, particularly in plants propagated vegetatively. Irradiation in combination with *in vitro* culture has proved to be a valuable method of producing desired variation and rapid propagation (Maluszynski *et al*., 1995). Both somaclonal variation and mutations result in the production of new genotypes with a limited change in the original genome. As a source of variation, somaclonal variation mimics induced mutation (Brown, 1991; Brown *et al*., 1993). Keeping in view the present study was carried out.

Materials and Methods

The present study was carried out during 1995 to 1997 at National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad and in the experimental fields of Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University, Dera Ismail Khan.

Callus culture was initiated from young leaves of 6-12 months old field grown sugarcane var. CP-43/33 (a hybrid of COL-54). A modified MS medium (Murashige and Skoog, 1962) was used in the experiment. The medium was supplemented with B5 vitamins and ph was adjusted to 5.8. The supplementing of medium corresponds to the media as used especially for sugarcane (Shahid *et al*., 1990, 1994; Siddiqui *et al*., 1994). Young leaves were taken from the internal 10 mm whorl and were sliced into 3 mm pieces. Induced calli were maintained/proliferated on the same medium by serial transfer after 2-3 weeks. The cultures were maintained at 26 + 2°C under dark condition. Five weeks old yellow to white embryogenic calli (75) were subjected to four different doses of gamma rays (0.5 Kr., 2.0 Kr., 4.0 Kr. and 6.0 Kr.) (60 Co-source at Niab). Post Irradiated material was maintained/proliferated by subculturing after every 2-3 weeks. One month old post-radiated and non-irradiated calli were transferred to the following regeneration medium: MS basal supplemented with Fe-EDTA 1 mg l^{-1}, Sucrose 60 g l^{-1}, Casein hydrolyzate 500 mg l^{-1}, L-Cystine free base 30 mg l^{-1}, which is often used for sugarcane (Shahid *et al*., 1990,1994). Four calli pieces (0.5 g) were placed in each bottle containing 50 ml of regeneration medium. Jars were placed in controlled temperature room set at 28-30C with 16 hours photoperiod. The regenerents were transplanted to sugarcane rooting medium. The regenerated plantlets were transferred to vermiculite under high humidity (>90%) by covering the plants with plastic envelops. Some of the regenerants were transferred to pots after hardening for growing under Local climatic conditions. The plants were transplanted to well prepared micro-plots, plant to plant distance was kept as 2 ft and row to row distance was kept as 2.5 ft. All the recommended cultural practices were applied and data was taken on 5 month old plants on some morphological

Table 1: Agronomic Characteristics of 5 months old sugarcane mutants

Characteristics	Genotypes			
	Control	I	II	III
Plant Height	47 cm	60 cm	38 cm	55cm
No. of Tillers/Plant	40-42	55-58	38	50-58
Cane Thickness (Diameter)	5 cm	5.2 cm	4.1 cm	3.9 cm
Cane Colour	Radish Green	Pinkish Green	Pinkish Red	Pinkish
Internode Shape	Conoidal	Conoidal	Conoidal	Conoidal
No. of Green Leaves/Plant	13-16	15-16	13	14
Leaf Breadth	3.9 cm	2.3 cm	1.4 cm	1.3 cm
Root Band	3.9 cm	2.2 cm	1.9 cm	1.3 cm

Control. Soma-clones I. Exposed to 0.5 Kr. II. Exposed to 2.0 Kr. III. Exposed to 4.0 Kr.

characteristics viz. plant height, number of tillers/plant, cane thickness (diameter), cane colour, internode shape, number of green leaves/plant, leaf breadth and root band (Table 1).

Results

The variety CP-43/33, one of the good variety of sugarcane was used in the experiment. The data is based on four to six plants per treatment. The calli exposed to 6Kr did not form plantlets, whereas plantlets regenerated well with other treatments i.e. 0.5 Kr, 2.0 Kr and 4.0 Kr. Plant height recorded in plants developed from call exposed to 0.5 Kr was maximum (60 cm) followed by plants developed from calli exposed to 4 Kr (55 cm), control (47 cm) and plants developed from call exposed to 2 Kr (38 cm). Number of tillers/plant was higher in plants developed from calli exposed to 0.5 Kr (55-58) followed by plants developed from call exposed to 4 Kr (5058), control (40-42) and plants developed form call exposed to 2 Kr (38). Cane thickness (diameter) was higher in plants exposed to 0.5 Kr (5.2 cm) followed by control (5.0 cm), plants developed from call exposed to 2 Kr (4.1 cm) and plants developed from call exposed to 4 Kr (3.9 cm). Cane colour was mostly pinkish in all the treatments except for control where it was observed as radish green.

The shape of internode was conoidal in all the treatments along with the control. Number of green leaves per plant were higher in plants developed from calli exposed to 0.5 Kr (15-16) followed by control (13-16), plants developed from calli exposed to 4 Kr (14) and plants developed from call exposed to 2 Kr (13). The breadth of leaves was higher in control (3.9 cm) followed by plants developed from calli exposed to 0.5 Kr (2.3 cm), plants developed from call exposed to 2 Kr (1.4 cm) and plants developed from calli exposed to 4 Kr (1.3 cm). Root band was much higher in control (3.9 cm) followed by plants developed from call exposed to 0.5 Kr (2.2 cm), plants developed from call exposed to 2 Kr (1.9 cm) and plants developed from call exposed to 4 Kr (1.3 cm) (Table 1).

Discussion

Sugarcane is one of the most complex and least characterized of the crop plants. It lends itself well to exploitation via cultured somatic variants that can be recovered as plants. This may be so because the genomic complexity provides an effective buffer against physiological imbalances caused by genetic changes (Maretzki, 1987).

Induced mutation through the use of mutagen has long been practiced in various crops and useful mutants have been selected to best suit the requirements in a particular area of work (Kanzak, 1984; Maluszynski, 1990; Micke, 1991; Micke *et al.*, 1990; Rutger, 1992). However, induced mutation in combination with *in vitro* culture technique is restricted to few crops (Novak *et al.*, 1990; Sonnino *et al.*, 1986; Ahloowalia, 1990). This technique enhances the chances of improvement in required set of characters which are otherwise not possible with either of induced mutation or *in vitro* culture when applied separately (Novak, 1991; Klu, 1993). The technique of mutation induction and *in vitro* culture seem to be ideally suited for the improvement of vegetatively propagated crops (Maluszynski *et al.*, 1995).

In the present study, exposure of calli to lower dose of radiation (0.5 Kr) was found useful as it caused increase in the height of plant, number of tillers/plant, cane thickness and number of green leaves/plant compared to control and other radiation doses (Table 1). These characters are directly concerned with yield and sugar content. Increase in these characters enhances the yield/acre and sugar content of the cane which is the ultimate goal in term of grower's interest. Mutants so developed have also advantage over plants developed from call exposed to radiation doses of 2 Kr and 4 Kr in leaf breadth and root band characters. However, they were no better than control in the later mentioned characters. Increase in the dose of radiation had a negative impact on there characters. The dose of 6.0 Kr is even very deleterious as plantlets did not develop from the callus exposed to it. Marked difference was also found in the colour of cane developed from call exposed to 0.5 kr. It will be interesting to note the maintenance of there characters till the maturity of plants. Similar kind of results have been found in other crops with exposure to lower dosage of radiation (Min *et al.*, 1989; Safo-Kantanka and Owusu-Nipa, 1992; Klu, 1993).

Recording of data is the first attempt on grown up plants. It will be worth while to follow up the research findings and study the stability or otherwise of improved characters in succeeding generations and find its impact on sugarcane breeding.

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