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# *IN VITRO* MUTAGENESIS IN SUGARCANE

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Abstract

Two sugarcane clones viz., AEC81-8415 and BL4 were tested for *in vitro* mutagenesis using irradiation doses of 0, 10, 20, 30, 40 and 50Gy. Maximum callus proliferation and shoot regeneration was observed in 10Gy in both clones with linear decrease in callusing and regeneration potential when the irradiation dose was increased upto 50Gy. Better response in root development was observed in MS medium containing 6% sucrose and 1mg/l IBA. Data on quantitative and qualitative traits were also recorded.

## Introduction

Sugarcane is one of the most important cash crops of Pakistan, grown over an area of 963,000 hectares with an average yield of 47 t/ha (Anon., 1997). Average cane yield and sugar recovery in Pakistan are the lowest among the sugarcane growing countries of the world (Alam, 1995; Hashmi, 1995). This dismal state of affairs demands evolution of new sugarcane varieties endowed with high yield, better sugar recovery and resistance to pests, pathogens and environmental stresses.

Selection from the available genetic variability for higher yield plays a major role in cane improvement and agronomic practices just trigger the inherent potential of varieties (Hensely *et al.*, 1973). Hence sustained improvement of sugarcane productivity mainly depends on continued inputs of new potential genes. Cane flowering and viable seed production has always been a problem in Pakistan and arrangements for cane hybridization under artificial conditions are scarce. Alternative methods like tissue culture and mutation have been used for induction of genetic variability (Heinz, 1973; Jagathesan, 1982). The ability to differentiate plantlets from callus tissue of *Saccharum* species was first demonstrated by Heinz & Mee (1969). The fascinating feature of the tissue and cell culture is that one can alter one or few character(s) of a genotype keeping the rest of the genome intact. Castillo Munoz (1989) and Lu (1990) combined tissue culture and induced mutation (*in-vitro* mutagenesis) for sugarcane improvement. Results of similar efforts for improvement of sugarcane clones in Sindh are reported in this paper.

## Materials and Methods

Two clones viz., AEC81-8415 and BL 4 were selected for this study. The explants were obtained from shoot apices consisting of meristematic dome with leaf primordia.

The sheath were peeled off till the spindle with a thickness of 5mm was obtained. Surface was sterilized by dipping in 95% ethanol for one minute followed by immersion in 4% Sodium hypochlorite for 20 minutes. To remove all the traces of sterilants, explants were rinsed thoroughly with sterilized distilled water. The sterilized apices were dissected by making cuts aseptically. The sliced tissues were explanted in 150mm x 25mm test tube containing 20 ml modified MS medium (Murashige & Skoog, 1962). The pH of medium was adjusted to 5.8 and solidified with 0.8% Difco bacto agar.

Callus collected from one month old explant was irradiated with gamma rays from Cesium 137 source (Nigo 5, Bulgaria). Doses applied were 0, 10, 20, 30, 40 and 50 Gy. Dose rate at the time of irradiation was 30.86 Gy/minute. One gram of callus of each clone was used for irradiation. Irradiated callus was placed on fresh MS medium supplemented with growth regulators and organic nutrients used for the proliferation of callus. At the time of sub-culturing, one gram of callus from each treatment was placed on the regeneration medium. Number of shootlets were counted. Plantlets 7-8 cm in height were transferred on rooting medium. Green as well as chlorophyll mutants plantlets were recorded visually.

The following media were used:

| | | |
|---|---|---|
| (a) medium for callusing | = | MS+3mg/l 2,4-D |
| (b) medium for differentiation | = | MS + 2mg/l IAA + 2mg/l IBA + 2mg/l Kinetin (Siddiqui *et al.*, 1988). |
| (c) medium for rooting | = | MS medium |
| | = | 1/2 MS medium |
| | = | 1/2 MS medium + 6% sucrose |
| | = | MS medium + 6% sucrose |
| | = | MS medium + 9% sucrose |
| | = | MS medium + 15% sucrose |
| | = | MS medium +6% sucrose + 1 mg/l IBA |
| | = | MS medium + 3% sucrose + 2mg/l IBA |
| | = | MS medium + 6% sucrose + 3mg/l IBA |
| | = | MS medium + 7% sucrose + 5mg/l NAA |

All the treatments were carried out under aseptic conditions. Cultures were incubated at 28 $\pm$ 2°C under 16 hours photoperiod. Rooted plantlets were acclimatized and transplanted to field for screening of somaclonal variation.

## Results and Discussion

**Callus induction:** Growth response, colour and friability of the callus are strongly influenced by the radiation doses (Bajaj *et al.*, 1970; Siddiqui & Javed, 1982). Callus was initiated 10 to 15 days after explanting. Both the varieties showed different response to callusing. Explants produced phenolic compounds which oxidized and cause their death (Vuylsteke & Langhe, 1985; Shamim *et al.*, 1994). The use of cystein (HCI 40 mg/l) in

**Table 1. Proliferation of irradiated callus and plantlets regeneration in sugarcane clones (after 4 weeks of irradiation).**

| Doses (Gy) | Proliferation of callus (gm) | Total no. of plants regenerated from irradiated callus | Proliferation of callus (gm) | Total no. of plants regenerated from irradiated callus |
|---|---|---|---|---|
| | AEC81-8415 | | BL 4 | |
| | Mean ± SD | | Mean ± SD | |
| Control | 1.935 ± 0.158 | 56 | 2.201 ± 0.158 | 144 |
| 10 | 1.386 ± 0.707 | 64 | 2.215 ± 0.158 | 127 |
| 20 | 0.974 ± 0.957 | 52 | 2.311 ± 0.141 | 107 |
| 30 | 0.710 ± 0.100 | 39 | 0.442 ± 0.071 | 88 |
| 40 | 0.521 ± 0.161 | 21 | 0.351 ± 0.070 | 39 |
| 50 | 0.231 ± 0.100 | 11 | 0.121 ± 0.007 | 10 |

the medium increased the survival rate of explants, as it might have prevented excessive production of polyphenolic compounds. Degree of blackening of the culture medium also affects callus proliferation (Khatri *et al.*, 1997).

After 4 weeks of explanting the callus was sub-cultured on a fresh medium (Table 1). Two types of callus were observed, yellowish white, compact, dry nodular (`A` type) (Fig 1) and whitish globular, non-compact and wet (`B` type) (Fig.2). Similar type of calluses were observed by Orton (1979) from tissue culture of *Hordeum vulgare*, *H. tubatum* and their interaspecfic hybrid. Irradiation doses of 10, 20 and 30 Gy produced `A` type callus only whereas 40 Gy and 50 Gy doses produced both types of

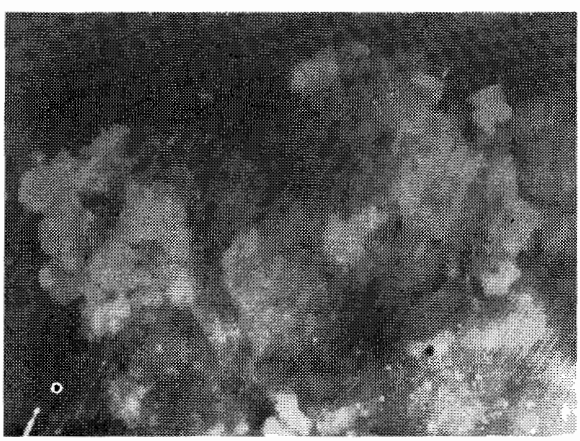

Fig.1. Yellowish white, compact, dry nodular 'A' type callus.

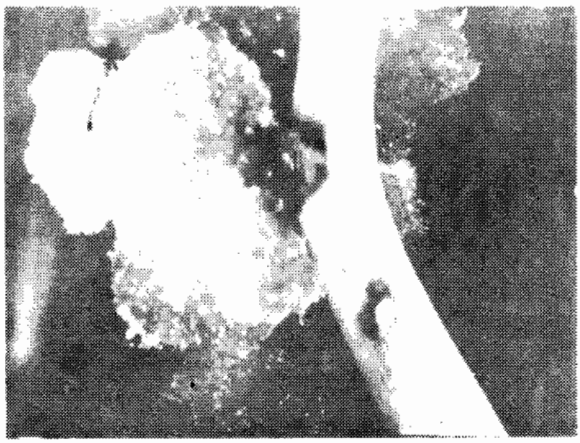

Fig.2. Whitish globular, non-compact, wet 'B' type callus.

calluses. `A` type callus has high potential of regeneration (Shaheen & Mirza, 1989) but 'B' type had no regeneration potential (Orton, 1979). In our study callus proliferation in BL 4 was stimulated in 10 and 20Gy treatments. Bajaj *et al.*, (1970), also reported stimulation in callus growth at low doses of gamma irradiation. However, no such stimulation was observed in AEC81-8415 clone. This could be due to the difference in genetic makeup of these two clones.

**Regeneration:** Differentiation of plants was observed when callus tissue was transferred to regeneration medium. Regeneration was 88, 74, 61, 27 and 7% in BL4 and 114, 93, 70, 37 and 20% in AEC81-8415 of control in 10,20,30,40, and 50Gy treatments, respectively (Table 1). Regeneration potential decreased with an increase in radiation dose but 10Gy had stimulating effect on regeneration potential in AEC81-8415 (Fig.3).

The plantlets regenerated from irradiated as well as non-irradiated callus (control) showed chlorophyll variants (Siddiqui *et al.*, 1994) (Table 2). Both clones showed

**Table 2. Chlorophyll mutant obtained in regenerated plantlets of irradiated callus of BL4 and AEC81-8415.**

| Doses (Gy) | AEC81-8415 | | | | BL 4 | | | |
|---|---|---|---|---|---|---|---|---|
| | Albino | Viridis | Others | Total | Albino | Viridis | Others | Total |
| 0 | 4 | - | - | 4 | 1 | 2 | 2 | 5 |
| 10 | 10 | 2 | 1 | 13 | 7 | - | - | 7 |
| 20 | 8 | 4 | 3 | 15 | 11 | 2 | 1 | 14 |
| 30 | 15 | 4 | - | 19 | 10 | 6 | 1 | 17 |
| 40 | 10 | - | - | 10 | 8 | - | 3 | 11 |
| 50 | 8 | - | - | 8 | 5 | - | - | 5 |


Fig.3 (a). Stimulation in regeneration in 10 Gy of AEC81-8415.

maximum number of chlorophyll variants at 30 Gy, followed by 20 and 40 Gy. The low production of chlorophyll mutants in 50 Gy was probably due to less regeneration at higher dose of radiation. Siddiqui & Javed (1982) reported that 15 to 30 Gy were the optimal doses in sugarcane because growth was drastically affected by doses higher than 40 Gy. The chlorophyll variants (Fig.4) were mostly albino and viridis. Chlorophyll mutants were 7.14, 20.31, 28.84, 48.71, 47.61 and 72.72% in AEC81-8415 clone and 3.47, 5.51, 13.08, 19.31, 28.20 and 50% in BL4 of total regenerated plantlets in 0, 10, 20, 30, 40 and 50 Gy respectively. The frequency of the chlorophyll variants were higher in AEC81-8415 as compared to BL4. This revealed that AEC81-8415 is more sensitive to irradiation doses as compared to BL4.

**Rooting:** Problems with root initiation from callus are greater as compared with shoot initiation (Siddiqui *et al.*, 1988). Roots grow from the nodal primordia only when the plantlets are well developed. Root initiation can be obtained by qualitative and quantitative manipulation of auxins.

In the present study 10 different combinations of auxins with different levels of sucrose were used in basic MS medium for root induction (Table 3). Vigorous root development (Fig.5) was achieved when the plantlets were separated, leaves trimmed and plantlets placed on the medium containing MS+6% sucrose + 1 mg/l IBA. Thrope & Biondi (1984) showed that use of IBA in medium induced vigorous root development in conifers. The plantlets with well developed shoots and roots were transferred to the jiffy pots containing sterilized perlite. After acclimatization, plantlets were first transferred to the earthen pots for hardening and then to soil.

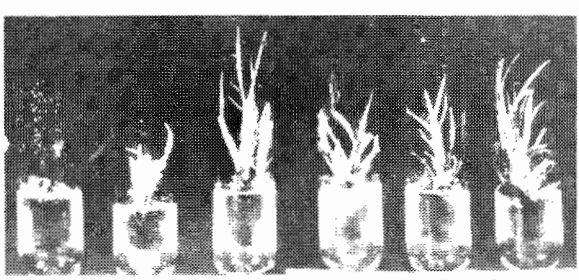


Fig.3 (b). No stimulation in regeneration in 10 Gy of BL4.

**Table 3. Effect of medium composition on root induction of sugarcane.**

| Medium | Rooting behaviour |
|---|---|
| MS medium | - |
| 1/2 MS medium | + |
| 1/2 MS medium + 6% sucrose | + |
| MS medium + 6% sucrose | ++ |
| MS medium + 9% sucrose | + |
| MS medium + 15% sucrose | + |
| MS medium + 6% sucrose + 1 mg/l IBA | +++ |
| MS medium + 3% sucrose + 2mg/l IBA | - |
| MS medium + 6% sucrose + 3mg/l IBA | + |
| MS medium + 7% sucrose + 5mg/l NAA | ++ |

-, No root, + weak rooting, ++, good rooting, +++ excellent rooting

Fig.4. Chlorophyll mutants.

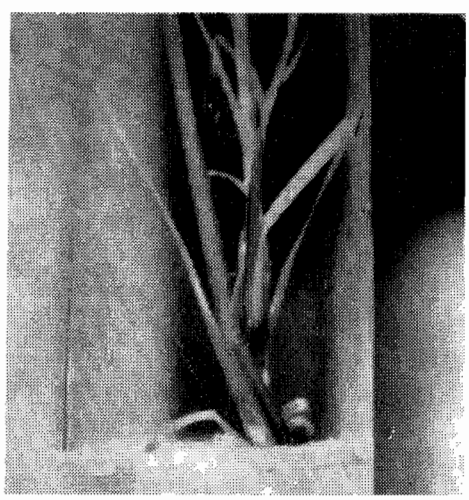

Fig.5. Vigorous rooting in 1 mg/L IBA.

**Table 4a. Variability observed in BL 4 after *in vitro* mutagenesis.**

| Characters | Parent | Somaclones | Variability | Irradiated population (10,20,30 & 40Gy) | variability |
|---|---|---|---|---|---|
| Plant scored | | 144 | | 361 | |
| Tillering (Nos.) | Medium | Low (1-4) | 1% | Medium (5-8) | 25% |
| | | Medium (5-8) | 8% | Heavy (9 & | 75% |
| | | Heavy (9 & | 91% | above) | |
| | | above) | | | |
| Cane thickness | Thick | Thin (2-2.5) | 99% | Thin (2-2.5) | 75% |
| (cm) | | Medium | 1% | Medium | 25% |
| | | (2.5-2.9) | | 2.5-2.9) | |
| Cane colour | Brownish | Red | 89% | Yellowish | 8% |
| | green | Yellowish | 5% | Red | 17% |
| | Yellowish | brown | | Reddish green | 58% |
| | green | Whitish green | 1% | Brownish green | 17% |
| | | Brownish | 5% | | |
| | | green | | | |
| Internode shape | Concave- | Concave- | 100% | Conoidal | 25% |
| | convex | convex | | Concave-convex | 75% |
| Root band | Broad | Medium | 100% | Medium | 100% |
| Bud Shape | Ovate | Ovate | 10% | Triangular | 17% |
| | | Round | 2% | Ovate | 83% |
| | | Triangular | 88% | | |
| Brix % (H.R.) | 20.12 | 18.13-22.93 | 82% better | 15.47 -24.27 | 33% |
| (% age) | | | than parent | | better |
| | | | | | than |
| | | | | | parent |

H.R. = Hand Refractometer

Variability: Plantlets of 10, 20, 30 and 40Gy treatments survived in the field. Variation was observed among somaclones for many characteristics such as tillering, cane colour, thickness, internode shape, root band, bud shape and brix %. Eighty two % (non irradiated material) and 33% (irradiated material) somaclones of BL4 produced higher brix than their parents (Table 4a). Seventy four percent (non irradiated material) and 24% (irradiated material) somaclones of AEC81-8415 produced higher brix than their parents (Table 4b). The somaclones with higher sugar contents than their parents have been advanced in the next generation for further screening. The causes of variation in non irradiated material are not known. They may be associated with variation in chromosome balance (Krishnamurthi & Tlaskal, 1974).

**Table 4b. Variability observed in AEC81-8415 after *in vitro* mutagenesis.**

| Characters | Parent | Somaclones | Variability | Irradiated population (10,20,30 & 40Gy) | variability |
|---|---|---|---|---|---|
| Plant scored | | 56 | | 176 | |
| Tillering (Nos.) | Medium (5-8) | Low(1-4) | 7% | Low(1-4) | 3% |
| | Heavy (9& above) | Medium (5-8) | 3% | Medium (5-8) | 22% |
| | | Heavy (9& above) | 90% | Heavy (9& above) | 75% |
| Cane thickness (cm) | Thin (2-2.5) | Thin (2-2.5) | 67% | Thin (2-2.5) | 83% |
| | Medium (2.5-2.9) | Medium (2.5-2.9) | 33% | Medium (2.5-2.9) | 17% |
| Cane colour | Yellowish green | Yellowish green | 100% | Yellowish green | 4% 96% |
| Internode shape | Conoidal | Conoidal | 90% | Conoidal | 35% |
| | Concave-convex | Concave-conoidal | 10% | Concave-convex | 5% |
| | | | | Cocave-conoidal | 60% |
| Root band | Medium | Medium | 100% | Medium | 100% |
| Bud shape | Triangular | Ovate | 30% | Triangular | 34% |
| | Ovate | Round | 2% | Ovate | 52% |
| | | Triangular | 68% | Round | 14% |
| Brix% (H.R.) (% age) | 20-22 | 16.65-23.13 | 74% better than parent | 15.47-24.27 | 24% better than parent |

H.R. = Hand Refractometer

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