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- 2 EFFICACY OF DIFFERENT WEEDICIDES TO CONTROL WEEDS IN SUGARCANE  
Muhammad Aslam and Muhammad Naseem
- 6 ECONOMIC POTENTIAL OF INTERCROPPING RAYA IN AUTUMN PLANTED SUGARCANE  
Muhammad Aslam, Arshad Ali Chattha and Muhammad Bakhsh
- 11 IN VITRO SELECTION OF SOMACLONES OF SUGARCANE UNDER DROUGHT STRESS CONDITION AND THEIR EVALUATION IN FIELD CONDITION  
M W Islam, M A S Miah, M H R Pramanik, M A Hossain, M K Begum and M S Islam
- 25 EFFICACY OF SPLIT FERTILIZATION FOR SUGARCANE INTERCROPPED WITH POTATO AT TWO IRRIGATION LEVELS  
M. A. B. Siddique, G. C. Paul, M. N. A. Miah and A. S. M. Amanullah
- 30 SUGAR INDUSTRY ABSTRACTS  
M. Awais Qureshi and Shahid Afghan

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# EFFICACY OF DIFFERENT WEEDICIDES TO CONTROL WEEDS IN SUGARCANE

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## ABSTRACT

A field study to determine the relative efficiency of cultural and chemical weed control in spring planted sugarcane was conducted at Sugarcane Research Station, Khanpur during 2007. Four weedicides namely Krismat 75 W.G, Prexmixtra gold 720 S.C, Authority 4 F, Atrazine+Ametryne 80WP and hand weeding were tested against weedy check. The results revealed that both control measures gave better weed control as compared to weedy check. Hand weeding excelled with 85.13 percent weed control comparably followed by Krismat and Atrazine+Ametryne. An increase in cane yield ranging from 4.02 to 33.43 percent was observed. Hand weeding, Krismat application and Atrazine+Ametryne spray gave matching maximum cane yields of 111.03, 110.63 and 102.88 tons per hectare, respectively.

**Key words:** Sugarcane, weedicides, weeding, yield.

## INTRODUCTION

The presence of weeds is a serious menace to the economic crop harvests. They give an ugly look to the fields and compete with the crop plants for nutrients, light, heat energy and space. In addition, weeds exert stress to the cultivated crops through their allelopathic effects and parasitism. Weeds also harbor insects and disease organisms (Anderson, 1983). In Pakistan yield losses in sugarcane are not less than 15-30 % due to weeds under normal conditions (Makhdoom, 1986). Shafi *et al.*, 1994 studied the comparative effect of herbicides and hand weeding on weed control in sugarcane. Gesapex Combi 80 WP and hand weeding controlled the weeds up to 75 and 70 percent and gave an increased cane yield of 65 and 68 tonnes per hectare, respectively against 41 tons per hectare of control plot. Aslam *et al.*, 2000 concluded from a field trial that manual and chemical control suppressed weed population up to 76.64 % and 73.00 %, respectively. They recorded 20.72 % increase in final cane yield. Deho *et al.*, 2002 determined the effect of mulching methods and hand weeding on weed management, growth and yield of sugarcane. They recorded lowest weed biomass in hand weeding treatment followed by trash mulched plot. A reasonable increase in cane yield was recorded due to weed control measures. In an other field study Aslam *et al.*, 2003 found 15.14 % increase in cane yield with wheat straw mulch, 14.87 % with cane trash, 14.34% with interculture and 14.02% with weedicides over control owing to better tillering, cane weight and cane density. An experiment conducted by Cheema and Mahmood, 2005 revealed that all the chemicals gave significantly better weed control, number of millable canes, stripped cane and sugar yields as compared to weedy check. The results of a field experiment conducted by Oad *et al.*, 2007 showed that both the manual and chemical weed control increased cane yield over weedy check. Two weedings with spade gave 51.28% more cane yield over control owing to increased germination, tillering and cane girth. Keeping in view the importance of weeds and their control in crop husbandry, the present study was undertaken to quantify the effect of cultural and chemical weed control practices on the growth and yield of sugarcane.

## MATERIALS AND METHODS

Relative efficacy of different weedicides in comparison with hand weeding was studied at Sugarcane Research Station, Khanpur during 2007. A commercial sugarcane variety SPF 234 was planted by dry method in 1.2 meter apart furrows in the third week of February using recommended seed rate of 75000 double budded setts per hectare. Fertilizer was applied @ 168-112-112 Kg, NPK per hectare. Three weeding were done at 15 days interval in hand weeding treatment. The weedicides were sprayed at post emergence level to crop and weeds according to the following treatments:

- T<sub>1</sub> = Krismat 75 W.G. @ 1 Kg ha<sup>-1</sup>
- T<sub>2</sub> = Preximixtra gold 720 S.C. @ Lha<sup>-1</sup>
- T<sub>3</sub> = Authority 4 F @ 1.5 Lha<sup>-1</sup>
- T<sub>4</sub> = Atrazine+Ametryne 80WP @ 2.5Kg ha<sup>-1</sup>
- T<sub>5</sub> = Hand weeding
- T<sub>6</sub> = Weedy check

The experiment was laid out in Randomized Complete Block Design with a net plot size of 3.6 x 12 m and four replications. All the agronomic practices were kept uniform except the treatments. Observations pertaining to weed population, cane germination and tillering were recorded during the course of study using standard procedures. Data on millable cane weight, density and yield were recorded at harvest during the first fortnight of December. The data so collected were analyzed statistically using analyses of variance technique. Least significant difference test was applied to compare the treatment means at five percent level of probability (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

### Weed Control

The data presented in table 1 show that all the treatments decreased weed population significantly over control. Statistically higher numbers of weed plants (186.18 m<sup>-2</sup>) were recorded in weedy check plot. Lowest number of weeds (27.67 m<sup>-2</sup>) was recorded for hand weeding which shows 85.13% weed mortality. It was non-significantly followed by Krismat and Atrazine + Ametryne sprayed treatments with 83.25 and 78.77% weed mortality, respectively. A significant weed mortality due to chemical and cultural control measures has also been reported by Aslam *et al.*, 2000, Oad *et al.*, 2007 and Shafi *et al.*, 1994.

### Germination and Tillering

The germination of sugarcane was not affected by the treatments to a significant level (table 1). The non significant average germination ranged from 48.66 to 51.23%. The similar number of germinates in all the plots may possibly be due to the fact that all the treatments were applied after the emergence of crop plumule.

The data on number of tillers per plant show a significant role of weed control treatments in promoting cane tillering. All the tested weed control measures gave significantly higher number of tillers per plant over control. Hand weeded plots produced maximum number of tillers per plant (2.84) which was followed by Krismat (2.81) and Atrazine + Ametryne(2.62). The later two were at par with each other. Minimum number of per plant tillers (1.72) was recorded for weedy check. The data show that weed control decreased crop-weed competition

and as such tiller formation was improved. Shafi *et al.*, 1994, Aslam *et al.*, 2000 and Oad *et al.*, 2007 also arrived at the similar conclusions.

### **Cane Weight and Density**

The weight of individual cane stalks is one of the most important yield attributing characters which directly contribute to the final cane yield. A perusal of the data given in table-2 showed that different treatments exerted a pronounced effect on cane weight. All the treated plots produced heavier canes than the untreated control. Hand weeding gave the maximum hundred cane weight of 101.14 kg which was comparably followed by Krismat and Atrazine + Ametryne sprayed plots while the control plot gave the minimum of 83.94 kg. It shows that low weed crop competition promoted crop growth which raised cane weight considerably in the treated plots.

Millable cane density directly reflects the final cane yield. The data embodied in table 2 revealed that the treatments gave measurable differences among the means of final cane stand. All the treatments improved cane formation. Matching maximum number of millable cane stalks of 111.21, 109.78 and 104.62 thousand per hectare were recorded for Krismat sprayed, had weeded and Atrazine + Ametryne applied plots, respectively. Minimum cane count of 99.14 thousand per hectare was recorded for untreated control. The increase in final cane stand may be attributed to the better nutrient uptake by the cane shoots in the treated plots due to reduced weed crop competition. As such tiller mortality was reduced and better crop stand was established. The results are in line to the findings of Aslam *et al.*, 2000, Aslam *et al.*, 2003 and Oad *et al.*, 2007.

### **Stripped Cane Yield**

The ultimate goal of grower is to harvest better cane tonnage per unit area. The cane yield data packed in table 2 indicate that the differences in the final cane yield of different treatments were measurable. All the chemical and manual weed control methods gave substantial yield increase over control. Matching highest cane yields of 111.03, 110.63 and 102.88 tons per hectare have been harvested from hand weeded, krismat and Atrazine + Ametryne sprayed plots, respectively. Untreated control plot fetched the bottom position by producing a cane yield of 83.21 t/ha. Hand weeding and krismat spray treatments on account of better tillering, increased cane weight and density gave 33.43 and 32.95 % increase in cane yield over control. These results are in agreement to those of Shafi *et al.*, 1994, Aslam *et al.*, 2000, Deho *et al.*, 2002, Aslam *et al.*, 2003, Cheema and Mahmood, 2005 and Oad *et al.*, 2007.

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Table 1 WEED POPULATION, CANE GERMINATION AND TILLERING AS AFFECTED BY WEED CONTROL TREATMENTS

Treatment	Weeds / m <sup>2</sup>	Germination %	Tillers / plant
Krismat 75 W.G	31.18d	51.23	2.81ab
Premixtra gold 720 S.C	47.46c	50.56	2.58c
Authority 4 F	65.19b	49.34	2.03d
Atrazine+Ametryne 80WP	39.51cd	48.66	2.62bc
Hand weeding	27.67d	50.63	2.84a
Weedy check	186.18a	49.48	1.72e
LSD 0.05	15.41	N.S	0.21

Values with different letter(s) differ significantly (P=0.05)

Table 2 CANE DENSITY, WEIGHT AND YIELD AS AFFECTED BY WEED \ CONTROL TREATMENTS

Treatment	Cane density (000 ha <sup>-1</sup> )	100-cane weight (Kg)	Cane yield Tons ha <sup>-1</sup>	Variation in yield %
Krismat 75 W.G	111.21a	99.48a	110.63a	32.95
Premixtra gold 720 S.C	101.42bc	89.74bc	91.01b	09.37
Authority 4 F	100.26bc	86.34c	86.56b	04.02
Atrazine+Ametryne 80WP	104.62abc	98.34ab	102.88a	23.64
Hand weeding	109.78ab	101.14a	111.03a	33.43
Weedy check	99.14c	83.94c	83.21b	---
LSD 0.05	9.63	8.91	9.86	---

Values with different letter(s) differ significantly (P=0.05)

## ECONOMIC POTENTIAL OF INTERCROPPING RAYA IN AUTUMN PLANTED SUGARCANE

\*Muhammad Aslam \*\*Arshad AH Chattha \*\*\*Muhammad Bakhsh ABSTRACT  
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### ABSTRACT

Agro-economic studies on intercropping raya by gupchat and drill method in autumn planted sugarcane were carried out at Sugarcane Research Station, Khanpur during the year, 2006. Sugarcane was sown alone in September and after harvesting Raya in March or in combination with raya varieties i.e., Khanpur raya and Anmol raya by gupchat and drill method. The results indicated non significant effect of intercrops on germination and significant on yield and its attributing characters. Cane planted alone superseded in yield and its allied components. However, the additional harvests of intercrop raised the gross and net income of intercropping treatments. Sugarcane intercropped with "Khanpur raya" by gupchat method gave the highest net income of Rs. 106.25 thousands per hectare followed by sugarcane sown in March after harvesting "Khanpur raya".

**Key words:** Sugarcane, intercropping, Khanpur raya and Anmol raya.

### INTRODUCTION

Economically high Agricultural productivity is a prerequisite to foster an efficient and competitive agricultural industry. As sugarcane production involves a heavy long term financial investments so there should be a source of interim income especially for the small growers which will reduce the sugar production cost also. Intercropping is a convincing approach to achieve a reasonable interim income. Autumn planted sugarcane passes a dormant growth phase of about four months in its early days due to low winter temperature and makes a little use of soil and water resources. In order to derive benefits from this early slow growth and to make better use of resources, an additional sweep of short duration intercrop can be harvested. However, adjustment in crop management practices is needed for successful maturity of either of the crops. Nazir *et al.* (9) received the highest yield of alone cane (91.13t/ha) closely followed by cane + mash (87.08t/ha) and cane + soyabean (86.71t/ha). Higher B.C.R. (3.02) was recorded from sugarcane intercropped with mash bean. Malik and Kamoka (8) observed that raya over shadowed the cane crop and affected the tillering and cane density adversely. Though yield reduction of 9.63 percent was reported but the net income from cane + raya was significantly higher than cane alone. Aslam *et al.* (2) found that intercropping mung and maize did not affect the germination and tillering of sugarcane. While cane formation and yield was measurably depressed. The cane + mung intercrop gave significantly higher net income with 23.23% gain over cane alone. Aslam *et al.* (3) conducted a field trial and reported higher cash returns (Rs.23197/ha) by intercropping mung in sugarcane. In another field experiment Aslam *et al.* (4) concluded that intercropped soyabean and mung bean did not affect the cane yield and its components significantly. Intercropping treatments gave slightly better net income than sole sugarcane. According to Aslam *et al.* (5), although intercropping raya and sunflower depressed cane yield, yet the net income was relatively greater than that from September planted alone cane. Afzal *et al.* (1) undertook a study on intercropping sunflower in spring planted sugarcane and recorded statistically similar cane yields in alone and intercropped sugarcane. Chattha *et al.* (7) planted sugar beet in sugarcane and recorded a magnificent increase in gross income due to intercropping.

The present field study was carried out to explore the economic feasibility of intercropping raya in autumn planted crop of sugarcane.

## **MATERIALS AND METHODS**

The trial was conducted at Sugarcane Research Station, Khanpur during year, 2006 to find out the economic potential of intercropping raya in autumn planted sugarcane. A commercial sugarcane cultivar SPF.234 was planted in the second week of September at 1.2m row distance, while raya was intercropped in the first week of October. The planting of sugarcane was done by dry method using a seed rate of 75000 DBS/ha, the field was fertilized at the rate of 168:112:112 Kg NPK/ha. Full dose of Phosphorous and Potash was applied at the time of sowing. Nitrogen was applied in three split doses, 1/3 at the completion of cane germination, 1/3 at tillering of sugarcane in the last week of January and the remaining 1/3 N was added after harvesting the intercrop in March. The experiment comprised of seven treatments as detailed below.

- T<sub>1</sub> = Sugarcane alone in September
- T<sub>2</sub> = Sugarcane after harvesting "Khanpur Raya".
- T<sub>3</sub> = Sugarcane after harvesting "Anmol Raya"
- T<sub>4</sub> = Sugarcane + "Khanpur Raya" sown by gupchat
- T<sub>5</sub> = Sugarcane + "Anmol Raya" sown by gupchat
- T<sub>6</sub> = Sugarcane + "Khanpur Raya" sown by drill
- T<sub>7</sub> = Sugarcane + "Anmol Raya" sown by drill

The experiment was planted in Randomized Complete Block Design with four replications and a net plot size of 3.6 x 12m. Raya was intercropped as per treatments. Thinning of intercrop was done twice, at 6 inches and 9 inches plant height. All other cultural operations were performed as and when required by the crops. The yield of intercrop was recorded after harvesting and drying the grains in the first week of March. In treatments 2 and 3 sugarcane was sown in the third week of March. Meanwhile observations were recorded on germination and tillering of sugarcane. Data on cane density, weight and yield were recorded at harvest during the last week of December. The recorded data were then analyzed by using Analysis of Variance techniques and Least Significant Difference test was applied at five percent probability level to compare the treatment means (10).

## **RESULTS AND DISCUSSION**

### **Germination and Tillering**

The data presented in table-1 indicate that different intercropping treatments did not affect the germination of sugarcane probably because it has emerged out during the germination phase of intercrop before the start of active plant competition. The tabulated data depict the depressing effect of intercrop on the tiller formation. The depressing effect was more pronounced where raya was sown by gupchat. However, the intercrop Varietal difference was non significant in this regard. Sugarcane planted alone in September produced maximum tillers/plant (3.11), followed by sugarcane sown alone in March at the harvest of raya (2.63). The lowest tillers per plant (1.30) have been recorded in the plots where raya was sown by gupchat method. Relatively more reduction in the expression of tillering potential in these treatments was probably due to the closer competition as compared to the drill sown treatments. The depressing effect of intercrops on cane tillering has also been reported by Aslam *et al.* (5) and Malik and Kamoka (8). Millable Cane Density and Weight Millable cane

density in an important yield attributing character and is the interaction of germination, tillering and tiller mortality. The mean cane stand differences were statistically significant as shown by the data embodied in table 2.

September planted sole cane gave significantly high cane density of 102.78 thousand canes per hectare. It was matching followed by the sugarcane sown in March after harvesting raya. The sugarcane intercropped with raya sown either by gupchat or drill, reduced cane formation significantly. The depressing effect of raya on cane formation may be attributed to the corresponding lower tillering and relatively more tiller mortality due to shading effect of the intercrop. Similar conclusions have also been drawn by Aslam *et al.* (5) and Malik and Kamoka (8).

The data recorded in table-1 for hundred cane weight reveal significant differences among the means of different treatments. The cane stalks planted in September either alone or with intercrops were heavier than the spring planted cane probably because of the prolonged growth period available to the former. The minimum hundred cane weights of 93.50kg were recorded for the cane planted after harvesting raya in March. Crop Yields

The final crop yield is the ultimate goal of each and every grower. A perusal of the data given in table 2 exhibit that the differences among the means of cane yield in the treatments were statistically significant. Autumn planted alone sugarcane produced the highest cane yield (115.33t/ha). None of the other treatments could match it. The sugarcane intercropped with raya sown by drill method gave slightly more yield than the gupchat treatments but these were statistically at par with one another. Sugarcane planted after the harvest of raya in March gave the least tonnage of 95.60 per hectare. These yield losses were compensated by the additional harvests of intercrop. The "Khanpur Raya" when planted alone gave a produce of 2.37t/ha. The same variety of raya produced 2.1 It/ha when intercropped by gupchat method and 1.72t/ha when sown by drill method. The raya variety Anmol produced lower than Khanpur Raya in all the treatments. The yield results are quite in line to those of Aslam *et al.*(4), Chattha *et al.*(7) and Malik and Kamoka(8). Economic Benefits

The economics of different crop combinations worked out in terms of gross income, cost of production and net income is given in table-3. The data show that the gross income received from either of the intercropping combinations was higher than the alone cane sown in September. The highest gross income of Rs. 214.10 thousands per hectare has been calculated for sugarcane intercropped with "Khanpur Raya" by gupchat method followed by the sugarcane planted after harvesting Khanpur Raya(Rs.208.57 thousands /ha). Net income was also greater for sugarcane intercropped with "Khanpur Raya" by gupchat method. The EMV of all the intercropping treatments was greater, which advocates the higher net returns from intercropping treatments. The highest EMVs of 1.51 and 1.42 show economic superiority of sugarcane + Khanpur Raya sown by gupchat and sugarcane sown after harvesting Khanpur raya, respectively. Similar economic gains due to intercropping have also been reported by Aslam *et al.*(2,3,4,5), Bahadar *et al.*(6) and Malik and Kamoka (8).

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Table-1 Germination, Tillering and Cane weight as affected by different intercropping systems

S. No.	Treatment	Germination %	Tillers /plant	100-cane weight (Kg)
1.	Sugarcane alone in Sept.	44.40	3.11 a	112.25 a
2.	Sugarcane after KPR Raya	41.59	2.63 b	95.00 b
3.	Sugarcane after Anmol Raya	42.30	2.55 b	93.50 b
4.	Sugarcane+KPR Raya Gupchat	44.63	1.33d	108.75 a
5.	Sugarcane+Anmol Raya Gupchat	44.65	1.30 d	107.25 a
6.	Sugarcane+KPR Raya drill	44.84	1.52 c	109.00 a
7.	Sugarcane+Anmol Raya drill	44.83	1.54 c	110.25 a
	LSD 0.05	N.S.	0.11	5.65

Treatments having no or same letter do not differ significantly (P=0.05)

Table-2 Cane Density and yield as affected by different intercropping stems

S.No.	Treatments.	Cane density (000/ha)	Cane yield (t/ha)	Variation (%)	Raya yield (t/ha)
1.	Sugarcane alone in Sept.	102.78 a	115.33 a	...	—
2.	Sugarcane after KPR Raya	100.58 a	95.60c	17.10	2.37
3.	Sugarcane after Anmol Raya	102.36 a	96.58 c	16.26	1.76
4.	Sugarcane+KPR Raya Gupchat	95.66b	104.05 b	9.78	2.11
5.	Sugarcane+Anmol Raya Gupchat	94.79 b	101.33 be	11.79	1.68
6.	Sugarcane+KPR Raya drill	96.35 b	105.09 b	8.87	1.72
7.	Sugarcane+Anmol Raya drill	95.48 b	105.32 b	8.67	1.58
	LSD 0.05	2.03	6.22	—	—

Treatments having no or same letters do not differ significantly (P-0.05)

Table-3 Economic analysis of different intercropping systems

s. No	Treatments	Mean yield (t/ha)		G. Income Rs.000/ha	Cost of production Rs.000/ha	Net income Rs.000/ha	Estimated Monetary Value (EMV)
		Cane	Raya				
1.	Sugar cane alone in Sept	115.33	—	172.99	102.49	70.50	—
2.	Sugarcane after KPR Raya	95.60	2.37	208.57	107.81	100.75	1.42
3.	Sugarcane after Anmol Raya	96.58	1.76	193.27	107.11	86.16	1.22
4.	Sugarcane+KPR Raya Gupchat	104.05	2.11	214.10	107.85	106.25	1.51
5.	Sugarcane+Anmol Raya Gupchat	101.73	1.68	198.79	106.61	92.18	1.30
6.	Sugarcane+KPR Raya drill	105.09	1.72	204.93	107.52	97.41	1.38
7.	Sugarcane+ Anmol + Raya drill	105.32	1.58	201.43	107.36	94.07	1.33

Sugarcane @ Rs.1500/ton and Raya @ Rs.27500/ton

# IN VITRO SELECTION OF SOMACLONES OF SUGARCANE UNDER DROUGHT STRESS CONDITION AND THEIR EVALUATION IN FIELD CONDITION

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## ABSTRACT

*In vitro* selection was conducted to select for drought tolerant somaclones in sugarcane variety Isd 28. Unexpanded spindle leaf sheath was used as explants. MS medium supplemented with 2, 4-D (3 mg l<sup>-1</sup>) and coconut water (10 %), BAP (2 mg l<sup>-1</sup>) with Kn (1 mg l<sup>-1</sup>), NAA (5 mg l<sup>-1</sup>) were used for callus induction, shoot regeneration and root formation respectively. To develop of drought tolerant sugarcane somaclones *in vitro* medium was stressed with different concentrations of PEG such as 5, 7.5 and 10 % along with control (without PEG). Callus growth was vigorous at control as well as at lower concentration of PEG. The frequency of callus induction decreased with the increase of PEG concentration. Shoot and root production capacity also decreased with the increase of PEG concentration. Most callus became reddish black and died within 40-45 d on the medium supplemented with 10 % PEG. Shoot and root formation observed on medium supplemented with 5 % PEG. Twenty somaclones were studied for drought tolerance. Biochemical parameters such as total chlorophyll, chlorophyll a, chlorophyll b, chlorophyll a:b ratio and CSI showed positive correlation as well as morphological parameters such as leaf number and leaf area showed negative correlation to drought tolerance in somaclones and parents. Tolerance level was measured based on greenness of leaves as well as data recorded on biochemical and morphological parameters. Close association with biochemical and morphological parameters was observed for drought tolerant reaction. Less variation on Brix per cent was observed among somaclones and their parents. Somaclones Isd 28Sc-3, Isd 28Sc-8, Isd 28Sc-11, Isd 28Sc-17 and Isd 28Sc-18 of parent variety Isd 28 were observed as highly tolerant somaclones.

## INTRODUCTION

Abiotic stress includes drought, salinity, extreme temperature; chemical toxicity and oxidative stress are serious threats to agriculture, and natural states of environment (Wang *et al.*, 2003). Abiotic stress significantly increased negative effects on both quantity and quality of crop production and limited plant growth and impaired productivity (Moshin, 2004). Drought is one of the principal environmental stresses limiting crop productivity around the world (Watanabe, 2002).

The sugarcane cultivars slowly deteriorate over a period of time due to vulnerability to pests, diseases, and especially to soil moisture stress during formative phase (Rao, 2003). Among environmental stresses, drought stress is one of the major problems in reducing crop yield. It hampers biochemical process of cane plant which affects growth and sugar synthesis. However, degree of damage varies with extent of drought and period it prolongs (Malik, 1992). In these circumstances, irrigation may not be considered a reasonable means of boosting up sugarcane yield (Miah, *et al.*, 1993). Sugarcane cultivation under drought stress received no attention in the past, but due to increased demand for sugar and gur, it has now become imperative to explore production potentiality of sugarcane.

Since conventional plant breeding methods are slow to create substantial improvement over drought stress, an alternative approach of utilizing plant cell culture regeneration of plants from potential cell mutant has received increased attention. Sugarcane varieties those are cultivated under drought stress do not have proven capabilities to grow under extreme stress condition (Miah, *et al.*, 1993).

We know that somaclonal variation brought desirable agronomic changes in the progeny and increased sugar yield in sugarcane. Variation in differentiation ability in *Saccharum spp.* has been reported elsewhere (Liu *et al.*, 1972; Banshali and kishan, 1982). Considering enrichment of sugarcane productivity under drought stress as well as to minimize demand of sugar, regeneration of drought tolerant varieties can play a vital role. To meet ever increasing demand of sugar and 'gur', regeneration of drought tolerant varieties is very important. To achieve, it is necessary to understand basic phenomenon of stress. Therefore, the present investigation was carried out to establish *in vitro* regeneration under induced drought stress condition, and *ex vitro* evaluation of regenerated somaclones for drought tolerance.

## **MATERIALS AND METHODS**

The experiment was conducted at the Biotechnology Laboratory and experimental farm of BSRI, Ishurdi during cropping season 2006-2007. The experimental materials used were unexpanded young leaf sheaths of sugarcane variety Isd 28. The explants (unexpanded leaf sheaths) were collected from 3-4 month-old-field grown sugarcane from BSRI experimental farm. The explants were cultured aseptically on MS (Murashige and Skoog, 1962) medium supplemented with different concentrations and combinations of growth regulators.

For the induction of callus, regeneration of shoots and roots MS medium was supplemented with 2,4-D ( $3 \text{ mg l}^{-1}$ ), BAP ( $2 \text{ mg l}^{-1}$ ) with Kn ( $1 \text{ mg l}^{-1}$ ) and NAA ( $5 \text{ mg l}^{-1}$ ) respectively (Islam, 2006). To develop drought stress tolerant sugarcane somaclones, *in vitro* medium was stressed with different concentrations of Polyethylene glycol (PEG) such as 5.0, 7.5 and 10.0 % along with a check (without PEG). Besides, agar (0.6 %) was added to make the medium semi-solid. At first callus was developed in MS medium supplemented with 2,4-D ( $3 \text{ mg l}^{-1}$ ) having different concentrations of PEG. To induce callus, 10 % coconut water along with PEG was also added to medium. Then calli were transferred to MS medium supplemented with BAP  $2 \text{ mg l}^{-1}$  and Kn  $1 \text{ mg l}^{-1}$  for shoot regeneration. Regenerated shoots were transferred to MS medium supplemented with  $5 \text{ mg l}^{-1}$  NAA for rooting. PEG was also added to both shooting and rooting media.

Rooted plants were washed under tap water to remove medium adhered with roots and planted in earthen pots containing sterilized soils and kept under polyethylene shed with higher moisture for 2-3 weeks to harden plants for successful establishment of transplanted somaclones in the field.

After hardening, plants were transplanted in the field of BSRI experimental farm for screening against field drought stress. Plants were transplanted in the field at line to line spacing 1m and pit to pit spacing 50 cm in the line. Plants were transplanted in pits containing soils and pressmud at 3:2 ratio. The soils-pressmud mixture of each pit was fertilized at the rate of 4, 3 and 3 g of Urea, TSP and MP respectively. Full TSP, MP and 2.0 g urea were applied in each pit as basal dose at transplanting time and the rest 2.0 g of urea was applied as top dressing at 45d after transplanting. Intercultural operations including irrigation, weeding and plant protection measures were done as and when required.

Data collection from *in vitro* drought stress stage:

To develop drought tolerant sugarcane variety, data were collected from *in vitro* drought stress stage on the following parameters:

- 1) Percent of explants induced callus
- 2) Degree of callus induction
- 3) Morphology of callus
- 4) Number of shoots per callus
- 5) Number of roots per shoot

In case of *ex vitro* drought tolerance studies, data were collected under field condition on the following parameters:

Chlorophyll a  
Chlorophyll b  
Total chlorophyll  
Chlorophyll a:b ratio  
Chlorophyll Stability Index (CSI)  
Leaf number  
Leaf area  
Visual tolerance rating  
Brix (%)

Amount of chlorophyll a, chlorophyll b and total chlorophyll were calculated on a fresh weight basis employing following formulae (Mahadevan and Sridhar, 1982):

$$\begin{aligned}\text{Total chlorophyll (mg g}^{-1}\text{)} &= \frac{20.2A_{645} + 8.02A_{663}}{a \times 1000 \times w} \times v \\ \text{Chlorophyll a (mg g}^{-1}\text{)} &= \frac{12.7A_{663} - 2.69A_{645}}{a \times 1000 \times w} \times v \\ \text{Chlorophyll b (mg g}^{-1}\text{)} &= \frac{22.9A_{645} - 4.68A_{663}}{a \times 1000 \times w} \times v\end{aligned}$$

Where,

A = Optical density in each sample

a = Length of light path in the cell (usually 1cm)

v = Volume of extract in ml and

w = Fresh weight of sample in 'g'

Chlorophyll Stability Index (CSI) is a good parameter for drought tolerance measurement in sugarcane. CSI was estimated following the method of Kilen and Andrew (1969). Number of green leaves of all canes from each pit was counted for this purpose. Ten leaves were randomly collected from each pit for this purpose. The leaf area (cm<sup>2</sup>) was measured by using leaf area meter (Model- CI-202 Area meter, USA). Brix readings were taken and recorded with the help of Hand Refractometer from randomly selected three canes of each pit. The Data collected were statistically analyzed following Completely Randomized Design (CRD) using computer software MSTAT-C. The analysis of variance was done and means were compared by Duncan's New Multiple Range Test (DNMRT) at 5% level of probability for discussion of results.

## RESULTS AND DISCUSSION

In the present investigation, techniques of *in vitro* callus induction, shoot regeneration and root formation of variety Isd 28 under control and PEG induced drought stress conditions, hardening of regenerated plants and their establishment in soil after transplanting, and *ex vitro* drought tolerance studies have carefully been described and discussed under following headings and sub-headings.

*In vitro* Regeneration under PEG Induced Drought Stress Condition Induction of callus under *in vitro* PEG induced drought stress condition:

For callus induction from leaf sheath explant of variety Isd 28, 2,4-D 3 mg l<sup>-1</sup> was used with different concentrations of PEG such as 5 , 7.5 and 10 % with a control (without PEG). Results of callus induction are presented in the Figure 1 and Tables 1-3. Calli were found to be initiated from cut edges of explants, and callus mass produced within 3-4 weeks of culture. Callus induction was affected by addition of PEG in the medium. The callogenic response of explants declined when PEG level was increased in MS medium. The highest percentage (95%) of explants responded to callus induction in control treatment (without PEG). With the increase of PEG level, frequency of callus induction was decreased. The lowest percentage (65%) of explants responded to callus formation when medium was supplemented with 10 % PEG. Taghian (2002) used three concentrations of PEG (50, 75, 100 g l<sup>-1</sup>) to determine selective concentration of PEG. It was reported that development of callus and its differentiation decreased in presence of PEG in medium. The formation of callus decreased from 88.33 % (in non-stressed medium) to 15.55 % when 50 g l<sup>-1</sup> PEG was added to medium. El-Aref (2002) observed that development of callus and its differentiation decreased with increase of PEG level from 2.5 to 10 %. At higher concentration (10 %), the most of cultured calli turned brown and failed to regenerate.

Table 1: *In vitro* effects of PEG % on callus induction in variety Isd 28

Treatments	Number of Explants	Response of explants for callus induction (%)
		Variety: Isd 28
Control	10	95
MS + 5 % PEG	10	90
MS + 7.5 % PEG	10	80
MS + 10 % PEG	10	60
Mean	-	81.3

Higher per cent of callus induction was recorded on medium without PEG as compared to medium fortified with various levels of PEG. Rate of callus induction decreased with increase of PEG level in the medium (Table 1). Poor callus induction was recorded at medium supplemented with 10 % PEG.

Table 2: *In vitro* effects of PEG on callus induction in variety Isd 28.

Treatments	Isd 28
Control	+++
MS + 5 % PEG	+++
MS + 7.5 % PEG	++
MS + 10 % PEG	+

\* Degree of callus induction: +++ = massive; ++ = moderate; + = poor

Whitish colour vigorous callus formed where no PEG was used in the medium. Most of callus formed became reddish in colour in medium where PEG was added. With the increase of PEG level callus became reddish black in colour. The callus became black and died after 40-45 d of incubation at the highest level (10 %) of PEG (Table 3).

Table 3: *In vitro* effects of PEG on morphology of callus formed in variety Isd 28.

Treatments	Variety: Isd 28
Control	Vigorous and whitish (Fig. 1a)
MS + 5 % PEG	Vigorous and reddish (Fig. 1b)
MS + 7.5 % PEG	Vigorous and reddish (Fig. 1c)
MS + 10 % PEG	Poor, reddish black and dead (Fig. 1d)

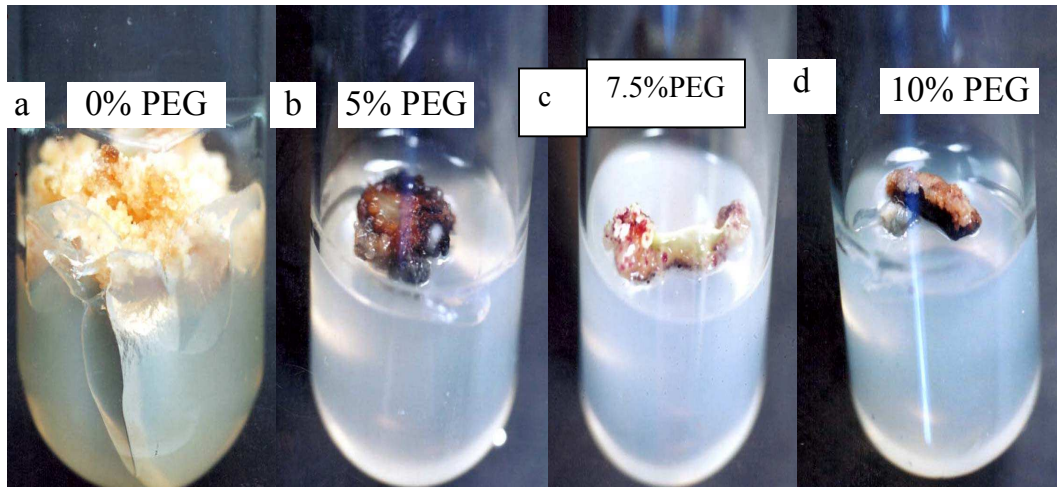
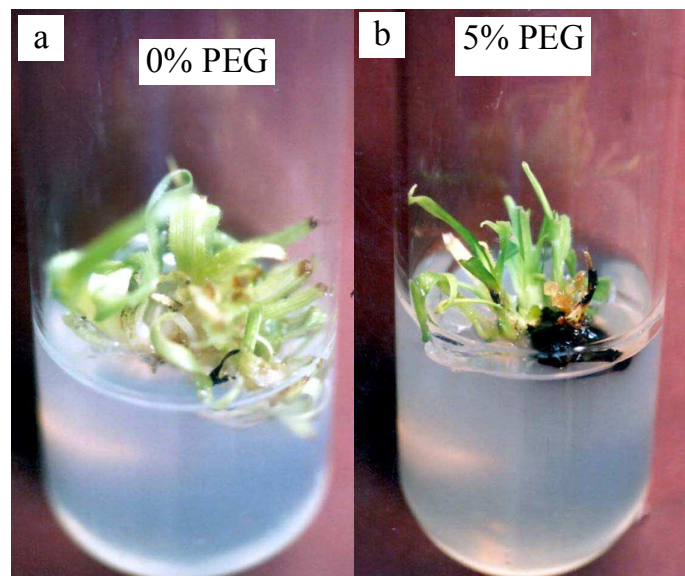


Figure-1 Effects of PEG concentrations (0, 5, 7.5, 10 %) on callus induction in variety Isd 28 (a-d).

Regeneration of shoots under PEG induced drought stress:

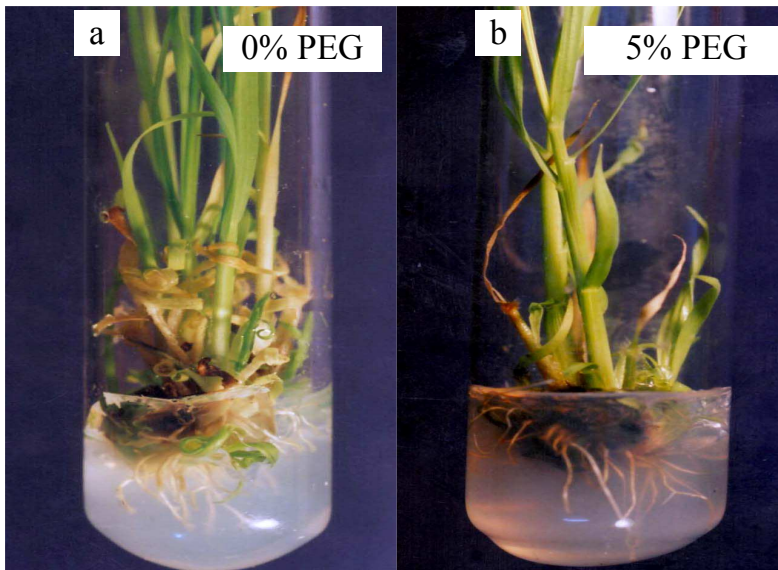
Results of shoot regeneration and multiplication have been presented in the Figure 2 and Tables 4-5. Higher number of shoots and usable shoots per culture were obtained where no PEG was used (Table 5). Similar results were reported by El-Aref, 2002. He stated that the regeneration of shoots decreased from 305.3 shoots per 100 calli in control medium to 14.5 shoots per 100 calli under 7.5 % PEG level. El-Tayeb and Hssanein (2000) observed that fresh and dry shoots of all tested lines were markedly decreased as level of PEG was increased.



**Figure 2. Effects of PEG levels (0 and 5 %) on shoot regeneration in variety Isd 28 (a-b)**

Root formation in shoots under PEG induced drought stress :

Results on root formation have been shown in the Figure 3 and Tables 4-5. The highest number of roots per shoot was observed where no PEG was used (Table 5). *In vitro* healthy and well established rooted plantlets were transplanted to earthen pots containing soils-pressmud mixture, and kept under hardening shed for 2-3 weeks (Fig 4).



**Figure 3. Effects of PEG levels (0 and 5 %) on root formation in variety Isd 28 (a-b)**

Table 4. Effects of PEG on shoot and root production under *in vitro* condition in variety Isd 28

Varieties	Total shoots number/culture	Usable shoots number/culture	Roots number/shoot
Isd 28	11.15	6.80	13.40

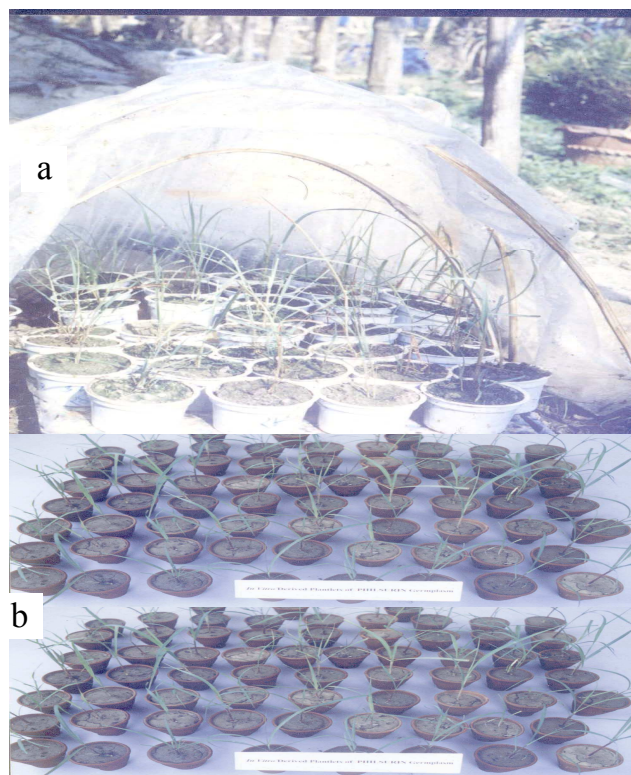
Table 5. Combined effects of PEG levels and variety on shoot and root production under *in vitro* condition

PEG × Varieties		Total shoots number/culture	Usable shoots number/culture	Roots number/shoot
PEG 0 %	Isd 28	13.00a	13.00a	14.00a
PEG 5 %	Isd 28	9.30c	7.50c	12.80b

\* Figures in the columns followed by different letters are significantly different by DNMR test at  $p=0.05$ .

#### Hardening of plantlets

Rooted plantlets were hardened for 2-3 weeks under locally made low cost polyethylene shed. After hardening, plantlets were directly transplanted to pits having soil-pessmud mixture in the field for drought tolerance studies.



**Figure 4.** Hardening of plantlets in locally made low cost polythene shade (a) hardened plantlets in small earthen pots; (b) plantlets are ready for transplantation to pits in the field.

## *Ex vitro* Evaluation of *In vitro* Regenerated Plants for Drought Tolerance

Results of Biochemical parameters such as chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a:b ratio, Chlorophyll stability Index (CSI) and Brix % as well as morphological parameters such as leaf number, leaf area and visual tolerance rating scale have been stated below:

Chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a:b ratio:

Biochemical parameters such as chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a:b ratio of different somaclones of parent variety Isd 28 have been presented in the Table 6. It is seen from the Table 6 that chlorophyll a, and total chlorophyll content was found significantly higher in somaclones Isd 28Sc-3 and Isd 28Sc-11. Chlorophyll b content was found significantly higher in Isd 28Sc-3 followed by Isd 28Sc-11. Chlorophyll a, chlorophyll b, and total chlorophyll content of all tissue culture PEG induced somaclones were recorded higher than their parent variety Isd 28. The chlorophyll a:b ratio was found significantly higher in Isd 28Sc-14. In somaclone Isd 28Sc-13, chlorophyll a:b was statistically identical to parent variety Isd 28. Results revealed that comparatively higher chlorophyll a, chlorophyll b, and total chlorophyll content, and lower chlorophyll a:b ratio was observed in PEG induced drought tolerant somaclones of variety Isd 28. Kamat *et al.* (2004) reported that chlorophyll a and chlorophyll b content are important characters for screening sugarcane varieties under rainfed situation due to their positive and significant correlation. However, Daneshmand *et al.* (2007) reported that drought stress increased chlorophyll a:b ratio because of greater decrease in chlorophyll b.

Chlorophyll Stability Index (CSI):

Chlorophyll stability index of all somaclones of Isd 28 regenerated from PEG induced drought tolerant callus have been presented in Table 7. In case of parent variety Isd 28, significantly the highest CSI was recorded in somaclone Isd 28Sc-3. Somaclones Isd 28Sc-11 and Isd 28Sc-17 showed statistically identical CSI values. The lowest CSI was recorded in their parent variety Isd 28. CSI of all tissue culture PEG induced somaclones were recorded higher than their parent variety Isd 28 (Table 7). Kaloyereas (1958) described a method of chlorophyll stability analysis for determining relative drought resistance. Chlorophyll stability index is the difference between colorimetric reading of chlorophyll extract from heated and unheated leaf samples. Table 7 show that higher the CSI, higher the drought tolerance and lower the CSI, lower the drought tolerance. Mohan *et al.* (2000) reported that the chlorophyll stability index is an indication of stress tolerance capacity of plants. A high CSI value means less drought stress effects on chlorophyll content of plants. A higher CSI helps plants to withstand stress through better availability of chlorophyll. This leads to increase photosynthetic rate, more dry matter production, and higher productivity. It indicates how well chlorophyll can perform under drought stress.

Higher positive correlation between CSI and field drought resistance in pine and rice were reported elsewhere (Kaloyereas, 1958; Murty and Majumder, 1962). Fanous (1967) also reported a close association between CSI and drought resistance in pearl millet.

Number of green leaves:

In case of parent variety Isd 28, it is seen from the Table 8 that the highest number of green leaf was recorded in parent variety Isd 28 followed by somaclone Isd 28Sc-15. The lowest number of green leaf was recorded in somaclones Isd 28Sc-3 and Isd 28Sc-8. Green leaf number of all tissue culture PEG induced somaclones were lower than their parent variety Isd

28. Malik (1992) reported that number and size of leaf were reduced in drought resistant varieties. Smith *et al.* (2005) studied effects of water stress on canopy development in two sugarcane cultivars viz N 22 and NCo 376 under rain shelter facility. They reported a simultaneous reduction in leaf appearance rate and increase in leaf senescence rate, resulting reduction in green leaf number in both cultivars.

Leaf area:

In case of parent variety Isd 28, it is seen from the Table 8 that the highest leaf area was observed in parent variety and somaclone Isd 28Sc-15 followed by Isd 28Sc-6. The lowest leaf area was observed in somaclone Isd 28Sc-3. The variation in leaf area might occur due to the variation in number of leaves and expansion of leaf. It is seen from the Table 8 that the higher leaf area was recorded in drought intolerant somaclones and their parent variety compared to drought tolerant somaclones. Sinclair *et al.* (2004) reported that leaf area development is critical in the establishment of a full canopy to maximize interception of solar radiation and achieve higher crop productivity. Vasantha *et al.*, (2005) reported that drought stress significantly reduced individual leaf area, leaf number, leaf area index and tiller population.

Tolerance rating:

In case of parent variety Isd 28, somaclones Isd 28Sc-3, Isd 28Sc-8, Isd 28Sc-11, Isd 28Sc-17 and Isd 28Sc-18 were found to be highly tolerant having tolerance rating scale 1; somaclones Isd 28Sc-1, Isd 28Sc-2, Isd 28Sc-4, Isd 28Sc-5, Isd 28Sc-6, Isd 28Sc-7, Isd 28Sc-9, Isd 28Sc-10, Isd 28Sc-12, Isd 28Sc-13, Isd 28Sc-14, Isd 28Sc-15, Isd 28Sc-16 and Isd 28Sc-19 showed tolerant reaction having tolerance rating scale 2. In the present investigation the parent variety Isd 28 showed moderately tolerant reaction having tolerance rating scale 3 (Table 8).

Brix %:

Brix reading of parent variety Isd 28 and its somaclones have been presented in the Table 9. The highest Brix was recorded in somaclones Isd 28Sc-15 and Isd 28Sc-17. The lowest Brix was observed in somaclones Isd 28Sc-5 and Isd 28Sc-8. Brix reading of all tissue culture PEG induced somaclones were found to be higher except somaclones Isd 28Sc-5 and Isd 28Sc-8 than their parent variety Isd 28. Hossain *et al.* (2005) reported higher Brix reading in all tissue culture derived clones compared to donor variety. They also reported that higher Brix readings were associated with tolerance level and Brix level increased as tolerance level increased.

Table-6 Variation in Biochemical parameters in somaclones of variety Isd 28 regenerated From PEG induced drought tolerant callus.

Parent variety	Somaclones	Chlorophyll a (mg g <sup>-1</sup> )	Chlorophyll b (mg g <sup>-1</sup> )	Total chlorophyll (mg g <sup>-1</sup> )	Chlorophyll a:b ratio
Isd 28	-	0.5567j *	0.1603k	0.717m	3.473ab
	Isd 28Sc-1	1.028g	0.3153h	1.343j	3.263bcd
	Isd 28Sc-2	1.189d	0.4020d	1.591de	2.960fgh
	Isd 28Sc-3	1.384a	0.4800a	1.864a	2.883gh
	Isd 28Sc-4	1.242bc	0.4327bc	1.675bc	2.890gh
	Isd 28Sc-5	1.178d	0.4110cd	1.589de	2.867h
	Isd 28Sc-6	1.075fg	0.3517fg	1.427hi	3.063cdefgh
	Isd 28Sc-7	1.149de	0.3583fg	1.507g	3.207cdef
	Isd 28Sc-8	1.054g	0.3670fg	1.421hi	2.877h
	Isd 28Sc-9	1.052g	0.3480fg	1.400ij	3.023defgh
	Isd 28Sc-10	1.148de	0.3663fg	1.514fg	3.133cdefg
	Isd 28Sc-11	1.392a	0.4523b	1.844a	3.077cdefgh
	Isd 28Sc-12	1.121ef	0.3460g	1.467gh	3.247bcd
	Isd 28Sc-13	1.279b	0.3673fg	1.646cd	3.483ab
	Isd 28Sc-14	0.7803i	0.2187j	.9990l	3.567a
	Isd 28Sc-15	0.9077h	0.2817i	1.189k	3.227cde
	Isd 28Sc-16	1.279b	0.4420b	1.721b	2.893gh
	Isd 28Sc-17	1.199cd	0.3753ef	1.574ef	3.197cdef
	Isd 28Sc-18	1.170de	0.3533fg	1.523fg	3.313bc
	Isd 28Sc-19	1.281b	0.3973de	1.678bc	3.227cde
Isd 28Sc-20	0.8660h	0.2907i	1.157k	2.977efgh	

Figures in the columns followed by different letters are significantly different by DNMR test at p= 0.05.

Table-7 Variation in Chlorophyll Stability Index in somaclones of variety Isd 28 regenerated From PEG induced drought tolerant callus.

Parent variety	Somaclones	CSI
Isd 28	-	1.007j *
	Isd 28Sc-1	2.577h
	Isd 28Sc-2	3.857de
	Isd 28Sc-3	5.030a
	Isd 28Sc-4	4.320bc
	Isd 28Sc-5	2.393h
	Isd 28Sc-6	4.390b
	Isd 28Sc-7	3.467ef
	Isd 28Sc-8	2.393h
	Isd 28Sc-9	2.593h
	Isd 28Sc-10	3.407f
	Isd 28Sc-11	4.610ab
	Isd 28Sc-12	3.337f
	Isd 28Sc-13	1.253ij
	Isd 28Sc-14	1.050ij
	Isd 28Sc-15	2.820gh
	Isd 28Sc-16	3.950cd
	Isd 28Sc-17	4.627b
	Isd 28Sc-18	4.223bcd
	Isd 28Sc-19	3.127fg
Isd 28Sc-20	1.457i	

\* Figures in the columns followed by different letters are significantly different by DNMR test at p= 0.05.

Table-8 Variation in morphological characters in somaclones of variety Isd 28 regenerated from PEG induced drought tolerant callus.

Parent variety	Somaclones	Number of leaf	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	Tolerance rating scale (1-5)**
Isd 28	-	15.00a *	5662.580a	3
	Isd 28Sc-1	12.33bcde	5259.143i	2
	Isd 28Sc-2	11.33de	5227.260j	2
	Isd 28Sc-3	10.67e	5021.567n	1
	Isd 28Sc-4	13.67abc	5569.330c	2
	Isd 28Sc-5	12.00bcde	5338.717h	2
	Isd 28Sc-6	13.67abc	5592.443b	2
	Isd 28Sc-7	13.33adcd	5538.110d	2
	Isd 28Sc-8	10.67e	5043.773m	1
	Isd 28Sc-9	12.67bcde	5349.073gh	2
	Isd 28Sc-10	12.00bcde	5092.857l	2
	Isd 28Sc-11	11.33de	5106.343l	1
	Isd 28Sc-12	13.67abc	5544.057d	2
	Isd 28Sc-13	13.00abcd	5495.677e	2
	Isd 28Sc-14	13.33abcd	5258.123i	2
	Isd 28Sc-15	14.00ab	5652.640a	2
	Isd 28Sc-16	13.67abc	5542.427d	2
	Isd 28Sc-17	12.67bcde	5251.460i	1
	Isd 28Sc-18	12.33bcde	5184.373k	1
	Isd 28Sc-19	13.00abcd	5454.613f	2
Isd 28Sc-20	13.33abcd	5366.597g	2	

\* Figures in the columns followed by different letters are significantly different by DNMR test at p= 0.05.

\*\* Tolerance rating scale (1-5) is based on greenness of plants and other data collected, where 1=highly tolerant, 2=tolerant, 3=moderately tolerant, 4=intolerant and 5=highly intolerant.

Table-9 Variation in Brix % in somaclones of variety Isd 28 regenerated from PEG induced drought tolerant callus.

Parent variety	Somaclones	Brix %
Isd 28	-	13.77i *
	Isd 28Sc-1	20.20ab
	Isd 28Sc-2	15.17ghi
	Isd 28Sc-3	17.83cdef
	Isd 28Sc-4	15.17ghi
	Isd 28Sc-5	10.77j
	Isd 28Sc-6	18.10bcdef
	Isd 28Sc-7	18.13bcdef
	Isd 28Sc-8	10.23j
	Isd 28Sc-9	19.07bcde
	Isd 28Sc-10	19.17bcde
	Isd 28Sc-11	16.53fgh
	Isd 28Sc-12	17.53def
	Isd 28Sc-13	19.73abcd
	Isd 28Sc-14	19.87abc
	Isd 28Sc-15	21.47a
	Isd 28Sc-16	16.53fgh
	Isd 28Sc-17	21.83a
	Isd 28Sc-18	17.27efg
	Isd 28Sc-19	14.77hi
Isd 28Sc-20	17.10efg	

\* Figures in the columns followed by different letters are significantly different by DNMR test at p= 0.05.

In the present investigation it was found that both Biochemical parameters such as chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a:b ratio and CSI as well as morphological characters such as number of leaves and leaf area of some somaclones showed superiority compared to their donor parent variety Isd 28. These results indicate feasibility and effectiveness of selection of drought tolerant sugarcane genotypes through tissue culture by using PEG in the medium. It was also observed that callus produced up to 10% PEG in medium. Shoots and rooting in shoots were possible at 5% PEG level. However it was also reported elsewhere that, recurrent *in vitro* selection can effectively be applied for further improvement of desired traits (Ochatt *et. al.* 1989; Lutts *et. al.* 2001).

In this work drought tolerant somaclones were selected in both *in vitro* and *ex vitro* conditions. Biochemical parameters were affected due to heat induced drought treatments of leaf samples. Adverse effects of drought stress have been observed higher in tolerant somaclones as compared to moderately tolerant somaclones of parent variety Isd 28. It indicates possibility of desired genetic manipulation to produce drought tolerant somaclones.

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## EFFICACY OF SPLIT FERTILIZATION FOR SUGARCANE INTERCROPPED WITH POTATO AT TWO IRRIGATION LEVELS

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### ABSTRACT

An experiment was conducted at Regional Sugarcane Research Station (RSRS), Thakurgaon during 2005-06 and 2006-07 cropping seasons to assess effects of different levels of irrigation and split application of N-K fertilizers in sugarcane (var. Isd 36) intercropped with potato (var. Dimond). The intercrop, potato received recommended fertilizer as usual. Application of Urea and Potash (N-K fertilizers) in two splits (B<sub>1</sub>) produced highest number of tiller, millable cane and also highest yield in both levels of irrigation in both years. Though the difference was not significant over B<sub>2</sub> and B<sub>3</sub>. But irrigation level A<sub>1</sub> (six light irrigation with 65 mm effective rainfall) had significant difference over A<sub>2</sub> (five comparatively deep irrigation with same effective rainfall). Highest number of tiller ( $215.3 \times 10^3 \text{ ha}^{-1}$ ), millable cane ( $114.7 \times 10^3 \text{ ha}^{-1}$ ) and cane yield ( $109.9 \text{ t ha}^{-1}$ ) were produced by the treatment B<sub>1</sub> under A<sub>1</sub> level of irrigation in the crop year 2006-07. Hence split application of N-K fertilizers with two equal splits applied at plantation and at 145 days after plantation and also light irrigation, 6 to 7 number with total amount of 460-500 mm including effective rainfall may be preferred for loamy and sandy loam soils.

**Key words:** Sugarcane, split fertilization, irrigation, intercropped.

### INTRODUCTION

Fertilizers are indispensable for the crop production systems of modern agriculture. Among the factors that affect crop production, fertilizer is the single most important one that plays a crucial role in yield increase, provided other factors are not too limiting.

Sugarcane being a long duration and high yielding crop removes large quantity of nutrients from soil. Parthasarathy (1972) reported that a 46 ton crop of sugarcane removes from soil 164 lbs nitrogen and 398 lbs potash. Timely fertilization is the key for maximum benefit from the added fertilizers. Parthasarathy (1972) found that 40-50% of N and K<sub>2</sub>O are taken up in October- December and 15-20% of P<sub>2</sub>O<sub>5</sub> taken up in the first 6 months of plantation. Bhoj *et.al.*, (1974) reported that the best practice would be to apply the fertilizer in two installments i.e. half at the time of planting and the rest half at tillering.

Intercropping increase the crop yield per unit area by intensifying the use of land. Growers achieve additional benefit from sugarcane, as the yield of sugarcane is increased by intercropping with potato by 64.26% (Imam *et.al.*, 1987). In the monsoon climatic region, the best period of planting of both sugarcane and potato is from October to 3<sup>rd</sup> week of November (Nankar, 1993). The combined return of cane and potato was always higher when both the crops were planted together and received their full rates of fertilizer (Imam *et. al.*, 1990).

For optimum growth of sugarcane 1500 to 1800 mm rainfall is necessary (Rashid *et.al.*, 1987). The annual rainfall in Bangladesh ranges from 1000-1500 mm, which is unevenly distributed throughout the year. Frequent irrigation promotes rapid canopy development of

both potato and sugarcane. Khalak and Kumaraswamy (1992) reported that tuber yield along with growth attributes of potato increase with the increase of irrigation frequency and found best yield at PR 1.00 (IW: CPE = 1). Islam *et al.*, (1990) found maximum total water use for optimum growth of potato to be 267 mm by 5 irrigation with 40 mm of average irrigation water (depth). Siddique *et al.*, (1998) found highest yield for sugarcane intercropped with potato with the application of premonsoon total irrigation water 420 mm having 96 mm effective rainfall by the period. The soil was Brahmaputra River Plain loamy soil. In silty clay loam soil of calcareous type highest yield of cane intercropped with potato was found with 480 mm premonsoon total irrigation water having effective rainfall 65 mm. Number of irrigation was seven (Siddique *et al.*, 2005). For this experiment Principal Investigator budgeted premonsoon irrigation water range 420-525 mm (average 473 mm) and 450-540 mm (average 495 mm) by seven and six irrigation respectively (including effective rainfall). The range was thought for giving relaxation to the farmers who are not habituated to measure or estimate the amount of irrigation water they apply in the field. Hence the experiment was conducted to find out the effect of split application of N-K fertilizers with the variation of irrigation levels on sugarcane with potato as intercrop.

## **MATERIALS AND METHODS**

The experiment was conducted at RSRS farm, Thakurgaon under Bangladesh Sugarcane Research Institute, Ishurdi, Pabna during the cropping seasons 2005-06 and 2006-07. The land selected had sandy loam soil of Old Himalayan Piedmont Plain as per research program. Sugarcane variety Isd 36 and potato variety Dimond were planted. Sugarcane was planted by Spaced Transplanting (STP) method. One row of potato was planted in the space of one meter between two rows of sugarcane. Two levels of irrigation and three levels split application of N-K fertilizers were tested. The experiment was set up in split plot design with the following treatments, having three replications [A (2) X B (3)] X 3:

Factor A: Irrigation

Level A<sub>1</sub>: Seven light irrigation (60-75 mm) at 0, 25, 55, 80, 110, 140 & 175 days after transplanting (DAT)

Level A<sub>2</sub>: Six comparatively deeper irrigation (75-90 mm) at 0, 25, 65, 110, 140 & 175 DAT respectively.

Factor B: Split application of top dressing fertilizers

Level B<sub>1</sub>: 50% N and K-fertilizer as basal + 50% N and K fertilizers at 145 DAT

Level B<sub>2</sub>: 30% N and K fertilizers basal + 35% N and K fertilizers at 115 DAT + 35% N and K fertilizers at 145 DAT

Level B<sub>3</sub>: 25% N and K fertilizers as basal + 25% N and K fertilizers at 115 DAT + 25% N and K fertilizers at 145 DAT + 25% N and K fertilizers at 180 DAT

Plantation was done with two budded settling raised in soil bed in the end of November 2005 and 2006 with 45 cm spacing between planted settlings. Necessary intercultural operations were done as and when required. Collection of data on yield and various yield contributing characteristics for cane and intercrop were done. The average values of all parameters were analyzed statistically.

## RESULTS AND DISCUSSION

Data of cane yield and yield attributes of cane and potato at two levels of irrigation and split application of N-K fertilizers for the crops of 2005-06 and 2006-07 are presented in the Table 1 and Table 2 respectively.

### Yield of Cane:

Non-significant difference was observed in cane yield among the treatments in both cropping seasons (Table 1 and Table 2). At 2005-06, the highest yield ( $70.6 \text{ t ha}^{-1}$ ) was found from the treatment  $B_1A_1$  followed by  $70.0 \text{ t ha}^{-1}$  from the treatment  $B_1A_2$  respectively. The lowest yield ( $56.1 \text{ t ha}^{-1}$ ) was recorded from  $B_2$  treatment under  $A_2$  level of irrigation. In case of Table 2,  $B_1$  showed the highest yield of  $109.9 \text{ t ha}^{-1}$  followed by  $108.8 \text{ t ha}^{-1}$  from  $B_2$  under  $A_1$  irrigation level. Lowest yield ( $100.0 \text{ t ha}^{-1}$ ) was found from  $B_3$  treatment under  $A_2$  level of irrigation.

### Tiller:

No significant difference was observed in tiller production among the treatments at  $A_1$  and  $A_2$  levels of irrigation (Table 1 and Table 2). From the Table 1, highest tiller population of  $162.6 \times 10^3 \text{ ha}^{-1}$  was produced from  $B_1$  followed by  $B_3$  treatments under  $A_2$  irrigation level. Lowest tiller of  $143.3 \times 10^3 \text{ ha}^{-1}$  was recorded from  $B_3$  under  $A_1$  irrigation level. From Table 2, the highest tiller population ( $215.3 \times 10^3 \text{ ha}^{-1}$ ) was produced from  $B_1$  followed by  $B_2$  under  $A_1$  level of irrigation.

### Millable Cane:

No significant difference was observed in millable cane production in both cropping seasons (Table 1 and Table 2). The maximum millable cane  $111.5 \times 10^3 \text{ ha}^{-1}$  was recorded from the  $B_1$  and the minimum ( $93.4 \times 10^3 \text{ ha}^{-1}$ ) was from the  $B_2$  treatment under  $A_2$  irrigation level (Table 1). From Table 2, the maximum millable cane was found ( $114.7 \times 10^3 \text{ ha}^{-1}$ ) from  $B_1$  treatment under  $A_1$  level of irrigation and did not differ with other treatments in both irrigation levels.

### Brix%:

The data presented in the Table 1 and Table 2 reveals that the different time of fertilizer applications and level of irrigation did not affect Brix%.

### Intercrop Yield (Potato):

No significant difference was observed in potato yield at both cropping seasons (Table 1 and Table 2). The maximum potato yield was found  $7.5 \text{ t ha}^{-1}$  from  $B_1$  under  $A_1$  irrigation level.

In the present experiment, split application of N and K fertilizers into two equal halves i.e.  $\frac{1}{2} \text{ N} + \frac{1}{2} \text{ K}$  at planting and the rest  $\frac{1}{2} \text{ N} + \frac{1}{2} \text{ K}$  at 145 days after planting ( $B_1$ ) under  $A_1$  level of irrigation was found superior to other treatments. This is evident from the results of the experiment that  $B_1$  level of N-K fertilizers (2 splits) with  $A_1$  level of irrigation (6 light irrigation) with 65 mm effective rainfall is superior in respect of cane yield improvement in sandy loam to loamy soil. As the difference between  $B_1$  and  $B_2$  is insignificant, we may assume that in case of heavy irrigation and heavy rainfall just after irrigation or fertilizer (N-K) application, three splits or additional split with additional dose of fertilizer to recover leaching loss of N and K elements may give higher yield. In that situation, three splits or an additional split with additional dose of N-K fertilizer or with only N-fertilizer may be recommended (hypothesis).

Table1. Yield and yield attributes of cane and potato at different levels of irrigation and split application of N-K fertilizers during 2005-06 cropping season.

Level of Irrigation	Level of N-K fertilizers	Tiller (x 10 <sup>3</sup> ha <sup>-1</sup> )	Millable cane (x 10 <sup>3</sup> ha <sup>-1</sup> )	Brix (%)	Cane Yield (t ha <sup>-1</sup> )	Potato Yield (t ha <sup>-1</sup> )
A <sub>1</sub> (6 light Irrigation)	B <sub>1</sub>	155.5	107.1	19.1	70.6	7.4
	B <sub>2</sub>	150.6	101.5	19.8	61.1	6.8
	B <sub>3</sub>	143.3	99.3	18.7	63.2	6.8
A <sub>2</sub> (5 deep Irrigation)	B <sub>1</sub>	162.6	111.5	19.3	70.0	6.1
	B <sub>2</sub>	140.0	93.4	19.3	56.1	6.8
	B <sub>3</sub>	157.0	106.0	19.1	60.1	6.6

Table2. Yield and yield attributes of cane and potato at different levels of irrigation and split application of N-K fertilizers during 2006-07 cropping season.

Level of Irrigation	Level of N-K fertilizers	Tiller (x 10 <sup>3</sup> ha <sup>-1</sup> )	Millable cane (x 10 <sup>3</sup> ha <sup>-1</sup> )	Brix (%)	Cane Yield (t ha <sup>-1</sup> )	Potato Yield (t ha <sup>-1</sup> )
A <sub>1</sub> (6 light Irrigation)	B <sub>1</sub>	215.3	114.7	18.3	109.9	7.5
	B <sub>2</sub>	211.9	114.6	19.0	108.8	6.9
	B <sub>3</sub>	210.3	113.8	18.3	106.6	6.5
A <sub>2</sub> (5 deep Irrigation)	B <sub>1</sub>	209.9	111.6	18.8	105.6	5.9
	B <sub>2</sub>	204.2	111.5	19.0	103.8	6.3
	B <sub>3</sub>	212.8	114.0	19.0	100.0	6.5

Irrigation level A<sub>1</sub> had significant difference over A<sub>2</sub> at 5% level of significance on cane yield in both the years. Other parameters had insignificant difference over each other.

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# SUGAR INDUSTRY ABSTRACTS

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## **Optimal scheduling of sugarcane harvest and mill delivery**

M. E. Salassi, F. G. Barker and M. A. Deliberto, (2009) Dept. of Agri. Economics and Agri. Bussines LSU, USA. Sugarcane International May-June Vol.27 issue 3, pp. 87-90.

As a major sugarcane producing state in the United States, Louisiana harvests more than 160,000 hectares of sugarcane annually. In general, sugarcane growers decide on harvest starting times on an individual basis, resulting in relatively inefficient unloading operations at many mills. A research project was initiated to develop a general mathematical programming model which could be used to optimally schedule harvest and transport operations for a group of farms delivering to a common mill with the goal of minimizing truck waiting time at the mill by spreading out deliveries of harvested sugarcane to a mill over a wider daily delivery time window. Results illustrate that linear programming models can be effectively utilized in developing optimal harvest and transport schedules for individual mill situations.

## **The effect of *Sugarcane yellow leaf virus* infection on yield of sugarcane in Louisiana**

M. P. Grisham, G. Eggleston, J. W. Hoy, and R. P. Viator, (2009) USDA-ARS-Sugarcane Research Laboratory, Houma LA-70360, USA Sugarcane International May-June Vol.27 issue 3, pp. 91-94.

A series of field experiments were conducted to determine the effect of *Sugarcane yellow leaf virus* (ScYLV) infection on cane and sucrose yield of four commercial sugarcane cultivars (LCP 85-384, Ho 95-988, HoCP 96-540 and L 97-128) that occupied 93% of the sugarcane production area in Louisiana in 2007. As in commercial fields, visual symptoms of sugarcane yellow leaf disease caused by ScYLV were not observed in the plants of this study. The results of this study, however, indicated that ScYLV infection can cause loss of cane and sucrose yields. In the first of two experiments that included cultivar LCP 85-384, cane and sucrose yields did not differ between control and ScYLV-infected plants across the crop cycle (plant-cane, first-ratoon, and second-ratoon crops). In the second experiment, cane yield was reduced in ScYLV-infected plants of LCP 85-384 across the crop cycle; however, sucrose yield was not reduced because of an increase in the sucrose content of infected plants. The only observed effect of ScYLV-infection in cultivar Ho 95-988 was an increase in sucrose content of the cane. Cane and sucrose yields were reduced in ScYLV-infected plants of cultivars HoCP 96-540 and L 97-128. Currently, all sugarcane cultivars recommended for planting in Louisiana are susceptible to ScYLV infection; however, the effect of the infection on cane and sucrose yield varies. To minimize the potential for yield loss from ScYLV infection, growers should plant seed cane free of the virus infection.

## **Management of sugarcane cultivars imported into Colombia**

J.I. Victoria, J.C. Angel, M.L. Guzman and M. Oicata (2009) Colombian Sugarcane Research Center-CENICANA, Calle 58 N # 3BN-110 PO Box 9138 Cali-Colombia <sup>2</sup>Instituto Colombiano Agropecuario, ICA, Colombia, pp. 95-98.

CENICANA imports sugarcane cultivars through a closed quarantine station (CQS) at ICA-Mosquera (Cundinamarca) and an open quarantine station (OQS) at Piedechinche (Cauca Valley). The cane cultivars that arrive at the CQS are sown in plastic pots and placed in separate cubicles where they are evaluated during two 9-10 month crop cycles. Plants are examined by several techniques including tissue blot immunoassay (TBIA), dot blot immunoassay (DBIA), polymerase chain reaction (PCR) and reverse transcriptase-polymerase chain reaction (RT-PCR) for Sugarcane *yellow leaf* virus (SCYLV), Sugarcane mosaic virus (SCMV), Fiji disease virus (FDV), Sugarcane streak mosaic virus (SCSMV), *Xanthomonas albifineans* and *Leifsonia xyli* subsp. *xyli*. Plants that have shown no symptoms of diseases are then sent to CENICANA and sown in small pots in a glasshouse, and then placed in a growth chamber for then no therapy followed by in vitro culture of apical meristems to eliminate microorganisms. This procedure prevents the introduction of plant pathogens to Colombia. Thereafter, plantlets are transplanted to the OQS, grown for nine months, and examined for *leaf scald* disease (LSD), ratoon stunt disease (RSD), yellow leaf, Fiji disease, streak mosaic and mosaic by the above mentioned techniques. Over a 26 year period, 1006 sugarcane cultivars from different countries have been imported, and 20.3% in the CQS and 6.4% in the OQS were eliminated due to sanitary problems. Thermotherapy and in vitro meristems culture have shown excellent results, producing disease-free materials of 774 cultivars. Nevertheless 16.2% of the material entering OQS was eliminated due to infection by pathogens or bad germination. The imported cultivars pass through this system and enter the germ bank. Agronomical and characterization trials are conducted to select those that may be incorporated into the sugarcane breeding and improvement program of CENICANA.

#### **Sugarcane water use and irrigation requirements in a semi-arid environment**

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Water for agricultural irrigation is becoming increasingly limited and, therefore, must be used as efficiently as possible. Sugarcane is a high biomass crop requiring lots of water. A field study was conducted to determine water requirements and crop water use by applying different levels of water. These were achieved by varying crop coefficients used with reference evapotranspiration to determine irrigation scheduling. Sugarcane yield responses to different water levels varied annually. The irrigation level producing maximum yields was not the same each year. Total water input including rainfall and irrigation to achieve maximum yields varied from 973 to 1328 mm/y; while water use efficiency (fresh weight of cane produced per unit of water uptake) varied from 6.0 to 11.71 cane/ML of water. These results suggest that the amount of water required to produce maximum yields in the Lower Rio Grande Valley of Texas may be less than the amount theoretically used by this crop under ideal conditions as reflected in the established crop coefficient curves. Deficit irrigation may lower yields, but may increase the amount of cane produced per unit of water used by the crop.

#### **Knowledge of soil biology to reduce nitrogen loss as N<sub>2</sub>O from sugarcane farming**

K. McGrath, R. Bracking P. Lakshmanan, P. Schenk and S. Schmidt (2009) School of Integrative Biology, University of Queensland, Brisbane, Australia <sup>2</sup> BSES Limited, Brisbane, Australia. pp.102-106.

Contemporary cropping systems have to combine high yields with environmental sustainability. The conversion of nitrogen (N) fertilizer to forms which are easily lost from soil (as nitrate or gaseous N compounds) is driven by soil biology, specifically the soil microbial community. Although knowledge of soil microbial activity is crucial for minimizing N loss, it is not known how soil conditions and associated microbes can best be

manipulated through farming practices. We have developed new tools for fingerprinting microbial activities in soil. Our new method quantifies, for the first time, the activities of microbial genes that are switched on or off under certain conditions in the soil. The method links soil parameters and microbial activities by using a new custom-made micro array. The advantage of this novel mRNA-based approach is that it (i) measures of the 'microbial activity' rather than the 'presence' of specific microbes, (ii) monitors 'activities' of many microbial groups simultaneously, (iii) provides insights into whole-system soil biology. We have applied this technique to monitor microbial activities in cane soils with different management histories. Our preliminary results indicate that the method can successfully detect differences in the microbial activities of soils with different fertilizer application and N<sub>2</sub>O emission rates (Mackay), and different cropping history (Ingham). Ultimately this research will provide information for farmers to effectively manipulate soil biology to reduce N loss and improve productivity.

### **Crop losses due to two sugarcane stem borers in Reunion and South Africa**

Francois-Regis Goebel and Michael Way (2009) CIRAD, Systemes de Cultures Annuels.TA B-102/02,34398 Montpellier Cedex 5, France. <sup>2</sup> South African Sugarcane Research Institute, P/Bag X02, Mount Edgecombe, 4300, South Africa. pp. 107-111.

The impact on sugarcane yield of two key stem borer species, *Chilo sacchariphagus* and *Eldana saccharina*, were investigated over a period of 10 years in Reunion Island and South Africa. Replicated and randomised field plot trials were conducted. Treatments consisted of pest exclusion using concentrated and repeated chemical applications, of natural infestations, and of artificial inoculations to enhance these infestations. The relationship between borer injury (measured as percent bored internodes) and the corresponding stalk length and diameter, biomass, fibre and sugar content were determined. Borer injury impaired the growth and reduced quality of sugarcane stalks. *C. sacchariphagus* decreased stalk biomass to a greater extent than sucrose content. *E. saccharina* injury reduced sucrose content and increased fibre level, and affected to a lesser extent stalk biomass. Since *E. saccharina* typically attacks sugarcane early during the main period of biomass accumulation and *C. sacchariphagus* attacks later during the maturation phase, the timing of borer infestations might explain these results. Numerous components of stalk quality were negatively correlated to injury from both species. *Chilo sacchariphagus* impacted mostly sugarcane biomass while *E. saccharina* decreased sucrose content. Crop loss models, as well as the formulation of any IPM recommendation, would need to be specific to the borer species.

### **Performance of cultivars on different row configurations**

B. Salter, A.L. Garside and N. Berding (2009) <sup>1</sup> BSES Limited, Central Experiment Station, Mackay, Australia <sup>2</sup> BSES Limited, c/o CSIRO Davies Laboratory, Townsville, Australia <sup>3</sup> BSES Limited, Meringa Experiment Station, Australia. pp. 112-117.

Controlled traffic has been promoted in order to reduce compaction. Controlled traffic can be achieved by widening row spacing from the conventional 1.5 m single row system to row spacing of 1.8-1.9 m. This is the width of current harvesting and haul-out machinery. Many growers are now producing cane on these wider row spacing. However, the crop improvement program uses 1.5 m singles rows when selecting clones. Are cultivars that are suitable for wider row spacing making it through the breeding program? This question was addressed by looking at whether a cultivar x row spacing interaction occurs when cultivars are grown on different row configurations. Three trials were planted in Queensland in 2006 (Mackay, Ingham and Meringa). Each trial contained four cultivars on three row configurations. Dual-row configurations tended to produce more early shoots but often this difference was lost later in the season when stalk development occurred. Cultivars used

different growth pathways to reach final yield. In many cases these different pathways did not result in significantly different yields at final harvest. However, understanding these pathways may allow for better cultivar recommendations for particular environments. A significant cultivar-by-row-configuration interaction was found for total fresh biomass at Ingham, one of the three sites. This interaction was mainly due to the good performance of Q135 on dual rows and the poor performance of Q174<sup>11</sup> on dual rows. The lack of an interaction at the other sites suggests that it is still unclear whether selection on wide rows would actually produce cultivars even better suited to wide rows compared to selection on narrow rows.

### **Developing optimal selection systems in sugarcane breeding programs**

Fengduo Hu, Phillip Jackson and Kaye Basford (2009) <sup>1</sup>BSES Limited, <sup>2</sup>CSIRO Plant Industry and <sup>3</sup>The University of Queensland, Australia. pp. 1189-123

Selection represents a costly and important part of sugarcane breeding programs. Previous research showed that cane yields in small single-row plots are affected strongly by competition effects, and that a high weighting in selection indices should be placed on CCS in small single-row plots to maximize gains for economic value. This led to a new selection system being suggested, involving initial screening of large numbers of clones in 5 m plots with heavy selection pressure for CCS followed by two stages of selection in multi-row plots. A stochastic simulation model using assumptions on relevant parameters (genetic, error, competition, and GE variances, genetic correlations) was developed to predict gains from alternative selection systems. Field trials were conducted in the Burdekin region to assess realized gains from alternative selection trial designs to validate and refine assumptions important in the model. The model was then used to predict genetic gains in selection systems with a wide range of configurations (e.g. plot size, replicate number, number of sites, selection criteria, selection intensity in each stage, and number of stages). Based on the results, it was recommended that three stages of Clonal selection (following current family selection in stage I be performed in core breeding programs. This should involve firstly screening clones in small (1 row x 5 m) plots, with a selection index biased strongly toward CCS, but also including cane yield estimated via visual grade. Selected clones should then be evaluated in two further stages -the first one consisting of 4-row plots at four sites with a single replicate per clone per site. Clones selected from this stage should then be evaluated in 4-row plots at four sites but with two replicates per site. The recommended system has been introduced in the Burdekin selection system for further practical evaluation and it is recommended that it be assessed in other regions. The research conducted here also emphasized the importance of using optimal selection indices in single-row plots, in order to maximize gains from selection.

### **Slow red rot resistance in sugarcane**

Narender Singh, Anil Kumar, Kushal Raj, K.S.Virk and Satyavir (2009) Department of Plant Pathology, CCS Haryana Agricultural University, Hisar 125 004, India, pp. 125-130.

Studies were undertaken to identify clones for slow red rot resistance in sugarcane. For initial identification, 17 clones viz., CoH 110, CoH 115, CoH 117, CoH 118, CoH 119, S-98-70, S-98-143, S-98-193, S-98-197, S-98-212, S-98-232, CoPant 84211, Co 97017, CoS 96258, CoJ 64, CoLK 9606 and CoL 29 were inoculated by two methods (plug and nodal injury) using pathotype Cf 08. Six clones viz. CoH M 9, CoH 110, CoH 115, CoPant 84211, Co 97017 and CoS 96258 which showed slow red rot development along with susceptible checks CoLK 9606 and CoL 29 were selected to study the rate of red rot development and

parameters of slow red rot resistance. Susceptible checks CoL 29 and CoLK 9606 recorded maximum rate of red rot development by both inoculation methods. The disease index as well as lesion length was significantly lower in clones viz<sup>^</sup> CoH 119, CoH 110, CoH 115, CoPant 84211, Co 97017 and CoS 96258. Parameters of slow red rot resistance vb<sup>^</sup>, drying of tops, sporulation over the rind, spindle infection and secondary spread of the disease were studied using nodal, nodal injury and plug methods of inoculation. Maximum drying of tops, sporulation over the rind and spindle infection were recorded in susceptible clones by all the three methods of inoculation, while drying of tops, sporulation over the rind and spindle infection remained absent in clones, CoH 119 and CoH 110. Sporulation over the rind was found to be positively correlated with drying of tops. There was no secondary spread of the disease in clones CoH 119, CoH 110 and CoPant 84211.