STUDIES ON IN VITRO REGENERATION AND SALT TOLERANCE IN SUGARCANE (*Saccharum officinarum* L.)

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DISSERTATION

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LIST OF ABBREVIATIONS

MS - Murashige and Skoog

BAP - Benzyl amino purine

NAA - 1-Napthalene acetic acid

IBA - Indole-3-butyric acid

2,4-D - (2,4-Dichlorophenoxy) acetic acid

INTRODUCTION

CHAPTER I

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is one of the most important cash crop of the Maharashtra state. A fine network of sugar factories has been developed which is one of the important factor in the economic development of the state. Sugarcane cultivation has been possible due to the most suitable climate and water supply from the various major and minor irrigation projects.

The indiscriminate use of irrigation water and continuous sugarcane cultivation on the same soil has led to the development of salinity particularly on the deep black cotton soils where the drainage is relatively poor. Insufficient drainage, over irrigation, underground saline watertable, poor management of soils and tropical climate conditions are the main factors which contribute the saline soil formation.

Excess salt, usually NaCl, is the most widespread chemical condition inhibiting plant growth in nature (Casey, 1972).

In some species the diversity of salt resistance among cultivars seems quite extensive, and conventional breeding techniques are being used to improve their salt resistance (Epstein, 1976). In many species with little diversity for salt resistance, promising approaches would be either to use variation existing in wild relatives or to use tissue culture techniques for salt resistance.

Genetic variability generated during tissue culture is termed as Somaclonal variation. The term somaclonal variation was coined by Larkin and Scowcroft (1981) to cover all those types of variations which occur in plants regenerated from cultured cells or tissues. Somaclonal variation is a novel source of variability for plant improvement. The spectrum of characters affected can be diverse and the frequency of variant occurence comparitively high, and hence may find its greatest application for plant improvement in selection for desirable mutant at the cellular level (Larkin and Scowcroft, 1981).

The potential usefulness of somaclonal variation for plant improvement first became apparent in sugarcane. Variability amongst plants derived from sugarcane tissue cultures have been reported by several workers (Annonymous 1967, 1968, 1969 and 1970)

Somaclonal variation is very easily obtained in sugarcane, it is extensive, affects many important characters and shows a promise for the improvement of varieties particularly those with single defects.

Salt tolerance is a widespread agricultural problem and the determination of 'absolute tolerance' under *in vivo* conditions has rather been imposible because of the compelx interactions between the plant system, and the environmental factors. However, the recent developments in the *in vitro* technology offer a meaningful tool for determining the tolerance and also for screening and developing the salt tolerant genotypes.

Like most of the other agronomic traits salt tolerance also seems to be under genetic control. Thus the efficient *in vitro* screening even at the differentiated stage could result in plant types capable of withstanding the saline environments (Iyengar and Kurian, 1977).

The use of genetic approaches in developing salt-tolerant crop plants is instrumental in expanding agriculture into areas of high salinity.

The use of cell culture methods to study salt tolerance offers several advantages. This approach permits characterization at the cellular level of the processes involved in salt tolerance. The relative lack of differentiation in cultured cells eliminates the complications arising from the morphological variability and highly differentiated state of various tissues of the whole plants. The growth of plant cells on defined culture media also permits a relatively uniform treatment of these tissues with salt (Croughan *et al.*, 1978)

The present investigation was undertaken on *in vitro* regeneration and salt tolerance in sugarcane using the genotypes viz: CO740 and CO7219 with following objectives,

1. To study the induction of callus in different varieties.
2. To study the regeneration ability and possibility of spontaneous mutations if any.
3. To study the regenerated plants (without roots) for tolerance to salinity in solid medium.
4. To study the rooting ability in the cell lines.
5. To study the rooted plants for tolerance to salinity in liquid medium.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Most of the work on varietal development in Sugarcane is carried out at the Sugarcane Breeding Institute (SBI) Coimbatore. Number of varieties have been evolved and released for cultivation in the country through hybridization programme.

Haberlandt (1902) developed a concept that a complete plant can be regenerated with the same/modified characteristics from any part of the plant. He demonstrated the totipotentiality of the cell. Since then the tissue culture work in different crops took a momentum and efforts are being made to develop variation and subsequently selection for better productivity, tolerance to biotic and abiotic stresses and quality.

In vitro techniques in saccharum species was started in Hawaii in 1961 by Nickell. The recent developments in the *in vitro* technology offer a meaningful tool for determining the tolerance and also screening and developing the salt - tolerant genotypes. The advantages of the tissue culture technique for selecting salt-resistant mutants was discussed by Melchers and Thompson (1972). The work conducted on *in vitro* techniques in Sugarcane improvement and other crops is briefly reviewed here.

2.1 Organogenesis

Tissue culture is now widely used in Sugarcane improvement and breeding programme. This has been made possible by the pioneering work of Heinz and Mee (1969), Barba and Nickell (1969). Who first demonstrated that plantlets could be developed from Sugarcane callus cultures.

Bantana (1983) conducted a trial on a method to obtain plantlets of Sugarcane from meristems *in vitro* and reported that shoot formation was obtained after 8 weeks of growing meristems of 8 month old variety C857 on MS medium with added 22.5 mg/l Kinetin. The shoot was then transferred to MS medium with added 10 mg NAA which induced strong root formation.

Lago *et al.* (1987) conducted a study on the *in vitro* callus formation in 15 Sugarcane cultivars and reported the differences in callus colour, texture and root regeneration between cultivars.

Grisham and Bourg (1989) studied on the efficiency of *in vitro* propagation of Sugarcane plants by direct regeneration from leaf tissue and by shoot tip culture. The two propagation methods were compared for the cultivars CP65-357 and CP70-321. Plant production by direct regeneration of leaf roll section was low (4-18 plants/leaf roll). In shoot tip culture shoot tips of the two cultivars CP 65-357 and CP 70-321 were cultured successfully to the shoot proliferation stage. The best of 4 liquid rooting medium formulations tested for variety CP65-357 contained half strength MS salts, 60 g sucrose/liter. Three regimes of day length and temperature were compared for 16 weeks for their ability to maintain *in vitro* cultures without transfer. All cultures remained viable after 16 weeks and most probably have survived longer.

Mohatkar *et al.* (1993) reported organogenesis in *Saccharum officinarum* L. variety CO740. The sterilized explants of Sugarcane variety were cultured on semisolid MS medium containing 100 mg myo-inositol, 3mg 2,4-D and 10%v/v coconut milk. Callus was obtained in 18 days from inoculation then differentiated into shoots in 22 days. Shoots of 6-7 cm of height were transferred into basal half strength MS liquid medium. Roots of 5-8 cm in length were obtained in 7-10 days. Complete plants were regenerated through organogenesis and cane matured in 11 months.

2.2. Salt tolerance studies

Hussain & Ramzan (1969) have observed reduction in yield and number of tillers in Sugarcane due to salt stress.

Devu and Bajwa (1972) have ranked the salt tolerance capacity of different cultivars in the order. (1) COJ 1328 (2) CO 1148 (3) COJ 46 (4) COJ 39.

Dix and Street (1975) selected number of cell lines of *Nicotiana sylvestris* and *Capsicum annum* capable of growing in liquid medium containing up to 0.34 M NaCl. Some of these lines retained resistance to salt after several subcultures in media lacking NaCl.

Nabors *et al*. (1975) selected NaCl-resistant cell lines from *Nicotiana tabacum L*. cell-suspension culture treated by the mutagen EMS and then grown in a medium containing 0.03M NaCl. Cell derived from these lines even resisted concentrations as high as 0.09 M NaCl in the medium. Most of the control cultures did not grow on the saline medium. Few of them, however, remained alive and after several transfers started to grow fast. This growth was explained by the appearance of dominant mutations.

Goldner *et al*. (1977) classified the possible mutations selected for resistance to salt stress in tissue culture into 3 major groups : (1) Mutations resistant to osmotic stress, (2) Mutations resistant to stress caused by high concentrations of total salts and (3) Mutations resistant to stress caused by specific ions.

Croughan *et al*. (1978) isolated NaCl-resistant cells, which could grow on a medium containing 0.17 M NaCl, from a cell culture of alfa (*Medicago sativa L*.). The selected line behaved like a halophyte in some respects, including the need for salt for optimal growth, maintenance of high level of K+ in the presence of high levels of Na^+ and increased level of $NO3^-$ at low salt concentrations, $NO3^-$ probably replacing Cl^-.

Greenway and Munns (1980) recommended that in breeding for salt resistance, concentration be placed as the ability of the plant to synchronize between an effective compartmentation of the absorbed solutes by the leaf cell and a high rate of transport of these ions to the shoot.

Kochba *et al.* (1980) reported the isolation of "Shamouti" orange callus lines with increased resistance to NaCl. The resistance was maintained in embryos obtained from these lines.

Nabors *et al.* (1980) reported the inheritance of salt resistance in plants regenerated from selected NaCl-resistant cells. Cell-suspension culture of *Nicotiana tabacum* was exposed to increasing levels of NaCl, and cell lines resistant to NaCl concentration up to 0.88% were selected. The resistance was maintained in the progeny of plants regenerated from culture for the two subsequent generations examined. The level of resistance in the progeny of the regenerated plant was higher than in the original resistant cells in culture.

Berssan *et al.* (1981) found that cultured cells selected for resistance to drought imposed by polyethylene glycol were also more resistant to NaCl than unselected cells. The possible use of non penetrable osmotica for selecting mutations resistant to osmotic effect of salt stress should therefore, be taken into consideration.

Smith and McComb (1981) have examined the salt tolerance in salt tolerant and sensitive sp. and shown that salt tolerance is expressed in callus cultures.

Gosal and Bajaj (1984) have studied the NaCl tolerance in some grain legumes by transferring callus cultures of chick pea, pea and green gram on MS medium supplemented with 0, 0.05, 0.5, 1.0 & 3 per cent NaCl. The increase in NaCl concentration beyond 0.5% caused browning and hence reduced growth which was drastically inhibited at 1 and 3% NaCl in all the 3 crop genotypes. They have also noticed emulation of growth at low salinity (0.05%) in Cicer and Pisum but not in Vigna.

Korneva and Maribona (1984) established callus culture on a salt free medium which was subsequently transferred to media containing salt concentrations. Previously salt-treated calluses were also transferred to a salt medium, allowed to differentiate and plantlets were subcultured. Plantlets were eventually transplanted into saline soils. Only 3% of salt free calluses differentiated and only at salt conc. ≤ 1%. Although the plants regenerated had high survival rates in saline soils (48-66%) saline calluses gave 7% differentiation. Survival of regenerants was significantly reduced when subjected to recurrent culture, thus allowing salt resistant mutants to be selected from among adopted plants.

Liu and Yeh (1984) studied the regeneration of NaCl-tolerant Sugarcane plants from callus reinitiated from NaCl preselected plantlets. Differentiated shoot buds of the *Saccharum* species hybrid F177 were subjected to two selection passages on medium containing 1.17% NaCl. Surviving plantlets were cultured on MS medium with 0.94 or 1.17% NaCl. Callus was reinitiated at the base of stem explants of these plantlets. Resulting shoots were transferred to soil containing salt at 10mm hos/cm. electrical conductivity. After 105 days, tolerant plants had accumulated more Na^{+} Cl^{-} ions than normal control, with no effect on growth vigour.

Mc Hughen and Swartz (1984) have studied the *in vitro* response of flax callus to saline conditions. After one month of transfer of callus to salt medium most of the cells had reported to be died.

Kavi Kishor and Reddy (1985) have studied the resistance of rice callus tissue to NaCl and PEG.

Bajaj and Gupta (1987) have studied the effect of NaCl during callus induction in Napier grass *Pennisetum purpureum* by cultivating young infloreseence segments on MS basal medium containing 2,4-D (5mg/lit). Various concentrations of NaCl (0.1 to 3%) were used. They have observed delayed initiation of callus and gradual decrease and complete inhibition of growth with increasing salt level. Completely regenerated plants were obtained from cell cultures tolerant to 0.5% salt.

Hamill and Moriotti (1987) have reported the sensitivity of *Medicago sativa* callus culture to chlorate salinity.

Kavi Kishor (1988) have studied the effect of salt stress on callus cultures of *Oryza sativa* L. He has reported that non.adopted callus showed a decrease in growth at all NaCl concentrations tested (50 to 200 mol/m-3). Increasing the concentration of NaCl (500 mol/m-3) reduced the growth of even salt adopted callus.

Naik and Babu (1988) conducted experiments on redifferentiation of NaCl tolerant Sugarcane plants from callus derived resistant lines. Actively growing callus of varieties Co740 and C062175 produced from shoot tips of young leaves was subcultured at 15-30 days interval, then transferred to MS basal medium containing 0.1, 0.2, 0.5, 1.0, 1.5 or 2.0% NaCl. Growth was completely inhibited at and above a concentration of 1%. Small sectors of actively growing cells were observed in some cultures upto 1.5%. By subculturing these aggregates of cells, callus derived salt-tolerant cell lines were obtained from both varieties. Shoots and roots were induced on modified MS medium and a completely regenerated plant of C062175 was obtained. Regeneration capacity from tolerant cell lines was higher in C062175 than in C0740.

Chen and Wang (1991) conducted a study on isolation and characteristics of a Sugarcane cell line resistant to hydroxyproline (HYP). Cultured *Saccharum sinensis* cell variant line R8968 was selected for its resistance to growth inhibition by HYP. The proline content of R8968 was 3.8 times higher than that of HYP-sensitive donor. Other free amino acid levels were also higher than those of donor. In addition, R8968 showed higher tolerance of NaCl, low temperature and PEG. It was concluded that proline accumulation may favour the increase of tolerance to environmental stress.

Shrivastava *et al.* (1993) studied the effect of NaCl induced salt stress on iron uptake, partitioning and accumulation in Sugarcane. The highest salinity level decreased overall Fe concentration in Sugarcane tissues but about doubled concentration in the stalk.

Somaclonal Variation

Heinz and Mee (1969, 1971) demonstrated that Sugarcane plantlets regenerated from callus showed wide variation in chromosome number and in several important characters.

Krishnamurthi and Tlaskal (1974) screened somaclones of a number of varieties for reaction to Fiji disease (a leafhopper transmitted virus) and downy mildew (*Sclesospora sacchari*). Among the 300 somaclones derived from varieties susceptible to Fiji disease, four were resistant and one had intermediate resistance. Similar screening for downy mildew resistance using susceptible varieties produced some variants that were more resistant than donor clones.

Liu and Chen (1978) screened high yielding variants. Replicated field tests for selected somaclones were completed each year since 1974. The results obtained in 1974-75 indicated that 70-6132 had a higher cane yield (34%) and stalk number (6%) than the donor, F-164. Differences between 70-6132 and the donor for cane and sugar yield were significant at the 5% probability level.

Liu and hen (1980) reported high-sucrose variants. The line 71-4829 is an example whose percentage sucrose has been consistently higher than its donor, H37-1933. The difference was significant at the 1% probability level, suggesting that 71-4829's high sucrose level is genetically stable.

Larkin and Scowcroft (1981) concluded from a review of literature that plant cell culture itself generates genetic variability (somaclonal variation). Substantial number of examples of somaclonal variation on crops like Sugarcane, potato, tobacco, Rice, Oats, Maize, Barley, Brassica sps, Pelargonium were discussed.

Larkin and Scowcroft (1981) tested 260 somaclones derived from a clone susceptible to an eye spot disease (*Helminthosporium sacchari*), viz; Q101, for disease resistance. They found that 8.9% of the somaclones were highly resistant or nearly immune.

Liu and Chen (1982) screened high yielding variants. Among the F160 derivatives, No. 74-3216 performed well and has been released in regional field trials. Derivative No. 74-3216 was higher in cane yield (6%), sugar yield (10%) and stalk no. (6%) than its donor.

Mauboussin and Chagvardieff (1985) observed somaclonal variations in Sugarcane. Twelve out of 78 somaclones produced *in vitro* from callus tissue showed a greater vigour of shoot development from cuttings than the parental cultivar.

Alonso *et al.* (1987) evaluated the variability in subclones obtained by tissue culture of variety B62163 at the plant cane stage.

Lourens and Martin (1987) evaluated the *in vitro* propagated Sugarcane hybrids for somaclonal variation. In a 2 year field study with the *Saccharum* interspecific hybrid cultivar CP65-37 and CP72-356, propogation by each of 2 tissue culture methods resulted in a slight increase in morphological variability (in characters such as tiller no, stalk length, leaf altitude, stalk diameter) over that in the original cultivar.

Gonzalez *et al.* (1988) evaluated the varietal differences in response to high concentrations of mannitol in *in vitro* culture of Sugarcane. Four cultivars were studied for callus development and plant regeneration in media containing solutions of 0.33 & 0.66 M mannitol. The cultivars My 55-14 and My 54129 were the most tolerant but were drought susceptible and drought resistant respectively in the field. Ja 6410 & B42231 were the least tolerant & were drought susceptible drought resistant respectively in the field.

Fahmy (1990) reported Sugarcane subclones resistant to Mosaic virus (MV) from callus tissue culture.

Liu (1990) reported the selection of mutant TC8201 from 46 plants from ROC5 through plant cell culture systems.

Chen (1992) evaluated three subclone populations derived from *in vitro* culture of different donors by a plantlet tracing method and concluded that tissue culture can provide general variation at the molecular or cell level which could prove useful for improving some agronomic characters of commercial Sugarcane cultivars.

Sreenivasan and Sreenivasan (1992) conducted experiments on micropropogation of Sugarcane varieties for increasing cane yield. A procedure for high rate *in vitro* plant multiplication from meristem is described. Media for meristem culture, shoot apex multiplication and rooting were based on white and MS media. Average increase of 10ton/ha in yield and 0.5% in sucrose in the juice were obtained and maintained for about 5 years of vegetative propogation.

2.4. Varietal differences for callus formation and regeneration

Singh and Sinha (1981) reported differential response of Sugarcane varieties CO419, CO740, COLK8001, to callus growth when different concentration of 2,4-D, Dicamba, & NAA were used in the medium.

Cheema *et al.* (1992) studied on response of different genotypes to callus induction and plant regeneration. Ten genotypes of Sugarcane comprising five varieties and five promising strains were used to determine their response to callus induction and subsequent plant regeneration. Callus cultures were established using spindle and axillary bu.ds as explants. The basal medium consisted of salts and vitamins of MS medium which was supplemented with different combinations of growth regulatiors. The per cent callus induction ranged between 56.2 and 100 on different media when young expanding leaves were used while it ranged between 36.6 and 100 when axillary buds were used as explants. In general, spindle exhibited better callus induction as compared to axillary buds on all the media used. Rooting on separated shoots was induced on MS + 7 % sucrose + NAA (5 mg/l). Differential response of the genotypes for callus induction has been observed.

2.5 Effect of harmones and growth regulators

Barba *et al.* (1979) reported that callus induction has been found to vary with the medium used Addition of cyptokinins particularly in the form of BAP gave significantly higher callus induction. Addition of coconut water added as supplement did not increase callus induction, however, it hastened the callus initiation.

Lal and Singh (1991) reported morphogenesis and growth studies in Sugarcane callus with different 2,4-D levels. Shoot buds can be induced from the callus by transferring it into a harmone free modified MS medium. Callus initiation and maintenance was achieved on the modified MS medium supplemented with 5mg/lit 2,4-D, however levels of 2,4-D showed occurrence of organogenesis and embryogenesis together. Concentration of 2,4-D at 5mg also showed highest rate of dry matter biosynthesis per unit weight of callus.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Material

The experimental material of the present study comprised of two sugarcane varieties viz., CO740 and CO7219. The apical meristematic tissues (shoot tips) were used for callus development. The explant material was collected from the multiplication plots of central farm, Marathwada Agriucltural University, Parbhani. The features of these genotypes are presented in Table 1.

3.2 Treatments

3.2.1 For callus induction the following treatments were used;

i. MS + 2,4-D 1 mg/l

ii. MS + 2,4-D 2 mg/l

3.2.2 For regeneration of plantlets;

MS + BAP 2 mg/l

3.2.3 The regenerated plantlets were tested with the following NaCl salt concentrations;

i. 0.5 %

ii. 1.0 %

iii. 1.5 %

iv. Control

3.2.4 For rooting the regenerated plantlets the MS + NAA 1 mg/l + IBA 0.5 mg/l media was used.

3.2.5 The rooted plantlets were tested with the following NaCl salt concentrations with modified MS media

i. 0 5 %

ii. 1.0 %

iii. 1.5 %

iv. Control

3.3 Experimental Methods

3.3.1 Preparation of media

The basal medium used in the present study was MS medium (Mruashige and Skoog, 1962). The composition of the medium is given in Table-2. Before the preparation of actual media for inoculation, the stock solutions of all major and minor elements (except calcium chloride) including vitamins were prepared, properly labelled and stored in refrigerator. The stock solutions of different harmones viz., 2,4 - Dichlorophenoxy acetic acid (2,4-D), Benzylaminopurine (BAP), Napthalene acetic acid (NAA), Indole Butyric acid were also prepared and stored in refrigerator. During the preparation of the basal medium all the above stock solutions of major and minor, elements were pipetted out in required quantity and mixed them to get the required concentration. The concentration of sucrose used was 3 per cent (30 gm/l). The glaxos agar was used @ 8 gm/l. The pH of the medium was adjusted to 5.6 by adding dilute HCl or NaOH before addition of agar. The

Table 1. Salient features of the genotypes used for tissue culture studies

Sr. No.	Varieties	Year of release	Salient features
1.	CO740	1956	Evolved from a cross between (CO421 x CO440) x (CO464 x CO440) Pale yellow cane, suberect, good tillering, thin, not breaking on lodging. Medium late to late maturity, most suitable for *adsali*. Unable to withstand long periods of moisture stress or waterlogging. It can tolerate alkalinity to a certain extent. It is suceptible to stemborer and highly suceptible to smut disease. Yield - 176 t/ha. Sucrose percentage - 16.4.
2.	CO7219 (Sanjeevani)	1982	Evolved by hybridization of (CO419 x CO658) Tolerant to smut and grassy shoot. Early variety, requires 9 - 10 months for maturity with good ratooning performance. Recommended for all 3 seasons. Yield - 173 t/ha. Sucrose percentage - 15.5.

Table 2 : Chemical composition of Murashige and Skoog's (MS) medium.

Sr.No.	Stock solutions	Constituents	Content (mg/l)
1.	**Mineral salts**		
	A	$NH_4 NO_3$	1650
	B	KNO_3	1900
	C	$KH_2 PO_4$	174
		KI	0.83
		$CoCl_2$	0.025
		$Na_2 MoO_4$	0.025
		$H_3 BO_3$	6.20
	D	$CaCl_2$	440
	E	$CuSO_4.5H_2O$	0.005
		$MgSO_4.4H_2O$	370
		$ZnSO_4.7H_2O$	8.72
		$MnSO_4.4H_2O$	23.3
	F	Na_2 EDTA	87.55
		$FeSO_4.7H_2O$	27.55
2.	**Vitamins**		
	G	Myo-Inositol	100
		Nicotinic acid	0.5
		Pyridoxine HCl	0.5
		Thiamine HCl	0.1
		Glycine	2.0
3.	**Organic constituents**		
		Sugar	30 g (3%)
		Agar agar	8 g

volume of the medium was adjusted to 1000 ml and boiled to dissolve agar. The media was then distributed uniformly into test tubes and closed with tight cotton bunks wrapped in muslin cloth. The media was then autoclaved at 15 pounds per square inch pressure (1.06 kg/cm^2) and temperature 121^{o}C for 20 minute. The tubes were ready for inoculation after cooling.

3.3.2 Cultural Conditions

All the cultural operations including sterilization, inoculation and transfer were carried in a horizontal laminar flow chamber using sterilized instruments. Before the inoculation the chamber was smeared with ethyl alcohol. The cultured tubes after inoculation were incubated under dark at 24 $\pm$ 1^{o}C.

3.3.3 Preparation of Explants

The apical meristematic tissue was excised by carefully stripping off the outer sheath. Then the shoot apices were cut and washed in sterile water 2-3 times. These shoot apices were surface sterilized with 0.1 per cent mercuric chloride solution for about 3-5 minutes, followed by 3-4 thorough washings with distilled sterile water. The material was made into pieces (explant) of 4-5 mm size with sterile forceps under aseptic conditions. The explants were inoculated on MS medium immediately.

3.3.4 Induction of callus

For induction of callus following two treatments were used,

MS + 1 mg 2,4-D /l

MS + 2 mg 2,4-D /l.

3.3.5 Inoculation of Explants

The explants as prepared above were inoculated aseptically on the callus inducing media in such a way that the explants remain in touch with the medium. Two hundred and fourty tubes were inoculated on MS + 2,4-D (1mg/l) and another 240 tubes on MS + 2,4-D (2mg/l). All the operations were carried out under horizontal laminar air flow chamber. The culture tubes after inoculation were incubated under dark at 24 ± 1°C.

3.3.6 Subculture

After inoculation the callus initiation and development was carefully watched in the inoculated varieties. A brown exudate was frequently observed in the apical dome. Frequent subcultures are therefore necessary to eliminate the brown exudate. After 10-15 days of callus initiation, the fresh calli were subcultred on the same medium containing the same levels of growth harmones. Three subculturing were carried out at an interval of 1-15 days. Each time callus cultures were incubated in dark at 24 ± 1°C.

3.4 Observations

Following observations were recorded in the present investigation.

3.4.1 Callus initiation

Each treatment was observed for callus initiation and its development. Callus was initiated within 6 - 8 days after inoculation. Callusing ability percentage of two cultivars was worked out.

3.4.2 Callus Growth

Growth measurements of calli were recorded as function of increase in their fresh and dry weights. Fresh and dry weights of six calli obtained from two different explants of each cultivar were recorded after 2 days of second subculturing. The compact and whitish uniform size calli were selected for weighing. The fresh weight of callus was measured and then oven dried at $50^{o}C$ till constant weight was recorded.

3.4.3 Regeneration of plants

In three subculturing the calli were sufficiently multiplied. Some calli showing luxuriant growth, compact and whitish colour were transferred to the regenerative medium (excluding 2,4-D), i.e. MS + 2 mg BAP/l. After regeneration (shoot initiation) it was subcultured twice in the same media. The calli obtained from two different varieties were transferred on each of the regenerative medium. These cultures were maintained at 24 ± 1°C temperature under cool white fluorescent light of an intensity of abut 3800 lux for 18 hr. photoperiod. The regeneration ability was studied. Percentage of regenerated plants was recorded.

3.5 Testing of the regenerated plantlets in various levels of NaCl salt concentrations (without roots)

After regeneration and shoot initiation, the plantlets were transferred twice in the same media i.e. MS + 2 mg BAP/l and then were transferred to the regenerative medium supplemented with three levels of NaCl and one control viz., 0.5, 1, 1.5 per cent and control.

The media MS + 2 mg BAP/l was prepared, and the respective amount of salt was added after boiling the media and then the media was autoclaved. The growth and development of the plantlets in the different concentrations was studied.

3.6 Rooting of regenerated plantlets

The regenerated plants (without roots) were transferred to the rooting media,

MS + 1 mg NAA/l + 0.5 mg IBA/l.

Rooting was observed after 15 - 20 days of transfer. Percentage of rooted plants was recorded.

3.7. Testing of the rooted plantlets in various levels of NaCl salt concentrations with modified MS media

After good rooting of the plantlets, they were transferred to the modified MS media (liquid media) supplemented with three levels of NaCl and one control, viz., 0.5, 1.0, 1.5 per cent and control.

Salt of a given concentration was added after boiling the media and then the media was autoclaved. The growth and development in different concentrations was studied.

3.8. Experimental design

The factorial randomised block design (FRBD) with replications was used. That is there were 2 factors with 3 different levels. The first factor was variety viz., CO740 and CO7219. The second factor was callusing treatment with two different treatment combinations of 2,4-D viz., MS + 1 mg 2,4-D/l and MS + 2 mg 2,4-D/l. There were six replications each consisting of 40 tubes of each treatment

3.8.1 Statistical Analysis

The data obtained in different experiments was analysed by using the appropriate statistical procedures. (Panse and Sukhatme. 1976).

RESULTS

CHAPTER IV

RESULTS

The results of the present investigation on callusing ability, callus growth, shoot and root initiation, effect of levels of salinity on the growth and mortality are presented below.

4.1 Callusing ability

The callusing ability of two sugarcane varieties viz., CO740 and CO7219 using the meristematic tissues (shoot tips) as the explant was studied on two levels of 2,4-D i.e.1 mg and 2 mg/l using MS media. The results are presented below.

4.1.1 Days required for callus initiation

It was seen from the Table 3 that there was no variation for number of days required for callus initiation in both the varieties at 1 mg/l 2,4-D level. The variety CO740 took around 8 days for callus initiation, whereas the mean for CO7219 is around 7.5. The 2,4-D treatment @ 2 mg/l showed that the mean number of days required are around 8 (8.23) for the variety CO740, whereas for CO7219 it was around 7.5 (7.62).

4.1.2 Callusing ability

The analysis of variance for the callusing ability presented in Table 4 showed that the treatment differences were significant, whereas the variety didnot show the

Table 3 : Mean days required for callus initiation in different treatments of the two varieties.

CO 740				CO 7219			
2,4-D (1mg/l)		2,4-D (2mg/l)		2,4-D (1mg/l)		2,4-D (2mg/l)	
No.of tubes showing callus	No.of days requ-ired	No.of tubes showing callus	No.of days requ-ired	No.of tubes showing callus	No.of days requ-ired	No.of tubes showing callus	No.of days requ-ired
6	6	16	6	33	6	13	6
87	7	84	8	69	7	75	7
28	8	21	9	49	8	32	8
14	10	8	10	9	10	21	9
23	11	9	11	10	11	8	11
Mean no. of days required for callus initiation	7.98		8.23		7.48		7.62

Table 4 : ANOVA for callusing ability, fresh weight and dry weights of different varieties and treatments.

Sr. No.	Source of variation	Df	MSS due to per cent explants callused	MSS for fresh wt. of callus after 25 days of second subculture	MSS for dry wt. callus after 25 days of second subcultre
1.	Replication	5	0.34033	0.004712	0.0000426
2.	Treatments	1	162.50*	0.11523**	0.000392
3.	Varieties	1	50.979	0.4991**	0.00246
4.	Treatment x varieties	1	0.32142	0.000018	0.000273
5.	Error	15	22.567	0.013115	0.000623

* and ** significant at 5% and 1% levels of significance respectively.

Table 5 : Mean performance of callusing treatments and varieties for callusing ability

Source of variation	No.of tubes inoculated	Mean percentage of explant callused
Varieties		
Co 740	240	51.829
Co 7219	240	54.74
SE ±		1.3713
CD at 5 %		4.1825
Treatments		
1mg 2,4-D/l	240	55.889
2mg 2,4-D/l	240	50.685
SE ±		1.3713
CD at 5%		4.1825

significant variation. The callusing ability in the variety CO7219 was higher than CO740 (Fig. 1 and 2). The treatment 1 mg 2,4-D /1 showed 55.88 % callusing as against 50.68 % in the treatment 2 mg 2,4-D/1, which was significantly superior (Fig.1). In terms of percentage of callusing ability CO7219 had shown 54.74 per cent callusing ability as against 51.82 per cent in CO740 (Fig. 2).

4.1.3 Fresh and dry weight of callus

The callus growth can be measured with the fresh and dry weight of the callus. In the present study the fresh and dry weight of the callus was measured 25 days after second subculturing. The analysis of variance (Table 4) showed that the differences due to treatments and also due to varieties were significant at 1 per cent level for fresh weight of callus, however, the treatment x variety interaction was non-significant.

The differences due to treatments, varieties and treatments x variety were non-significant for dry weight of callus.

The mean fresh weight in the variety CO7219 was significantly superior than the mean fresh weight of CO740 (Table 6), Similarly the mean fresh weight in the treatment 1 mg 2,4-D/1 was significantly higher than the mean fresh weight of the treatment 2 mg 2,4-D/1.

Table 6 : Mean performance of callusing treatments and varieties for fresh and dry weight of callus.

Source of variation	Mean fresh weight (g)	Mean dry weight (g)
Varieties		
Co 740	1.4870	0.14675
Co 7219	1.986	0.1670
SE ±	0.03305	0.0072
CD at 5 %	0.1008	0.02197
Treatments		
1mg 2,4-D/l	1.4121	0.15283
2mg 2,4-D/l	1.2735	0.16092
SE ±	0.03305	0.007205
CD at 5%	0.1008	0.02197

The variety CO7219 had shown higher mean dry weight of the callus than the variety CO740, similarly the treatment 2 mg 2,4-D/1 had shown more mean dry weight than 1 mg 2,4-D/1, however, they were on par.

4.2 Plant regeneration and proliferation

After sufficient proliferation of the callus the fresh calli were transferred to a regenerating medium (without 2,4-D) supplemented with 2 mg BAP. The cultures of both the varieties were maintained at 16 hours light alternating with 8 hours dark at 24 ± 1°C. The callus turned green and subsequently showed shoot initiation. The results of regeneration of shoots are presented in Table 7. It was observed that the per cent green plantlet formation was highest in the variety CO740 (96.6 %) (Fig 3 and 4).

4.3 Root initiation and proliferation

The regenerated plants (without roots) in both the varieties were cultured on the media supplemented with NAA (1 mg/1) and IBA (0.5 mg/1). The rooting percentage was 97.14 in the variety CO740 (Fig.5), whereas 88.57 in CO7219 (Fig.6) (Table 8). After the proliferation of the roots, the plantlets were used for testing salt tolerance.

4.4 Testing of regenerated plantlets (without roots) on salt media

The regenerated plantlets (without roots) were cultured on three different levels of sodium chloride salt i.e. 0.5, 1.0 and 1.5 per cent with one control. Ten

Table 7 : Mean performance of regeneration ability in two genotypes of Sugarcane

Sr.	Varieties	Regenerating medium	No.of tubes kept for regeneration	No.of tubes showing green plants	% green plantlets formed
1.	CO 740	MS + 2 mg BAP	120	116	96.66
2.	CO 7219	MS + 2 mg BAP	120	112	93.3

Table 8 : Mean rooting performance of the two genotypes

Sr.	Varieties	Rooting medium	No. of shoots cultured	No.of shoots showing rooting	per cent rooting
1.	CO 740	MS + 1mg/l NAA + 0.5mg/l IBA	70	68	97.14
2.	CO 7219	MS + 1mg/l NAA + 0.5mg/l IBA	70	62	88.57

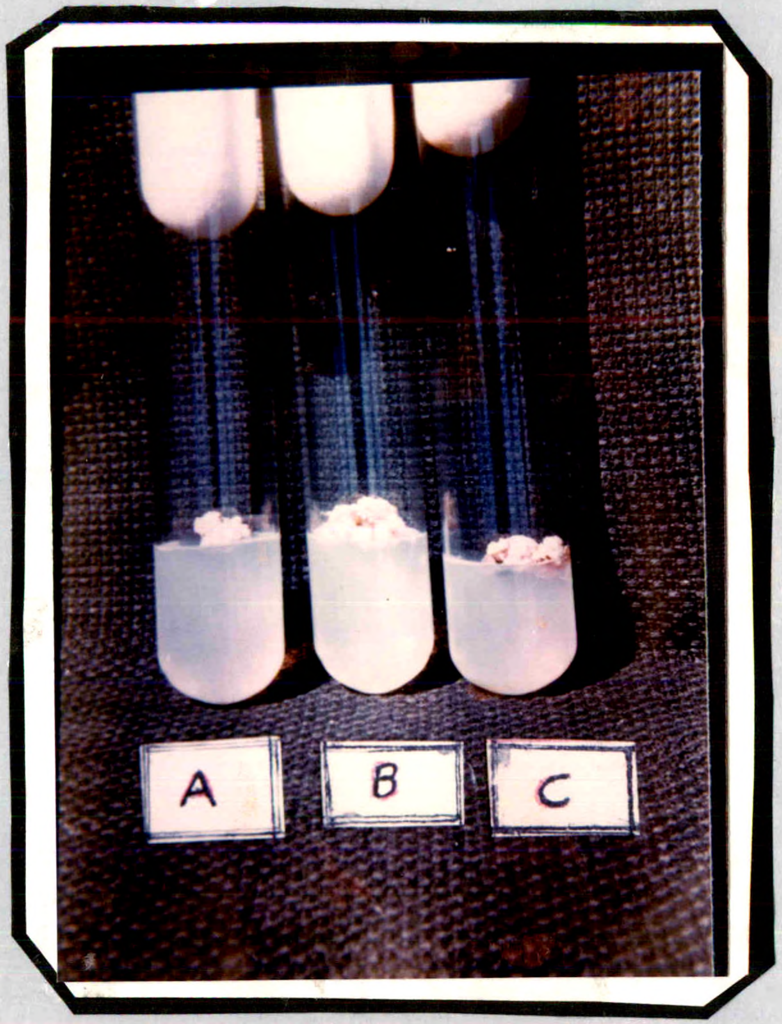


Fig.1. Callus proliferation in the variety CO740 at different levels of 2,4 D.

Fig.2. Callus proliferation in the variety CO7219 at different levels of 2,4-D.

A - 2 mg/l 2,4-D

B - 1 mg/l 2,4-D

C - 2 mg/l 2,4-D

Fig.3. Shoot regeneration in the variety CO740.

MS + 2 mg/l BAP

Fig.4. Shoot regeneration in the variety CO7219

MS + 2 mg/l BAP

Fig.5. Root initiation in CO740.

MS + 1 mg NAA + 0.5mg/l IBA

Fig.6. Root initiation in CO7219

MS + 1 mg NAA + 0.5mg/l IBA

plantlets were inoculated on MS media in each replication on these salt levels and maintained in the laboratory for 16 hours of light alternating with 8 hours dark at 24 ± 1 °C. Total fourty plants were inoculated in each treatment. Observations were recorded after 10 days and 20 days of inoculation.

The analysis of variance from Table 9 for the survived plants after 10 days showed that the differences due to treatments (salt concentrations) and varieties were significant at 1 per cent level.

After 20 days of observation the treatment differences (salt concentrations) were significant at 1 per cent whereas, differences due to varieties were significant at 5 per cent. The interaction effects (treatment x variety) were non-significant.

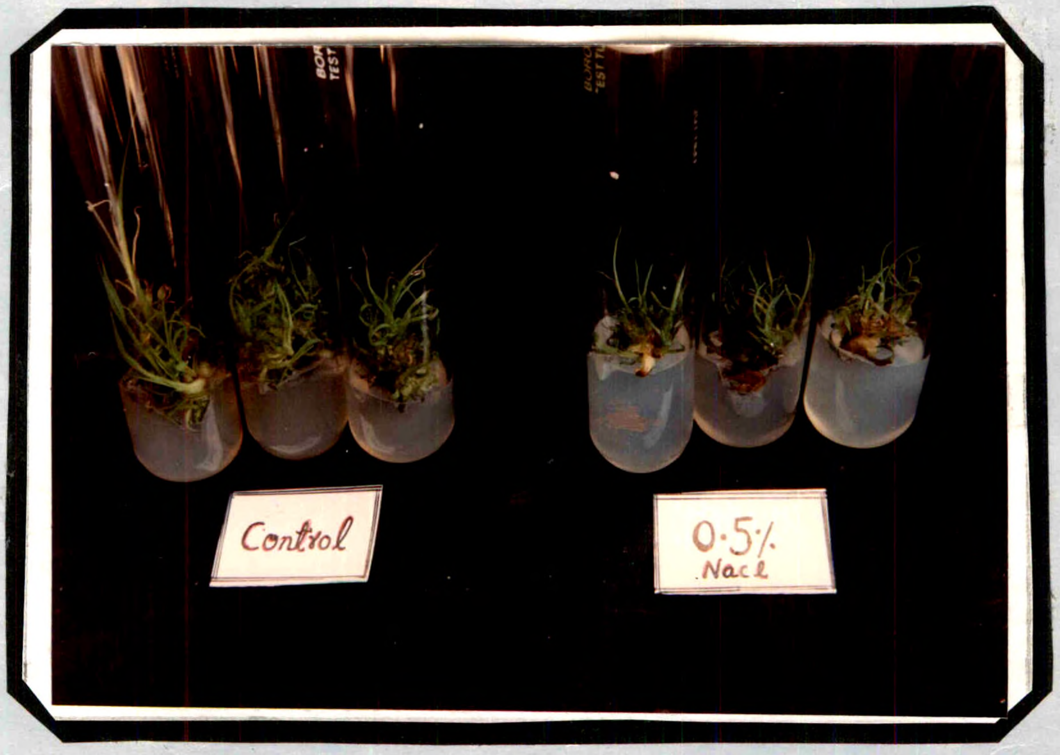
The data presented in Table 10 showed that the survival percentage was more in 0.5 per cent salt treatment followed by 1.0 and 1.5 per cent (Fig 7 and 8). The survival percentage in 0.5 per cent was 61.86 per cent whereas in 1.5 per cent it was 37.49 per cent. The suviving plants in the higher level of concentration i.e. 1.5 and 1.0 per cent showed reduced growth, reduced tillering and pale green colour. In the 0.5 per cent treatment the growth was reduced than the normal and showed sparced tillering. Among the varieties in CO740, the growth was observed in 64.67 per cent of the tubes as

Table 9 : ANOVA for the regenerated plantlets (withour roots) tested at different concentrations of salt levels in soild medium

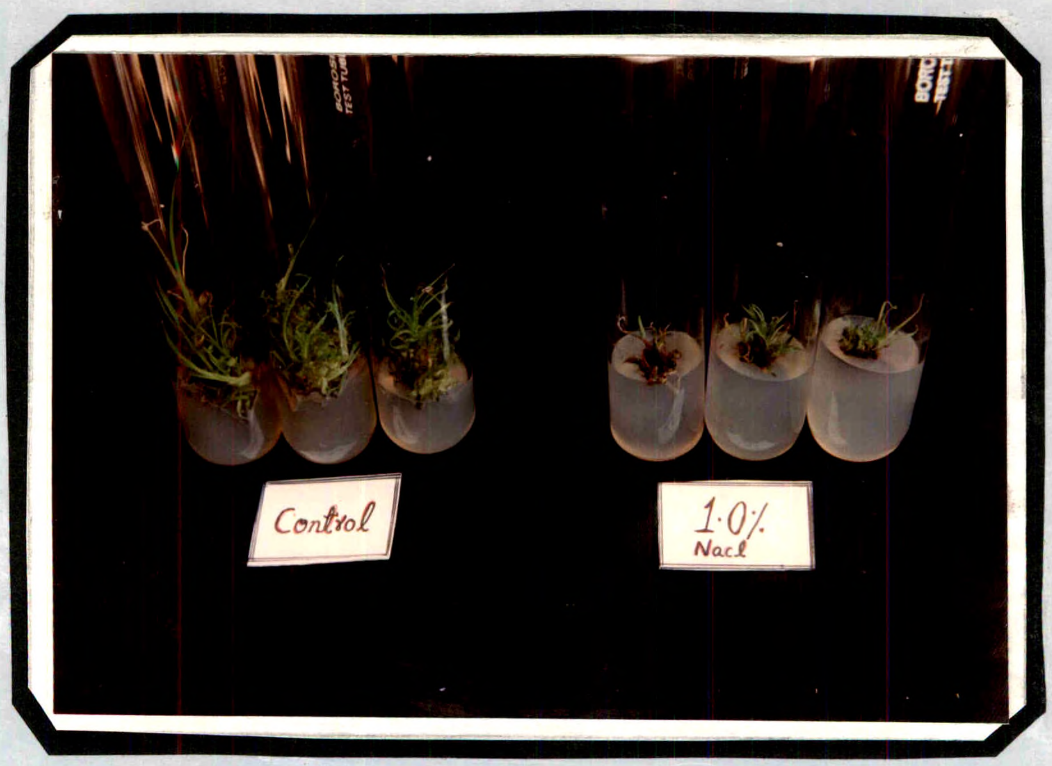
Sr. No.	Source of variation	Df	MSS due to per cent of regenerated shoots showing good growth after 10 days	MSS due to per cent of regenerated shoots showing good growth after 20 days
1.	Replication	3	271.80	46.863
2.	Treatment	3	4208.3**	6101.5**
3.	Variety	1	1012.2**	112.47*
4.	Treatment x varieties	3	131.22	37.49
5.	Error	21	127.20	20.084

* and ** significant at 5% and 1% levels of significance respecively.

Fig.7. Effect of NaCl salt concentrations on growth of the regenerated shoots variety CO740.



A



B

C

Table 10: Mean performance of regenerated plantlets (without roots) cultured on different concentrations of salt levels in solid medium

Source of variation	Mean percentage of regenerated shoots showing growth after 10 days	Mean percentage of regenerated shoots showing growth after 20 days
Varieties		
Co 740	64.679	51.556
Co 7219	53.431	47.806
SE ±	2.8196	1.1204
CD at 5 %	8.5397	3.3933
Treatments		
Control	89.988	89.988
0.5 %	61.867	44.994
1.0 %	46.869	33.746
1.5 %	37.495	29.996
SE ±	3.9875	1.58454
CD at 5 %	12.077	4.7989

against 53.43 per cent in CO7219 after 10 days of observation. After 20 days of the culture only 29.99 per cent of the plants survived in 1.5 per cent treatment with pale green colour and without any tillering. There was increasing trend of survival and growth in 1.0 and 0.5 per cent level.

The analysis of variance showed that the treatment 1.0 and 1.5 per cent were on par. The variety CO740 was significantly superior in showing green plantlets than CO7219.

After 20 days of inoculation, it was observed that again the treatments 1.0 and 1.5 per cent were on par The treatment, 0.5 per cent salt concentration was superior in retaining the growth of the plantlets. The per cent survival in 0.5 per cent was highest 61.86 whereas it was 46.86 and 37.49 per cent in 1.0 and 1.5 per cent salt concentrations. Among the varieties, CO740 was significantly superior than CO7219 in retaining the growth of the plants. The per cent survival in CO740 was 51.55 per cent against 47.80 per cent in CO7219.

4.5 Testing of rooted plantlets on salt concentrations in liquid medium

The rooted plantlets of the varieties CO740 and CO7219 were cultured on the treatment viz., control, 0.5, 1.0 and 1.5 per cent on liquid medium (Fig.9 and 10). . In each replication ten tubes were inoculated. A total of

A

B

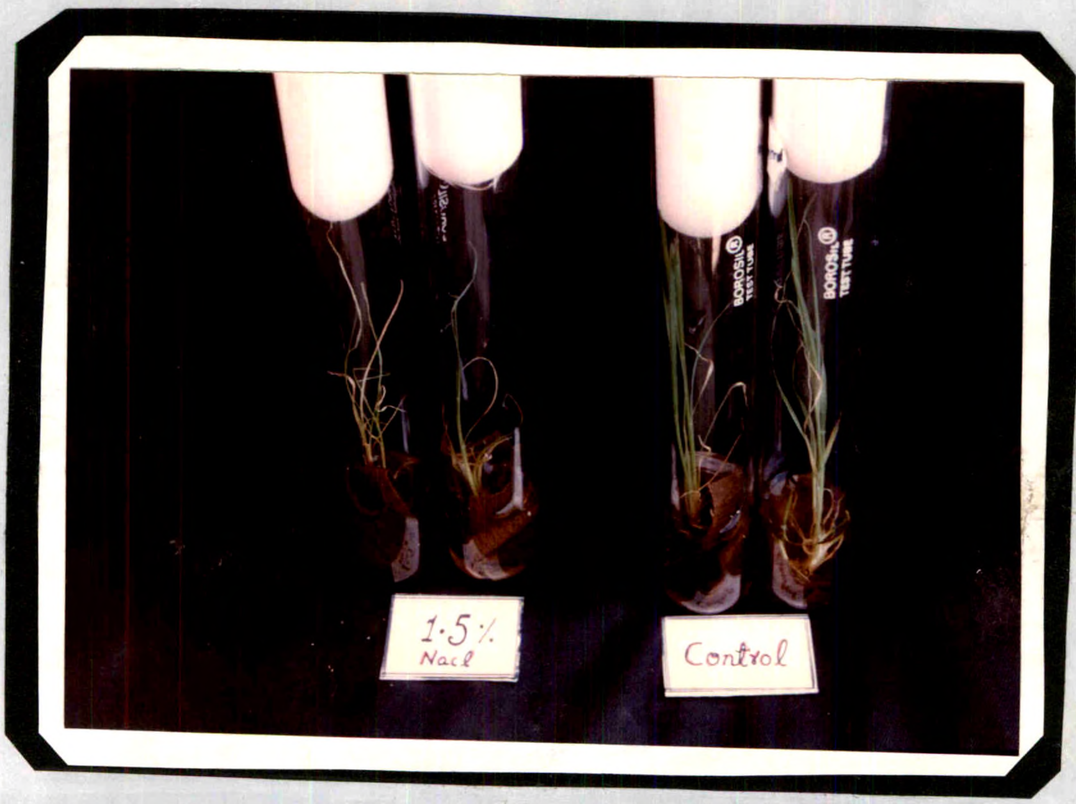
Fig.8. Effect of NaCl salt concentrations on growth of the regenerated shoots variety CO7219.

C

Fig.9. Effect of NaCl salt concentrations on growth of the rooted plantlets in liquid medium of the variety CO740.

A

B



C

A

B

Fig.10. Effect of NaCl salt concentrations on growth of the rooted plantlets in liquid medium of the variety CO7219

C

forty tubes were kept for observation and maintained in the laboratory for 16 hours light alternating with 8 hours dark at 24 ± 1°C.

The analysis of variance (Table 11) of the plants survived showed significant differences for treatments, varieties and treatment x variety interaction at 1 per cent level of significance. After second inoculation the treatment and treatment x variety interaction effects were significant at 1 per cent whereas the variety effects were non-significant. Observations from Table 12 showed that after one week in the treatment 1.5 per cent only 31.87 per cent plants survived as against 43.11 per cent in 1.0 per cent and 58.11 per cent in 0.5 per cent. The plants showed reduced growth and pale yellow leaf colour. The tillering was not observed in 1.0 and 1.5 per cent salt concent rations. Among the varieties maximum mortality was observed in CO7219 i.e. 49.39 per cent and in CO740 the mortality was 39.07 per cent.

The mean performance of growing plantlets showed that, the difference after 1 week were significant among treatments and also in the varieties, however, after 2 weeks of inoculation, the treatments 1.0 and 1.5 per cent were on par and showed significant mortality than 0.5 per cent. The varietal differences were not evident.

Table 11 : ANOVA of the rooted plantlets tested at different concentrations of salt levels in liquid medium

Sr. No.	Source of variation	Df	MSS due to per cent of rooted plantlets showing growth after 1 week	MSS due to per cent of rooted plantlets showing growth after after 2 weeks
1.	Replication	3	231.97**	100.76*
2.	Treatment	3	5087.0**	6267.9**
3.	Variety	1	850.56**	63.265
4.	Treatment x varieties	3	194.48**	25.775**
5.	Error	21	33.808	31.13

* and ** significant at 5% and 1% levels of significance respecively.

Table 12: Mean performance of rooted plantlets cultured on different concentrations of salt levels in liquid medium.

Source of variation	Mean percentage of rooted plantlets showing growth after 1 week	Mean percentage of rooted plantlets showing growth after 2 weeks
Varieties		
Co 740	60.93	50.618
Co 7219	50.618	47.806
SE ±	1.4536	1.3949
CD at 5 %	4.4026	4.2247
Treatments		
Control	89.988	89.988
0.5 %	58.117	44.994
1.0 %	43.119	31.871
1.5 %	31.871	29.996
SE ±	2.0557	1.9726
CD at 5 %	6.2263	5.974

DISCUSSION

CHAPTER V

DISCUSSION

Salt tolerance is a widespread agricultural problem due to several reasons. The insufficient drainage, over irrigation, underground saline watertable, poor management of soils and tropical climatic conditions are some of the main factors contributing to saline soil formation. Salt affected soils are rich in Na^+, Mg^{++}, Cl^- and SO_4^{--} ions. Heavy losses in the productivity in different crops due to increased salinity has been reported in different crops, including Sugarcane.

The determination of absolute tolerance under *in vitro* conditions has rather been impossible because of the complex interactions between the plant systems and the environmental factors. However, the recent development in the *in vitro* technology offer a meaningful tool for determining the tolerance and also for screening and developing salt tolerance genotypes. Like most of other agronomic traits salt tolerance also seems to be under genetic control. Thus, the efficient *in vitro* screening even at the different seedling stages could result in plant types capable of withstanding the saline environment. The cells even from the same plant in culture, exhibit great natural variation which could be further increased by treating the cells with various mutagens.

Sugarcane (*Saccharum officinarum* L.) is widely grown in Maharashtra and suffered due to the increasing levels of salinity in different regions. In some of the parts the indiscriminate use of irrigation water resulted very high salinity level and even the crop could not germinate, even if it germinated the growth was poor and the tillering was very poor (Anonymous 1993).

Looking to the importance of growing problems of salinity an attempt was made in the present investigation on *in vitro* regeneration and salt tolerance in sugarcane using two varieties viz., CO740 and CO7219 with the following objectives,

1. To study the induction of callus in different varieties,
2. To study the regeneration ability and possibility of spontaneous mutations if any,
3. To study the regenerated plants (without roots) for tolerance to salinity in solid medium,
4. To study the rooted plants for tolerance to salinity in liquid medium

5.1 Callus initiation and proliferation

The observations on the two varieties of Sugarcane viz., CO740, and CO7219 with two concentrations of 2, 4-D i.e. 1 mg and 2 mg/l revealed that there were no differences for number of days required for callus initiation in these two varieties. About 7-8 days are required for callus initiation. Naik and Babu (1988) reported initiation of callus in between 5-7 days after inoculation in different varieties. Lal and Singh (1991) observed callusing, approximately 90% explants within two weeks in the treatment 5 mg 2, 4-D/l.

Differential response of the genotypes for callus induction has been reported by Cheema *et al.* (1992) and Singh and Sinha (1987). In the present study the differences in callus initiation were not evident.

The mean number of days required for callus initiation were not significant in the treatments of 2,4-D however, the mean was more for 2 mg/l of 2,4-D in both the varieties. Lal and Singh (1991) reported extensive callus formation with 2mg and 5mg 2,4-D/l, whereas it was moderate at 1 mg/l.

The treatment differences for the callusing ability were significant. The treatment 1mg 2,4-D/l showed significantly higher callusing than the treatment 2 mg 2,4-D/l, however, the varietal differences were non-significant and similarly the treatment x variety interaction was non-significant.

The callus growth can be measured with the fresh and dry weight of the callus. The treatment and variety differences in the fresh weight of callus after 25 days of second subculturing were significant, however, for the dry weight it was non-significant.

The mean fresh weight as well as the dry weight was more in the variety CO7219 than CO740. Similarly for fresh weight the treatment 1 mg 2,4-D/l was significantly superior over 2mg 2,4-D. There was a direct relationship between the volume of callus to fresh weight. Lal and Singh (1991) also reported similar results.

5.2 Plant regeneration and proliferation

Organogenesis can be brought out in callus by controlled initiation of an organ primordia through manipulation of the nutrients and hormonal constituents in the culture media. The phenomenon is dependent on a number of factors such as source of origin of callus, its genotype and endogenous hormone level. (Corneso and Primo, 1979). Development of green spots (shoot primordia) occured after 4-5 days of culturing and shoots appeared during the second week of incubation in the present study. Shoot regeneration ranged from 93.3 per cent in CO7219 to 96.6 per cent in CO740 and the number of shoots ranged from 10 - 30 per callus mass. It was interesting to note that the whole callus mass got differentiated into shoots resulting into heavy crowding. It was necessary to separate out these shoots for their subsequent normal growth.

Cheema *et al.* (1992) observed a range of 85-100 per cent shoot regeneration and 15-35 shoots per calli in ten genotypes of sugarcane, They also reported that the shoot apex is good for callus induction and shoot regeneration than the axillary buds. Similar results are also reported by (Larkin 1982).

Use of cytokinins particularly in the form of BAP was also reported by Singh and Sinha (1987), Barba *et al.* (1979) and Cheema *et al.* (1992).

5.3 Root initiation and proliferation

The regenerated shoots of both the varieties were cultured on the media supplemented with 1 mg/l NAA and 0.5mg/l IBA. In the variety CO740 highest rooting was observed 97.14 per cent, which was 88.57 per cent in CO7219.

Cheema *et al.* (1992) reported 97.8 per cent of shoots in the media supplemented with 5 mg/l NAA. There were no differences in the root initiation and it's growth in both the varieties.

5.4 Studies of regenerated plantlets (without roots) on salt media

Most of the salt stresses in nature are due to Na^+ salts, particularly NaCl. Inspite of the several difficulties in estimating salt tolerance limits of various plants, Levitt (1980) has reported the wide range of limits of stress survival. Spinach and garden beet are tolerant and often show a stimulation of growth by levels of NaCl that would be lethal for the pea (Bernstien and Hayward, 1958).

Naik and Babu (1988), reported differential response of salt tolerance in sugarcane varieties. The growth of the callus was inhibited beyond 0.5 per cent salt concentration. In the present study, the differences for growth among the varieties and concentrations of salt were significant for the normal growth after ten days of observation. After twenty days of observation also, the variety and treatment differences for the normal growth were significant.

Among the varieties CO740 showed better growth and reduced mortality as compared to CO7219 in both the sets of observations. Naik and Babu (1988) also reported slightly higher limit of salt tolerance in the variety CO740 and differential response of the genotypes under saline conditions. In the present studies reduction of shoot height, growth, leaf area, tillering was much more in the variety CO7219 than CO740. Similar results are also reported by Joshi and Naik (1981).

Among the treatments, the highest mortality of the plants was observed in the treatment 1.5 per cent followed by 1 per cent both after 10 and 20 days of observations. Gosal and Bajaj (1984) also reported the lethal effects beyond 0.5 per cent in grain legumes. The possible mutations in the tissue culture for resistance to salt stress can be classified in 3 major groups, 1) Mutations resistant to osmotic stress, 2) Mutations resistant to stress caused by high concentration of total salts, 3) Mutations resistant to stress caused by specific ions (Goldner *et al* .,1977). In the present study, the normal growing shoots were studied on different treatments of salt concentrations and possibility of *in vitro* spontaneous mutations was explored. The plants growing on high concentrations of salt needs to be studied further for chances if any for tolerance to salt.

5.5 Studies on rooted plantlets

The rooted plantlets of the two varieties viz., CO740 and CO7219 were cultured on 0.5, 1.0, 1.5 per cent of the salt concentration in addition to control treatment. The differences due to varieties, treatments and the Treatment x variety interactions were significant after one week of observations. There was differential response of the varieties to the varying salt concentrations in the first week of observations, however, the varietal differences were non-significant after second week of observations. This indicatedthat the testing is to be continued beyond second week of observation also. The treatment differences were significant. The treatment x variety interaction effects were significant.

The variety CO740 showed better percentage of survival and growth than the CO7219 in both the sets of observations. In the treatment of salt concentration the mortality was very high in 1.5 per cent treatment followed by 1.0 and 0.5 per cent. There was heavy reduction of height, tillering, root proliferation and plants turned pale yellow and died in the high concentrations of the salt. Joshi and Naik (1977) studied the variety CO740 for tolerance to salinity in liquid medium. Greenway and Munns (1980) recommended that in breeding for salt-resistance, concentration be placed as the ability of the plant to synchronize between an effective compartmentation of the absorbed solute by the leaf cell and high rate of transport of these ions in the shoot. In the present study, a chance was explored to isolate a spontaneous mutant for tolerance to salinity. The surviving plants need to be studied further.

5.6 Implication in the breeding approach

In vitro technique offers a very good source of creation of variability if a proper approach is followed to tackle the given problem (Larkin and Scowcroft, 1981). Induction of callus from the young plant leaves and apical buds and study of this callus/cell suspension on different concentrations of sodium salt and further selection of the resistant cell lines may lead to achieve the goal. This can be supplemented with mutagenesis at the callus level.

The plants obtained in the present study which showed growth in 0.5 and 1.0 per cent of the concentrations needs to be cultured further and studied for the growth parameters. The simulated salinity in the pots will be useful to indicate tolerance to salinity. It is further proposed that the callusing be initiated on simulated salt medium, the callus should be exposed to different salt concentrations and cell lines be established having tolerance to salinity. These plantlets also be studied in the simulated salt concentrations in field or pots.

SUMMARY

CHAPTER VI

SUMMARY

The present investigation was undertaken to study the callusing ability, callus growth, shoot and root initiation and the effect of levels of salinity on the growth and mortality in the Sugarcane varieties viz., CO 740 and CO 7219 using apical meristematic tissue (shoot tip) as explant on MS medium. The results of the present investigation are summarised below.

1. The callus initiation in both the Sugarcane varieties required almost same time in between 7 -8 days.

2. Varieties did not show significant difference for callusing ability, however, the callusing ability in the variety CO 7219 was higher than CO 740.

3. The treatment differences were significant for callusing ability and the treatments MS ± 1mg 2,4-D/1 proved most effective for inducing callus.

4. The growth measurement of calli were recorded on the function of increase in their fresh and dry weight which was more in the variety CO 7219 than CO 740.

5. Callusing treatment MS ± 1mg 2,4-D/1 was superior over fresh and dry weight over MS ± 2mg 2,4-D/1.

6. The ability of regeneration was found to be higher in CO 740 than CO 7219.

7. Better shoot regeneration was observed in CO 740 than CO 7219.

8. The response of regenerated plantlets (without roots) tested on different levels of salt concentrations showed significant differences due to the salt treatments. The highest mortality of the plants was observed in the treatments 1.5 per cent followed by 1.0 per cent, both after ten and twenty days of observations. In 0.5 per cent treatments, the growth was reduced than the normal and showed sparced tillering.

9. The response of regenerated plantlets (without roots) tested on different levels of salt concentrations also showed significant differences due to varieties. Among the varieties CO 740 showed better growth and reduced mortality as compared to CO 7219.

10. The response of the rooted plantlets tested on different levels of salt concentrations also showed significant differences for treatments. In the treatment of salt concentrations, the mortality was very high in 1.5 per cent followed by 1.0 and 1.5 per cent. Heavy reduction of height, tillering and root proliferation was observed. The plants turned pale yellow and finally died.

11. The response of the rooted plantlets tested on different levels of salt concentrations also showed significant differences for varieties after first week of observation, however, the varietal differences were non-significant after second week of observations. The treatment x variety interaction effects were significant. The variety CO 740 showed better percentage of survival and growth than the CO 7219 in both the sets of observations.

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