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SCHEDULING IRRIGATION FOR SUGARCANE WITH CABBAGE AS INTERCROP

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ABSTRACT

An experiment was conducted in Bangladesh Sugarcane Research Institute (BSRI) farm at Ishurdi during 1997-98 cropping seasons to develop an irrigation schedule for sugarcane with cabbage as intercrop using pan ratio (PR) method. Irrigation was applied during pre-monsoon only. No irrigation was applied during monsoon or post-monsoon. The highest yield of sugarcane as well as cabbage were obtained when irrigation was applied at PR= 1.0. With this PR, the highest yield of sugarcane (109.79 t ha⁻¹) was obtained with 430 mm irrigation water in addition to 176.43 mm of effective rainfall. The highest yield of cabbage (21.66 t ha⁻¹) was obtained with 310 mm of irrigation water.

Key words: Irrigation schedule, sugarcane, cabbage, pan ratio

INTRODUCTION

Farmers grow large number of winter vegetables as sole crop occupying an area of about 220 X 10³ ha. On the other hand, about 20% of sugarcane land (160 X 10³ ha) is intercropped with various short growing winter crops viz. cabbage, cauliflower, potato, carrot, radish etc. Successful intercropping of various crops with sugarcane has been reported by many researchers (Rathi *et al.*, 1974; Behli and Narwal, 1977). Without intercropping, the poor cane growers cannot wait for such a long time to get economic return from sole crop of sugarcane. Intercropping increased the crop yield per unit area by intensifying the use of land.

The nutritive value of vegetables is high owing to the presence of mineral salt and vitamins. In Bangladesh, the vegetables production is not evenly distributed throughout the year. Most of the important vegetables are produced in winter, which amount 367 thousand tons. Only in the summer 243 thousand tons of vegetables are produced (Anon., 2001). Nutritionist suggests that an adult person should eat at least 285 g of vegetables per day for maintaining good health. But in Bangladesh an individual consumes an average of 32 g per day and if potato and sweet potato are excluded, the quantity becomes just 25 g (Hossain *et al.*, 1990), which result in chronic malnutrition. All vegetables can't be grown in kharif season due to adverse climatic condition. As a result, late variety cabbage (Atlas-70) plays an important role to supplement this shortage during the lag period. Late variety of cabbage harvesting in the end of rabi season and beginning of the kharif season (month of March) which easily meet up the vegetables crisis during the lag period.

The average yield of this crop is very low due to various reasons including the management problems. Among these, irrigation, fertilization and plant spacings are the most important agronomic practices, which have 12 to 49% contribution in sugarcane production (Ali *et al.*, 1989). 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. Around 70% of the total rainfall occurs during the monsoon period of July to September, where as, a very little rainfall occurs in the months of November to May which is the establishment and tillering phases of sugarcane. Imam *et al.*, (1988) reported that irrigation always played a vital role for increasing cane yield at tillering stage. On the other hand, irrigation increased the yield of cabbage (cv. Prva zetev) by 11% compared with no irrigation (Cerne, 1991). While conducting experiment with cultivar Gloria, Fischer and Nel (1987) found that high soil moisture levels during vegetative growth improved leaf growth but not necessarily increased yields. Even at low moisture the crop gave satisfactory yields indicating considerable drought resistance.

In an experiment in Germany, Hartmann and Zengerle (1987) observed that quick maturing (85 days) white cabbage cv. Grenit and slow maturing (136 days) red cabbage cv. Autoro required 396 and 611 mm water and yielded marketable heads weighing 1.40 and 2.18 kg, respectively with soil moisture maintained at 75-100% of capacity. According to Saha *et al.*, (1998) total water use by cabbage for high yield, irrigation schedules ranged from 158.44 to 183.23 mm. Maximum efficacy of irrigation water in terms of marketable yield (602.35 kg/ha per mm) was achieved when irrigation led to 30% DASM (depletion of available soil moisture).

MATERIALS AND METHODS

The experiment was conducted at BSRI farm, during 1997-98 cropping seasons. Sugarcane variety Isd 28 and cabbage (Atlas-70) were used as planting material. Sugarcane was planted by STP method, while single row of cabbage seedlings were transplanted in between two rows of sugarcane spaced by 90 cm. The experiment was set in a randomized complete block design with three replications.

The treatments of the experiment were as follows:

I_B = only two base irrigation at 1 and 15-20 DAT (days after transplanting) respectively.

I_1 = I_B + irrigation at PR= 0.6

I_2 = I_B + irrigation at PR= 0.9

I_3 = I_B + irrigation at PR= 1.0

Where pan ratio (PR) is the ratio of irrigation water (IW) to the cumulative open pan evaporation (CPE) from USWB class-A open pan i.e. $PR = IW/CPE$. Irrigation water (IW) was calculated considering 40 cm effective root depth at the start and 100 cm during tillering stage and assuming 50% depletion of available soil moisture allowable. Then irrigation interval was determined by the following equation:

$$CPE = \frac{IW + ER}{PR}$$

Where, ER is the effective rainfall between two irrigation. In each irrigation a prefixed amount of irrigation water (in proportion to effective root depth) was applied. No irrigation was needed during and after monsoon.

RESULTS AND DISCUSSION

The effects of irrigation on yield and yield components of sugarcane as well as cabbage during 1997-98 are given in table 1 & 2. It is evident from the table 1 that irrigation had significant effect on tiller count, millable cane and yield of sugarcane. It had no effect on recovery of sugar. The highest yield was obtained from treatment I₃ (109.79 t ha⁻¹) followed by treatment I₂ (104.08 t ha⁻¹). The yields of cane, tillers count and also millable cane under the treatments differed significantly from each other except I₂ & I₃ for yield of sugarcane.

From the table 2, it is seen that treatment I₃ that gave highest yield of sugarcane, received 8 (eight) irrigations including 6 (six) during intercrop period. It received 430 mm of irrigation water including 310 mm during intercrop period. It is also seen that irrigation had significant effects on the yield of cabbage. The highest yield of cabbage obtained by treatment I₃ (24.66 t ha⁻¹) followed by the treatment I₂ (22.05 t ha⁻¹). But I₃ and I₂ differed insignificantly and both differed from I_B significantly.

Table-1 Effect of irrigation on tillering, millable cane, recovery and yield of sugarcane during 1997-98 cropping season

Treatment	Tiller count (x 10 ³ ha ⁻¹)	Millable cane (x 10 ³ ha ⁻¹)	Recovery (%)	Yield (t ha ⁻¹)
I _B	255.11 a	135.37 a	8.37	80.43 a
I ₁	274.58 b	142.00 b	9.24	99.33 b
I ₂	288.54 c	154.70 c	9.53	104.08 bc
I ₃	304.21 d	163.54 d	8.69	109.79 c
LSD at 5%	13.75	6.53	NS	6.90

Table-2 Irrigation water, effective rainfall, number of irrigation and yield of cabbage and sugarcane during 1997-98 cropping season

Treatment	Irrigation Water		Number of irrigation		Effective rainfall (mm)	Yield of cabbage (t ha ⁻¹)	Yield of sugarcane (t ha ⁻¹)
	Intercrop period	Total	Intercrop period	Total			
I _B	70	70	2	2	Nil	13.35 a	80.43 a
I ₁	190	310	4	6	98.72	16.65 b	99.33 b
I ₂	250	430	5	8	176.43	20.05 bc	104.08 bc
I ₃	310	430	6	8	176.43	21.66 c	109.79 c

* Figures in a column accompanied by similar letter (s) do not differ significantly at 0.05 level of probability as per DNMRT.

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RESPONSE OF SUGARBEET *BETA VULGARIS* (VARIETY KAWETERMA) TO DIFFERENT FERTILITY LEVELS

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ABSTRACT

The response of sugarbeet variety Kaweterma to different fertility levels was ascertained in the field. The treatments included six N-P levels viz: 0-0 (control), 50-50, 75-75, 100-100, 125-125 and 150-150 N-P kg ha⁻¹. Application of N-P fertilizers in higher quantities (150-150 kg ha⁻¹) resulted 93230 ha⁻¹ plant population, 62.50 cm leaves length, 37.38 leaves plant⁻¹, 2.22 kg sugarbeet weight plant⁻¹, 83.142 m.t ha⁻¹ beetroot yield, 22.37% brix content and 11.34% sugar recovery. N-P application at the rate of 125-125 and 100-100 kg ha⁻¹ produced 92367 and 90053 ha⁻¹ plant population, 61.25 and 58.75 cm leaves length, 35.91 and 31.95 leaves plant⁻¹, 2.21 and 2.13 kg sugarbeet weight plant⁻¹, 80.588 and 79.484 m.t ha⁻¹ beetroot yield, 22.34 and 22.11% brix content and 11.31 and 11.19% sugar recovery. It was observed that there was a gradual improvement in all the growth and yield components with increasing fertility levels. However, this increase was uneconomical when applied beyond 100-100 kg ha⁻¹, because differences amongst 100-100, 125-125 and 150-150 kg N-P ha⁻¹ for all the parameters were statistically non-significant. Hence, 100-100 kg N-P level was considered to be an optimum level for economical production of sugarbeet under Tandojam conditions.

Keywords: Sugarbeet, fertility levels, beetroot yield, brix, sugar recovery.

INTRODUCTION

Sugar Beet, *Beta vulgaris* L. belongs to the family *chenopodiaceae*, is grown throughout the world mainly wild *Beta maritima* for food and medical purpose and its roots used as vegetable. There are three types of beetroot, the globe beet (which is the most popular); the intermediate or cylindrical beet and the long beet. Although the long beet is still grown commercially and for exhibition, it has been superseded to a great extent by the other two kinds. For early sowing always use the globe beet. The intermediate beet is bigger but it grows more slowly. So it is more suitable for main crop use (Martyn, 1978). Sugarbeets are believed to be native to the Mediterranean area of Europe, Egypt and North Africa, and secondary area of development was located in the near East. Many members of the beet family are found in areas with elevated salt leaves. Beets have been grown as a potherb throughout the recorded history. The roots of wild beets, however, were used by ancient civilizations only for medicine. These wild forms did not resemble the modern enlarged beet (Hamilton, 2005).

Sindh is the only other province where sugarbeet is cultivated but on a very small scale. The reported area under cultivation in Sindh is about 100 hectares. Sugarcane is the main source of sugar production in the country. Our sugar industry is entirely dependent on the availability of sugarcane. However, it is a high delta crop notorious for its lavish water use and occupies land

for 10-14 months. In this respect, sugarbeet has a comparative advantage, as it is a low delta crop and occupies land for 4-5-months (Syed, 2002).

Proper management of fertilizer applications to sugarbeets still remains a challenge for growers. The researchers have quantified rates of fertilizer uptake, and estimated the amount of N, P and K in sugarbeet crops that was derived from the soil itself, compared to that derived from fertilizers. Use of chemical fertilizers has become essential for good beet crop harvest. The macronutrient (N.P.K) has their individual role in development of root zone and good crop stand. Moreover, these elements are vitally needed to establish different properties in the beet root juice and similarly needed for satisfactory crop growth (Pakissan.com, 2004). Plant analysis is associated with nutrient's concentration in a specific plant part to the growth of the plant (George and Schmitt, 2002; Mortvedt *et al.*, 2005; Cattanach *et al.*, 1993). Sugarbeets responded to N deficiency by an increase in sucrose percentage of storage roots and the change results from an inhibition of vegetative growth, which permits a higher proportion of the sucrose produced in the leaves to accumulate in the roots rather than be used in growth (Mortvedt *et al.*, 2005). The crop responses to P fertilization are quite common. The P deficiency is by far the most difficult to recognize. An overall stunting of the plant and a slight deepening of the green foliage color are the only visual signs (Mortvedt *et al.*, 2004). In case of Potassium, a 30-ton sugarbeet root crop contains about 180 pounds of K₂O (150 pounds K) in its tops and roots. The experiment was conducted to examine the response of sugarbeet to different fertility levels in terms of its growth, yield and sugar recovery under Tandojam conditions.

MATERIALS AND METHODS

The experiment was laid out in a four replicated randomized complete block design (RCBD), the sub-plot size kept was 4.2m x 6m (25.2m²). A fallow left (in the off-season) piece of land was selected and prepared by giving cross wise deep ploughings. After soaking dose, when the land came in condition, the seedbed was prepared by using cultivator (cross-wise) and rotavator. Thereafter, clods were broken completely by clod crusher followed by thorough levelling. After seedbed preparation 60 cm apart ridges were prepared with tractor drawn ridger. Pure seed of sugarbeet variety "Kaweterma" was obtained with the courtesy of from Sugarcane Specialist, Agriculture Research Institute, Tandojam and the sowing was completed in the third week of October 2005. Before sowing, a certain amount of chemical fertilizers (nitrogen and phosphorus) was applied by mixing in the soil to improve the soil for any sort of deficiency in these essential nutrient elements. After completion of the germination, the plants were thinned to maintain plant to plant spacing of 20 cm. Nitrogen was applied in the form of urea and Phosphorus in the form of Diammonium Phosphate (D.A.P). All P and 1/3rd of nitrogen were applied at the time of sowing, while the remaining nitrogen was divided into two equal doses and applied at third and fourth irrigation. All the agronomic practices were carried out uniformly in all the plots. Eight irrigations were applied from sowing upto the crop maturity. After completion of field observations, the samples were brought to the laboratory of Sugarcane Section, Agriculture Research Institute, Tandojam, where the beetroots were sliced and juice was extracted to record Brix and sugar recovery. Finally, the data so collected were subjected to statistical analysis to analyze the treatment variation, while L.S.D. (Least Significant Difference) test was applied to observe the significance of difference within treatments as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Plant population ha⁻¹

The plant population ha⁻¹ was significantly higher (93230) in plots fertilized with highest fertility level of 150-150 kg N-P ha⁻¹, closely followed by plant population of 92367 and 90053 plants ha⁻¹, recorded in plots fertilized with 125-125 kg and 100-100 kg N-P ha⁻¹, respectively. The sugarbeet crop fertilized with 75-75 kg and 50-50 kg N-P ha⁻¹ had mean plant population of 78743 and 71389 plants ha⁻¹, respectively. However, the lowest plant population of 69173 plants ha⁻¹ was recorded in plots left untreated (control). This higher plant population in plots treated with higher fertility levels was mainly associated with the better germination of seeds, because due to application of nitrogen and phosphorus in higher quantities, the soil fertility improved to an adequate level and hence increased plant population resulted. A comprehensive research on various aspects of sugarbeet has been carried out world over and is published in various research journals. Jaszczolt (1998) studied different fertilizer sources and concluded that application of N-P fertilizers improved the plant growth substantially and when applied at sowing it resulted better crop stand and higher plant population.

Length of leaves (cm)

The length of leaves was highest (62.50 cm) in plots fertilized with highest fertility level of 150-150 kg N-P ha⁻¹, closely followed by 61.25 and 58.75 cm length of leaves, recorded in plots fertilized with 125-125 kg and 100-100 kg N-P ha⁻¹, respectively. The crop fertilized with 75-75 kg and 50-50 kg N-P ha⁻¹ produced leaves of 52.25 cm and 44.25 cm in length, respectively. However, the lowest length of leaves of 33.75 cm on average was recorded in plots given zero fertilizer (control). This higher length of leaves in plots treated with higher fertility levels was mainly associated with adequacy of soil in essential nutrient elements i.e. N and P due to their application in higher quantities. Moreover, the results suggested that there was a successive increase in length of leaves with each increase in N-P level, but the increase in N-P level beyond 100-100 kg ha⁻¹ was not economical, because the differences amongst 150-150, 125-125 and 100-100 kg N-P ha⁻¹ were statistically non-significant. Similarly, Khan *et al.*, (1998) from Pakistan reported that plant height with 200 kg N was higher than with 150 kg N from sugarbeet variety KaweTerma.

Number of leaves plant⁻¹

The number of leaves plant⁻¹ was significantly highest (37.38) in plots fertilized with highest N-P level of 150-150 kg ha⁻¹, closely followed by 35.91 leaves plant⁻¹, recorded in plots fertilized with 125-125 kg N-P ha⁻¹, and under N-P level of 100-100 kg ha⁻¹ the number of leaves plant⁻¹ was reduced to 31.95 on average. In case of 75-75 kg and 50-50 kg N-P ha⁻¹ fertility levels, the mean number of leaves recorded were 25.36 and 21.58, respectively. However, the minimum number of leaves plant⁻¹ of 13.48 was recorded in plots left untreated (control). This higher number of leaves plant⁻¹ in plots treated with higher fertility levels was mainly associated with improved soil fertility of the experimental plots due to N-P application at higher quantities as compared to lower N-P levels or control plots. The results further indicated that there was a gradual increase in the number of leaves with each increase in fertility level, but this increase was uneconomical when N-P level exceeded 125-125 kg ha⁻¹, because differences in between 125-125 and 150-150 kg N-P ha⁻¹ was non-significant. Similar results have also been reported by Safronovskaya (1998) who recommended 60 t farmyard manure + 120 kg N, 80 kg P, 180 kg K for higher sugarbeet yields, while Wyszynski *et al.*, (1999) recommended application of 130-160 kg N ha⁻¹ required to achieve optimum plant growth in sugarbeet.

Sugarbeet weight per plant⁻¹

The sugarbeet weight plant⁻¹ was significantly maximum (2.22 kg) in plots fertilized with highest N-P level of 150-150 kg ha⁻¹, closely followed by 2.21 kg and 2.13 kg plant⁻¹, recorded in plots fertilized with 125-125 kg and 100-100 N-P ha⁻¹, respectively. The results further showed that the fertility levels of 75-75 and 50-50 kg N-P ha⁻¹ resulted mean sugarbeet weight of 1.63 and 1.22 kg ha⁻¹, respectively. However, the minimum sugarbeet weight of 0.66 kg plant⁻¹ was recorded in plots left untreated (control). This higher sugarbeet weight plant⁻¹ in plots treated with higher fertility levels was resulted due to healthy growing plants which obviously further resulted in healthy beetroots. However, this all happened due to improved soil fertility of the experimental plots due to N-P application at higher quantities. These results have further been supported by Wyszynski *et al.*, (1999) recommended application of 130-160 kg N ha⁻¹ required to achieve a sucrose yield of 10 t ha⁻¹ and found that root quality decreased at higher N rates.

Sugarbeet weight per ha⁻¹

The beetroot yield was significantly maximum (83.142 m.t ha⁻¹) in plots fertilized with highest N-P level of 150-150 kg ha⁻¹, closely followed by 80.588 and 79.484 m.t ha⁻¹, recorded in plots fertilized with 125-125 kg and 100-100 N-P ha⁻¹, respectively. The results further showed that the fertility levels of 75-75 and 50-50 kg N-P ha⁻¹ resulted mean beetroot yield of 65.499 and 54.638 m.t ha⁻¹, respectively. However, the minimum beetroot yield of 35.255 m.t ha⁻¹ was recorded in control plots where no fertilizer was applied. This higher beetroot yield ha⁻¹ in plots treated with higher fertility levels was mainly associated with greater sugarbeet weight plant⁻¹ which has direct effect on the accumulated beetroot yield ha⁻¹. Supporting the above experiences of present investigation, Asad *et al.*, (2000) concluded that 120 kg N ha⁻¹ was economical than 150 kg ha⁻¹, while Barik (2001) used 120 kg N ha⁻¹ and 150 kg K ha⁻¹ treatment at 110 days of the crop. Similarly, Barlóg (2003) mentioned that the nitrogen is the basic mineral nutrient determining the yield and quality of the sugarbeet root. The efficiency of nitrogen-based nutrition can be increased by balanced application of nutrients directly controlling the yielding functions of nitrogen. It should be mainly focused on phosphorus and potassium, since these nutrients form the physiological basis of high yields. In a similar study, Uvarov *et al.*, (2004) from Russia concluded that 90-90-90 kg NPK + 40 t ha⁻¹ manure was most effective combination in sugarbeet production.

Brix percentage

Brix percentage was significantly higher (22.37 %) in plots fertilized with highest N-P level of 150-150 kg ha⁻¹, closely followed by 22.34% and 22.11%, recorded in plots fertilized with 125-125 and 100-100 kg N-P ha⁻¹, respectively. The brix percentage in sugarbeet juice from the plants fertilized with 75-75 and 50-50 kg N-P ha⁻¹ was 20.20 and 19.09 %, respectively. However, the lowest brix content of 17.70% was recorded in control plots where no fertilizer was applied. This higher brix percentage in plots treated with higher fertility levels may have association with the nitrogen and phosphorus which are needed for formation of sugar in the juice. It was observed that there was successive increase in brix percentage with increasing fertility level, but this increase was uneconomical when N-P level exceeded 100-100 kg ha⁻¹, because differences amongst 100-100, 125-125 and 150-150 kg N-P ha⁻¹ were statistically non-significant for brix percentage. The results of the present investigation are fully confirmed by the findings of Shahani *et al.*, (2005) brix content in sugarbeet was 22.50 percent under 100-75 kg N-P ha⁻¹. Similarly, Usmanikhail *et al.*, (2005) concluded that 100-100 kg N-P ha⁻¹ fertility level proved to be an optimum level for producing significantly economical results.

Sugar recovery

The sugar recovery was significantly highest (11.34 %) in plots fertilized with higher N-P level of 150-150 kg ha⁻¹, closely followed by 11.31% and 11.19%, recorded in plots fertilized with 125-125 and 100-100 kg N-P ha⁻¹, respectively. The sugar recovery in sugarbeet juice from the plants fertilized with 75-75 and 50-50 kg N-P ha⁻¹ was 10.34 and 9.78 %, respectively. However, the lowest sugar recovery of 8.98% was recorded in control plots where no fertilizer was applied. This higher sugar recovery in plots treated with higher fertility levels probably have association with the N-P fertilizers, because application of these chemical fertilizers upto adequate levels helps the plant to form more glucose and hence recovery improved. It was observed that sugar recovery was improved consecutively with increasing fertility level, but this increase was not so pronounced when N-P level exceeded 100-100 kg ha⁻¹, because differences amongst 100-100, 125-125 and 150-150 kg N-P ha⁻¹ were statistically non-significant for sugar recovery. The results of the present investigation are fully confirmed by the findings of Shahani *et al.*, (2005) recovery in sugarbeet was higher under 100-75 kg N-P ha⁻¹. Similarly, Usmanikhail *et al.*, (2005) concluded that 100-100 kg N-P ha⁻¹ fertility level proved to be an optimum level for producing significantly economical results

CONCLUSIONS

It was concluded from the results of the present investigation that there was a consecutive improvement in all the quantity and quality components of sugarbeet, but statistically the differences among 100-100, 125-125 and 150-150 kg N-P ha⁻¹ were non-significant for all the growth, beetroot yield and recovery parameters. Thus, fertilizer application beyond 100-100 kg N-P ha⁻¹ was not economical and the above fertility level was considered as an optimum level for economical sugarbeet production under Tandojam conditions.

Table-1 Mean plant population ha⁻¹ of sugarbeet as affected by different fertility levels

Treatments (NP kg ha ⁻¹)	Plant population ha ⁻¹	Length of leaves (cm)	No.of leaves plant ⁻¹	Beetroot weight (kg plant ⁻¹)	Beetroot yield (kg ha ⁻¹)	Brix (%)	Recovery (%)
T1= Control	69173c	33.75a	13.48e	0.66d	35.255d	17.70d	8.98d
T2=50-50	71389c	44.25c	21.58d	1.22c	54.638c	19.09c	9.78c
T3=75-75	78743b	52.25b	25.36c	1.63b	65.499b	20.20b	10.34b
T4=100-100	90053a	58.75a	31.95b	2.13a	79.484a	22.11a	11.19a
T5=125-125	93367a	61.25a	35.91a	2.21a	80.588a	22.34a	11.31a
T6=150-150	93230a	62.50a	37.38a	2.22a	83.142a	22.37a	11.34a
S.E±	558.2330	0.9923	0.6362	0.0511	0.9290	0.0822	0.0620
LSD 0.05	3190.00	3.547	2.275	0.1846	2.972	0.2955	0.2213
LSD 0.01	3945.00	4.807	3.082	0.2502	4.027	0.4005	0.2999
CV%	4.66	5.21	5.66	7.36	3.38	1.09	1.45

Values followed by same letters do not differ significantly at 0.05 probability level.

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EFFECT OF MEDIUM COMPOSITION ON CALLOGENESIS AND SOMATIC EMBRYOGENESIS IN DIFFERENT VARIETIES OF SUGARCANE (*SACCHARUM OFFICINARUM* L.) I.E. S-2002-US-302, HSF-240 AND HSF-242

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ABSTRACT

Segments from inner young leaf whorls (of 3-9mm length) of sugarcane were cultured on MS growth medium supplemented with auxin alone i.e. 2,4-D (1-4 mg/l) and auxin-cytokinin combinations in different ratios. Leaf tissue explants had great potential for callus induction, somatic embryoid induction and supported plant regeneration. After inoculation embryogenic callus (of varied texture and color) was formed while in some cases direct embryoid emergence on cut edges of explant was also exhibited. Among MS media with auxin concentrations 2,4-D 3mg/l was more appropriate for callus and somatic embryoid induction as well as plant regeneration in all three varieties. Each variety showed different response to different ratios of auxin-cytokinin combinations. 2,4-D+BAP (2+1, 2+2 & 2+3mg/l) and IAA+BAP (2+2 & 2+3mg/l) were more appropriate for callus and embryo induction and plant regeneration.

Abbreviations: BAP: 6-Benzyl aminopurine, 2,4-D: 2,4-Dichlorophenoxyacetic acid, IAA: Indole acetic acid, MS: Murashige and Skoog's growth medium, EDTA: Ethylene diamine tetra acetate.

Key words: Sugarcane, *Saccharum officinarum*, Callogenesis, Somatic embryogenesis. Leaf whorls as explants

INTRODUCTION

Sugarcane is a member of family *Gramineae* and is commonly farmed, well known and important cash crop of Pakistan. It is cropped in Pakistan mainly for sugar production. It is second largest cash crop of Pakistan after cotton and the multibillion sugar industry of Pakistan entirely based on sugarcane crop.

Sugarcane is an important source of income and employment for the farming community of Pakistan along with sugar and sugary production. It also forms essential items for industries like sugar, chip board, paper, baggase, confectionary, and use in chemicals, plastics, paints, synthetics, fiber, insecticides and detergents (Alam & Khan, 2001).

It is cultivated on about 7.009 million hectares with 48.8 tons per hectare average yield and 8.33% sugar recovery. This is far below the world average of 63.7 tons per hectare yield and 10.6% sugar recovery (PSMA Annual report-2005). There may be many reasons for low cane production and sugar recovery but lack of extensive research studies in sugarcane crop technology & contamination of elite, transgenic varieties during multiplication by common methods are major causes. Yield decline due to pathogen is most threatening factor.

Production losses in cane crop due to plant diseases can be stopped or lowered by cropping sugarcane varieties with good agronomic characteristics i.e. tolerant to pests, diseases and

weather fluctuations. Disease tolerant or disease resistant varieties, produced by biotechnology technique can be rapidly multiplied (*in vitro* propagated) by plant tissue culture technique. Callogenesis, organogenesis and micropropagation techniques can be used to multiply transgenic elite species in very short time. These techniques also eliminate the threats of disease entry in a species during multiplication.

Sugarcane propagation by tissue culture technique is well appropriate because the main method of plant production is vegetative in nature by stem cuttings called 'setts'.

To get benefit from tissue culture technique, in this research work three varieties (S-2002-US-302, HSF-240 & HSF-242) of sugarcane were *in vitro* propagated through somatic embryoids via intervening callus phase.

MATERIALS AND METHODS

Plant material: The explants of three varieties S-2002-US-302, HSF-240 and HSF-242 were obtained from Ayub Agriculture Research Centre, Faisalabad. Inner whorls of leaves were used as explants. The field collected plant material was washed several times with tap water with a few drops of liquid soap followed by rinsing with autoclaved double distilled water. The outer mature leaves were removed carefully the explant was surface sterilized to escape the threat of contamination and inner leaf whorls of 3-9 mm were excised and inoculated on MS (Murashige and Skoog, 1962) medium supplemented with different ratios of auxins and cytokinins each singly and in combinations to find the optimum growth medium for *in vitro* propagation of sugarcane via callogenesis and somatic embryogenesis.

Media formulation: Growth medium was prepared on MS formulation (MS basal salts Macronutrients, Micronutrients, Iron EDTA and vitamins) with 3% sucrose and the media was gelled with 1% Difco-Bacto agar after adjusting the pH at 5.5 to 5.7. The media was autoclaved at 121°C for 20 min at 105 kPa and was inoculated with explants in complete aseptic conditions. The cultures were incubated at 23±2°C under 16-18hr light period (from fluorescent light tubes) with 6-8hr dark period with light intensity of 3000-4000 lux.

Cultures were shifted to fresh media, regularly after three weeks, either with same supplementation or with different. Proliferation rate and percentage of callus formation and somatic embryoid formation was noted after each subculturing.

Callus induction: To study the sugarcane propagation via callogenesis, leaf tissue explants of all varieties were cultured on MS media supplemented with four different concentrations of auxin alone (in concentrations of 2,4-D 1- 4mg/l) and auxins in combination with cytokinins i.e. 2,4-D & BAP (1+1, 2+1, 2+2, 2+3 & 1+2 mg/l) and BAP & IAA (2+2 & 3+2 mg/l). Data about percentage of callus, form and color of callus was recorded after three weeks of incubation and the time (in days) to induce callus formation was noted for each medium separately.

Somatic embryoid induction and plantlet formation:

Direct somatic embryogenesis: In this study, leaf tissue explants were cultured on MS medium supplemented with different concentrations of auxin (2,4-D 1-4mg/l) and auxin-cytokinin combinations in different ratios i.e. 2,4-D & BAP (1+1, 2+1, 2+2 & 2+3 mg/l) and BAP & IAA (2+2 & 3+2 mg/l). Time taken (in days) to produce direct somatic embryos without intervening phase of callus was noted and the percentage of direct somatic embryo induction and plant regeneration from somatic embryos was calculated after six weeks of incubation in all three varieties.

Indirect somatic embryogenesis: However indirect somatic embryogenesis via intervening phase of embryogenic callus and number of regenerated plants were observed on the same type of culture medium and after shifting into new selected media with supplementations different from initial culture medium (auxin-cytokinin combinations). Percentage of indirect embryos induction and plant regeneration was calculated after ten weeks of incubation.

The embryos produced were germinated into roots and shoots after certain weeks of inoculation. To mediate plant regeneration through somatic embryos different media containing different concentrations of hormones and their combinations were used and percentage of plant regeneration was recorded.

Subculturing: Cultures were shifted to fresh media, regularly after three weeks, either with same supplementation or with different. Proliferation rate, percentage of callus and somatic embryoid formation was noted after each sub culturing.

RESULTS AND DISCUSSION

Effect of Auxin alone and Auxin-Cytokinin combinations on callogenesis

In immature leaf segments callus induction was observed within first week of incubation. HSF-242 was observed to be latent in response to callus induction as compared to other two varieties; HSF-240 responded earlier than others with higher percentage of callus formation. Among four concentrations of auxin alone (2,4-D 1-4 mg/l) used, MS medium supplemented with 2,4-D 3mg/l proved to be the best for callus response with highest percentage of callus formation (100% in S-2002-US-302 & HSF-240 and 90% in HSF-242) with least time (5,3 & 9 days respectively) taken to induce callus formation (Table-1). 14 days old calli of S-2002-US-302, HSF-240 & HSF-242 in 2,4-D 3mg/l are shown in Fig-1(a) to (c).

Many researchers like Liu *et al.*, (1980), Ho and Vasil (1983) and Virupakshi *et al.*, (2002) have also used young leaf tissues of sugarcane as explant for callogenesis. Alam *et al.*, (2003) used MS with supplementation of 2,4-D (1-4 mg/l) and found 2,4-D 3mg/l more appropriate for callus induction. Kharinarain *et al.*, (1996) and Mannan & Amin (1999) found MS+2,4-D 3mg/l as best medium for callusing in sugarcane. Fitch and Moor (1990), Somashekhar *et al.*, (2000), Lal, (2003) and Gandonou *et al.*, (2005) maintained callus on MS medium with 2,4-D at 3 mg/l and obtained good results. Gill *et al.*, (2006) reported swelling in cultured leaf segments of sugarcane before callus induction.

Two types of auxin-cytokinin combinations (2,4-D & BAP in concentration of 1+1, 2+1, 2+2 & 2+3 mg/l and BAP & IAA in 2+2 & 3+2 mg/l) were tested to check the potential of sugarcane for callogenesis. S-2002-US-302 responded better to

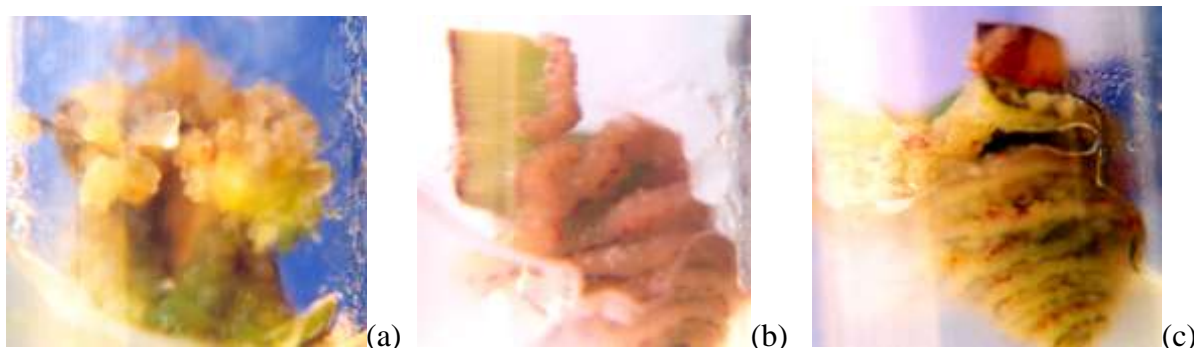


Fig.1 (a) callus of S-2002-US-302, (b) callus of HSF-240 & (c) callus of HSF-242 (all are 14 days old).

MS medium supplemented with IAA+BAP 2+3mg/l than any other combination. In HSF-240 & HSF-242 best callusing responses were exhibited by 2,4-D & BAP in ratios of 2+2mg/l and 2+1mg/l.

Similar work was done by Kumari (2000) and has reported callus formation in sugarcane on MS basal medium with various concentrations and combinations of different auxins and cytokinins. Of the different media tested, the best response was found on MS with BAP + 2,4-D 1+2 mg/l for callus formation.

Significant variability was observed in the form and colour of callus even in viability (embryogenic calli produced plenty of plants through somatic embryoids) and proliferation rate (the rate with which explant turned into callus tissue) of calli of different varieties developed in growth medium with different supplementation as a result of hormonal action (detailed observations are given in (Table-1).

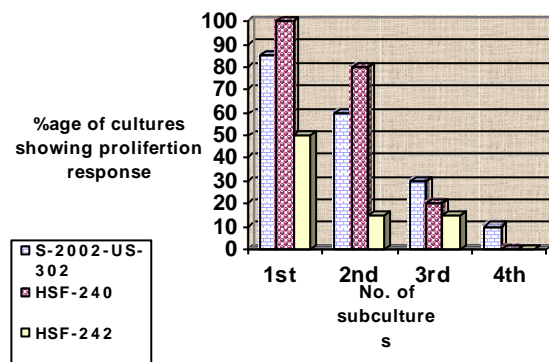
Many researchers have reported variety of callus colors and texture types in many varieties of sugarcane on different media from different explants i-e. Guiderdoni and Demarly (1988) found nodular and friable, Zhou *et al.*, (1995) reported compact and granular, Escalona *et al.*, (1995) observed compact and nodular, Fitch and Moore (1990) noted white and green colored and Anbalagan *et al.*, (2000) reported (a) loose, friable and non embryogenic, and (b) compact, white, nodular and embryogenic calli.

Response to growth media with different supplementations lead to make two assumptions: first that the time taken by explant to induce callus formation goes on decreasing with the increase in concentration of auxin in medium upto specific concentration (3 mg/l) i-e, variety S-2002-US-302 took 7, 6, 5 and 9 days, variety HSF-240 took 9, 7, 3 and 5 days and variety HSF-242 took 15, 15, 9 and 11 days in 2,4-D 1mg/l, 2mg/l, 3mg/l & 4mg/l respectively (Table-1); second that auxins alone were more effective to produce fresh and viable calli as compared to auxins in

combination with cytokinins i-e, percentage of callus induction was higher in auxins than auxins+cytokinins (Table-1).

Effect of subculturing on callus proliferation rate

The calli formed from leaf tissue explant of all varieties, called as the main cultures, were subcultured on fresh MS medium with same supplementation as the main cultures, after every three weeks upto four subcultures and the data of observations was collected upto fifteen weeks of incubation. When the calli (the main cultures), formed in different media, were subcultured they proliferated at their maximum rate after first and second subculture but turned brown, at the end of third subculture and died after fourth subculture. The argument is supported with the percentage of cultures those showed positive response (proliferated better than before subcultured) i-e variety S-2002-US-302 showed 85, 60, 30 & 10%, variety HSF-240 showed 100, 80, 20 & 0%, and variety HSF-242 as 50, 15, 15 & 0% proliferation after first, second, third and fourth subculture respectively (Text fig.1).



Text fig-1 Percentage response of subculturing on callus proliferation in varieties S-2002-US-302, HSF-240 & HSF-242

Effect of Auxins alone and Auxin-Cytokinin combinations on direct somatic embryogenesis:

Initially the explants (young leaf segments) developed nodular outgrowths on the cut edges when cultured on MS medium, which transformed into pro-embryoids. These pro-embryoids developed into well-developed bipolar embryos within six weeks of incubation.

Direct embryogenic response was observed within 12-35 days of incubation in four different concentrations of 2,4-D. Pro-embryos were observed at cut edges of leaf tissue (Fig.2) in 2,4-D 3mg/l within 12 and 18 days after incubation with 40% and 20% embryo induction (Table-2) in varieties S-2002-US-302 and HSF-240 respectively. In other concentrations of 2,4-D embryos induced later than 2,4-D 3mg/l and didn't support plenty of plantlets. The same behaviour was true for both varieties. In variety HSF-242 no embryo induction was observed directly rather whole of the explant cultured turned into embryogenic callus.



Fig.2 Direct embryogenesis in S-2002-US-302 in 2,4-D 3mg/l.

Among hormonal combinations, embryo induction was observed within 16-39 days and 2,4-D+BAP 2+1 mg/l found most appropriate with 5% and 50% embryo induction within 39 and 16 days in S-2002-US-302 and HSF-240 respectively. 2,4-D+BAP 2+2 mg/l, induced embryoids within 22 and 37 days with 5% and 10% in S-2002-US-302 and HSF-240 respectively (Table-2). IAA+BAP combination didn't support direct somatic embryos at all in any variety.

Some other researchers have also used young leaf segments as explant for embryogenesis i.e. Gill *et al.*, (2006) used young leaf segments. Manickavasagam and Ganapathi (1998) also used leaf segments and cultured on MS media supplemented with auxins alone (2,4-D 1-4mg/l) and in combination with 0.5, 1.0 & 2.0mg/l BAP. He found that 2,4-D+BAP 2+1mg/l among different combinations was best for direct embryogenesis. Niaz & Quraishi (2002) and Franklin *et al.*, (2006) obtained considerable results in 3-mg/l 2,4-D for embryogenesis.

Effect of Auxin alone and Auxin-Cytokinin combinations on indirect somatic embryogenesis:

In variety S-2002-US-302 MS medium supplemented with 2,4-D 1- 4 mg/l was used to investigate the effect of auxins alone on indirect somatic embryogenesis. In all concentrations of 2,4-D, 1-4mg/l the calluses were observed to induce somatic embryos in main culture (before first sub-culture) within 16, 15, 14 & 20 days with 25, 50, 100 and 30% embryo induction respectively (Table-3) but these embryos died with passage of time in successive subcultures and showed no plant regeneration except in 2,4-D 3mg/l that seemed more appropriate and produced plenty of plants Fig.3 (a) shows brown colored somatic embryoids on yellowish green callus and root emergence is very clear and (b) shows shoot emergence through somatic embryoids.

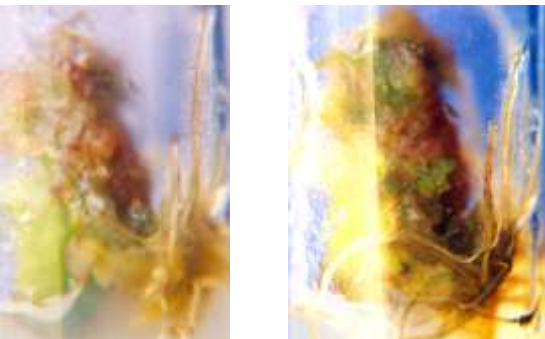


Fig.3 (a) Somatic embryoids in 21 days old culture in S-2002-US-302 in 2,4-D 3mg/l (b) plant emergence through somatic embryoids in same medium in 40 days old culture.

Among different auxin-cytokinin combinations 2,4-D+BAP (2+3 mg/l) and IAA+ BAP (2+3mg/l) showed best results. In 2,4-D+ BAP (2 + 3 mg/l) embryo formation started after 14 days of incubation with 65% embryo formation (Table-3).

In HSF-240 2,4-D 3mg/l was more appropriate among 2,4-D 1-4mg/l for embryo induction (70 %) and took least time (among auxins alone) to induce somatic embryoids (second day after 1st subculture and 23 days of incubation) but these were not viable for plant regeneration. 2,4-D+BAP 2+1 and 2+2 mg/l media proved best with 90 and 100% induction of most viable embryoids within 20 & 18 days (Table-3), Fig.4 (a) shows yellow colored nodular embryoids and Fig.4 (b) plantlet emergence through these embryoids. IAA+ BAP (1+2 and 2+2 mg/l) also showed good results.

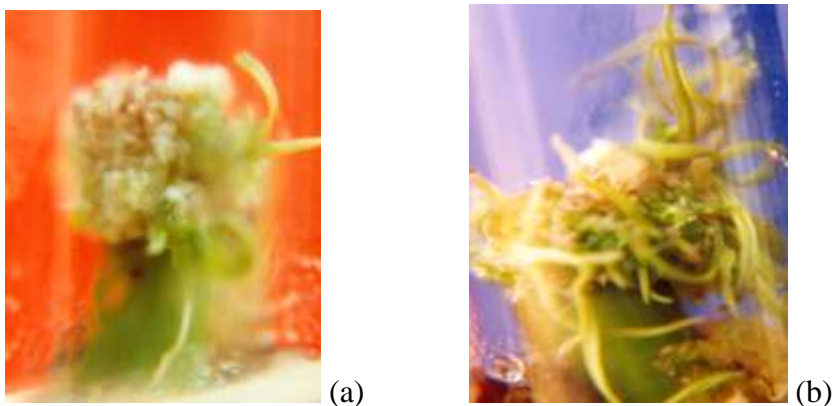
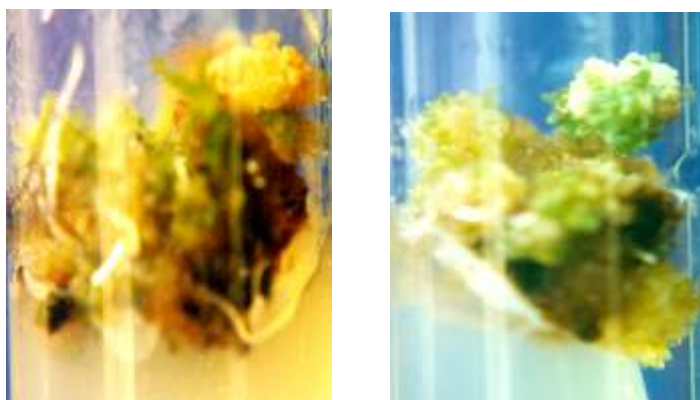


Fig. 4 (a) Somatic embryoids in 50 days old culture of HSF-240 in 2,4-D+BAP (2+2) mg/l, (b) plant emergence through somatic embryoids in same medium in 70 days old culture.

In HSF-242, in contrary to rate of callus formation the rate of embryo induction and shoot formation was too slow in different concentrations of 2,4-D as compared to auxin-cytokinin combinations. None of the culture was observed to induce somatic embryos before subculture (Table-3). 2,4-D+BAP (2+2 & 2+3 mg/l) combinations proved best for embryo induction (30 & 35% respectively, Table-3) and plant regeneration in this variety (Fig.5 a-b).

It was inferred that the potential of leaf tissue explants for somatic embryo induction and plant regeneration was found to be very high in all varieties, cultured on MS medium supplemented with auxin-cytokinin combination and least in auxin alone except S-2002-US-302 which showed very good percentage, rate of embryo induction and plant regeneration in auxin (2,4-D) also. Direct embryogenesis showed rapid differentiation than that of indirect but the rate of survival was higher in plants generated through indirect somatic embryogenesis.

Some other researchers i.e. Niaz and Quraishi (2002), Gill *et al.*, (2004) and Ahloowalia & Maretzki (1983) have also found that among auxins alone 2,4-D 3mg/l is most appropriate medium for indirect somatic embryogenesis and plant regeneration in sugarcane. Ho and Vasil (1983) has used young leaves of sugarcane for somatic embryogenesis on MS medium with 2,4-D.



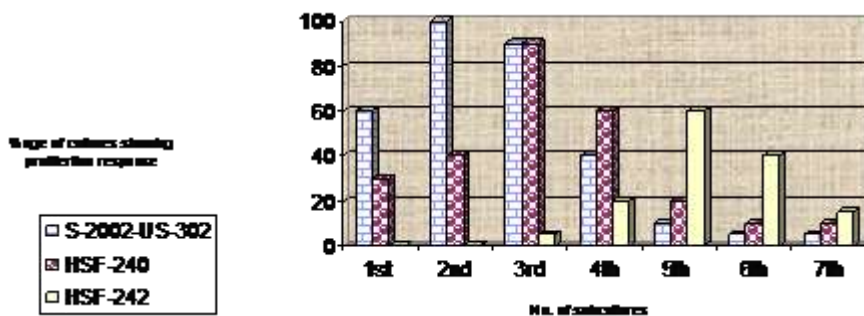
(b)

Fig.5 (a) Somatic embryoids in 105 days old culture of HSF-242 in 2,4-D+BAP (2+2) mg/l (b) plant emergence through somatic embryoids in 2,4-D+BAP (2+3) mg/l in 155 days old culture.

Ahloowalia and Maretzki (1983) reported somatic embryogenesis on MS medium with 3mg/l 2, 4-D after 10 weeks of culture. Effect of auxin-cytokinin combination on indirect somatic embryogenesis was studied by Manickavasagam and Ganapathi (1998) and they used 2, 4-D alone (1-4mg/l) and in combination with BAP (0.5, 1.0 & 2.0mg/l) and found best results with 2, 4-D+BAP (2+1 mg/l).

Effect of sub-culturing on somatic embryogenesis and plant regeneration

Sub-culturing strongly affects the somatic embryogenesis and rate of plant regeneration through somatic embryoids. Auxin (2,4-D) at the concentration of 3mg/l formed calli with highest rate of proliferation and good percentage but these calluses didn't induce any embryo or plantlet formation, even after several subcultures on medium of same supplementation. These calluses were sub-cultured and shifted to new media with different supplementations i.e. supplemented with auxin-cytokinin combinations. It was generally observed that sub-culturing favors embryo induction and plant regeneration especially when cultures were transferred from auxin alone (2,4-D) to auxin-cytokinin combination i.e. 2,4-D + BAP (1+1, 2+1, 2+2, 2+3mg/l) and IAA+BAP (2+2, 2+3 mg/l) in S-2002-US-302, 2,4-D + BAP (3+1, 1+1, 2+1, 2+2 mg/l) in HSF-240 and HSF-242. Two combinations, 2, 4-D + BAP (2 + 1, 2 + 2 & 2 + 3) and BAP + IAA (2 + 1 & 2 + 2) showed best results.



Test fig-2 Percentage response of sub-culturing on embryo induction and plant regeneration in varieties S-2002-US-302, HSF-240 & HSF-242

In S-2002-US-302 1st, 2nd and 3rd with 60, 100 and 90% response, in HSF-240 3rd and 4th with 90 & 60% (Fig.6 a & b) and in HSF-242 5th and 6th with 60 & 40% (Fig.7 a & b) subcultures were more effective to enhance the rate of embryogenesis and plant regeneration (Text fig.2).

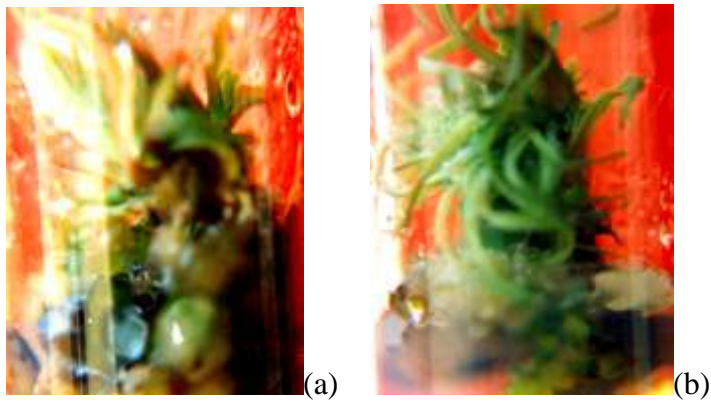


Fig.6 Effect of subculturing on somatic embryoids and plant formation in HSF-240 (a) Response to 3rd subculture 70 days old culture in 2,4-D+BAP 2+1mg/l (b) Response to 4th subculture 85 days old culture in same medium.

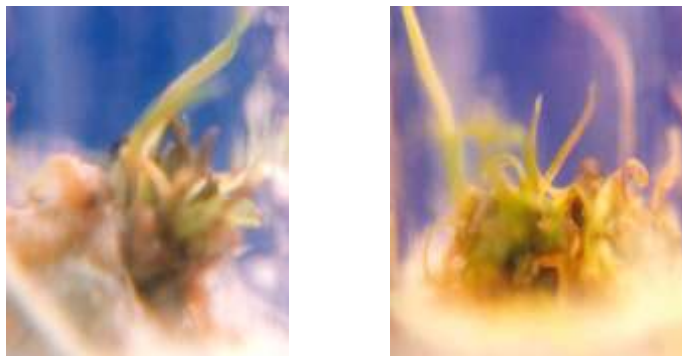


Fig.7 Effect of subculturing on somatic embryoids and plant formation in HSF-242 (a) Response to 5th subculture 120 days old culture in 2,4-D+BAP 2+2mg/l (b) Response to 6th subculture 145 days old culture in same medium.

Table-1 Effect of different concentrations of phytohormones and combinations on callus initiation and its development in variety S-2002-US-302, HSF-240 and HSF-242

Medium	Conc. mg/l	No. of explant cultured	Age of culture (weeks)	Days to callus initiation after incubation			%age of callus induction			Proliferation rate			Color of callus			Form of callus			Viability		
				30	240	242	30	24	24	30	24	24	30	24	24	30	240	24	30	24	24
Varieties →				2			2	0	2	2	0	2	2	0	2	2	0	2	2	0	2
MS+2,4-D	1.0	20	3	7	9		30	15	35	P	P	P	B	B	B	F	LF	LC	N	N	N
	2.0	20	3	6	7	15	35	20	40	E	F	P	G	D	Y	C	LF	LF	V	V	V
	3.0	20	3	5	3	15	10	10	90	E	E	F	Y	Y	B	LC	LF	LC	L	L	N
	4.0	20	3	9	5	9	0	0	25	G	F	G	B	B	P	LF	F	LF	V	V	V
						11	45	60				B	Y	Y	LC		LF-NF	V	V	V	
													Y	Y			NF	V	V	L	V
																		N	V	V	
MS +2,4-D+ BAP	1+1	20	3	10		12	30	-	25	P	-	F	D	-	F	LF	-	NF	L	-	N
	2+1	20	3	8	-	10	45	85	60	F	G	F	G	O	Y	LF	L	NF	V	V	V
	2+2	20	3	5	4	9	40	90	45	F	E	P	FG	Y	F	LF	F	LC	V	V	V
	2+3	20	3	10	7	18	20	95	25	F	F	P	B-	FY	Y	NF	N	NF	V	V	V
	1+2	20	3	-	3	-	-	35	-	-	P	-	Y	Y	Y	-	F	LC	L	L	V
					5							G	LB	Y		F	NF	V	V	N	
												Y				F			V		V
MS +IAA+ BAP	2+2	20	3	-	-	-	40	-	-	F	-	-	D	-	-	LF	-	-	V	-	-
	2+3	20	3	14	-	-	90	-	-	E	-	-	B	-	-	NF	-	-	V	-	-
				20								G	Y						V		

Abbreviations:

Conc. concentration

- B: brown DB: dark brown LC-LF: less compact to less friable
P: poor YB: yellowishbrown FG: fresh green
LF-NF: less friable to nodular friable F: fair PY: pale yellow
DG: dark green LC-NF: less compact to nodular friable G: good
Y: yellow OY: orange yellow NF: nodular friable
E: excellent FY: fresh yellow BY: brownish yellow
GY: greenish yellow B-Y: brown to yellow DY: dark yellow
BY: brownish yellow

Table-2 Effect of different concentrations of phytohormones and combinations on direct embryogenesis in variety S-2002-US-302, HSF-240 and HSF-242

Medium	Conc. mg/l	No. of explant cultured	Age of cultures (weeks)	Days to embryo induction after incubation			%age of embryo induction		
				302	240	242	302	240	242
Varieties →				302	240	242	302	240	242
MS+2,4-D	1.0	20	6	18	25	-	5	5	-
	2.0	20	6	20	29	-	5	5	-
	3.0	20	6	12	18	-	40	20	-
	4.0	20	6	35	31	-	5	5	-
MS+2,4-D+BAP	1+1	20	6	-	-	-	-	-	-
	2+1	20	6	39	16	-	5	50	-
	2+2	20	6	22	37	-	5	10	-
	2+3	20	6	-	-	-	5	-	-
MS+IAA+BAP	2+2	20	6	-	-	-	-	-	-
	2+3	20	6	-	-	-	-	-	-

Table-3 Effect of different concentrations of phytohormones and combinations on callus initiation and its development in variety S-2002-US-302, HSF-240 and HSF-242

Medium	Conc. mg/l	No. of explant cultured	Age of culture (weeks)	Days to embryo induction after incubation			Days to embryo induction after transfer			%age of embryo induction			Color of callus		
				302	240	242	302	240	242	302	240	242	302	240	242
Varieties →				302	240	242	302	240	242	302	240	242	302	240	242
MS+2,4-D	1.0	20	10	16	-	-	-	-	-	25	5	-	+	-	-
	2.0	20	10	15	(50)	-	-	8(2)	-	50	55	-	+	+	-
	3.0	20	10	14	(23)	-	-	2(1)	-	100	70	-	3+	2+	-
	4.0	20	10	20	(40)	-	-	19(1)	-	30	60	-	+	+	-
MS+2,4-D+BAP	1+1	20	10	(44)	-	-	-	-	-	35	-	-	0	-	-
	2+1	20	10	(37)	20	(150)	2(2)	-	3(7)	45	90	15	+	3+	+
	2+2	20	10	(25)	18	(110)	16(1)	-	15(5)	20	100	30	+	3+	2+
	2+3	20	10	14	-	(108)	4(1)	-	3(5)	65	-	35	3+	-	2+
	3+1	20	10	-	(50)	-	-	8(2)	-	-	40	-	-	0	-
MS+IAA+BAP	1+1	20	10	-	(91)	-	-	13(4)	-	-	40	-	-	+	-
	2+1	20	10	-	(96)	-	-	18(4)	-	-	40	-	-	+	-
	3+1	20	10	-	(106)	-	-	1(5)	-	-	20	-	-	+	-
	1+2	20	10	-	(31)	-	-	10(1)	-	-	70	-	-	2+	-
	2+2	20	10	(37)	(23)	-	16(1)	2(1)	-	55	90	-	2+	3+	-
	2+3	20	10	(27)	-	-	6(1)	-	-	70	-	-	3+	-	-

Abbreviations:0: less than previous +: same as before subculturing

2+: doubled than previous 3+: tripled than previous

Days to embryo induction after incubation: In the () the age of cultures is given and represents induction after 1st subculture while without parenthesis represents before subculture.

Days to embryo induction after transfer: no. of subcultures is given in parenthesis.

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DETERMINATION OF SITE SPECIFIC FERTILIZER REQUIREMENT OF SUGARCANE AND INTERCROP (POTATO) UNDER SUGARCANE-BASED CROPPING SYSTEM

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ABSTRACT

A field experiment was conducted at Ishurdi under High Ganges River Floodplain (AEZ 11) and Jamalpur under Young Brahmaputra and Jamuna Floodplain (AEZ 9) soils with a view to determine the fertilizer requirements of cane and intercrop (potato) and their economics under sugarcane based cropping systems. Cane yield was enhanced (6-8%) when it was intercropped with potato for the residual effect of applied fertilizer to intercrop. Gross return and gross margin was higher in cane intercropped with potato over sole crop at both the locations. Intercropping potato with sugarcane using BSRI'98 rates of fertilizer for cane and potato followed by in situ dhaincha incorporation gave higher economic benefit at Ishurdi and Jamalpur.

INTRODUCTION

Substantial advantage in yield from intercropping system is achieved compared to sole cane due to synergistic effects of companion crops on each other by making better use of agronomic management and residual advantage of applied fertilizer for intercrops (Imam *et al.*, 1990; Bokhtiar *et al.*, 1995 and Willey *et al.*, 1979). Sugarcane plants have large lateral spread when fully-grown but initial rate of increase in horizontal spread is rather slow. Generally sugarcane is cultivating with a row spacing of 90 cm or more and much of this space remains unutilized for a period of 100-120 days. Hence there is an ample scope to grow one or more intercrops in the vacant space in between the rows. The sugarcane crop depletes a considerable amount of fertilizer from soil. As a consequence soils lose its inherent ability to supply nutrient for sustainable production. Yadav *et al.*, 1987 reported that the organic matter content of sugarcane soil increased due to companion cropping of pulses. 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). The importance of these intercropping systems is known, the trial was designed to verify how different nutrient management options based on medium and high yield goals influence its productivity, profitability and change in soil fertility.

MATERIALS AND METHODS

A field experiment was conducted in 2000-2001, 2001-02, 2002-03 and 2003-04 cropping seasons at Ishurdi under High Ganges River Floodplain (AEZ 11) and Jamalpur under Young Brahmaputra and Jamuna Floodplain (AEZ 9) soils. The soil characteristics of the experimental sites are given in table 4a,b. Each treatment replicated three times in a randomized complete block design. Details of treatments

and the amount of fertilizers used for each treatment are given in Table 1. A 30-day old single budded soil bed settlings with a row spacing of 1m and interplant spacing of 0.5m were transplanted at Ishurdi (var. Isd 30) and Jamalpur (var. Isd 28) in December and it was harvested after 13-months. The unit plot size was 8 m x 6m. One line of potato (var. cardinal) tuber as intercrop was planted in between cane rows and harvested after 90 days of plantation. Full dose of P, S, Zn as TSP, Gypsum and Zinc sulphate were applied in trenches and thoroughly mixed with soils by spade prior to transplanting of sugarcane settlings. One third of the quantities of N and K were top-dressed after 20-30 days of settling transplantation. The rest amount of N and K fertilizers was applied in two equal splits at tiller completion stage and grand growth stage. Half of N, K and all other fertilizers for potato were applied at the vacant space of two cane rows and mixed thoroughly with soils and remaining N and K were applied as top dressing at 45 days of planting. The recommended crop management practices were followed when required for cane and intercrop potato.

Table-1 Details of treatments and the amount of fertilizers (kg ha⁻¹) used in each treatment in sugarcane + potato intercropping systems

Treatments	Locations											
	Ishurdi						Jamalpur					
	N	P	K	S	Zn	GM (Green biomass)	N	P	K	S	Zn	GM (Green biomass)
T ₁ Cane	130	35	60	20	3	-	120	40	70	25	2	-
T ₂ Cane	196	27	97	0	0	-	177	60	115	9	-	-
T ₃ Cane	150	52	90	35	3	-	150	42	100	30	2	-
T ₄ Cane	130	35	60	20	3	-	120	40	70	25	2	-
Potato	<u>50</u>	<u>10</u>	<u>20</u>	<u>0</u>	0	-	<u>50</u>	<u>10</u>	<u>25</u>	<u>10</u>	-	-
T ₅ Cane	150	52	90	35	3	-	150	42	100	30	2	-
Potato	<u>69</u>	<u>16</u>	<u>40</u>	<u>0</u>	0	-	<u>50</u>	<u>10</u>	<u>40</u>	0	0	-
T ₆ Cane	150	52	90	20	3	13, 500	150	42	100	30	2	15, 000
Potato	<u>50</u>	<u>10</u>	<u>20</u>	<u>0</u>	0	-	50	10	40	0	0	-

T₁-Sole sugarcane (Fertilizer used for MYG as per FRG'97 on AEZ basis), T₂- Sole sugarcane (Fertilizer used for HYG as per STB), T₃- Sole sugarcane (Fertilizer used as per BSRI'98), T₄- Sugarcane + potato (as per FRG'97), T₅- Sugarcane + potato (as per BSRI'98) and T₆- Sugarcane + potato (as per BSRI'98) followed by Dhaincha.

MYG-Moderate yield goal; STB- Soil test basis; BSRI-Bangladesh Sugarcane Research Institute; FRG- Fertilizer Recommendation Guide

RESULTS AND DISCUSSION

Yield of sugarcane and intercrop (potato)

The effect of different fertilizer management options on the yield of cane and intercrop (potato) is presented in Table 2. Fertilizers applied for MYG gave high yield of sole cane (95.56 t ha⁻¹). Further increase the amount of fertilizer either soil test basis or BSRI'98 basis did not increase cane yield remarkably. Cane yield was higher in intercropping system than sole crop and the highest cane yield was found in T₅ (113.86 t ha⁻¹) which was 6% higher than the corresponding sole cane (T₃). The

residual value of fertilizers applied in potato might be helped in increasing cane yield. This finding was in close conformity with the finding of Verma and Bhoj (1980) who reported that plots intercropped with potato yielded more cane than plots with cane alone and net profit from this plot was significantly higher than any other intercropping combination. Highest potato yield of 7.99 t ha⁻¹ was obtained in T₆ treatment that received fertilizer as per BSRI'98 followed by dhaincha. Cane equivalent yield was influenced by cane yield but small variation in potato yield (0.84 t ha⁻¹ between T₅ and T₆) largely influenced the corresponding cane equivalent yield. The effect of GM in increasing cane yield was not evident.

Cost and return analysis

Gross margin increased with increase of cane equivalent yield and the highest was with T₆. BCR was lower in intercropping systems than sole crop because of higher total variable cost with former systems (Table 3).

Soil fertility status

The status of soil pH, organic matter, total N, available P, K and S in initial soil as well as post harvest soil are presented in Table 4a. There were not conspicuous changes in organic matter and total N contents in soils. A little negative change in available P with a positive change in available S was observed. However, the change in available K was not conspicuous for the different fertilizer treatments.

Yield of sugarcane and intercrop (potato)

The effect of different fertilizer management options on the yield of cane and intercrop (potato) is presented in Table 2. Fertilizers applied for MYG gave high yield of sole cane (113.32 t ha⁻¹). Further increase the amount of fertilizer either soil test basis or BSRI'98 basis did not increase cane yield remarkably. Cane yield was higher in intercropping system than sole crop and the highest cane equivalent yield was found in T₆ (167.33 t ha⁻¹) which was 40% higher than the corresponding sole cane (T₃). The residual value of fertilizers applied in potato might be helped in increasing cane yield. This finding was in close conformity with the finding of Verma and Bhoj (1980) who reported that plots intercropped with potato yielded more cane than plots with cane alone and net profit from this plot was significantly higher than any other intercropping combination. Highest potato yield of 8.17 ha⁻¹ was obtained in T₆ treatment that received fertilizer as per BSRI'98 followed by dhaincha. Cane equivalent yield was influenced by cane yield but small variation in potato yield (0.62 t ha⁻¹ between T₅ and T₆) largely influenced the corresponding cane equivalent yield. This result was also in good agreement with the finding of several workers (Sinha *et al.*, 1990; Ricaud, 1982 and Nanker 1990). The effect of GM in increasing cane yield was evident.

Cost and return analysis

The highest gross margin was with T₆. BCR was lower in intercropping systems than sole crop because of higher total variable cost with former systems (Table 3).

Soil fertility status

The status of soil pH, organic matter, total N, available P, K and S in initial soil as well as post harvest soil are presented in Table 4b. There were considerable increases in organic matter in soils. A little negative changes in total N and available P with a

positive change in available S was observed where the changes in available K were not conspicuous for the different fertilizer treatments.

In conclusion, it may be suggested that fertilizer application as per soil test value (STV) basis against targeted yield would produce higher cane yield by 6 per cent over MYG as per AEZ basis. In sugarcane-potato intercropping system, an increase of 6 to 8 per cent higher cane yield could be ensured over sole cane.

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Table-2 Yield of sugarcane and potato intercrop as affected by different fertilizer management options

Treatment	2000-01			2001-02			2002-03			2003-04			Mean
	Yield (t ha ⁻¹)		Cane equivalent yield (t ha ⁻¹)	Yield (t ha ⁻¹)		Cane equivalent yield (t ha ⁻¹)	Yield (t ha ⁻¹)		Cane equivalent yield (t ha ⁻¹)	Yield (t ha ⁻¹)		Cane equivalent yield (t ha ⁻¹)	
	Cane	Potato		Cane	Potato		Cane	Potato		Cane	Potato		
Ishurdi													
T ₁	105.47	-	105.47	90.93	-	90.93	93.93	-	93.93	91.92	-	91.92	95.56
T ₂	108.07	-	108.07	96.87	-	96.87	87.02	-	87.02	112.24	-	112.24	101.05
T ₃	112.45	-	112.45	104.61	-	104.61	103.21	-	103.21	110.00	-	110.00	107.57
T ₄	115.47	7.38	154.78	126.40	4.95	152.80	79.94	6.28	113.43	97.60	6.52	132.37	138.35
T ₅	126.46	8.06	169.13	121.14	5.88	152.50	88.04	7.21	126.49	119.79	7.43	159.42	151.89
T ₆	120.94	8.50	166.27	118.90	6.41	153.09	85.18	8.77	131.95	115.36	8.28	159.52	152.71
Jamalpur													
T ₁	102.00	-	102.00	96.61	-	96.61	124.44	-	124.44	130.21	-	130.21	113.32
T ₂	112.80	-	112.80	95.15	-	95.15	136.66	-	136.66	139.11	-	139.11	120.93
T ₃	110.50	-	110.50	98.21	-	98.21	139.31	-	139.31	128.96	-	128.96	119.25
T ₄	106.30	5.05	133.33	103.12	5.78	133.95	147.36	7.91	189.55	152.29	7.88	194.32	162.79
T ₅	114.90	5.26	142.95	103.44	6.16	136.29	133.33	9.13	182.02	138.07	9.63	189.43	162.67
T ₆	110.60	6.19	143.61	101.95	6.38	135.98	137.36	9.55	188.29	145.31	10.56	201.63	167.33

Table-3 Cost and return analysis of different fertilizer management options for sugarcane and potato intercropping systems (four years average)

Treatment	Cane equivalent yield (t ha ⁻¹)	Gross Return (Tk. ha ⁻¹)	Total variable cost (Cane + Potato intercrop) (Tk. ha ⁻¹)	Gross Margin (Tk. ha ⁻¹)	BCR
Ishurdi					
T ₁	95.56	107505	29156	78349	3.69
T ₂	101.05	113681	31036	82645	3.66
T ₃	107.57	121016	31322	89694	3.86
T ₄	138.35	156644	46030	110614	3.40
T ₅	151.89	170876	48576	122300	3.52
T ₆	152.71	171799	49376	122423	3.48
Jamalpur					
T ₁	113.32	127485	29,660	97825	4.30
T ₂	120.93	136046	31,208	104838	4.36
T ₃	119.25	134156	30,732	103424	4.37
T ₄	162.79	183139	46,851	136288	3.91
T ₅	162.67	183004	48,208	134796	3.80
T ₆	167.33	188246	49,308	138938	3.82

Price (Tk. / kg): Input: Urea = 6.50, TSP =14.00, MP =9.50, Gypsum =4.00, Zinc sulphate = 60.00, MgO = 40.00, Potato = 11.00, Dhaincha=20.00, Labor = Tk 70 person¹day⁻¹ Output: Sugarcane =1.125, Potato = 6.00

Table-4a Status of initial and post harvest soil as affected by different fertilizer management options for sugarcane and intercrop (potato) at Ishurdi

Cropping season	Treatments	pH	Organic matter (%)	Total N %	Available P (ppm)	Available K (meq 100 ⁻¹ g soil)	Available S (ppm)
2000-2001		Initial soil					
		7.8	1.30	0.06	23.0	0.19	12.0
		Post harvest soil					
		7.5	1.28	0.065	21.0	0.17	15.0
		7.6	1.29	0.07	21.5	0.18	16.0
		7.5	1.28	0.07	21.0	0.18	16.5
		7.7	1.31	0.075	22.0	0.20	16.5
		7.7	1.29	0.07	22.5	0.18	17.0
		7.5	1.30	0.07	21.0	0.19	17.0
2001-2002		Initial soil					
		7.3	1.68	0.07	20.0	0.20	30.0
		Post harvest soil					
	T ₁	7.5	1.45	0.065	21.0	0.19	28.0
	T ₂	7.6	1.50	0.06	19.0	0.20	27.0
	T ₃	7.5	1.43	0.07	19.5	0.18	28.0
	T ₄	7.4	1.53	0.065	18.5	0.18	30.0
	T ₅	7.4	1.50	0.065	19.0	0.419	29.0
	T ₆	7.5	1.55	0.07	18.0	0.18	18.0
2002-2003		Initial soil					
		7.7	0.96	0.070	21	0.17	17.0
		Post harvest soil					
	T ₁	7.5	0.91	0.070	20	0.17	14.0
	T ₂	7.6	0.85	0.070	20	0.18	14.0
	T ₃	7.5	0.91	0.074	21	0.17	15.0
	T ₄	7.7	0.85	0.074	23	0.18	16.5
	T ₅	7.8	0.91	0.078	23	0.18	16.5
	T ₆	7.7	0.97	0.078	21	0.18	14.0
2003-2004		Initial soil					
		7.5	1.01	0.07	20.0	0.18	15.0
		Post harvest soil					
	T ₁	7.5	0.91	0.06	19.0	0.18	16.0
	T ₂	7.6	0.93	0.07	19.5	0.19	17.0
	T ₃	7.5	0.98	0.07	19.0	0.18	17.0
	T ₄	7.6	1.05	0.08	20.0	0.20	16.5
	T ₅	7.6	1.01	0.08	21.0	0.19	17.0
	T ₆	7.5	1.00	0.08	20.0	0.20	17.0

Table-4b Status of initial and post harvest soil as affected by different fertilizer management options for sugarcane and intercrop (potato) at Jamalpur

Cropping season	Treatments	pH	Organic matter (%)	Total N %	Available P (ppm)	Available K (meq 100 ⁻¹ g soil)	Available S (ppm)
2000-2001		Initial soil					
		5.5	0.78	0.07	24.0	0.17	25.0
		Post harvest soil					
	T ₁	5.6	0.80	0.06	20.0	0.16	26.0
	T ₂	5.6	0.82	0.065	21.0	0.17	26.0
	T ₃	5.6	0.81	0.065	21.0	0.17	25.0
	T ₄	5.7	0.82	0.07	21.5	0.19	27.0
	T ₅	5.5	0.85	0.07	22.0	0.18	26.0
2001-2002		Initial soil					
		5.5	0.78	0.07	24.0	0.17	25.0
		Post harvest soil					
	T ₁	5.6	0.80	0.06	22.0	0.18	22.0
	T ₂	5.5	0.75	0.06	23.0	0.09	23.0
	T ₃	5.7	0.76	0.065	21.0	0.17	22.0
	T ₄	5.6	0.75	0.07	22.0	0.18	24.0
	T ₅	5.5	0.78	0.065	23.0	0.17	23.0
2002-2003		Initial soil					
		5.5	0.78	0.06	22.0	0.14	18.0
		Post harvest soil					
	T ₁	5.6	0.78	0.054	18.0	0.16	16.5
	T ₂	5.6	0.78	0.060	20.	0.17	15.0
	T ₃	5.7	0.71	0.066	20.	0.17	16.5
	T ₄	5.7	0.71	0.072	21.0	0.16	18.0
	T ₅	5.5	0.78	0.066	23.0	0.17	18.0
2003-2004		Initial soil					
		5.7	0.82	0.06	21.0	0.15	18.5
		Post harvest soil					
	T ₁	5.6	0.80	0.07	20.0	0.16	19.0
	T ₂	5.6	0.82	0.07	21.0	0.17	20.0
	T ₃	5.7	0.81	0.06	22.0	0.16	18.0
	T ₄	5.7	0.83	0.06	21.5	0.17	18.5
	T ₅	5.6	0.85	0.07	22.0	0.17	19.0
T ₆	5.7	0.87	0.07	22.0	0.16	19.0	

SUGAR INDUSTRY ABSTRACTS

By

M. Awais Qureshi & Dr. Shahid Afghan

AGRICULTURAL ENGINEERING

Sugarcane trash collection at the small farms in Southern India

H. Vincent Paul and M. Krishnamurthi

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To extend the operation of a bagasse based co-generation power plant, both the procurement of supplementary fuels and the extending of the crushing season in the sugar mill were required. The collection of sugarcane trash for use as supplementary fuel was taken up at EID Parry sugar mill at Nellikuppam in Tamil Nadu, India on a trial basis, to determine the viability of this strategy. Trials were done by windrowing trash in the field and collecting and baling it, initially using small rectangular and later, round hay balers. Windrowing was initially undertaken manually. However, a mechanical rake was later used to windrow the trash for trials. Different baler types were tested to find the factors impacting on baler productivity. Modifications were undertaken to adapt the balers to suit Indian field conditions, and appropriate field operating procedures were developed. Manual collection of trash was also introduced at places near to the mill to increase the supply of trash to the mill. Both the small rectangular balers and the round baler had similar outputs in the actual field conditions experienced. The transport efficiency was lower with round bales as they required specialized loading equipment and longer low bed trailers, which could not effectively negotiate the narrow rural roads. Rectangular bales were manually loaded and were simple to handle and transport. While the performance of balers was initially good, performance declined in the second year primarily due to maintenance and wear issues. Although the ability to bale trash was established, the throughput of the equipment must be increased to achieve a viable operation. Apart from small field size, several other field factors were also identified as obstacles to the baling and transport of residues. Farm layouts and farming practices needed to be changed to suit mechanised operations.

Mill-scale supply chain and logistics model integration for improved decision support

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The management of cane procurement and general logistics at a mill scale remains complex and contains valuable opportunities for improved efficiencies and cost savings. In the past, several researchers have focused on different logistical and supply chain issues. This paper reviews these works and attempts to synthesise valuable modeling contributions towards a more holistic supply chain decision

support system. Three previously independent research projects that focused on modeling supply chain and logistics issues at the Sezela sugar mill in South Africa are reviewed. Firstly, a simulation logistic model was used to assess the impacts of logistics on harvest-to-crush delays. Secondly, an optimisation model was used to quantify the impacts of different management rules on transportation and mill performance. Thirdly, a weekly supply chain model was used to estimate the potential benefits of different management strategies on capacities, utilisation and sugar production. The integration of short time stepped logistical models and larger time stepped supply chain models are discussed in order to create a suitable decision support environment that will assist mill scale management to assess the impacts of their decisions at various levels across the supply chain and over extended periods of time. An integrated modeling system, therefore, has the ability to assess the logistical impacts of longer-term supply chain decisions, and vice versa. Integrated systems, however, pose challenges in finding suitable input data and software interfaces. It is concluded that an integrated supply chain modelling system, although difficult to conceptualize and construct, offers real potential benefits to the optimal management of sugar cane supply chains.

AGRICULTURAL AGRONOMY

Improving cane productivity with dual row planting in Mauritius

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Dual row planting, consisting of pairs of cane rows 0.5 m apart with 1.8 m between their centres, was studied in twelve field trials between 1999 and 2004. Dual rows were compared to the standard row spacing of 1.6 m. Two N fertiliser rates (normal and + 25%) and two cane sett densities (normal and a reduced amount) were included as treatments in the early trials whereas the response of sugarcane varieties to the new spacing was evaluated later. Increases in cane yields with dual row planting varied between 3% and 28% depending on cane varieties; M 1400/86 and R 579 were the most responsive with a mean increase varying between 8% and 16%. Yields of plant and ratoon cane showed that dual rows could be planted with an equivalent amount of cane setts and using the same rate of fertiliser as for the conventional spacing. No difference in sucrose content has been observed between the two spacings. Weed management improved with dual rows; and critical periods of weed competition were shortened by at least four weeks. Mechanised harvest of dual rows at some of the sites showed that the pairs of rows can be cut simultaneously without any difficulty; the efficiency of the machines also improved with less driving distance per hectare, less turning time and a higher pour rate. Dual row planting is being increasingly adopted as initial results from commercially planted fields are confirming the gain in productivity.

New herbicide tank-mix, krismat® + dinamic®: a cost-effective broad-spectrum pre- & post-emergence treatment for managing weeds in sugarcane

S. Seeruttun, C. Barbe and A. Gaungoo
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New weed management strategies are being developed to reduce the amount and cost of herbicides used within the Mauritian sugar industry. One approach consists of applying herbicides a few weeks after planting or harvest at the beginning of the critical period of weed competition, with the goal of controlling emerged weeds and providing long-term residual activity. With these objective, new herbicides Krismat (trifloxysulfuron + ametryn) and Dinamic (amicarbazone) were tested alone or in tank-mixes in both plant and ratoon cane. When applied pre-emergence to weeds, Krismat (1.5.1.8 kg a.i./ha) and Dinamic (1.05 and 1.4 kg a.i./ha) were found to be comparable to the standards. Krismat was less effective on *Digitaria horizontalis* and *D. timorensis* while Dinamic did not control *Cyperus rotundus*, *Paspalum* spp. and *Kyllinga* spp.; tank-mixing lower rates of the two herbicides overcame their weaknesses while maintaining a residual activity over 14 to 16 weeks. With early post-emergence applications, both Krismat and Dinamic were effective on many broad-leaved weeds and some grasses. The efficacy of Krismat on *Paspalum* spp., *C. rotundus* and other sedges, and that of Dinamic on *Digitaria horizontalis* compensated for their individual inefficacies when they were tank-mixed. The tank-mix, Krismat + Dinamic (1.5 + 0.875.1.05 kg a.i./ha) did not cause crop injury in young plant or ratoon cane. The efficacy (pre- and post-emergence) of the new tank-mix offers a new perspective for managing weeds in sugarcane, as delaying of the first herbicide application will result in savings of at least one herbicide treatment per season.

SUGARCANE BREEDING

Utilising genetic dissimilarity for planning of crosses in sugarcane

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One of the most important sugarcane breeding issues is choice of parental genotypes. Breeders have concentrated efforts in the search of the best method of parental characterisation, both *per se* and by analysis of their progeny. They also have estimated heritabilities, general and specific combining abilities, coefficient of parentage and, more recently, genetic dissimilarity measures using molecular markers. In this direction, we evaluated genetic relationships among a group of 45 parents, taken at random from the available parental genotypes in Camamú, BA, Brazil. We estimated genetic dissimilarities using the Jaccard similarity index and 206 alleles obtained from 20 microsatellite loci (SSR). Sixty-four biparental crosses were planted in a field trial. The families, and the parents of the families, were evaluated for height, stalk diameter, stalk number, Brix, tonnes of cane, tonnes of Brix, and pithiness in a three-replicate experiment with 56 seedlings per plot. We calculated plant cane heterosis and correlations using genetic dissimilarities among the family data. The

results suggest that use of SSR genomic molecular markers as an auxiliary tool has potential for prediction of performance of sugarcane crosses for stalk height, stalk number, Brix, tonnes of cane, and tonnes of Brix.

Multivariate analysis to characterize cenicaña's parental collection

H. Rangel, C. Moreno, A. Amaya, C. Viveros and J. Victoria
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There are 1174 clones in Cenicaña's parental collection which are used to preserve genetic variability and create new combinations. Each clone is replicated three times in plots of one row, 5 m long. The objectives of this research were to use statistical analyses to characterise the collection and to divide the clone population into subsets or genetic groups. Through the characterisation the genetic variability for 25 quantitative and 4 qualitative variables was determined. Using multivariate statistical methods such as principal component analysis, canonical discriminant analysis, cluster analysis and multiple correspondence analysis, six groups of cultivars with similar characteristics within a group were found. According to the principal component analysis, the first five components explained 66.5% of the total variability. The cluster analysis accounted for 76.2% of the variation in the data with six groups. Two canonical variables related to quality of the soluble solids and initial development of the crop generated most of the differences between groups. Population improvement is conducted using such groups to explore combinations for specific environments according to the agro- ecological zones for the sugarcane crop in the Cauca Valley, Colombia.

Sugarcane entomology crop losses due to two sugarcane stem borers in reunion and South Africa

Francois-Regis Goebel and Mickael J. Way
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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 Réunion 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.

Impact of the lesser cornstalk borer, *elasmopalpus lignosellus*, on sugarcane in Tucuman, Argentina

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During the shoot stage, sugarcane in Argentina may be affected by the lesser cornstalk borer, especially under high temperatures and low rainfall conditions. Larvae bore into the shoot's basal area, producing galleries, and killing the shoot. The objective of this work was to quantify the percentage of attacked shoots in different crop conditions and to determine the impact of damage on growth and production of sugarcane crops. Field observations showed differences in damage and impact according to the cultural practices. Irrigated crops and those following green-cane harvesting suffered almost no attack, while those under conventional practices showed 7–57% of shoots attacked. After attack, stalk weights were reduced by 43% and pol reduced by 13%, resulting in a loss of sugar yield of 55%. We conclude irrigation and/or green-cane harvesting provide adequate ways to manage this pest.

MOLECULAR BIOLOGY

Identification and characterisation of sugarcane proteins required for posttranscriptional gene silencing

J. W. Park, R. Huang and T.E. Mirkov
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HC-Pro is a well-known plant viral protein that suppresses RNA silencing in plants. We confirmed that *Sorghum mosaic virus* (SrMV) infection in a transgenic sugarcane line, showing posttranscriptional gene silencing, induced the expression of the silenced transgene (GUS). Also, the introduction of SrMV P1/HC-Pro into another transgenic line that is posttranscriptionally silenced for the SrMV coat protein (CP) resulted in the accumulation of SrMV CP RNA. These data confirmed that SrMV P1/HC-Pro acts as a suppressor of RNA silencing in sugarcane, one of the most economically important crops worldwide. In order to investigate cellular component(s) involved in RNA silencing and its suppression in sugarcane, HC-Pro was used as bait in a yeast-two-hybrid assay to screen a cDNA expression library constructed from a transgenic line of the sugarcane hybrid cultivar CP65-357 showing RNA silencing for the SrMV CP gene. Yeast-two-hybrid screening identified several cellular proteins as interactors with HC-Pro. One of these proteins is a ca. 22 KDa protein that preferentially binds to ssRNA. *In vitro* binding assays such as pull-downs and far-western assays further confirmed that SrMV HC-Pro interacts with the 22 KDa protein. Yeast-two-hybrid screening of the same cDNA expression library with the 22 KDa protein identified a ca. 33 KDa protein as an interactor, which shows high identity with 14-3-3 proteins. The involvement of these and other HC-Pro interacting sugarcane proteins in RNA silencing and its suppression will be presented.

Understanding the concern about gm crops within the Australian population and among sugarcane growers

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The adoption of a novel technology, such as gene manipulation, in the improvement of agricultural crops could contribute significantly to economic development and reduce environmental pressure in sensitive areas. However, consumers in a number of countries have expressed various concerns about the introduction of genetically modified crops into the food chain. In order to establish the level of public understanding about genetic modification of agricultural plants and assess the degree of concern about using genetically modified organisms in Australian food production, a mail survey of a sample of the general population and the cane growing community in Queensland, Australia, was undertaken. There were 400 completed questionnaires out of 1000 from the general public and 196 completed questionnaires out of 500 from cane growers. Results were analysed using Intercooled Stata 9 statistical software and showed that, in general, respondents have a low level of knowledge about the use of gene technology. The level of approval for the use of gene technology in agriculture and in food production varied according to the type of application and the benefits it might generate. Applications that involved non-food products were more readily accepted than those involving food products. Cane growers were also more likely to approve of the technology than respondents from the general public. However, a large percentage of people remain neutral in regard to the use of such technology, probably due to their low level of knowledge about it. As a result, there is a need to inform the public about this technology so they can make their own decisions about the use of gene technology in agriculture and in food production.

SUGARCANE PATHOLOGY

Sugarcane leaf yellows phytoplasma in Mauritius: molecular characterisation, transmission and alternative hosts

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Sugarcane leaf yellows phytoplasma (SCYP), which causes leaf yellows (LY) disease, is commonly encountered in Mauritius. The objectives of this study were (1) to identify the vector transmitting the phytoplasma (2) to characterise the phytoplasma and (3) to identify alternative hosts harbouring the phytoplasma in sugarcane fields. Screening of nucleic acid extracts from the sugarcane Delphacid planthopper, *Perkinsiella saccharicida* by nested polymerase chain reaction (PCR) using universal primers revealed the presence of a DNA fragment related to phytoplasmas. Molecular characterisation using restriction enzymes showed that infection in the planthopper could be due to the presence of more than one phytoplasma. Two PCR products amplified from *P. saccharicida* were sequenced and found to belong to two separate groups, namely the 16SrIII-Western X group and the aster yellows 16SrI group of

phytoplasmas. The former group was also observed in sugarcane and was not previously recorded in this crop in Mauritius. These results indicate the possible involvement of *P.saccharicida* as a vector of SCYP. The PCR technique was also used to diagnose phytoplasmas in graminaceous weeds growing in sugarcane fields. Fragments related to the Western X group were amplified from *Sorghum verticilliflorum*.

Management of sugarcane cultivars imported into Colombia

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Cenicaña 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 albilineans* and *Leifsonia xyli* subsp. *xyli*. Plants that have shown no symptoms of diseases are then transferred to CENICAÑA and sown in small pots in a glasshouse, and then placed in a growth chamber for thermotherapy 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* meristem 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 characterisation trials are conducted to select those that may be incorporated into the sugarcane breeding and improvement program of Cenicaña.

COPRODUCTS

Simulation and design of a bagasse gasifier

P.A. Hobson and J.A. Joyce
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An evaluation of options for the practical implementation of a novel biomass-reforming concept has been carried out. This concept utilizes the potassium that occurs naturally in biomass as a catalyst in cracking high molecular weight tars

produced during gasification to deliver a high quality syngas. One of the primary objectives was to develop a process, which yielded a gas with the highest possible fuel heating value, relative to that of the original bagasse fuel. This ratio is termed cold gas efficiency (CGE). Initially, a first law of thermodynamics analysis (simple mass/atomic species and energy balances) was carried out in which the preferred operating regimes were identified in terms of basic process parameters. Both deterministic and Monte Carlo analyses were performed using the first law models. Key findings from this preliminary analysis included: beyond the minimum steam requirement, maximum cold gas efficiency (CGE) is achieved by avoiding any further increase in the ratio of steam to dry ash free (d.a.f.) bagasse; the theoretical minimum steam requirement to fully convert all of the carbon in the bagasse to a gas of maximum heating value is approximately 13.5 wt% of the fuel (d.a.f. basis); without chemical recuperation, char/coke yields in excess of 12 wt% on a d.a.f. basis (characteristic of the process in question) will lead to suppression of the CGE. The Monte Carlo sensitivity analysis indicated a 90% probability of achieving a CGE of between 62% and 85% given the range of process parameters considered. Five basic concepts were evaluated for thermodynamic performance and engineering feasibility. A preferred hybrid of these concepts was selected and a process model developed. A second law approach (so-called 'Pinch Analysis') was then applied to elucidate the best heat recovery strategies. The optimised model was used to establish key specifications in terms of bagasse and steam flows, heat transfer areas, pump capacities and parasitic power requirements for a notional 5 MWth pilot plant. The predicted performance figures of greater than 80% CGE and 12 MJ/m³ product gas Lower Heating Value compare favourably with the values targeted at the onset of the project and represent a major improvement on the performance expected from the commonly proposed gasification designs.

Activities at sugar research and innovation

P. A. Hobson

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Sugar Research and Innovation (SRI) has been involved for some years in developing aspects of bagasse gasification technology. This has been undertaken with the long-term aim of implementing advanced cycle, high efficiency power generation in the sugar industry or the production of a gaseous feedstock for alcohols and other industrial commodities. Many of the R&D outcomes achieved at SRI in the area of gasification can be traced back to the formation in 1998 of the Queensland Biomass Integrated Gasification (QBIG) program. This program was set up to develop gasification for boosting power generation in the sugar industry. Under the QBIG program, major research projects were undertaken in the areas of bagasse and cane trash gasification kinetics, ash characteristics in bagasse gasifiers, the development of a continuous pressurised bagasse feeder, cane harvest residue recovery systems to improve gasifier utilisation and economies of scale as well as a financial appraisal of gasification for power generation. It became evident from this latter study that there was significant scope for reducing costs via a staged and highly factory integrated introduction of this technology, and a study was initiated to determine optimum strategies for the large scale adoption of gasification technology across the industry. On a more fundamental level, SRI has been collaborating with Hokkaido and Monash

universities in a project aimed at utilising the potassium that occurs naturally in biomass as a catalyst in cracking high molecular weight tars produced during gasification. This catalysed reforming process occurs at relatively low temperatures (500°C to 700°C) and has the potential to deliver significant gasifier cost and efficiency advantages. The role of SRI in this project has been to develop computational fluid dynamics (CFD) and process models with which to implement laboratory data in a thermodynamically optimised gasification cycle. This paper draws together the R&D activities undertaken at SRI to provide a positive picture with respect to the technical feasibility of implementing gasification technology for power generation and highlights some of the financial barriers to the large scale adoption of the technology in Australia.

FACTORY ENGINEERING

Simulation of the dynamic behaviour of a compartment of a continuous pan

D. J. Love

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A full grasp of continuous pan performance requires an understanding of the dynamic behaviour of individual pan compartments. A dynamic mathematical model of a continuous pan compartment has been developed and structured to be compatible with the state space format used for control system studies. Using model parameters obtained by fitting a steady state version of the model to tests on an industrial scale pan, it is possible to use the dynamic model to simulate dynamic behaviour. The simulated compartment performance shows complex behaviour, which is difficult to interpret in terms of a conceptual understanding. A simpler heuristic analysis is able to explain most of the detailed simulation in terms of three simple components, a balance between feed and evaporation, the effect of crystal growth and the 'washout' effect of massecuite flowing into and out of the compartment. The dynamic model is also useful for investigating the behaviour of automatic control. This is demonstrated by first simulating a tuning procedure that estimates appropriate tuning parameters for a feedback controller. Using these parameters in the simulation of the behaviour of feedback control using a PI controller shows that the physical limitation of a fully closed feed valve results in a more damped response than the quarter amplitude damping that would normally be expected.

Options for retrofitting white sugar milling (WSM) technology into existing raw sugar factories

C. R. C. Jensen and S. M. Kitching

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Sugar Mill (WSM) technology produces EEC2 quality white sugar directly from raw cane juice, thereby eliminating the refining process and its associated costs. In addition, the WSM process unlocks the value of the sugars currently found in molasses and produces a liquid fertiliser that is high in nitrogen and potassium. The technology has been piloted successfully in South Africa and Brazil and, in 2005; the

first commercial scale WSM plant was built and commissioned at Felixton Mill in South Africa. The technology applies membrane filtration, refrigeration, ion-exchange demineralization and decolourisation to produce a high quality white juice. The WSM technology can either be applied at a greenfield installation or, as is the case with Felixton, retrofitted to an existing factory. The WSM technology effectively bridges the gap between sugarcane milling and raw sugar refining. However, in doing so it has to make refined quality sugar from a raw material with significantly higher levels of impurities and of variable composition. Sugar factory designs vary from region to region depending on the technology employed, cane quality, season length and market requirements. Similarly, in designing the WSM process for a specific application, these and other factors need to be taken into account. Furthermore, in contrast to the seasonal operation of raw sugar mills, refineries generally operate all year round. This paper presents a generalised WSM flow sheet that allows the back-end of the WSM factory to be decoupled from the juice front-end, thereby facilitating year round operations. Thereafter, various options for incorporating the generalised WSM flow sheet into an existing sugar mill are discussed. The WSM Slipstream plant at Felixton is presented as a specific example.