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PLANT REGENERATION FROM IRRADIATED EMBRYOGENIC CALLUS OF SUGARCANE

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Abstract

Three sugarcane clones viz., NIA-98, NIA-0819 and BL4 were used for induction of genetic variability through *In-vitro* mutagenesis. Apical meristametic region was used for callus induction on 4mg/l 2,4-D. Actively growing callus was treated with four different doses of gamma rays (10Gy, 20Gy, 30Gy and 40Gy). Maximum callus, proliferation and plantlets regeneration growth was observed in control and minimum at 40Gy. Maximum chlorophyll mutation frequency was recorded in 30 and 40 Gy. Maximum number of roots was observed in control followed by 10Gy. The maximum root length (10.3cm) was obtained in control at 2 mg/l IBA. The maximum number of tiller in the irradiated population was observed in 10Gy. The treatments 30Gy and 40Gy exhibited negative impact on the tillering potential of the plant.

Introduction

Saccharum sp., hybrid sugarcane, is one of the major crop of the Sindh province after wheat, rice and cotton. The genus *Saccharum* comprises of 6 species viz, *Saccharum officinarum, Saccharum spontaneum, Saccharum robustum, Saccharum edule, Saccharum, sinense* and *Saccharum barbari*. The commercial canes have been derived mostly form the germplasm of 20 noble *Saccharum officinarum* and 10 *Saccharum spontaneum* species (Arcenaux, 1967). The present sugar cane varieties under cultivation world over are inter species hybrids of three or more species (Tai *et al.,* 1995). In the world, Pakistan ranks 5th in cultivated area and 15th in cane yield (Wains, 2008). Thus there is a big gap between ranking in cultivated area and cane yield. An extensive breeding work and management practices are required to narrow down this big gap. Non sporadic flowering and viable fertile seed production has been a problem in Pakistan. Therefore, low fertility hinders their genetic improvement through conventional breeding.

Hence, alternative methods such as *In vitro* culture techniques and induce mutations are being employed to create the new genetic variability for the selection of the desired genotypes. Variation among the regenerated plantlets termed as somaclonal variation (Larkin & Scowcroft, 1981) and this has been considered a source of new plant genotype for crop improvement. The variation poll can be broadened by the use of tissue culture along mutagenesis (Samad *et al.,* 2001). The first use of induced mutations in sugarcane has been reported in the twenties of twentieth century by the researchers at Hawaiian sugar planter's Association, Hawaii, USA (Anon., 1928). The availability of large population for mutagenesis is one of the basic pre-requisites to obtain sufficient variation. The *In vitro* techniques provide the mechanism to generate large population for mutation induction and rapid multiplication of the selected mutants (Siddiqui *et al.,* 1994; Khatri *et al*., 2002). Khan, *et al.,* (2004) reported 20Gy as the optimal dose for irradiation of the sugar cane crop. Considerable work on selecting agriculturally useful somaclones has been carried out in different countries such as Taiwan (Liu, 1971), Fiji (Krishnamurthi, 1974), Philippines (Lat & Lantin, 1976), Florida (Vasil *et al*., 1979), Brazil (Evans *et al*., 1980), France (Sauvarie & Galzy, 1980) and Australia (Larkin &Scowcroft, 1981). Crop breeding programme can be speeded up by combining the radiation technique with *In vitro* culture methods (Lee *et al* 2002). *In vitro* mutagenesis has contributed to genetic improvement in several crop plants such as pineapple (Lepade *et al.,* 1995), banana (Rao *et al.,* 1995) and grape (Kuksova *et al*., 1997). Mutagenesis through radiation in combination with tissue culture technique seems suitable for the improvement of vegetatively propagated crops (Ahloowalia *et. al.,* 1995).

The present research work was carried out to develop new genetic architecture in sugarcane endowed with high cane yield and high sugar content.

Materials and Methods

Three sugarcane (*Saccharum* spp. Hybrid) clones, NIA-98, NIA-0819 and BL4 were used for tissue culture studies. Ten explants containing leaf primordia were taken from each genotype, sterilized by standard procedure (Siddiqui *et al.*, 1994; Khan *et al*., 2009) and cultured on modified MS medium (Murashige & Skoog, 1962) containing 4mg/l 2,4-D for callusing. Media were solidified with 3g/l gelrite. One month old callus were irradiated with 4 different doses of gamma rays (10Gy, 20Gy, 30Gy and 40Gy). Irradiated callus were immediately transferred on the fresh medium for further proliferation. Weight of callus were recorded before and after irradiation and after four weeks of incubation, irradiated callus were weighted and cultured on regeneration medium MS +2 mg/I IBA + 2 mg/I IAA + 2 mg/I kinetin. The regenerated shoots were scored for chlorophyll mutations. When the plantlets attained 7-8 cm height, these were subjected to rooting by culturing on different media viz.; i) ½ MS medium, ii) MS medium + 2mg/l IBA + 4% sugar, iii) MS+1mg/l IBA+ 4% sugar. The rooted plantlets were acclimatized and transplanted in the field for evaluation.

Results

Callus induction and proliferation: Callus induction is a very important phenomenon in tissue culture (Chen *et al.,* 1988; Heinz & Mee, 1969). Morphologically three different type of callus was observed after irradiation, type 'A' yellowish white, compact dry nodular callus capable of plant regeneration (Fig. 1), type 'B' friable, globular and non-compact and type 'C' mucilaginous type of callus which could revert to other two types depending on the concentration of 2,4-D in the culture medium. Maximum callus production was observed in the control and callus production decreases as the irradiation doses increases (Table 1). Khan *et al.,* (2009) reported stimulation in callus growth at low doses whereas, no such stimulation was recorded in our experiment and this may be due to the

difference in the age of explant. Bajaj e*t al.,* (1970) and Siddiqui & Javed (1982) also reported the stimulation in callus growth at low doses of gamma irradiation. The best callus proliferation was observed in the control followed by 10Gy in all three genotypes (Table 2). The minimum callus proliferation was recorded in 40Gy (Fig. 2).

Type of callus

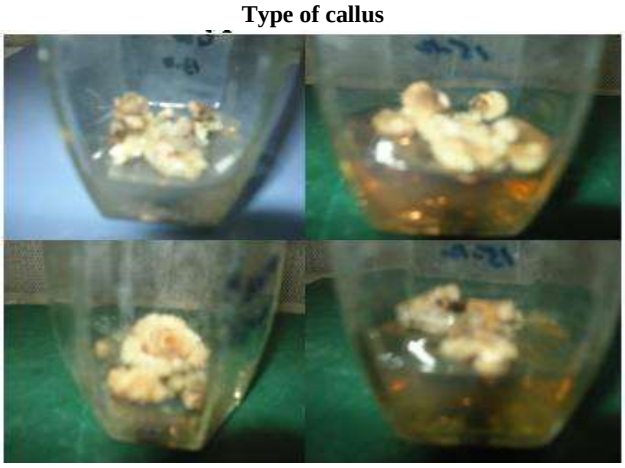

Fig. 1. Dry nodular compact and highly embryogenic callus.

Callus proliferation

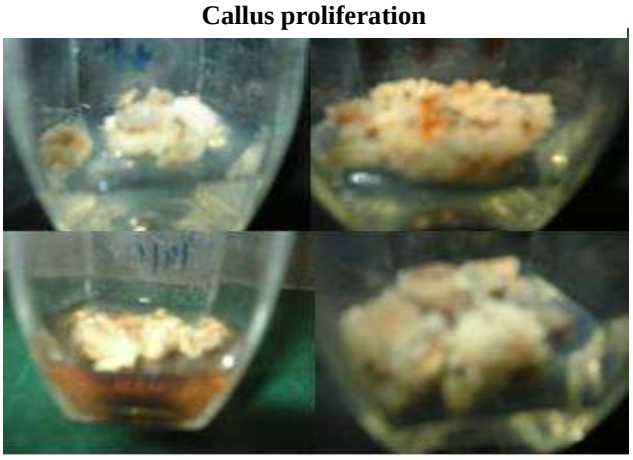

Fig. 2. Callus proliferation under different irradiation doses.

Table 1. Influence of different irradiation dose on callus production (gm).

Gamma radiation dose	NIA-98	NIA-0819	BL4	Mean
Control	1.88a	1.87ab	1.73de	1.82a
10Gy	1.82c	1.83bc	1.70ef	1.78b
20Gy	1.77d	1.72de	1.67gf	1.72c
30GY	1.72de	1.69ef	1.61h	1.67d
40GY	1.63gh	1.62h	1.55i	1.60e
Mean	1.76a	1.74a	1. 5b	

DMR test: Means denoted by similar letter showed non significant differences

Table 2. Effect of different irradiation dose on callus proliferation (gm).

Gamma radiation doses	NIA-98	NIA-0819	BL4	Mean
Control	2.52ab	2.86a	2.81ab	2.73a
10Gy	2.38bc	2.38bc	2.45ab	2.40b
20Gy	1.96cd	1.93cd	2.37bc	2.09c
30GY	1.87e	1.86e	1.93de	1.89cd
40GY	1.74e	1.77e	1.82e	1.77d
Mean	2.27a	2.16a	2.09a	

DMR test: Means denoted by similar letter showed non significant differences

Plantlet regeneration: The maximum number of plantlets regeneration was observed in the control in all three genotypes (Table 3). In the irradiated population maximum plant regeneration was observed in 20Gy of NIA-98 (110) and NIA-0819 (87) whereas no such stimulation was recorded in BL4 (Fig. 3). This may be due to the genotype effect. NIA-98 and NIA-0819 having NCo-310 blood whereas, BL4 was not derived from NCo-310. The minimum number of plantlets was regenerated in 40Gy. Alain *et al.,* (2002) reported that regeneration potential was inversely proportional to the mutagenic treatment but 20Gy had stimulating effect on regeneration potential. Maximum number of chlorophyll variants was observed at 40Gy followed by 30Gy (Table 3). Siddiqui & Javed (1982) reported that 15 to 30 Gy were the optimal doses in sugarcane because growth was drastically affected by doses higher than 40Gy. Our results are in agreement with the results of Siddiqui & Javeed (1982). The frequency of the chlorophyll variants was higher in NIA-98 and NIA-0819 as compared to BL4 (Table 3). This revealed that NIA-98 and NIA-0819 were more sensitive to irradiation doses as compared to BL4. The chlorophyll variants were mostly *albino* and *viridis* type (Fig. 4). The maximum number of tillers was observed in control followed by 10Gy and minimum in 40Gy (Table 4). The different irradiation doses showed significant impact on average number of tillers, as the dose increased the tillering potential decreased.

Table 3. Effect of different irradiation doses on plant regeneration (Nos.).

Gamma irradiation doses	NIA-98		NIA-0819		BL4	
	Green plants	Chlorophyll mutants	Green plants	Chlorophyll mutants	Green plants	Chlorophyll mutants
Control	130^{a}	5.00	108^{ab}	5.00	124^{ab}	4.00
10Gy	$94b^{c}$	4.66	79^{de}	3.33	99^{bc}	4.66
20Gy	110^{ab}	3.00	87^{cd}	4.33	87^{dc}	3.66
30GY	47^{gf}	13.33	50^{fg}	13.33	60^{ef}	12.00
40GY	39^{fg}	14.00	31^{g}	14.66	30^{g}	12.66
Mean	84^{a}	8.13	80^{ab}	8.00	71^{b}	7.40

DMR test: Means denoted by similar letter showed non significant differences

Fig. 3. Plantlet regeneration.

Fig. 4. Chlorophyll mutants.

Rooting: The maximum rooting was observed in NIA-98 followed by NIA- 0819 on media containing MS + 2mg/1 IBA + 4% sucrose (Fig. 5). Minimum numbers of root were recorded in BL4. Significantly differences were observed for root induction among different concentration of IBA (Table 5). Wains (2008) elaborated the use of IBA in medium for the induction of vigorous rooting in the regenerated plantlets. Alain *et al.*, (2002), reported that rooting medium containing NAA (5.0mg/litre) + 7% sucrose induces good rooting in sugarcane. The maximum root length was observed in control and minimum in 40Gy (Table 6). Rashid *et al.*, (2009) and Karim *et al.*, (2002) documented maximum root number and root length on1.0mg/l IBA. The plantlets with well developed shoots and roots were transferred to the jiffy pots (Fig. 6). After acclimatization, plantlets were first transferred to the earthen pots for hardening and then to soil for field evaluation.

Table 4. Effect of different irradiation doses on number of tillers.

Gamma radiation doses	NIA-98	0819	BL4	Mean
Control	11.67^{a}	10.33^{b}	9.33^{b}	10.44a
10Gy	10.00^{b}	8.00^{c}	6.66^{d}	9.22b
20Gy	10.33^{b}	9.33^{b}	8.00^{c}	8.22c
30GY	6.66^{d}	4.66^{efg}	5.00^{ef}	5.44d
40GY	5.33^{e}	3.66^{g}	4.00^{fg}	4.33e
Mean	8.80a	7.20b	6.60c	

DMR test: Means denoted by similar letter showed non significant differences.

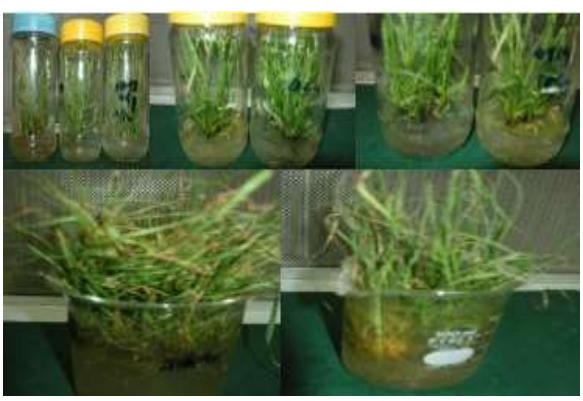

Fig. 5. Root formation on MS medium containing 2mg/l IBA.

Fig. 6. Plantlets in the earthen pots.

Table 5. Effect of different concentration of IBA on root induction in sugarcane.

Concentration of phytohormone (mg/l)	NIA-98	NIA-0819	BL-4
MS + (½) mg/l	+	+	-
2mg/1 IBA,+ 4% sucrose,	+++	++	+
1 mg/1 IBA+ 4% sucrose	++	++	+

DMR test: Means denoted by similar letter showed non significant differences

Table 6. Effect of different irradiation doses on root length (cm).

Gamma radiation doses	NIA-98	NIA-0819	BL4	Mean
Control	103^{a}	8.3^{b}	8.0^{b}	8.8^{a}
10Gy	8.3^{b}	7.0^{bc}	6.3^{cd}	7.2^{b}
20Gy	8.0^{b}	7.0^{bc}	6.3^{cd}	7.1^{b}
30GY	6.3^{cd}	4.6^{ef}	5.0^{de}	5.3^{c}
40GY	5.3^{de}	3.3^{g}	3.6^{fg}	4.1^{d}
Mean	7.6^{a}	6.0^{b}	5.8^{b}	

DMR test: Means denoted by similar letter showed non significant differences

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