ISSN 0970-3233 Volume 32, No. 2 December 2017

Indian Journal of Sugarcane Technology

The Association of Sugarcane Technologists of India
Lucknow 226 002 (UP) India

INDIAN JOURNAL OF SUGARCANE TECHNOLOGY

Frequency of Publication : Half Yearly (June & December)

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INDIAN JOURNAL OF SUGARCANE TECHNOLOGY

ISSN 0970-3233 Issue: Volume 32 No.2 December 2017

CONTENTS

Indian Journal of Sugarcane Technology **32(02)**: 45-49, December 2017

Impact of irrigation methods, water and soil quality on rhizosphere and sugarcane- A review

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ABSTRACT

Rhizosphere is the main surrounding, where plants and microbes complement each other nutritionally and protect each other from stresses. Adequate soil moisture in the rhizosphere is essential for the growth of plants and also soil microbiota. In the present paper efforts have been made to elaborate the effect of irrigation methods, water and soil quality on rhizosphere and sugarcane crop. It has been found that different soil microbes can tolerate different levels of moisture stress. Bacteria can tolerate moisture stress up to -10 MPa whereas, actinomycetes can tolerate moisture stress up to -70 MPa. With surface irrigation methods, only 35 to 40 per cent of the total quantity of water applied is utilized by the plants. On the other hand, with drip irrigation, water is applied in desired quantity, at desired place and at desired time, therefore, the plant and rhizospheric micro-organisms never face water and nutrient stress. However, use of drip irrigation with saline water should be avoided in arid regions to prevent accumulation of salts in rhizosphere. Fertigation with bio-fertilizers can considerably improve water and fertilizer use efficiencies. *Azospirillum*, phosphor-bacteria and methylotrophs colonizing in the rhizosphere region have the ability to fix nitrogen, solubilize phosphorus and stimulate plant growth. It has been reported that soils irrigated with sewage exhibited a significant decrease in microbial biomass carbon (-78.2%), soil respiration (-82.3%), phosphatase activity (-59.12%) and dehydrogenase activity (-59.4%). Long-term use of arsenic (*As*) contaminated irrigation water could result in *As* accumulation in the soil which will in turn result in loss of crop yield and human health risk. Deficit irrigation can be effectively used to reduce irrigation water use. It was observed that, partial root zone drying irrigation technique increased crop yield by more than 15%. Irrigation of sugarcane with paper mill effluent increased the populations of *Rhizobium* and *Azotobacter* in its rhizosphere. Flooding of sugarcane fields resulted in significant deterioration in physico-chemical properties of rhizospheric soil. Soil respiration has been found to be positively correlated with soil moisture content.

Key words: Rhizosphere, Irrigation, Saline Water, Soil Moisture, Deficit Irrigation, Sugarcane

Rhizosphere is broadly defined as the soil volume under the influence of plant roots, enriched with exudates, secretions and mucilaginous materials. It supports an active microbial population distinctive from the bulk soil. Subsequently, there are many interactions between plant-microflora, plant-macrofauna, microflora-macrofauna and soil-plant-water-microflora. Some relationships are positive and others parasitic. Crop management practices like planting methods, weed management practices, irrigation and nutritional options have significant impact on the rhizosphere soil.

Irrigation is an artificial application of water to the soil which is applied to avoid water deficits, for growing crops in dry areas and during periods of inadequate rainfall. More than 40 per cent food production comes from irrigated area which is only about 17 per cent of the total cultivated area in the world (Fereres and Connor, 2004). Nevertheless, irrigated agriculture is still practiced in many areas without caring for basic principles of resource conservation and sustainability. Therefore, irrigation water management will have to be carried out most efficiently, aiming at saving water and at maximizing its productivity. Irrigation with distillery effluent increased the populations of bacteria and fungi in the rhizospheric soil substantially. In irrigated plants, nitrogen additions appeared to decrease nitrogenase activity while phosphorus and potassium levels had no effect.

Soil salinity and sodicity are one of the limiting factors of crop production in arid and semi-arid areas. Awareness of rhizosphere bacterial diversity and use of salinity-resistant bacteria is considered as a critical strategy to increase plant growth in these areas. Salinity has the highest effect on bacterial community structure with the higher diversity of microorganisms in saline soils. Many of the sugarcane rhizosphere bacteria in saline and non-saline soils have some growth-promoting properties.

The quality of soil and, quality and quantity of irrigation water play an important role in altering rhizospheric environment. Some factors have positive effect and some have negative effect on rhizospheric environment. The present review has therefore, been taken up to study the effect of irrigation methods, water and soil quality on rhizosphere and sugarcane crop.

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Soil water and nutrient supply to the rhizospheric soil biota

Soil water distribution is critical to the growth and survival of the soil biota. Typical ranges of tolerance to water stress for soil microbes are given in Table 1. Soil water not only directly affects the growth and activity of the soil biota but also mediates effects through the supply of nutrients to the organisms. This supply occurs through both mass flow and diffusion. The relative importance of the two processes depends on the amount of movement of the soil water. Where this movement is low, the dominant supply of nutrients is by diffusion. With increasing movement of soil water, however, mass flow dominates the nutrient supply. This is particularly true for highly soluble nutrients such as nitrate. Significant flow of nutrients towards the root is observed due to the demand for water by plant roots. Diffusion of root-exuded soluble carbon supplies the growth substrate to rhizosphere microbial population.

Table 1 Tolerance of soil microbes to water stress

Microbes	Water potential (MPa)
Bacteria	0 to -10
Yeast	0 to -20
Fungi	0 to -60
Actinomycetes	0 to -70

Effect of soil water on rhizospheric soil air and nutrient content

The amount of water filled in pore space has a prominent influence on both the gaseous composition of the soil air and on the amount of soil air. Excess water creates anaerobic conditions in the soil. Plant roots are unable to respire anaerobically and prolonged anaerobic conditions lead to death of many plants. In water logged conditions, it is oxygen supply to roots that tends to limit the growth of most plants, because only a few plants have developed a provision of their root oxygen supply. The soil pores >30 μm drain rapidly under gravity and, become the main oxygen suppliers to roots and, smaller soil pores < 30 μm drain slowly under gravity and become the main water suppliers to roots. Soil water being held at tensions of 5 kPa or less will tend to drain from the soil under the influence of gravity. Nutrients, particularly those not attracted to soil particles by ion exchange, may well be lost from the soil as drainage process occurs. Nitrate, in particular, is readily leached in this way.

Effect of irrigation methods on rhizospheric soil moisture

In the conventional irrigation method, plants are irrigated at an interval of 10 to 20 days depending on the soil, climate and plant type. With surface irrigation methods, only 35 to 40 per cent of the total quantity of water applied is utilized by the plant. During initial two to three days of irrigation, soil pores are saturated with water. In this condition, total air in the soil is replaced by water and field capacity level is not maintained in the soil. Though sufficient nutrients are available in the soil, excess water condition suffocates roots of the plants and water absorption by roots is totally ceased. As plant is under suffocation, the growth is hampered. During last few days of irrigation, moisture level in soil goes below the root zone; hence, plant is under stress condition. Even though air and nutrients are sufficiently available in the root zone they cannot be taken easily by plant for the want of sufficient soil moisture. As the plant is under stress due to short supply of water and nutrients and hence growth is restricted. Micro-irrigation water is applied in predetermined quantity precisely near the root zone of plant at frequent intervals. Because water is applied in desired quantity, at desired place and at desired time, plant never faces water and nutrient stress. Drip irrigation helps in maintaining soil moisture at optimum level in the rhizosphere which results in better growth of soil micro-organisms.

Irrigation with saline water

All irrigation waters contain salts and, as water evaporates, salts concentrate in the soil profile and must be leached below the root zone before they reach to toxic concentration that limits crop production. Salt leaching is achieved by the movement of water applied in excess of evapotranspiration (ET). Thus, some of the water losses are unavoidable and are needed to maintain the salt balance; however, they can be minimized with efficient irrigation methods and by appropriate management. The use of poor quality water for irrigation can lead to problems with regard to soil degradation resulting from accumulation of salts. Extent of salt accumulation depends on the quality of irrigation water, the nature of soils, climate and the irrigation method used. The electrical conductivity of soil samples from areas irrigated with saline water was found to be significantly higher than in non-irrigated soils. Seasonal variations were also observed due to differences in water supply and in evaporation demands. With continued irrigation with salty water, soil showed a significant accumulation of salt. Salinity and pH are the most influential factors determining the diversity of bacteria in the rhizosphere of plants. It was found that the impact of low pH on microbial community was more severe during initial stages of plant development. Therefore, soil pH must be properly managed during the early stages of plant development as this may have severe impact on nutrient availability to plants. For maintaining suitable pH of irrigation water, it may either be treated with gypsum or be blended with non-saline water to bring down the pH to permissible limits.

Use of drip irrigation with poor quality water

Use of drip irrigation systems in an arid climate with strong evaporative demand, results in accumulation of salt in the rhizosphere. Such salinization of the soil could result in reduced yields. The irrigation system should be so selected that the salt concentration of the soil remains within limits that can be tolerated by crops. Surface irrigation systems (border, furrow and check basin) when used with poor quality

water, does not allow accumulation of salts in the rhizospheric soils as the amount of water applied with these irrigation methods is sufficient enough to effect leaching of excess salts.

Bio-fertigation

Bio-inoculants constitute an important component in integrated plant nutrient system. The organic and biological sources provide essential nutrients to the crop and also enhance the positive interaction with chemical fertilizers by increasing their efficiency. Bio-fertigation has an added advantage when these microbial inoculants are supplied through irrigation water with the help of drip irrigation system as it has more water and fertilizer use efficiencies. Effective micro-organisms can also be applied in the field along with organic or inorganic materials. Beneficial bacteria such as *Azospirillum*, phosphor-bacteria and methylotrophs colonizing the rhizosphere region have the ability to fix nitrogen, solubilize phosphorus and stimulate plant growth. Co-inoculation of *Methylobacterium* spp. with *Rhizobium* spp. increased plant growth, nodulation and yield attributes in groundnut significantly as compared with individual inoculation. Bio-fertigation can precisely deliver the bioinoculants in the root zone.

Sewage water irrigation

Irrigation with sewage water has potential benefits of meeting the water requirements but the sewage irrigation may also harm the soil health. Results have shown that use of sewage water for irrigation improved the clay content to 18-22.7%, organic carbon to 0.51-0.86% and fertility status of soils. Build up in total N was up to 2,713 kg ha^{-1}, available *N* (397 kg ha^{-1}), available *P* (128 kg ha^{-1}), available *K* (524 kg ha^{-1}) and available *S* (65.5 kg ha^{-1}) in the surface (0.15 m) soil. Long-term sewage irrigation has also resulted in a significant build-up of DTPA extractable Zn (314%), *Cu* (102%), *Fe* (715%), *Mn* (197.2), *Cd* (203%), *Ni* (1358%) and *Pb* (15.2%) when compared with the adjacent rain-fed soil. A significant decrease in microbial biomass carbon (-78.2%), soil respiration (-82.3%), phosphatase activity (-59.12%) and dehydrogenase activity (-59.4%) has been observed when soils were irrigated with sewage water (Masto *et al*., 2008).

Irrigation with arsenic rich ground water

Higher levels of arsenic (*As*) in groundwater is confirmed in seven Indian states, namely West Bengal, Bihar, Uttar Pradesh, Assam, Jharkland, Chattisgarh and Madhya Pradesh. The extent of the problem is not fully known in these states except West Bengal. In West Bengal, investigations suggest that eight districts show *As* content in well-water to be above 0.050 mg/l. According to United Nations Children's Fund (UNICEF), over 13.8 million people are at risk due to higher concentration of *As* in water. Irrigation water with high levels of *As* may result in land degradation in terms of loss of yield and food safety. Long-term use of *As*-contaminated irrigation water could result in *As* accumulation in the soil (Heikens 2006). If absorbed by the crops, this may add substantially to the dietary *As* intake, thus posing additional human health risks. Over time, *As* accumulation in the soil could reach soil concentrations toxic to crops, thus reducing yields (Figure 1).

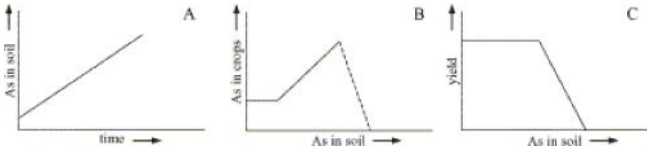

Fig.1. Effect of continuous use of arsenic rich irrigation water on arsenic build up in (A) soil, and effect of soil arsenic concentration on (B) arsenic concentration in crops and (C) yield reduction.

Deficit irrigation for reducing agricultural water use

Generally irrigation strategy is to supply sufficient irrigation water so that the crops transpire at their maximum potential and the full evapotranspiration (ET) requirements are met throughout the season. But under the situations of shortage of water, farmers often receive water less than the maximum ET needs, and either have to concentrate the supply over a smaller land area or have to irrigate the total area with levels below full ET. Application of water below the ET requirements is termed deficit irrigation (DI). Irrigation supply under DI is reduced relative to that needed to meet maximum ET (English, 1990). Therefore, water demand for irrigation can be reduced and the water saved can be diverted for alternative uses. A water supply constraint that decreases transpiration below the rate dictated by the evaporative demand of the environment reduces the biomass production. Due to insufficient water supply for irrigation, the objective of irrigation management will shift from emphasizing production per unit area to maximizing the production per unit of water consumed (water productivity). Deficit irrigation is an important tool to achieve the goal of reducing irrigation water use. The level of irrigation water supply under deficit irrigation should be relatively high and should permit achieving 60–100% of full evapotranspiration. Regulated deficit irrigation (RDI) not only increases water productivity, but also farmers' profits. Experiments have been conducted on application of deficit irrigation for enhancing water productivity in sugarcane at the ICAR- Indian Institute of Sugarcane Research, Lucknow. These deficit irrigation techniques include: irrigation at critical growth stages, application of irrigation water in alternate furrows, ring-pits or trenches (deep furrows). By adopting these deficit irrigation techniques, 20 to 40 per cent irrigation water can be saved without sacrificing the yield.

Partial root zone drying irrigation technique

Partial root zone drying (PRD) is the deficit-irrigation technique in which transpiration is restricted (Dry *et al.* 1996). The aim in this irrigation technique is to ensure that some roots are always exposed to dry soil, thus altering the production of chemical signals and their transmission to the

shoots to restrict water use. This is achieved by alternately irrigating only one side of the crop row at a time and allowing the other to dry the soil. Adoption of PRD induces beneficial agronomic and physiological responses that differ from DI, when the same volume of water is applied. The yield responses of PRD and DI plants are also different. It was observed that, PRD significantly increased yield by more than 15%. PRD promoted earlier crop maturity in tomato (Zegbe-Dominguez *et al.* 2003), increased fruit size in mango resulting in a more favourable fruit size distribution (Spreer *et al.* 2007), and increased berry skin anthocyanin concentrations independently of whether vines had greater (Antolin *et al.* 2006) or lesser (dos Santos *et al.,* 2003) vegetative vigour. At the end of a growing season during which both treatments received the same irrigation volumes, more water remained at depth in the soil profile under PRD than DI (Leib *et al.* 2006), despite PRD inducing greater root proliferation (Mingo *et al.* 2004). However, smaller evaporative losses from the soil under PRD plants (as less surface area of soil is wetted during each irrigation event) could only partially account for this (Leib *et al.* 2006), suggesting a greater restriction of water loss from leaves of PRD plants. Leaf stomatal conductance can be lower in PRD plants (de Souza *et al.* 2003; Du *et al.* 2006), implying that irrigation placement causes differences in root-to-shoot signalling.

Effect of soil moisture and irrigation on sugarcane and its rhizosphere

The rhizosphere is the main surrounding, where plants and microbes complement themselves nutritionally and protect each other from stresses. Rhizosphere is the ultimate soil portion in the roots' vicinity, where microbial community assemblage and activity are modulated by the release of root exudates (Drigo *et al.* 2013; Jiang *et al.* 2017). Kannan *et al.* (1990) observed that irrigation of sugarcane grown soils with paper mill effluent increased the populations of *Rhizobium* and *Azotobacter* for a particular time and further increase in the duration of irrigation did not significantly contribute to the increase in populations. Prolonged irrigation of the effluent affected the rhizosphere effect (R:S ratio) of these organisms. Populations of *Rhizobium* and *Azotobacter* were more in the rhizosphere of sugarcane and increased with the age of the crop in such soils. Gaddanakeri *et al.* (2006) reported that water stagnation or flooding durations of sugarcane fields for different periods did not influence pH and EC of soil. Physico-chemical properties of soil were significantly deteriorated in all flooding durations compared to control. With increase in flooding duration, there was proportionate decline in the major available nutrients (*N, P* and *K*) in the soils. The cane yield and quality parameters were found to deteriorate, as the flood duration increased from 7 days to 21 days. Mauri *et al.* (2017) studied effect of different water deficit level on sugarcane grown in the soils of different depths. They observed that for soil depths of 10 and 20 cm, irrigation at 160-mm cumulative pan evaporation caused total plant death. On the other hand, for soil depths of 30 and 40 cm, irrigation at 200-mm cumulative pan evaporation promoted plant death. They also observed that irrigation at more than 40 mm cumulative pan evaporation significantly reduced plant growth regardless of soil depth. Sornpoon *et al.* (2013) observed that soil respiration was positively correlated with soil moisture content. The obtained data showed that the respiration rate is increasing with the age of the plant, accounting for up to 29% of the total soil respiration before harvesting. The root to soil respiration ratio increased rapidly during the young seedling stage, *i.e.* first five months, then declined and finally got stabilized during yield formation and ripening stages. Yadahalli *et al.* (2007) studied the effect of soil moisture levels on sugarcane sett rot disease incidence. They observed that maximum germination (65.60%) and least sett rot incidence coupled with least rhizosphere population of *Ceratocystis paradoxa* was observed at soil moisture equal to 60 per cent moisture holding capacity (Table 2).

Table 2 Effect of soil moisture on germination, sett rot development and population of *Ceratocystis paradoxa* in rhizosphere

Soil moisture (% of water holding capacity)	Germination percentage	Sett rot percentage	Rhizosphere population of *Ceratocystis paradoxa* (cfu$\times 10^3$/g)		
			15 days after planting	25 days after planting	35 days after planting
20	38.50 (38.35)	31.63 (34.20)	12.70	15.60	18.70
40	46.70 (43.11)	22.20 (28.11)	9.90	12.80	14.90
60	65.60 (54.09)	5.50 (13.56)	6.53	8.43	10.40
80	55.30 (48.04)	15.70 (23.34)	9.40	11.40	15.05
100	28.60 (32.33)	41.80 (40.28)	17.80	19.60	23.60
SEm±	0.30	0.78		SEm±	CD (0.01)
CD (0.01)	1.30	3.36	Days (D)	0.06	0.24
			Soil moisture (M)	0.08	0.31
			Interaction (DxM)	0.14	0.54

CONCLUSIONS

Proper management of rhizospheric environment is essential to sustain health and fertility status of the soil. Irrigation water quality, quantity and methods of water application significantly influence the rhizosphere. Irrigation water alters temperature, microbial population and chemical composition of rhizospheric soil. Soil respiration has been found positively correlated with soil moisture content. Different soil microbes can tolerate different levels of moisture stress. Bacteria can tolerate moisture stress up to -10 MPa whereas actinomycetes can tolerate moisture stress up to -70 MPa. To maintain salt balance in the rhizospheric soil, some amount of water is to be applied in excess of evapotranspiration. Use of sewage water for irrigation improved organic carbon to 0.51-0.86%, build up in total *N* was up to 2,713 kg ha^{-1}, available *N* (397 kg ha^{-1}), available P (128 kg ha^{-1}), available *K* (524 kg ha^{-1}) and available *S* (65.5 kg ha^{-1}) in the surface (0.15 m) soil. Long-term irrigation with sewage water has also resulted in a significant decrease in microbial biomass carbon (-78.2%), soil respiration (-82.3%), phosphatase activity (-59.12%) and dehydrogenase activity (-59.4%) in rhizospheric soil. The cane yield and quality parameters were found to deteriorate, as the flood duration increased from 7 days to 21 days. Partial root zone drying irrigation technique increased crop yield by more than 15%. By maintaining moisture of rhizospheric soil at optimum level, activity of beneficial microbes in the soil can be increased manifold which will in turn result in higher productivity of land.

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Indian Journal of Sugarcane Technology **32(02)**: 50-59, December 2017

Ratooning ability of some promising Egyptian sugarcane varieties

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ABSTRACT

The study was carried out at Mattana Agricultural Research Station, Luxor Governorate, Egypt (lat 25° 17' N, long 32° 33' and alt 76 m ASL) during 2015, 2016 and 2017 harvesting seasons. Sixteen promising sugarcane varieties (*Saccharum* spp.), representative of selections from final stages in the sugarcane breeding program in Egypt, constituted the study material and check variety in randomized complete block design with three replications. Results revealed significant differences among evaluated genotypes for cane and sugar yield traits and its components in plant cane (PC), first ratoon (FR), second ratoon (SR) and across crops (OC). The genotype by crop cycle interaction was significantly affected by studied traits, all studied genotypes with check variety show ratooning ability for stalk length except two genotypes, 'G2004-27' and 'G99-103'. Twelve genotypes were good ratooner for cane yield and five genotypes, 'G.2010-26' (109.72%), 'G.2011-74' (117.81%), 'G.2010-8' (121.45%), 'G.2011-13' (127.14%) and 'G.2011-79' (111.56%) recorded high ratooning ability value for cane yield with compared check variety 'GT54-9' (107.69%). The genotype 'G.2011-74' recorded highest ratooning ability value 123.61% and 108.20% for sugar recovery and pol percentage respectively. Eight genotypes; 'G2011-74' (145.45%), 'G2010-8' (123.59%), 'G2011-13' (126.75), 'G2011-79' (113.74%), 'G2007-61' (115.31%), 'G2004-27' (116.36%), 'G2003-44' (116.33%) and 'G2003-49' (111.35%) recorded high ratooning ability value for sugar yield with compared check variety 'GT54-9' (106.58%). Four genotypes; 'G2011-74', 'G2010-8', 'G2011-13', 'G2011-79' recorded high ratooning values for cane and sugar yield together with compared check variety. The relative influences of genotypic variance (σ^2g) in determining phenotypic variance were more important than other components for most studied traits. Genotypic variance decreased from plant cane crop to second ratoon crop for stalk, stalk weight and °Brix%. Phenotypic coefficient of variation (PCV) decreased from plant crop to second ratoon crop for stalk length and sugar yield, while PCV increased for stalk diameter and purity percentage. Genotypic coefficient of variation (GCV) decreased from plant cane to second ratoon crop for stalk length, stalk weight and °Brix%, while increased for stalk diameter. Heritability decreased for stalk length and stalk weight and increased for stalk diameter with older crops. Crop cycle did not appear to affect heritability for sucrose percentage especially in second ratoon crop because of decreasing error variance**.** The results also indicate that high estimates of genotypic and phenotypic coefficients of variation GCV and PCV were recorded for stalk weight (52.23 and 50.34), sugar yield (49.59 and 48.64) and cane yield (33.68 and 32.97).

Key words: *Saccharum*, Ratoonability, PCV, GCV, Heritability

The primary goal of most sugarcane breeding programmes is to develop cultivars with improved cane and sugar yields. Sugarcane is a clonally propagated crop and is typically harvested for plant cane and a number of ratoon crops. First ratoon yields are commonly equal to plant cane yields but subsequent ratoons show yield decline. Because second ratoon yield potential is an essential cultivar characteristic, a study investigating crop effects should include the second ratoon to be meaningful. The development of new varieties of sugarcane from controlled crosses has been greatly extended and accelerated during recent decades with the development of many present commercial varieties.

Economics of sugarcane cultivation depends on the ratooning ability of the variety chosen for cultivation. A variety with good ratooning ability will reduce the cost of cultivation. In this context, sugarcane breeders should understand the importance of ratooning ability of sugarcane genotypes in order to identify a stable variety for a particular region. Ratooning ability is an important integral component of sugarcane production worldwide as it not only reduces the cost of cultivation but also dispenses with the requirement of seed material and some cultural practices like preparation and preparatory irrigation. In addition, it results in early maturity of canes at least by one month and thus adds to the effective crushing period (Shrivastava *et al.* 1992). A variety is considered to have good ratooning ability if it can maintain yield and it has a high yield potential over the normal plant crop. The plant characters of sugarcane associated with ratoonability were studied for possible use as selection criteria in breeding (Ferraris *et al.*1993). The major cane growing countries normally take two or more ratoons (Bashir *et al.*2013; Singh and Dey 2002; Yadav 1991). Yield of ratoon crop usually decreases with age and, hence, limits the economic production of sugarcane (Johnson *et al.*1993; Mirzawan and Sugiyarta 1999; Ricaud and Arceneaux 1986). Resources and programmes

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specific statistics guiding selection for breeding new sugarcane varieties with improved ratoonability vary in different countries. Selection in the ratoon crop was most effective for genetic gain for ratoonability in Louisiana and South Africa; whereas, in Australia, moderately high genetic correlations for sugar yield between plant and ratoon crops suggested that emphasis should be more on testing in more environments, more replications or more genotypes in early stage trials rather than testing for ratoonability. Comparison with key check varieties with enhanced ratoonability were important for advancing the goal of improved ratoonability(Gravois *et al.* 2016).The crop cycles had no effect on the juice quality traits (Mehareb *et al.* 2015).

Ratooning ability (RA) was defined as the second ratoon (SR) crop yield percent of the plant cane yield. Breeding programmes decisions commonly rely on knowledge of the underlying genetic structure of the breeding population and an understanding of the relative importance of genotype x environment (GE) interactions. Such knowledge includes accurate estimates of the genetic variance and covariance of pertinent traits, optimization of available resources, development of selection plans and indices, and prediction of the most fruitful parental combinations (Skinner 1971; Henderson 1984; Milligan *et al.* 1990; Chaudhary 2001 and Masri 2004). Results showed high genetic variance (σ^2g) as compared to environmental variance for all traits under study across seasons. Moderate values of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were coupled with high heritability for Brix, sucrose, sugar recovery and sugar yield (Mehareb and Abazied 2017).

The objectives of this study therefore, were to estimate the ratooning ability, performance and broad-sense heritability and genetic parameter of some sugarcane genotypes under three different crop cycles; plant crop, first and second ratoon crops.

MATERIALS AND METHODS

Plant material and experimental conditions

The study was carried out at Mattana Agricultural Research Station, Luxor Governorate, Egypt (lat 25° 17' N, long 32° 33' and alt 76 m ASL) during 2015, 2016 and 2017 harvesting seasons. Sixteen promising sugarcane varieties (*Saccharum* spp.) that were representative of selections from final stages in the sugarcane breeding program in Egypt, constituted the study material and a commercial variety namely 'GT. 54-9' was used as control (Table 1). Each sugarcane genotype was planted in three rows of 5 m length and 1m width in randomized complete block design with three replications and 25 three budded setts per row. The field was irrigated right after planting and all other agronomic practices were carried out as recommended. In order to study the crop cycle effects on ratooning ability, plant crop was ratooned for two consecutive years. Plant crop was harvested at 12 months after planting. The crop raised from the stubble of the first plant crop (PC) represented first ratoon crop and re-growth from first ratoon crop (FR) was considered as the second ratoon crop (SR).

Table 1 Genotypes used and their parentage

Varieties	Pedigree	
	Female	Male
'G. 2010-26'	'EH 94/134-1'	Selfing
'G. 2006-6'	'70-3898'	'82-4510'
'G. 2010-7'	'IN 94/116-3'	Selfing
'G. 84-47'	'NCo310'	??
'G. 2011-82'	'CP 57-614'	'G 85-37'
'G. 2011-74'	'CP 57-614'	'G 85-38'
'G. 2010-8'	'EH 94/72-5'	Selfing
'G. 2011-13	'G 85-37'	Selfing
'G. 2012-50'	'Mix 58-1866'	'Ph 8013'
'G. 2011-79'	'CP 57-614'	'G 85-38'
'G. 2003-47'	'CP 55-30'	'CP 85-1697'
'G.2007-61'	'CP 67-412'	'SP 71-1406'
'G.2004-27'	'CP 55-30'	'RoC 22'
'G.2003-44'	'CP 55-30'	'CP 85-1697'
'G.99-103'	'US.74-3'	'CP.76-1055'
'G. 2003-49'	'CP 55-30'	'CP 85-1697'
'G. T. 54-9' (check)	'NCo310'	'F. 337/925'

Phenotypic evaluation

Data were recorded on cane yield and juice quality traits. A sample of 10 stalks was used to measure stalk length and diameter. A sample of 20 stalks was crushed for juice analysis to determine quality traits. Stalk length (cm) was measured from soil surface to the visible dewlap and stalk diameter (cm) was measured at mid-stalk with no reference to the bud groove. Stalk weight (kg) was calculated by dividing cane yield per plot by the number of stalks per plot and cane yield per plot was converted to t/ha values. °Brix (percent soluble solids) was measured using hydrometer and sucrose percentage of clarified juice was determined using automated Sacharimeter according to A.O.A.C. (1980). Juice purity was calculated as:

Purity (%) = (Sucrose % /°Brix %) x 100

Sugar recovery % was calculated according to the formula described by Yadav and Sharma (1980) as:

Sugar recovery % = [Sucrose % - 0.4 (°Brix% -Sucrose %)] x 0.73

Cane yield (t/ha) was calculated on plot basis.

Sugar yield (t/ha) was estimated by multiplying net cane yield (t/hectare) with sugar recovery %.

Estimates of ratooning ability

Ratooning ability (RA) was calculated according to the formula described by Dunckelman 1982 as: RA = [(1R/PC) + (2R/PC)] /2

Where:

1R = yield in first ratoon

2R = yield in second ratoon

PC = yield in plant cane

The formula gives the ratooning ability of a variety as the average performance of the first and second ratoons in comparison to the plant crop.

Statistical analysis

Analysis of variance and variance component estimates were performed for each crop (reduced model) and across crops (using the full model). Except for specific crop, all factors (genotype, replicate and interaction) were considered random. Variance components were calculated by equating appropriate mean squares to their expectations and solving for the components.

Broad-sense heritability (H%) was estimated using variance components following the formula according to Johnson *et al.* (1955) :

$H= \sigma^2g/ (\sigma^2g + \sigma^2e /r + \sigma^2gy /ry)$.

Where, (σ^2g) and (σ^2e) refer to genotypic and error variance, respectively. The divisor (r) refers to number of replications. σ^2gy refers to genotype by year interaction variance. The divisor y refers to number years.

Genetic coefficients of variance (GCV) % provide measure of trait's genetic variation relative to its mean as per Burton and De Vane (1953). The GCV facilitating comparisons among traits with different units and scales, and giving perspective to the variation was estimated:

GCV %=(σg/general mean) ×100.

Phenotypic Coefficient of Variation (PCV) % was estimated as:

(PCV) % = (δp / general mean) x 100.

Where,

δg = genotypic standard deviation

δp = phenotypic standard deviation

RESULTS AND DISCUSSION

Crop cycle effects on cane yield traits

Data presented in Table 2 revealed significant differences among evaluated genotypes for stalk length, stalk diameter, stalk weight and cane yield in plant cane (PC), first ratoon (FR), second ratoon (SR) and across crops (OC). The genotype by crop cycle interaction was significantly affected by studied traits, indicating that performance of the genotypes differ among the crop cycles. It has been reported that genotype by crop interaction was important for sugarcane yield and its component traits (Milligan *et al.*1990; Orgeron *et al.* 2007 and Mehareb *et al.*2015).

Stalk diameter ranged from 2.24, 1.91, 1.77 and 1.98 cm for the genotype 'G.2011-13' to 2.96, 2.81, 2.77 and 2.85 cm for the genotype 'G.2010-26' in plant cane, first ratoon, second ratoon and across crops, respectively. The genotype'G.2010-26' gave significantly better stalk diameter in first ratoon and second ratoon as compared to the check cultivar 'GT.54-9' (2.49 and 2.37 cm). Stalk diameter was reduced for most of the tested genotypes in subsequent crops, which is in agreement with the studies of Mehareb *et al.* (2015).

Table 2 Mean performance of studied sugarcane genotypes for stalk diameter, stalk length, stalk weight and cane yield (t/ha) in plant cane (PC), first ratoon (FR), second ratoon (SR) and over crops (OC)

Variety	Stalk diameter (cm)				Stalk length (cm)				Stalk weight (kg)				Cane yield (t/ha)			
	PC	FR	SR	OC	PC	FR	SR	OC	PC	FR	SR	OC	PC	FR	SR	OC
'G. 2010-26'	2.96	2.81	2.77	2.85	205.00	214.33	225.67	215.00	1.13	1.05	0.70	0.96	102.43	123.68	101.10	109.07
'G. 2006-6'	2.87	2.49	2.47	2.61	215.00	231.67	228.67	225.11	1.24	0.87	0.57	0.90	105.88	121.60	96.39	107.96
'G. 2010-7'	2.63	2.57	2.33	2.51	235.67	267.33	260.67	254.56	1.06	1.27	0.63	0.99	119.00	136.44	106.39	120.61
'G. 84-47'	2.42	2.29	2.22	2.31	261.17	285.33	254.33	266.94	1.21	0.81	0.54	0.85	129.09	134.95	101.15	121.73
'G. 2011-82'	2.44	2.36	2.27	2.36	238.00	269.00	263.33	256.78	1.17	0.97	0.51	0.88	128.04	140.81	102.11	123.65
'G. 2011-74'	2.39	2.13	2.12	2.21	228.00	265.00	244.44	245.81	0.84	0.82	0.55	0.74	82.73	105.43	89.49	92.55
'G. 2010-8'	2.57	2.42	2.25	2.41	240.33	277.00	264.33	260.56	1.11	1.02	0.70	0.94	101.39	129.63	116.65	115.89
'G. 2011-13	2.24	1.91	1.77	1.98	231.00	289.33	278.67	266.33	0.69	0.79	0.59	0.69	92.66	135.42	100.20	109.43
'G. 2012-50'	2.25	1.95	1.90	2.03	224.00	245.33	240.67	236.67	1.02	0.65	0.56	0.74	119.10	113.29	95.44	109.27
'G. 2011-79'	2.39	2.27	2.24	2.30	241.00	269.33	273.89	261.41	0.71	0.88	0.49	0.69	101.86	129.47	97.82	109.72
'G. 2003-47'	2.67	2.33	2.15	2.38	257.33	262.33	275.00	264.89	1.19	0.84	0.73	0.92	129.72	135.41	118.77	127.97
'G.2007-61'	2.67	2.27	2.02	2.32	250.00	298.33	284.17	277.50	1.04	0.79	0.72	0.85	107.08	117.62	108.62	111.11
'G.2004-27'	2.83	2.57	2.45	2.62	293.33	283.33	268.33	281.67	1.27	1.12	0.93	1.11	135.94	145.22	136.29	139.15
'G.2003-44'	2.77	2.38	2.30	2.48	276.33	283.33	262.50	274.05	1.25	0.94	0.85	1.01	124.61	132.28	121.44	126.11
'G.99-103'	2.87	2.78	2.63	2.76	318.75	314.33	301.67	311.58	1.61	1.27	0.96	1.28	153.05	146.76	136.94	145.58
'G. 2003-49'	2.63	2.38	2.21	2.41	250.87	252.67	271.33	258.29	1.13	1.10	0.69	0.97	120.18	131.46	113.81	121.82
'G. T. 54-9'	2.71	2.49	2.37	2.52	260.83	277.00	276.33	271.39	1.33	1.26	0.64	1.08	127.85	144.22	131.15	134.41
LSD at 5%																
Variety (V)	**0.31**	**0.27**	**0.23**	**0.15**	**20.61**	**26.26**	**23.27**	**13.22**	**0.27**	**0.24**	**0.24**	**0.14**	**15.81**	**27.11**	**12.69**	**11.00**
Crop age (C)			**0.09**				**5.86**				**0.10**				**4.33**	
V x C			**NS**				**22.89**				**0.24**				**NS**	

The promising variety 'G.99-103' recorded highest stalk length (318.75, 314.33, 301.67 and 311.58 cm.), significantly better than the check cultivar 'G.T54-9' (260.83, 277, 276.33 and 271.39 cm) in plant cane, first ratoon, second ratoon and across crops respectively, which were 122.20%, 113.48%, 109.17% and 114.81% of the mean stalk length values respectively of the check cultivar 'GT.54-9'. Stalk length of most of the genotypes fluctuated among crops. It decreased in two genotype'G.2004-27' and 'G.99-103'and increased in four genotypes ('G.2010-26', 'G.2011-79', 'G.2003-47' and 'G.2003-49')with older crop cycles.

Stalk weight varied from as low as 0.69 kg for genotype 'G.2011-13' to 1.61 kg in the plant cane, while it ranged between 0.65 kg for genotype 'G.2012-50' and 1.27 kg for 'G. 99-103' in the first ratoon. Stalk weight varied from 0.49 and 0.69 for genotype 'G. 2011-79' in second ratoon and over crops to 0.96 and 1.28 kg for 'G. 99-103'. The genotype 'G. 99-103' recorded highest stalk weight in all crop cycles. It gave significantly high stalk weight values as compared check variety 'GT.54-9' (1.33 kg and 0.64 kg) in plant cane and second ratoon, respectively. The superiority of 'G.99-103' for stalk weight could be attributed to high mean values for both stalk diameter and stalk length across all crop cycles. The same was observed by Mehareb *et al.* (2015).Twelve genotypes exhibited decreasing trend for stalk weight from plant cane to first ratoon, which was in agreement with previous results (Mehareb *et al.* 2015; Chapman *et al.* 1992; Hunsigi 1982) where a reduction in stalk weight in the ratoon crop was observed.

Cane yield of five genotypes, 'G. 84-47' (129.09 t/ha), 'G.2011-82' (128.04 t/ha), 'G.2003-47' (129.72 t/ha), 'G.2004-27' (135.94 t/ha), 'G.99-103' (153.05 t/ha) was almost at par to the control 'GT.54-9' (127.85 t/ha) in plant cane, whereas cane yield of two genotypes, 'G.2004-27' (145.22& 136.29 t/ha) and 'G.99-103' (136.29 & 146.67 t/ha) was only slightly higher than the check variety in first ratoon and second ratoon. Cane yield in the first ratoon increased significantly by 12.27% as compared to the plant cane (Table 2). Cane yield ranged from as low as 82.73, 105.43, 89.49 and 92.55 t/ha, respectively in plant cane, first ratoon, second ratoon and across crop cycles for 'G.2011-74' to as high as153.05, 146.76, 136.94 and 145.58 t/ha, respectively for 'G.99-103', which is in accordance to the results of Mehareb *et al.* (2015) who reported that the genotype 'G.99-103' was top cane yielder due to its taller and thicker stalks. Cane yield of two genotypes 'G.2012-50' and 'G.99-103' decreased in consecutive crop generations form plant cane to second ratoon which is in agreement with Orgeron *et al.* (2007),who reported that at final selection stages, cane yield and sugar yield decreased from plant cane to third ratoon crop.

Crop cycle effects on sugar yield and juice quality traits

Data presented in Table 3 revealed significant differences among evaluated genotypes for total soluble solids (°Brix), sucrose%, pol%, juice purity%, sugar recovery% and sugar yield. Lowest °Brix percentage was 17.51%, 15.70%, 17.82% and 17.01 for genotype 'G. 2012-50' in plant cane, first ratoon, second ratoon and over the crops, respectively as compared to the highest value of 21.49% for genotype 'G.2011-79' in plant cane, 21.16% for genotype 'G.2003-47' in second ratoon and 21.34% and 20.83% for genotype 'G.84-47' in first ratoon and over the crops, respectively. Genotype 'G.2012-50' recorded lowest values in all crop cycles for sucrose% whereas, new promising variety 'G.2003-47' recorded highest values for this (17.44%, 18.59% and 17.40%) in plant cane, second ratoon and over crops(Table 3), however, the genotype 'G.84-47'recorded highest sucrose% (18.62%) in first ratoon.

Sucrose percentage fluctuated among crop cycles in all studied clones except genotype 'G. 2011-74', which showed an increase in consecutive crop generations. Purity percentage of juice (Table 3) varied from as low as 71.03, 71.47 and 73.17% for genotype 'G.2012-50' in plant cane, first ratoon and over crops, respectively and 73.71% for genotype 'G. 2010-26' in second ratoon to as high as 82.82% for 'G.2003-47' in plant cane, 87.18% for genotype 'G84-47' in first ratoon and 91.61% and 84.48% for genotypes 'G.2003-49' in second ratoon and over crops, respectively. Juice purity of one genotype 'G.2010-26' decreased in consecutive crop cycles but of six genotypes increased in consecutive crop cycles and for rest of the genotypes, it fluctuated among crop cycles. Overall purity percentage increased in first ratoon by 2.01% and in second ratoon by 5.78% as compared to the plant cane.

Significant differences were observed among the studied genotype for sugar recovery percentage across the crop cycles (Table 3); it ranged from 7.60%, 6.88%, 8.82% and 7.77% for genotype 'G.2012-50' in PC, FR, SR and OC, respectively to 11.67%, 12.82% and 11.98% for genotype 'G.2003-47' in PC, SR and OC, respectively and 12.79% for 'G.84-47' in FR . The Pol percentage varied significantly among evaluated genotypes across the crop cycles; Pol percentage of two genotypes'G.2010-7' and 'G.2011-13' decreased in consecutive crop cycles, while increased in only one genotype 'G.2004-27', however for most genotypes, it fluctuated among crop cycles.

In general, the crop cycle had no effect on juice quality traits. Chapman (1988) reported that clones in consecutive crop generations tend to mature earlier than previous crops, but final sucrose concentration and its components, °Brix, sucrose content, juice purity and sugar recovery are generally not affected by generations. These results are in agreement with Mehareb *et al.* (2015).

Sugar yield in plant cane varied from 6.89 t/ha for the genotype 'G.2011-74'to 15.20 t/ha for genotype 'G.99-103', while it varied from 7.80, 8.41 and 8.44 (t/ha) in first ratoon, second ratoon and over crops, respectively for 'G.2012-50'to 17.23 t/ha for the genotype 'G.84-47' in first ratoon and 15.32 & 15.25 t/ ha('G.T.54-9') and 15.20 & 15.24 t/ha ('G.2003-47') in second

Table 3 Mean performance of studied sugarcane genotypes for °Brix%, sucrose%, purity%, sugar recovery%, pol% and sugar yield (t/ha) in plant cane (PC), first ratoon (FR), second ratoon (SR) and over crops (OC)

Variety	°Brix%				Sucrose%				Purity%				Sugar recovery%				Pol%				Sugar yield (t/ha)			
	PC	FR	SR	OC	PC	FR	SR	OC	PC	FR	SR	CC	PC	FR	SR	OC	PC	FR	SR	OC	PC	FR	SR	OC
'G. 2010-26'	20.54	19.19	19.96	19.90	16.44	14.88	14.69	15.34	80.05	77.50	73.71	77.08	10.81	9.61	9.18	9.87	13.80	12.75	13.03	13.19	11.08	11.80	9.29	10.72
'G. 2006-6'	20.99	19.81	20.14	20.31	16.89	15.56	15.80	16.08	80.48	77.99	78.54	79.01	11.13	10.11	10.27	10.51	14.12	13.22	13.43	13.59	11.80	12.30	9.90	11.33
'G. 2010-7'	19.86	19.58	18.56	19.33	15.33	14.92	15.50	15.25	77.19	76.13	83.83	79.05	9.87	9.53	10.42	9.94	13.17	12.93	12.65	12.92	11.74	13.07	11.08	11.96
'G. 84-47'	20.81	21.34	20.33	20.83	16.29	18.62	16.99	17.30	78.28	87.18	83.51	82.99	10.57	12.79	11.43	11.60	13.87	14.78	13.87	14.17	13.65	17.23	11.50	14.13
'G. 2011-82'	20.41	19.19	20.17	19.92	15.52	15.05	17.35	15.97	76.11	78.33	85.99	80.14	9.91	9.78	11.84	10.51	13.47	12.80	13.90	13.39	13.06	13.77	12.16	13.00
'G. 2011-74'	18.63	19.59	19.31	19.18	13.47	15.60	15.66	14.91	72.39	79.28	81.11	77.59	8.33	10.22	10.37	9.64	12.09	13.14	13.03	12.75	6.89	10.77	9.27	8.98
'G. 2010-8'	20.01	19.43	19.76	19.73	14.88	14.43	15.48	14.93	74.43	74.20	78.41	75.68	9.37	9.07	10.05	9.50	13.11	12.73	13.17	13.00	9.50	11.76	11.71	10.99
'G. 2011-13'	20.20	19.89	19.76	19.95	15.84	15.52	15.61	15.66	78.27	77.75	78.99	78.34	10.29	10.05	10.18	10.17	13.47	13.24	13.21	13.31	9.52	13.92	10.20	11.21
'G. 2012-50'	17.51	15.70	17.82	17.01	12.44	11.22	13.72	12.46	71.03	71.47	77.01	73.17	7.60	6.88	8.82	7.77	11.30	10.15	11.81	11.09	9.11	7.80	8.41	8.44
'G. 2011-79'	21.49	18.84	19.98	20.10	16.00	14.42	17.11	15.84	74.48	76.15	85.64	78.76	10.08	9.24	11.65	10.32	14.08	12.46	13.75	13.43	10.27	11.96	11.40	11.21
'G. 2003-47'	21.10	20.17	21.16	20.81	17.44	16.16	18.59	17.40	82.82	80.15	87.87	83.61	11.67	11.44	12.82	11.98	14.34	13.55	14.70	14.19	15.12	15.40	15.20	15.24
'G.2007-61'	20.50	20.05	19.95	20.17	15.78	17.07	16.04	16.30	77.01	85.24	80.32	80.86	10.14	11.59	10.57	10.77	13.59	13.77	13.42	13.59	10.86	13.63	11.41	11.97
'G.2004-27'	19.48	19.78	20.04	19.77	14.69	16.00	15.87	15.52	75.48	80.89	79.32	78.56	9.33	10.57	10.37	10.09	12.82	13.34	13.41	13.19	12.69	15.35	14.18	14.07
'G.2003-44'	20.74	21.27	20.35	20.79	15.92	17.68	17.07	16.89	76.79	83.05	83.98	81.27	10.21	11.86	11.51	11.19	13.73	14.48	13.90	14.04	12.75	15.70	13.95	14.13
'G.99-103'	19.11	18.61	19.25	18.99	15.18	13.99	15.46	14.88	79.44	75.13	80.32	78.31	9.93	8.87	10.18	9.66	12.80	12.24	12.95	12.66	15.20	13.01	13.92	14.04
'G. 2003-49'	20.20	20.17	20.13	20.17	16.34	16.32	18.42	17.02	80.96	80.83	91.61	84.48	10.80	10.79	12.94	11.51	13.62	13.60	14.20	13.81	13.00	14.18	14.77	13.98
'G. T. 54-9'	20.89	19.98	20.89	20.59	17.15	16.44	17.41	17.00	82.12	82.47	83.32	82.64	11.43	10.97	11.69	11.36	14.16	13.55	14.23	13.98	14.61	15.82	15.32	15.25
LSD at 5%																								
Variety (V)	**0.95**	**2.40**	**1.27**	**0.93**	**1.31**	**2.61**	**1.57**	**1.08**	**5.93**	**5.81**	**8.50**	**3.86**	**1.25**	**2.04**	**1.55**	**0.92**	**0.67**	**1.78**	**0.81**	**0.67**	**2.72**	**3.82**	**2.16**	**1.68**
Crop age (C)			**NS**				**NS**				**2.33**				**0.62**				**NS**				**1.04**	
V x C			**NS**				**1.86**				**6.69**				**1.60**				**NS**				**NS**	

ratoon and over the crops. Sugar yield in plant cane, first ratoon and second ratoon crops, varied significantly among genotypes with each crop cycle and also among the crop cycles. Sugar yield for studied genotypes varied (did not show a trend) among the crop cycles except the genotype 'G.2003-49' which showed an increase in consecutive crop cycles. Sugar yield increased by 13.27% in first ratoon and by 1.42%, in second ratoon as compared to the plant cane.

Ratooning ability

All genotypes showed decreased stalk diameter in consecutive crop generations affecting ratooning ability adversely which was in agreement with the results obtained by Mehareb *et al.* (2015) and, Masri and Amein (2015)who reported that the stalk diameter of some genotypes decreased with consecutive crop cycles.

All studied genotypes along with the check variety showed better ratooning ability for stalk length except two genotypes *viz.*,'G2004-27' and 'G99-103' (Figure1 a). The highest RA value (122.94%) for stalk length was recorded by the genotype 'G.2011-13', indicating the superiority of stalk length in second ratoon crop over plant cane crop, which is in accordance with the results of Mehareb *et al.* (2015) and, Masri and Amein (2015)who reported that the stalk length increased with consecutive crop cycles. While one genotype 'G.2011-13' was a good ratooner with respect to stalk weight, other genotypes showed a decrease in consecutive crop cycles, which was in agreement with previous results (Mehareb *et al.* 2015; Chapman *et al.* 1992; Hunsigi 1982) where a reduction in stalk weight in the ratoon crop was observed.

Twelve genotypes were good ratooner for cane yield (Figure 1 b). Five genotypes, 'G.2010-26', 'G.2011-74', 'G.2010-8', 'G.2011-13' and 'G.2011-79' recorded high ratooning ability value for cane yield as compared to the check variety 'G.T.54-9'. Four genotypes; 'G.84-47', 'G.2011-74' (104.43%), 'G.2004-27'and 'G.2003-44'had good ratooning ability for °Brix percent ranging from 100.13 to 104.43%.

Good ratooning ability for sucrose (Figure 1 c) was recorded in eight genotypes (from 100.23-116.03%): 'G.84-47', 'G.2011-82' and 'G.2011-74' recorded highest values of ratooning ability and, 'G.2010-8', 'G.2012-50', 'G.2007-61', 'G.2004-27', 'G.2003-44' and 'G.2003-49' showed high ratooning ability sucrose as compared to the check variety 'G.T.54-9' (98.69%).

For purity percentage, all genotypes showed an increase in second ratoon as compared to the plant cane except three genotypes *viz.*, 'G.2010-26', 'G.2006-6' and 'G.99-103';the highest RA value (110.78%) for purity percentage was recorded by the genotype 'G.2011-74'.

The genotype 'G.2011-74' recorded highest ratooning ability value of 123.61% and 108.20% for sugar recovery and pol percentage respectively.

High ratooning ability value for sugar yield as compared to the check variety 'G.T.54-9' (106.58%) was recorded by eight genotypes; 'G.2011-74'(145.45%), 'G.2010-8'(123.59%),'G.2011-13'(126.75), 'G.2011-79'(113.74%), 'G.2007-61'(115.31%), 'G.2004-27'(116.36%), 'G.2003-44' (116.33%)and 'G.2003-49'(111.35%) (Figure 1 e).

Four genotypes *viz.*, 'G.2011-74', 'G.2010-8', 'G.2011-13', 'G.2011-79' recorded high ratooning values for cane and sugar yield together, as compared to the check variety. These results are in agreement with Mehareb *et al.* (2015) and Masri and Amein (2015) who reported that the cane and sugar yield of some genotypes increased with consecutive crop cycles.

Crop cycle effects on genetic parameters

The relative influence of genotypic variance (σ^2g) in determining phenotypic variance was more important than other components for most of the studied traits. Genotypic variance decreased from plant cane to second ratoon crop for stalk length, stalk weight and °Brix% (Table 4). Phenotypic coefficient of variation (PCV) decreased from plant crop to second ratoon crop for stalk length and sugar yield, while PCV increased for stalk diameter and purity percentage in these crop cycles. Genotypic coefficient of variation GCV decreased from plant cane to second ratoon crop for stalk length, stalk weight and °Brix%, while increased for stalk diameter. Heritability decreased for stalk length and stalk weight and increased for stalk diameter with consecutive crop cycles. Crop cycle did not appear to affect heritability for sucrose percentage especially in second ratoon crop due to decreasing error variance. Examination of variance component, calculated from full model analysis across crops showed the important contribution of σ^2gc in determining the phenotypic variance for stalk length, stalk diameter, stalk weight, stalk number and cane yield (Table 5). Therefore, it is necessary to test for more than one year to estimate the components of variance if the genotype x year, genotype x location, or genotype x year x location interaction is of importance (Dudley and Moll 1969).

Table 5 shows high genetic variance ($\sigma 2g$) relative to environmental variance for all traits under study, which is important as it describes the amount of genetic variation present for the trait. The results also indicate that high estimates of genotypic and phenotypic coefficients of variation GCV and PCV were recorded for stalk weight (52.23 and 50.34), sugar yield (49.59 and 48.64) and cane yield (33.68 and 32.97). These results are in agreement with Mehareb *et al.* (2015), who also reported high estimates of GCV and PCV for sugar and cane yield. The differential potential improvement of the traits over the years has resulted partially from the selection methodology adopted prior to this selection stage (Breaux 1972). Selection programme in Egypt tends to concentrate on sucrose quality and stalk diameter in its early stages. Therefore, genetic variability for these trait may be limited in consecutive generations (Gravois 1988 and Milligan 1988).

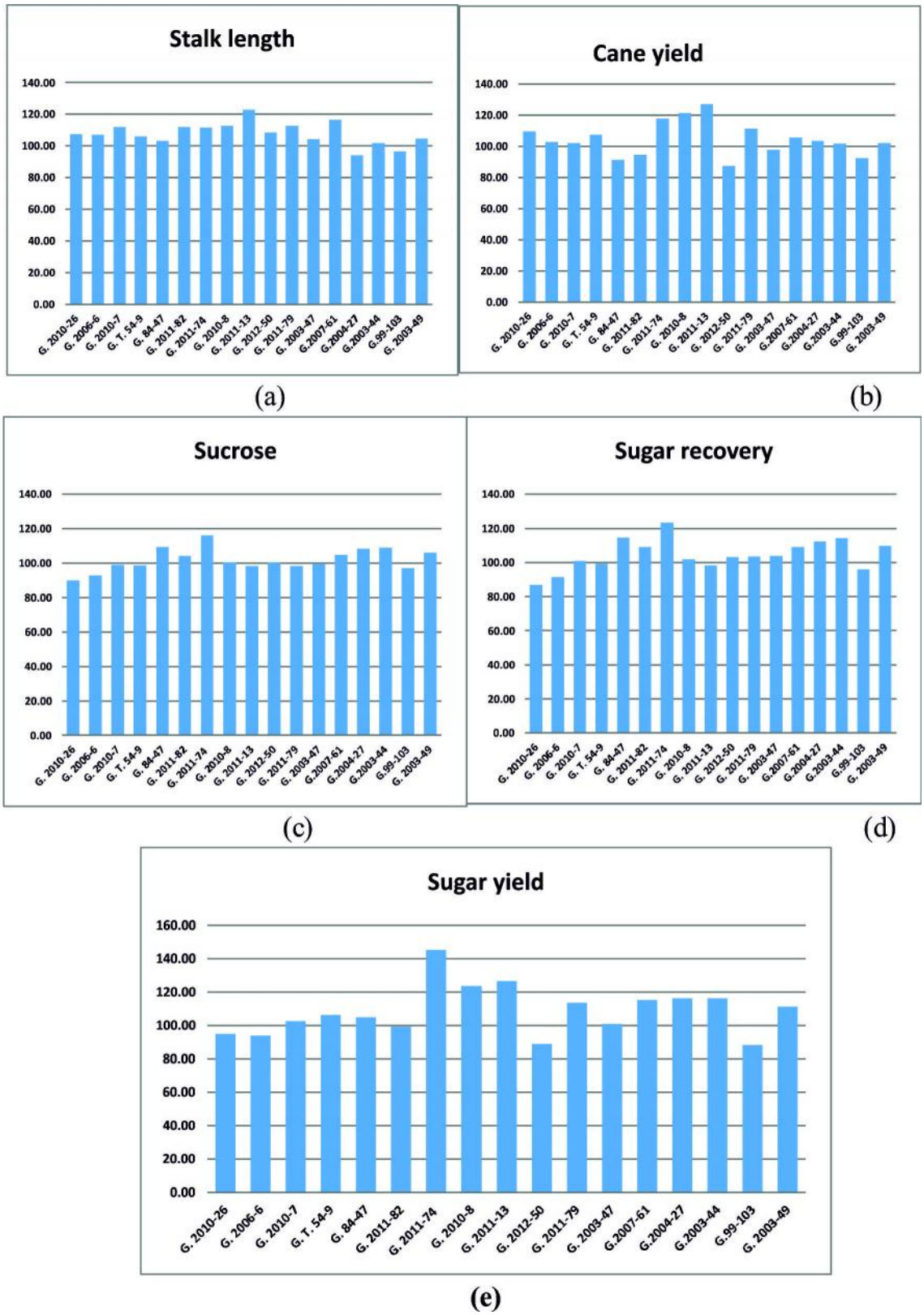

Fig. 1 (a-e). Ratooning ability percentage for (a) stalk length, (b) cane yield, (c) sucrose, (d) sugar recovery and (e) sugar yield.

Table 4 Variance components, heritability (H%), genotypic coefficient of variation (GCV%) and phenotypic coefficient of variation (PCV%) for all studied traits in plant cane (PC), first ratoon (FR) and second ratoon (SR)

	Stalk diameter			Stalk Length		
	PC	FR	SR	PC	FR	SR
σ^2e	0.03	0.03	0.02	153.54	249.21	195.81
σ^2g	0.04	0.05	0.05	732.81	504.34	323.77
σ^2ph	0.07	0.08	0.07	886.35	753.56	519.58
H%	76.72	85.67	89.50	93.47	85.86	83.22
GCV%	7.43	9.50	10.22	10.90	8.33	6.84
PCV%	10.25	11.63	11.90	11.99	10.18	8.66
	Stalk weight			^{o}Brix		
	PC	FR	SR	PC	FR	SR
σ^2e	0.03	0.02	0.02	0.33	2.08	0.58
σ^2g	0.04	0.03	0.01	0.89	0.81	0.44
σ^2ph	0.07	0.05	0.03	1.22	2.89	1.02
H%	83.14	80.43	66.01	89.11	54.07	69.17
GCV%	18.36	17.35	17.13	4.69	4.61	3.33
PCV%	23.23	22.73	27.24	5.48	8.69	5.09
	Sucrose			Purity		
	PC	FR	SR	PC	FR	SR
σ^2e	0.62	2.46	0.89	12.72	12.22	26.09
σ^2g	1.40	1.87	1.35	6.72	12.10	10.58
σ^2ph	2.01	4.33	2.25	19.43	24.32	36.67
H%	87.17	69.48	82.01	61.31	74.82	54.88
GCV%	7.57	8.80	7.15	3.34	4.40	3.97
PCV%	9.09	13.40	9.20	5.69	6.24	7.39
	Sugar recovery			Pol		
	PC	FR	SR	PC	FR	SR
σ^2e	0.56	1.50	0.87	0.16	1.14	0.24
σ^2g	0.89	1.29	1.01	0.57	0.63	0.39
σ^2ph	1.45	2.79	1.88	0.73	1.77	0.62
H%	82.55	72.05	77.82	91.32	62.39	83.08
GCV %	9.33	11.18	9.29	5.63	6.06	4.63
PCV%	11.93	16.45	12.65	6.38	10.16	5.87
	Cane yield			Sugar yield		
	PC	FR	SR	PC	FR	SR
σ^2e	90.41	265.75	58.20	2.67	5.26	1.68
σ^2g	279.38	42.63	194.79	4.38	3.16	4.34
σ^2ph	369.79	308.38	253.00	7.05	8.42	6.03
H%	90.26	32.49	90.94	83.14	64.29	88.55
GCV %	14.35	4.99	12.66	17.72	13.34	17.39
PCV%	16.50	13.42	14.43	22.48	21.78	20.49

Table 5 Overall variance components, heritability (H%), genotypic coefficient of variation (GCV%) and phenotypic coefficient of variation (PCV%) for all traits

Parameter	Stalk diameter	Stalk Length	Stalk weight	oBrix	Sucrose
σ2 e	0.03	199.52	0.02	1.00	1.32
σ2gy	0.01	365.83	0.04	0.62	1.87
σ2g	0.46	4368.63	0.21	7.41	12.93
σ2ph	0.47	4512.75	0.23	7.72	13.70
H%	98.66	96.81	92.90	95.89	94.37
PCV%	28.42	25.80	52.23	14.00	23.42
GCV%	28.23	25.38	50.34	13.71	22.75
	Purity	Sugar recovery	Pol	Cane yield	Sugar yield
σ2 e	17.01	0.98	0.51	138.12	3.20
σ2gy	25.14	1.54	0.48	153.18	3.21
σ2g	74.17	8.55	4.92	1544.26	36.21
σ2ph	84.44	9.17	5.14	1610.66	37.63
H%	87.84	93.22	95.78	95.88	96.21
PCV%	11.56	29.23	17.02	33.68	49.59
GCV%	10.83	28.22	16.66	32.97	48.64

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Indian Journal of Sugarcane Technology **32(02)**: 60-67, December 2017

'CoP 112': An early maturing and high yielding sugarcane variety for commercial cultivation in Bihar

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ABSTRACT

A early maturing and high yielding clone 'CoP 112' was developed from a cross combination of 'BO91' x 'Co 62198'at Sugarcane Research Institute, Dr. R.P.C.A.U., Pusa. It was initially tested in three different clonal generations at Pusa and was included in multi-location trials against the checks 'BO 130' and 'BO145',which were conducted for three consecutive years in RBD with three replications at different locations *viz.*, Harinagar, Narkatiaganj, Majhaulia, Riga and Sidhwalia in sugarcane growing area of Bihar. Besides, from 2008 to 2013, three plant and two ratoon crops trials were conducted at Pusa, whereas only plant crop trials were conducted at other five locations against the checks 'BO 130' and 'BO 145' for three consecutive years. The observations were recorded for CCS, cane yield, sucrose% in juice, yield attributing traits, resistance to diseases (red rot, smut and wilt) and tolerance to insects(root, shoot, stalk and top borers) during the crop periods. The clone'CoP 112'recorded 34.79% and 36.61% more cane and sugar yield (t/ha), respectively than the check 'BO 130' while 18.82% and 19.64% more cane and sugar yield, respectively than the check 'BO 145', while in ratoon crops 'CoP 112' showed 28.11% and 30.86% more cane and sugar yield, respectively over 'BO 130' while 14.73% and 17.71% more cane and sugar yield respectively over 'BO 145' during 2010-12 at Pusa. In multi-location trials for 3 years during 2009-12 at different sugar factory areas *viz.*, Harinagar, Narkatiaganj, Majhaulia, Riga and Sidhwalia the clone 'CoP 112' showed 34.31-38.47% and 33.37-36.27 % more cane and sugar yield, respectively, than the check 'BO 130', 25.05-29.53% and 21.02-21.45 % more cane and sugar yield respectively than the check 'BO 145' over the years across the locations. The clone 'CoP 112' also showed resistance against red rot, smut and wilt under artificial inoculation. Low insect pest incidence was observed during the crop period. Erect stool, greenish yellow colour of stem which turns pink on exposure to sunlight, straight and cylindrical inter-nodes having low wax, rare growth split, presence of ivory marking. Swollen node, two eye rows arranged in a regular fashion on root primordial zone, presence of bud groove and weather mark, are the distinguishable features of the 'CoP 112'. Based on superiority over the checks in three plant and two ratoon crops for CCS t/ha, cane yield and sucrose % in juice 'CoP 112' has been recommended for commercial cultivation in Bihar. This outcome will help the sugarcane, growers as well as sugar industry of the state as it has high tonnage and high sugar recovery.

Key words: Sugarcane, Early Variety, Commercial cultivation in Bihar

Sugarcane is an important agro-industrial crop of India with the total area of 5.307 million ha under this crop with a production of 366.80 million tonnes and productivity 69.1 tonnes per ha, of which share of Bihar is only an area of 0.302 million ha, production of 14.90 million tones and productivity of 50.00 tonnes per ha. Low productivity of sugarcane in Bihar has been recorded since last five decades which can be enhanced by increasing area of stable, early maturing and high yielding varieties. Reddy and Madhuri (2014) reported that subtropical zone contributes more than 55% area of the sugarcane, however, cane production and sugar recovery (%) is lower in comparison to tropical India. It is due to lack of coverage area under high yielding early maturing varieties specially in Bihar. In last 3 decades, it was observed that due to this reason, the sugar factories closed one by one in Bihar. Recently, some early maturing varieties are gaining popularity among farmers and 'CoP 112'is performing best for its productivity as well as recovery. 'CoP 112'an early maturing sugarcane variety has been developed from a bi-parental cross 'BO 91' X 'Co 62198'. The resistance ability of this variety against biotic and abiotic stresses has been imparted from 'BO 91'and higher yield and sucrose from 'Co 62198'. It also showed resistance against red rot, smut and wilt diseases under artificial inoculation and has low insect pest incidence. Distinguishable features of 'CoP 112'are erect stool, greenish yellow colour of stem, straight and cylindrical inter-node having low wax, rare growth split, presence of ivory marking, bud groove and weather mark.

MATERIALS AND METHODS

The plant material of this experiment *viz.*, 'X 03112', 'X 03116', 'X 03125', 'X 03146', 'CoX 03172', 'CoX 03178', 'CoX 03664', 'CoX 03672', 'BO 130'(C) and 'BO 145'(C)

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were planted at Pusa, Harinagar, Narkatiaganj, Majhaulia, Riga and Sidhwalia during 2008-2012 in RBD with three replications. In this paper, mean performance of summary data of 'CoX 03178' along with checks ('BO 130'and 'BO 145') has been discussed. The clone 'CoX 03178' (now 'CoP 112') was selected as early maturing clone. All the recommended agronomical package and practices were followed in all multi-locational trials. Further, three plant crops and two ratoon crops were planted in randomized block design (RBD) with three replications at Pusa while at other centres, only plant crops for three successive years from 2008 to 2013. Patel and Patel (2014) procedure was followed for sugarcane sett size, seed rate and sett treatment. Observations were recorded by selecting five random plants per genotype per replication for cane yield and juice quality characters. The red rot score (0-9 scale) was also observed after splitting of five randomly selected plants of each genotype per replication and insects incidences were also recorded. The reaction to red rot, wilt and smut was observed in field condition and rated under artificial condition also. The action to insect pest was observed in natural field condition. Juice quality tests were conducted as per standard procedure (Meade and Chen 1971). The morphological data were observed as per standards suggested by Dutt *et al.* (1974).

RESULTS AND DISCUSSION

Cane yield per hectare and percent sucrose in juice are factors of prime importance in sugarcane breeding. Sugarcane variety is the pivot around which its entire production system revolves. All the details *viz.*, distinguishing morphological features (Figs. 1 to 5), cane and sugar yield, performance of yield component traits, reaction to diseases and pests of clone 'CoP 112' are presented in Tables 1 to 9.

Distinguishing morphological features

As per standards suggested by Dutt *et al.*(1947), the clone 'CoP 112' could be identified by its erect stool , greenish yellow colour of stem which turns pink on exposure to sunlight, medium to long straight and cylindrical inter-node having low wax, growth split, presence of ivory marking swollen node, oval and small bud, two eye rows arranged in a regular fashion on root primordial zone, presence of bud groove and weather mark. Table 1 shows the information about distinguishing morphological features of identified sugarcane variety 'CoP 112' while important distinguishing characters can be seen in Fig. 1 to 4.

Table 1 Distinguishing morphological features of released sugarcane variety 'CoP 112' as per DUS characteristics

Sl. No.	Characters	State
1.	Plant growth habit	Erect
2.	Leaf Sheath: hairiness	Present
3.	Leaf Sheath: Shape of ligule	Crescent
4.	Leaf Sheath: Shape of inner auricle	Incipient
5.	Leaf Sheath: Colour of dewlap	Dirty Green
6.	Leaf Blade: Curvature	Arched
7.	Leaf Blade: Width	Medium
8.	Plant: Adherence of leaf sheath	Strong
9.	Internode: Colour (Not exposed to sun)	Greenish yellow
10.	Internode Colour: (Exposed to sun)	Pink colour
11.	Internode: Diameter	Medium
12.	Internode: Shape	Cylindrical
13.	Internode: Zigzag alignment	Absent
14.	Internode: Growth crack (Split)	Present
15.	Internode : Rind surface appearance	Smooth
16.	Internode: Waxiness	Low
17.	Node: Shape of bud	Oval
18.	Node: Size of bud (Measured from base of bud to the tip)	Small
19.	Node: Bud groove	Present
20.	Node: Bud cushion (Space between bud base and leaf scar)	Absent
21.	Node: bud tip in relation to growth ring	Above the growth ring
22.	Node: Prominence of growth ring	Swollen
23.	Node: Width of root band (opposite to bud)	Medium
24.	Internode Cross section	Oval
25.	Internode: Pithiness	Present
26.	Plant: Number of millable canes (NMC) per stool	High
27.	Plant: Cane height	Medium

Cane and sugar yield

Data given in tables 2, 3 and 4 showed consistent performance of clone 'CoP 112'for cane and sugar yield in plant and ratoon crops across the six locations over the years. Clone 'CoP 112' showed 34.79% and 36.61% more cane and sugar yield (t/ha), respectively than the check 'BO 130' while 18.82% and 19.64% more cane and sugar yield, respectively than the check 'BO 145'. In ratoon crop 'CoP 112' showed 28.11% and 30.86% more cane and sugar yield, respectively over 'BO 130', 14.73% and 17.71% more cane and sugar yield respectively over 'BO 145' during 2010-12 at Pusa. In multi-location trials for 3 years during 2009-12 at different sugar factory areas *viz.*, Harinagar, Narkatiaganj, Majhaulia, Riga and Sidhwalia, the clone 'CoP 112' showed 34.31 to 38.47% and 33.37 to 36.27% more cane and sugar yield, respectively, than

Fig. 1. Internodes of 'CoP 112'

Fig. 2. Leaf carriage of ''CoP 112'

Fig. 3. Bud of 'CoP 112'

Fig. 4. Bud groove of 'CoP 112'

Fig. 5. Field view of 'CoP 112'

Table 2 Mean performance of clone 'CoP 112' along with checks for cane and sugar yield in plant crop at Pusa, Samastipur over the years

Variety	1st year trial (2008-09)			2nd year trial (2009-10)			3rd year trial (2010-11)			Mean			% Improvement in 'CoP 112'		
	Cane yield (t/ha)	Sucrose % in juice	Sugar yield (t/ha)	Cane yield (t/ha)	Sucrose % in juice	Sugar yield (t/ha)	Cane yield (t/ha)	Sucrose % in juice	Sugar yield (t/ha)	Cane yield (t/ha)	Sucrose % in juice	Sugar yield (t/ha)	Cane yield	Sucrose % in juice	Sugar yield %
'CoP 112' ('CoX 03178')	94.32	17.42	11.86	98.73	17.56	12.05	96.63	17.46	11.92	96.56	17.48	11.94	-	-	-
'BO 130' (Check)	70.33	17.10	8.62	71.21	17.06	8.71	73.38	17.18	8.88	71.64	17.11	8.74	34.79	2.16	36.61
'BO145' (Check)	82.12	17.20	10.04	80.14	17.10	9.88	81.59	17.30	10.02	81.2	17.20	9.98	18.82	1.53	19.64
CD at 0.05	9.77	NS	2.07	9.19	NS	1.92	9.32	NS	1.86	-	-	-			
CV %	9.62	3.01	7.91	10.31	2.17	9.88	9.95	2.13	9.23	-	-	-			

Table 3 Cane and sugar yield performance of ratoon crop at Pusa of the clone 'CoP 112' from 2009 to 2011

Variety	1st year trial (2009-10)			2nd year trial (2010-11)			Mean			% Improvement in 'CoP 112'		
	Cane yield (t/ha)	Sucrose % in juice	Sugar yield (t/ha)	Cane yield (t/ha)	Sucrose % in juice	Sugar yield (t/ha)	Cane yield (t/ha)	Sucrose % in juice	Sugar yield (t/ha)	Cane yield	Sucrose % in juice	Sugar yield (%)
'CoP 112' ('CoX 03178')	88.23	17.40	10.56	86.87	17.36	10.97	87.55	17.38	10.77			
'BO 130' (Check)	65.57	17.00	8.04	69.11	16.80	8.42	68.34	16.90	8.23	28.11	2.84	30.86
'BO145' (Check)	78.35	17.10	9.28	74.26	16.90	9.02	76.31	17.00	9.15	14.73	2.23	17.71
CD at 0.05	9.21	ns	1.96	8.24	ns	1.89	-	-	-	-	-	
CV %	9.86	2.94	8.87	9.14	2.99	10.32	-	-	-	-	-	-

Table 4 Mean performance of 'CoP 112' and checks over years and locations for cane yield (t/ha) in plant crop

Variety	Year	Pusa	Harinagar	Narkatiaganj	Majhaulia	Riga	Sidhwalia	Mean	% Improvement in 'CoP 112'	Pooled Mean	Mean % increase over the checks
'CoP 112' ('CoX 03178')	2008-09	94.32	-	-	-	-	-	94.32	-	-	-
	2009-10	98.73	100.57	94.68	95.73	93.27	97.23	96.70	-	96.10	
	2010-11	96.63	99.92	97.79	95.53	94.87	101.78	97.75	-		
	2011-12	-	98.79	95.42	92.47	95.33	96.21	95.64	-		
'BO 130' (Check)	2008-09	70.33	-	-	-	-	-	70.33	34.11		
	2009-10	71.21	72.55	67.89	74.32	71.57	70.77	71.39	35.45	71.03	35.29%
	2010-11	73.38	71.45	68.32	69.87	71.72	72.42	71.19	37.31		
	2011-12	-	70.32	69.45	68.89	72.89	73.51	71.21	34.31		
'BO145' (Check)	2008-09	82.12	-	-	-			82.12	14.86		
	2009-10	80.18	79.53	76.78	73.56	75.37	79.81	77.54	25.05	78.11	23.03%
	2010-11	81.59	74.78	78.53	72.38	75.12	77.39	76.66	29.53		
	2011-12	-	78.78	75.53	71.88	76.72	77.58	76.10	25.68		
CD at 0.05	2008-09	9.77	-	-	-	-	-				
	2009-10	9.19	9.27	8.77	9.33	8.53	9.87				
	2010-11	9.32	10.28	9.32	9.48	9.23	10.12				
	2011-12	-	10.28	9.32	9.48	9.23	10.12				
CV%	2008-09	9.62	-								
	2009-10	10.31	10.14	10.06	9.89	10.23	10.51				
	2010-11	9.95	11.57	10.11	10.57	10.21	11.83				
	2011-12	-	11.57	10.11	10.57	10.21	11.83				

Table 5. Performance of 'CoP 112'in multi-location trials conducted during 2009-12 for sucrose % in juice

Variety	Year	Pusa	Harinagar	Narkatiaganj	Majhaulia	Riga	Sidhwalia	Mean Across the location	% Improvement in 'CoP 112'	Mean across the year & location	Mean % increase over the checks
'CoP 112'	2008-09	17.42	-	-	-	-	-	17.42			
('CoX 03178')	2009-10	17.56	17.44	17.22	17.30	17.46	17.48	17.41		17.42	
	2010-11	17.46	17.40	17.32	17.38	17.44	17.46	17.41			
	2011-12		17.48	17.44	17.38	17.40	17.46	17.44			
'BO 130'	2008-09	17.10	-	-	-	-	-	17.10			
(Check)	2009-10	17.06	16.70	16.92	16.90	16.70	16.82	16.85	3.45	16.91	0.03
	2010-11	17.18	16.60	16.90	16.82	16.74	17.00	16.87	3.51		
	2011-12		16.82	16.86	16.64	16.84	16.96	16.82	3.69		
'BO145'	2008-09	17.20	-	-	-	-	-	17.20			0.02
(Check)	2009-10	17.10	17.10	17.02	17.08	17.12	17.16	17.10	1.70	17.13	
	2010-11	17.30	17.10	17.04	17.10	16.90	17.10	17.09	1.93		
	2011-12		17.20	17.14	17.12	16.96	17.14	17.11	1.93		
CD at 0.05	2008-09	ns	-	-	-	-	-				
	2009-10	ns	ns	ns	ns	ns	ns				
	2010-11	ns	ns	ns	ns	ns	ns				
	2011-12	-	ns	ns	ns	ns	ns				
CV%	2008-09	3.01	-	-	-	-	-				
	2009-10	2.17	2.11	1.96	1.89	2.04	2.32				
	2010-11	2.13	1.91	2.73	2.95	1.44	1.31				
	2011-12		2.11	2.03	2.36	2.44	1.91				

Table 6 Performance of 'CoP 112'at six locations over the years 2009-12 for sugar yield (t/ha)

Variety	Year	Pusa	Harinagar	Narkatiaganj	Majhaulia	Riga	Sidhwalia	Mean over the year & locations	% Improvement in 'CoP 112'	Pooled mean	Mean % increase over the checks
	2008-09	11.86	-	-	-	-	-	11.86			
'CoP 112'	2009-10	12.05	12.42	11.83	11.58	12.18	12.39	12.08		12.02	
('CoX 03178')	2010-11	11.92	12.12	11.83	11.67	12.24	12.48	12.04			
	2011-12	-	12.19	11.89	11.78	12.21	12.36	12.09			
'BO 130'	2008-09	8.62	-	-	-	-	-	8.62	37.59		
(Check)	2009-10	8.71	8.74	8.42	9.12	9.02	8.96	8.83	36.81	8.83	36.13%
	2010-11	8.88	8.71	8.52	9.10	9.01	8.93	8.86	35.89		
	2011-12	-	8.91	8.63	9.13	9.21	9.23	9.02	34.04		
'BO145'	2008-09	10.04	-	-	-	-	-	10.04	18.12		
(Check)	2009-10	9.88	10.05	9.86	9.58	10.03	10.14	9.92	21.77	9.96	20.68%
	2010-11	10.02	10.16	9.87	9.68	9.96	10.15	9.96	20.88		
	2011-12	-	10.27	9.67	9.88	9.93	10.18	9.97	21.26		
CD at 0.05	2008-09	2.07	-	-	-	-	-				
	2009-10	1.92	2.40	1.53	1.42	1.79	1.32				
	2010-11	1.86	2.41	1.54	1.43	1.81	1.33				
	2011-12	-	2.81	1.61	2.13	3.10	2.41				
CV%	2008-09	7.91	-	-	-	-	-				
	2009-10	9.88	11.43	9.89	8.34	9.27	8.53				
	2010-11	9.23	11.23	9.67	8.02	9.21	8.39				
	2011-12		12.14	8.52	9.10	9.01	10.13				

the check 'BO 130' while 25.05 to 29.53% and 21.02 to 21.45% more cane and sugar yield respectively than the check 'BO 145' over the year across the locations.

This variety recorded 12.02 t/ha commercial cane sugar yield as pooled mean which was 36.13%, 20.68 % and 15.35% higher than the checks 'BO 130' (8.83 t/ha) and 'BO 145' (9.96 t/ha) respectively (Table 6) over the year across the six locations. The pooled mean for cane yield of 'CoP 112' was 96.1 t/ha which was 35.29% and 23.03% higher than the checks 'BO 130' (71.3 t/ha) and 'BO 145' (78.11 t/ha), respectively over the years across the six locations as indicated in the Table 4. 'CoP 112' surpassed the checks 'BO 130' and 'BO 145' under multi-location trials conducted at all six locations *viz.*, Pusa, Harinagar, Narkatiaganj, Majhaulia, Riga and Sidhwalia by a margin of more than 20% for cane and sugar yield the two most important features for release of a variety Singh *et al.* (2001) for 'CoSe 96436', Pandey *et al.* (2009) for 'BO 146', Kumar *et al.* (2015) for 'CoP 2061' and Sanghera *et al.* (2016) for 'CoPb 08212' also emphasized upon the importance of cane and sugar yield for identification and release of sugarcane variety.

Performance of yield component

'CoP 112' had higher number of millable canes (108630/ha) than the checks in different trials over the years across the centres. 'CoP 112' (0.860 kg) recorded 13.15% and 10.26 % higher single cane weight than the checks; 'BO 130' (0.76 Kg.) and BO 145(0.78 Kg.), respectively. The Cane diameter of 2.52 cm was recorded for 'CoP 112' followed by the checks 'BO 145' (2.5cm) and 'BO 130' (2.46 cm) over the years. The cane length was 300cm for 'CoP 112'which was 11.11% and 10.29% higher than 'BO 130' (270 cm) and 'BO145' (272cm) over the year across locations. Singh *et al.* (2001), Pandey *et al.* (2009), Kumar *et al.*(2015) and Sanghera *et al.* (2016) for 'CoPb 08212' have also highlighted the importance of yield components traits for identification and release of a sugarcane variety.

Reaction to diseases

An overview given in table 8, indicates that no disease appeared in 'CoP 112' over the year 2008-2013 at Pusa after artificial inoculation of isolates of red rot. For wilt and smut diseases this variety 'CoP 112' showed resistant reactions. Probably the disease resistance ability comes from its parent 'BO 91' which is a known source of resistance to red rot, smut and wilt diseases. The check 'Co 1148' was highly susceptible to red rot and moderately susceptible to wilt. The check 'Co 1158'was also moderately susceptible against wilt. The clone 'CoP 112', will certainly be helpful to the farmers as well as sugar mills for enhancing the productivity as well as sugar recovery (%) of the sugarcane in the state of Bihar, especially due to its red rot resistance.

Reaction to insects and pests

An overview given in table 8 indicated that low incidences of shoot/root borer, stalk borer and top borer appeared in 'CoP 112' as well as check 'BO 130' over the years 2008-2013 under natural condition. Percentage incidence based on dead hurt recorded in post germination phase at 30 days interval up to 120 days after planting was found very less. Incidence of pyrilla, black bug and whitefly were also in traces. It means that 'CoP 112' has insect tolerance ability which favours low input management.

CONCLUSION

The variety 'CoP 112' exhibited taller cane (300 cm), moderate single cane weight (0.86 kg.), thicker cane diameter (2.52cm), and millable canes (108000/ha) as compared to most of the check, with high tonnage and sugar yield. The variety was found resistant to red rot disease, wilt and smut under field conditions. The resistance ability against red rot was supposedly contributed from parent 'BO 91'. This variety is expected to play a great role in boosting the productivity of sugarcane in Bihar and also enhance the recovery of sugarcane in different sugar factories by crushing it early and for a longer period i.e. from October-November to January-February. Its green top can be used as best fodder for cattle. The early maturing high yielder and high sucrose containing clone 'CoP 112' will be a better option for enhancing the productivity and sugar recovery (%) of sugarcane in Bihar for cane grower and sugar industry. The yield advantage was 24.92 and 15.30 tonne over checks 'BO 130' and 'BO 145' respectively. The sugar factory will also be benefitted by a start in early crushing season.

ACKNOWLEDGEMENT

The authors are thankful to the Director Research, RPCAU, Pusa for providing grant and facilities for research work. We

Table 7 Mean performance of yield attributing traits of 'CoP 112' over the year across the locations

Genotypes	Cane height (cm)	Cane diameter (cm)	Single cane weight (kg)	NMC (000/ha)
'CoP 112' ('CoX 03178')	300	2.52	0.86	108.63
'BO 130' (Check)	270	2.46	0.76	95.37
'BO145' (Check)	272	2.50	0.78	102.36
CD at 0.05	24.31	0.49	0.91	11.16
CV %	6.87	6.23	6.81	8.24

Table 8 Reaction against major sugarcane diseases *viz*., red rot, wilt and smut*

Year	'CoP 112' ('CoX 03178')			'BO 130' (Check)			'Co 1148' Check for red rot & wilt		'Co 1158' Check for smut
	Red rot (cm)	Wilt (%)	Smut (%)	Red rot (cm)	Wilt (%)	Smut (%)	Red rot (cm)	Wilt (%)	Smut (%)
2008-09	16.4	0.00	0.00	17.2	0.00	0.00	59.9	11.2	11.5
2009-10	15.2	0.00	0.00	16.1	0.00	0.00	62.8	11.6	9.8
2010-11	14.4	0.00	0.00	15.3	0.00	0.00	66.5	10.2	11.4
2011-12	15.6	0.00	0.00	17.2	0.00	0.00	62.0	11.6	10.8
2012-13	14.3	0.00	0.00	14.5	0.00	0.00	63.2	10.2	9.8
Mean	15.2	0.00	0.00	16.1	0.00	0.00	62.9	10.9	10.7
Rating	R	R	R	R	R	R	HS	MS	MS

Inference: 'CoP 112' ('CoX 03178') was resistant to red rot , wilt and smut diseases.

*Rating scale of major sugarcane diseases followed at SRI, Pusa

Red rot			Smut and wilt		
Linear spread in cm.	:	Rating	% Infestation	:	Rating
0-10	:	HR	0 - 0.0	:	R
10.1-20	:	R	0.1-10	:	MR
20.1-30	:	MR	10.1-20	:	MS
30.1-40	:	MS	20.1-30	:	S
40.1-50	:	S	Above 30	:	HS
Above 50	:	HS			

Table 9 Reaction against important sugarcane insects and pests*

Year	'CoP 112' ('CoX 03178')				'BO 130' (Check)			
	Shoot stage		Cane stage		Shoot stage		Cane stage	
	Shoot+ Root borer	Top borer	Top borer	Stalk borer	Shoot+ Root borer	Top borer	Top borer	Stalk borer
2008-09	9.7	9.6	12.9	5.2	9.6	7.6	13.7	6.3
2009-10	7.4	9.2	14.4	5.9	9.2	8.4	14.3	8.5
2010-11	8.2	7.2	13.7	6.2	8.7	9.1	15.2	6.0
2011-12	8.6	7.4	12.6	6.1	9.3	8.2	13.2	5.2
2012-13	9.1	8.6	14.7	5.6	8.7	7.9	14.5	6.2
Mean	8.6	8.2	13.7	5.8	9.1	8.2	14.2	5.9
Rating	Low	Low	Low	Low	Low	Low	Low	Low

*Rating scale of Sugarcane Insect Pest followed at SRI, Pusa

Sl. No.	Sugarcane pests	Rating (%)		
		Low	Medium	High
1.	Shoot + Root borer	Below 15%	15.1-30%	Above 30%
2.	Top borer	Below 15%	15.1-30%	Above 30%
3.	Stalk borer	Below 15%	15.1-30%	Above 30%

Inference: 'CoP 112' ('CoX 03178') recorded lowest incidence of shoot/root borers, top borers and stalk borers.

also wish to acknowledge the help rendered by the Director, Sugarcane Breeding Institute for hybridization programme and scientists of NHG, SBI, Coimbatore for proper fluff supply. The help and support in the development of this variety by all the scientists of S.R.I., Pusa are also duly acknowledged.

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Indian Journal of Sugarcane Technology **32(02)**: 68-78, December 2017

Biometric markers for nitrogen use efficiency (NUE) *vis-a-vis* productivity and quality of mid-late maturing sugarcane genotypes grown with and without organics under Indian sub-tropics

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ABSTRACT

Field experiments were conducted for three consecutive years (two plant-ratoon cycles) during 2008-09 to 2011-12, starting from February, 2008 at the Research Farm of ICAR-Indian Institute of Sugarcane Research, Lucknow. The soil of the experimental site is categorized in order *inceptisol* under the group *Udic Ustochrepts*, neutral in reaction (pH 7.4), low in organic carbon (0.34%) and available N (158.5 kg/ha), medium in available P (16.6 kg/ha) and K (265.9 kg/ha). The treatments consisted of eight mid-late maturing sugarcane genotyes *viz*., 'CoJ 20193', 'CoS 99259', 'CoS 96275', 'CoPant 99214', 'CoH 110', 'CoH 119', 'CoLk 9616' and 'CoJ 99192'. The genotypes were planted in furrows at 75 cm row spacing during spring season (in the month of February) along with four nitrogen levels *viz*., control, 150 kg N ha^{-1}, Farm Yard Manure (FYM) @ 10 t ha^{-1} and 150 kg N ha^{-1} + FYM 10 t ha^{-1}. The genotypes were allocated to main plots and nitrogen levels to sub-plots in split plot design replicated thrice. The nitrogen use efficiency (NUE) of CoH 110 was found to be the highest (304.7 kg cane/kg N), followed by 'CoLk 9616' (267.7 kg cane/kg N) and 'CoJ 99192' (207.7 kg cane/kg N) at 150 kg N/ha. Genotype 'CoH 110' also accounted for the highest N uptake (134.8 kg N/ha) at 150 kg N + 10 t FYM/ha indicating that NUE was enhanced by inclusion of organics in fertilizer schedule. The N recovery was also observed to be the highest (52.5%) for the genotype 'CoH 110'. The wider root spread (29.2 cm), feeding zone (3.18m3/stool), high root volume (78.0 cc) longer length of roots (34.5 cm) and number of root hairs (1942.6/cm root length/clump) were recorded for the genotype 'CoH 110', which was followed by 'CoLk 9616' and 'CoJ 99192'. The root parameters were directly influenced by application of FYM @ 10 t/ha along with 150 kg N/ha. The biometric markers identified for higher NUE showed positive responses and measured strong relations (R^2 values found near to 0.9). The NUE is directly correlated with the number tiller *vis-à-vis* number of millable canes. Root volume of the genotypes and root hairs showed the strong correlation (R^2=0.749, 0.864 respectively) with nitrogen use efficiency. The genotypes possessing biometric markers such as high tillering (R^2= 0.85) with high root volume (R^2=0.82), more root hairs/cm/clump (R^2=0.89) and broader feeding zone (R^2=0.69) can be tagged for higher nitrogen use efficiency.

Key words: Apparent Recovery, Biometric Markers, Mid-late Genotypes, Millable canes, NUE, N-uptake, Sub-tropics

Sugarcane is an important agro-industrial crop of India. The area under sugarcane during 2015-16 soared to 4.95 million hectares producing 351.9 million tonnes of cane with an average productivity of 71.1 tonnes/hectare (NFCSF 2017). Competing sinks of vegetative growth fibre and stored sucrose in sugarcane undergo complex physiological regulations that largely depend on crop nutrition. Sugarcane is a high biomass-producing crop that requires substantial quantities of nitrogen from soil (Singh and Yadav 1992; Peter *et al*. 2005). The primary function of nitrogen in sugarcane is to increase the photosynthetic apparatus like tiller formation, leaf development and leaf expansion. It increases the leaf surface area and functional duration of leaves. Indian soils are mostly deficient in nitrogen hence the application rates are much higher. The yield potential of different genotypes varies with their inbuilt characters. Consequently the uptake of nitrogen by different genotypes also varies.

Low fertilizer N recovery has been reported from many sugarcane areas (Lal and Singh 1998 and 2002; Hartemink 2008). Efficient use of fertilizer N is, therefore, critical (Uribelarrea *et al*. 2007). All these point out to greater opportunity for using more balanced fertilizers for enhancing cane yield, improving produce quality and maintaining system sustainability.

The 'Soil-Cane-Sugar' system operates in an interlinked manner under two biological sub-systems *viz.*, 'Soil-Cane' and 'Cane-Sugar' which determine the efficacy of 'Produce to Product Chain'. Therefore, the production of sugar in terms of 'sugar bags' in factories depends upon the quantity and quality of sugarcane produced in the fields. The statement 'sugar is manufactured in the field and not in the factory' or 'sugar is synthesized in the field and recovered in the factory' clearly brings out this fact. As such, the fertilizers account for lion's share among the external production inputs.

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Productivity and quality of different sugarcane varieties are largely dependent upon the quantity and quality of millable canes. Studies have recorded a direct contribution of 40% of the number of millable canes to the agronomic yield of sugarcane crop followed by the weight (30%), length (27%) and girth (3%) of stalk (Yadav and Sharma 1978). Contribution of these yield attributing characters are mainly the function of nutrients. Varying N use efficiency coupled with the various biometrical characters of different genotypes necessitated to identify key markers which are responsible for high nitrogen recovery, so, that the fertilizer N can be efficiently utilized. Considering these points in view, present experiment was undertaken to find out suitable biometric markers responsible for high nitrogen use efficiency of different mid-late maturing sugarcane genotypes.

MATERIALS AND METHODS

Experimental site

Field experiment was conducted for three consecutive years (two plant-ratoon cycles) during 2008-09 to 2011-12, starting from February, 2008 at the Research Farm of ICAR-Indian Institute of Sugarcane Research, Lucknow located at 26°50' N latitude, 80°52' E longitude and 111 m above mean sea level in Uttar Pradesh state falling in subtropical belt of sugarcane cultivation. The soil of experimental field is categorized in order *inceptisol* under the group *Udic Ustochrepts*, neutral in reaction (pH 7.4), low in organic carbon (0.34%) and available N (158.5 kg/ha), medium in available P (16.6 kg/ha) and K (265.9 kg/ha). The soil texture was sandy loam (15.2 % clay, 21.4 % silt and 63.4 % sand) of Gangetic alluvial origin. The depth of the soil is about 2.6 metres, well drained and well levelled (slope is about 1%). The climate of the location (Lucknow) is semi-arid subtropical with dry hot summers (April to June) and cold winters (November to January). The average annual rainfall is 987 mm and nearly 85% of the total rainfall is received through south-west monsoon from second fortnight of June to mid September. The average monthly minimum and maximum temperatures fluctuate from 6.8 to 7.9 and 20.4 to 22.8°C in winter and from 22.3 to 25.5 and 39.8 to 41.7°C in summer, respectively.

Treatments and their execution

The experimental treatments consisted of eight mid-late maturing genotypes *viz*. 'CoJ 20193', 'CoS 99259', 'CoS 96275', 'CoPant 99214', 'CoH 110', 'CoH 119', 'CoLk 9616' and 'CoJ 99192' in main plots and four nitrogen levels *viz.*, control, 150 kg Nha^{-1}, Farm Yard Manure (FYM) @ 10t ha^{-1} and 150 kg N ha^{-1} + FYM 10t ha^{-1} in sub-plots under split plot design replicated thrice. The field was prepared by tilling with cultivator and harrows twice each after pre-planting irrigation followed by running of the wooden plank to conserve soil moisture.

The genotypes were planted in 10 cm deep furrows at 75 cm spacing during spring season on 6th and 8th February in the years 2008 and 2009 respectively. About 47000 three bud cane setts ha^{-1} (7 t ha^{-1}) were placed horizontally end to end in the furrows. The fertilizer was placed in the furrows below the setts. Nitrogen was applied as per the treatments through urea (46.6% N). The recommended doses of P and K were 60 kg P_2O_5 and K_2O ha^{-1} each. The sources of P and K were Diammonium Phosphate-DAP (18% N and 46% P_2O_5) and Muriate of Potash (60% K_2O). Full amount of P and K fertilizers and 1/3rd N were applied as basal. Remaining amount of the nitrogen was applied in two equal splits at initial (60days after planting) and final stages (120 days after planting) of tillering.

The crop was grown under assured irrigation supply. Six pre-monsoon irrigations were given in addition to pre-planting irrigation. One post-monsoon irrigation in the month of September in first year and two irrigations during September and October in the second year were given. The harvesting of crop was done manually during third week of January in both the years with the help of spade followed by de-trashing and de-topping using sickle.

Soil and plant sampling and Analysis

Initial soil samples were collected before commencement of the experiment in February 2008 for 1st crop cycle and 2009 for 2nd crop cycle. For chemical properties, samples from 0-20 cm profile depth were taken and analyzed for organic carbon (Walkley and Black'S rapid titration method), available N (alkaline $KMNO_4$ method), available P (0.5 M $NaHCO_3$, pH 8.5 extractable) as described by Olsen and Sommers (1982) and extractable K using NH_4OAC (1:6 soil: solution) following Page *et al.* (1982).

Three healthy clumps (stools) per treatment were selected for root studies. Each stool was dugout carefully up to 60 cm depth making all efforts to minimize loss of roots. The entire stool was then suspended in a water tank to wash-off the clinging soil. After washing, horizontal and vertical spread of roots was measured from base. Thereafter, the root mass was separated from the stalk and the fresh weight of the roots was recorded. The measurement of root spread (vertical/horizontal) led to derivation of a cone shaped 'feeding zone' and was calculated by the volume of a cone represented as

$$\text{Feeding zone} = 1/3\pi h^2V \quad \text{(i)}$$

(where h = one way (1/2 of the diameter) horizontal spread from the core/stalk base to the tip of longest lateral root and V is the vertical spread)

'Root intensity' which encompasses vertical and horizontal spread of the roots and the roots mass was calculated on fresh weight basis as :

$$\text{Root intensity} = \frac{\text{Root mass}}{\text{Feeding zone}} \quad \text{(ii)}$$

The ratio of above ground plant weight to the weight of below ground plant part (i.e. root mass) was taken as measure of shoot: root ratio and also termed as 'root efficiency' computed as:

$$\text{Root efficiency} = \frac{\text{Above ground plant fresh weight}}{\text{Below ground plant fresh weight}} \quad \text{(iii)}$$

Five millable canes (ripen canes ready to send to sugar mills) were randomly sampled for observations on yield attributes (length, girth and average cane weight) and juice quality parameters (^{0}brix, pol and purity). Juice purity and commercial cane sugar were calculated by the formulae as described by Gupta (1977):

Juice purity (%) = (Sucrose (%) in juice/corrected brix) x 100 (iv)

CCS (%) = {S-(B-5) x 0.4} x 0.73 (v)

Where S is sucrose % in juice, and B is corrected Brix (%), determined as per the method of Meade and Chen (1977)

The apparent N recovery and Nitrogen Use Efficiency have been envisioned by Yadav *et al.* (1997):

$$\text{Apparent N recovery, } AR_n = \frac{N_t - N_c}{Na} \quad \text{(vi)}$$

$$\text{Nitrogen Use Efficiency, NUE} = \frac{y_n - y_c}{Na} \quad \text{(vii)}$$

Where :

N_t = N uptake in treated plots

N_c = N uptake in control plot

y_n = cane yield kg ha^{-1} in treated plot

y_c = cane yield kg ha^{-1} in control plot

Na = applied N, kg ha^{-1}

Statistical analysis and calculation

Computing the ratio of the mean square concerned to the error mean square did the comparison of the treatments. The data were statistically analyzed for various characters as described by Panse and Sukhatme (1985). The standard error of mean is determined by dividing standard error by number of observations entered into the calculation of the mean. The standard error of difference multiplied further by √2 and t value (at 5% level of significance) at error degree of freedom gives the value of CD for statistical interpretation.

RESULT

The nitrogen use efficiency (NUE) of 'CoH 110' was found to be the highest among mid-late maturing genotypes (304.7 kg cane/kg N), followed by 'CoLk 9616' (267.7 kg cane/kg N) and 'CoJ 99192' (207.7 kg cane/kg N) at 150 kg N/ha. The nitrogen uptake by different genotypes varied according to their yield potential. 'CoH 110' accounted for the highest N uptake (95.5 kg N/ha) at 150 kg N + 10 t FYM/ha (Table 1). Uptake of nitrogen was significantly increased at 150 kg N and 150 kg N + 10 t FYM/ha application as compared to control. The N recovery was also the highest (52.5%) with the genotype 'CoH 110'. The wider root spread (29.2 cm), feeding zone (3.18 m^3/stool), high root volume (78.0 cc) longer length of roots (Fig. 1a & b), and number of root hairs (1942.6/cm root length/clump) were recorded for the genotype 'CoH 110', which was followed by 'CoLk 9616' and 'CoJ 99192'. The root parameters were directly influenced by application of FYM @ 10 t/ha along with 150 kg N/ha.

The highest percentage of germination was observed by 'CoLk 9616' (42.4%). The lowest germinating ability was recorded under 'CoJ 99192' (Table 2). The effect of application of nitrogen and its fortification by FYM was not found significant for enhancing germination. However the tillering behavior of the genotypes showed significant variation at

Table 1 Nitrogen uptake, use efficiency, apparent recovery and root characters of different sugarcane genotypes

Genotypes	*N uptake (Kg/ha)	*NUE (Kg cane /Kg N)	*Apparent N recovery (%)	Root spread (cm)	Feeding zone (m^3/stool)	Root Intensity (g/m^3)	Root volume (cc)	Root length (cm)	Number of root hairs/cm root length/clump
'CoJ 20193'	76.00	146.67	21.08	24.3	1.72	180.54	45.87	26.92	758.93
'CoS 99259'	64.75	138.67	26.04	22.3	1.46	189.17	43.80	27.22	524.89
'CoS 96275'	75.77	217.33	37.41	23.3	2.82	128.47	57.76	53.31	1332.75
'CoPant 99214'	55.45	197.33	19.27	20.8	1.40	177.67	41.98	29.70	750.94
'CoH 110'	95.49	304.67	52.45	29.2	3.18	114.34	78.03	34.48	1942.62
'CoH 119'	83.39	170.33	29.48	21.7	1.52	226.42	43.79	29.88	738.06
'CoLk 9616'	94.39	267.67	44.15	25.8	2.54	119.54	65.57	35.23	1491.17
'CoJ 99192'	96.06	207.67	40.49	25.5	2.17	136.91	45.81	31.05	1308.54
CD (P=0.05)								6.78	354.6
N levels									
0- Control				20.8	1.80	202.94	42.33	38.20	581.76
150 kg N /ha				25.4	2.13	145.93	55.64	30.57	1208.33
10 t FYM				22.3	1.64	177.52	51.56	30.82	1133.93
150kg N +10t FYM				27.8	2.84	110.14	61.77	34.31	1499.92
CD (P=0.05)								3.26	286.9

*****observed at 10 t FYM/ha + 150Kg N/ha

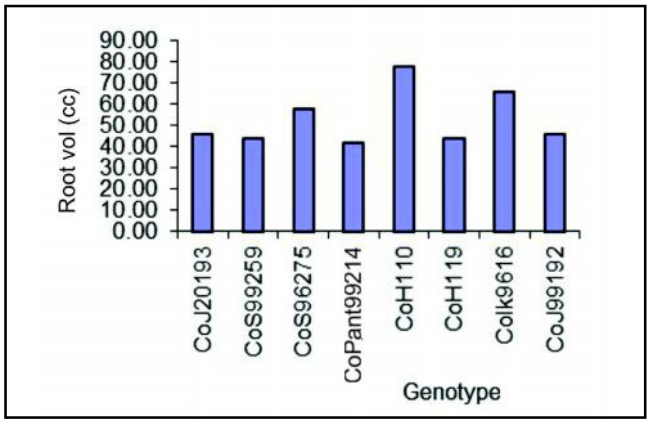

Fig.1a: Root volume of genotypes

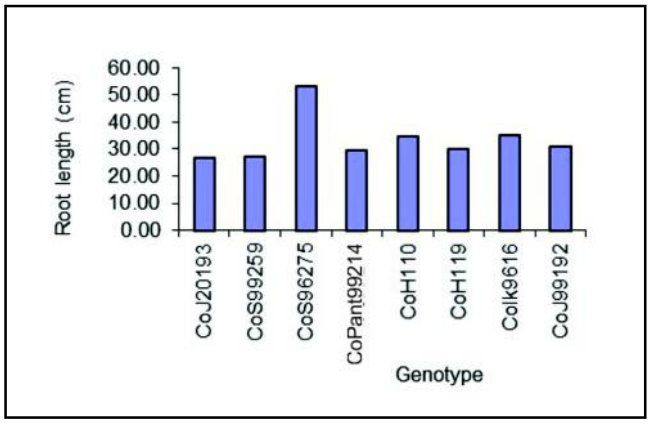

Fig.1b: Root length of genotypes

different period of growth starting from May to August. At initial stage (May) highest number of tillers were recorded by the genotype 'CoLk 9616' (131.86 thousand/ha).

At grand growth phase which lies in the month of August, the highest number of tillers (246.35 thousand/ha) were recorded by genotype 'CoH 110'. Application of FYM along with 150 kg N/ha significantly increased the tiller population. The percent increase in the number of tillers due to nitrogen and FYM was found maximum with the genotype 'CoS 99259' (29.9%), however, the increase in 'CoH 110' was not found significant.

The number of millable canes (NMC) was significantly highest in genotype 'CoH 110' (128.5 thousand/ha) which was comparable to 'CoLk 9616'. Genotypes 'CoJ 20193' and 'CoS 99259', were found at par in yield with each other. Significantly highest yield (61.8 t/ha) was observed with 'CoH 110' which was, however, practically equal to 'CoS 9616'. The yielding capacity of 'CoH 110' was significantly improved by using 150 kg N with FYM. Among the quality parameters, pol %, purity % and CCS % were found to be significantly higher in 'CoH 119' and was comparable to the genotypes 'CoJ 20193', 'CoS 99259', 'CoS 96275'. Although application of nitrogen was not found significant in increasing the quality parameters, however, the total sugar yield was significantly increased by application of 150 kg N with FYM.

The dry matter partitioning of the genotypes at maturity indicated clear cut variations (Table 3). The highest stalk dry matter (60.61%) was produced by genotype 'CoLk 9616'

Table 2 Growth, yield and quality of different sugarcane genotypes and effect of N levels

Treatment	Germination (%)	Number of tillers (000/ha)				NMC (000/ha)	Yield (t/ha)	°Brix	Pol (%)	Purity (%)	CCS (%)	CCS (t/ha)
Genotype		May	June	July	Aug							
'CoJ 20193'	33.40	103.38	136.55	202.55	197.48	100.01	48.50	20.40	18.02	88.33	12.45	6.05
'CoS 99259'	30.40	94.61	126.38	183.90	177.25	93.04	43.53	20.86	18.01	86.41	12.31	5.36
'CoS 96275'	34.59	104.84	158.88	232.45	222.73	104.08	58.86	20.14	18.12	90.00	12.64	7.44
'CoPant 99214'	31.33	108.03	149.95	230.75	218.45	98.93	53.08	19.58	17.04	87.04	11.70	6.20
'CoH 110'	36.18	128.76	172.20	261.70	246.35	128.51	61.80	20.59	17.96	87.30	12.34	7.64
'CoH 119'	30.33	105.06	145.30	235.95	220.25	99.93	54.61	21.10	18.13	86.10	12.37	6.74
'CoLk 9616'	42.44	131.86	174.40	268.23	232.33	125.49	61.31	19.89	16.74	84.09	11.30	6.88
'CoJ 99192'	28.15	108.69	144.95	236.78	219.38	101.50	54.93	19.48	16.97	87.12	11.66	6.34
C D (P=0.05)	4.36	8.68	11.63	18.89	14.55	10.76	7.86	NS	0.78	2.65	0.84	1.16
N levels												
0- Control	33.39	92.64	123.16	205.98	197.39	86.19	36.53	20.25	17.68	87.33	12.16	4.44
150 kg N /ha	33.29	117.38	168.55	245.05	222.09	112.84	63.21	20.01	17.42	87.08	11.96	7.54
10 t FYM	33.01	100.49	135.15	213.19	202.94	97.84	51.10	20.41	17.77	87.11	12.20	6.22
150kg N +10t FYM	33.71	132.09	177.44	261.94	244.69	128.86	67.47	20.35	17.62	86.68	12.07	8.13
CD (P=0.05)	NS	5.26	9.69	14.37	8.39	6.83	5.13	NS	NS	NS	NS	2.77

Table 3 Dry matter production of sugarcane genotypes and effect of N levels at harvest

Treatment	Dry matter production (t/ha)					Dry matter partitioning (%)				
Genotype	Root	Green leaf	Dry leaf	Stalk	AGP	Root	Green leaf	Dry leaf	Stalk	AGP
'CoJ 20193'	0.74	3.80	3.89	9.31	17.00	4.16	21.41	21.95	52.49	95.84
'CoS 99259'	0.80	3.58	3.64	8.83	16.05	4.77	21.22	21.62	52.39	95.23
'CoS 96275'	0.93	4.66	4.34	11.73	20.74	4.30	21.52	20.02	54.16	95.70
'CoPant 99214'	0.74	4.45	4.25	10.51	19.21	3.73	22.30	21.31	52.66	96.27
'CoH 110'	1.35	4.48	4.23	14.80	23.51	5.44	18.02	17.01	59.53	94.56
'CoH 119'	0.81	4.39	4.04	10.89	19.33	4.00	21.82	20.07	54.11	96.00
'CoLk 9616'	0.88	3.99	4.20	13.96	22.15	3.84	17.31	18.24	60.61	96.16
'CoJ 99192'	0.75	4.09	4.16	11.72	19.97	3.62	19.72	20.09	56.57	96.38
C D (P=0.05)	0.46	0.78	0.27	2.48	2.78	-	-	-	-	-
N levels										
0- Control	0.81	3.00	2.71	7.54	13.25	5.76	21.33	19.30	53.61	94.24
150 kg N /ha	0.83	4.55	4.40	12.69	21.64	3.69	20.26	19.56	56.49	96.31
10 t FYM	0.87	4.33	3.97	10.99	19.29	4.29	21.47	19.71	54.52	95.71
150kg N +10t FYM	1.00	4.84	5.30	14.65	24.79	3.88	18.76	20.54	56.83	96.12
CD (P=0.05)	0.14	0.86	1.25	1.95	3.16	-	-	-	-	-

which was very close to 'CoH 110'. The percentage dry matter produced by genotype 'CoLk 9616' was 17.31% (green leaf), 18.24% (dry leaf). The fraction of dry matter produced by the stalk of sugarcane genotypes was lower with control and FYM application without inorganic N application. NUE of Mid-late genotypes showed positive correlation with tiller (R^2=0.80) and millable cane (R^2=0.87) population (Fig. 2a, b, c and d), number of root hairs (R^2=0.89) and root volume (R^2=0.82) (Fig 3 a-n) and above ground part dry matter (AGPDM) and yield (Fig. 4 a & b and 5a & b) with and without organics. The shoot: root ratio (Fig 6a & b) did not show any relation with NUE.

The rate of photosynthesis was found almost static during tillering to grand growth phase for all the genotypes (Table 4). However, highest photosynthetic rate (28.16 µ mole/m²/S) for the genotype 'CoH 110' was recorded during highest tillering stage (July). Significant increase in photosynthesis was observed by application of 150 kg N along with FYM. The

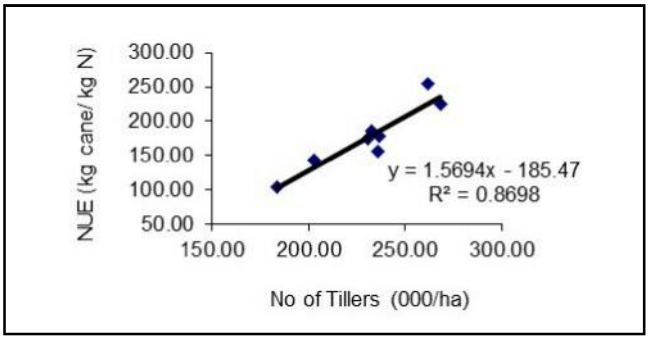

Fig. 2a: Tiller *vs* NUE (150 kg N/ha)

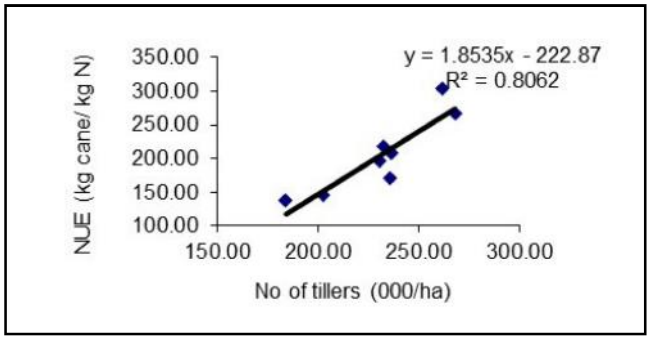

Fig. 2b: Tiller *vs* NUE (10 t FYM+150 kg N /ha)

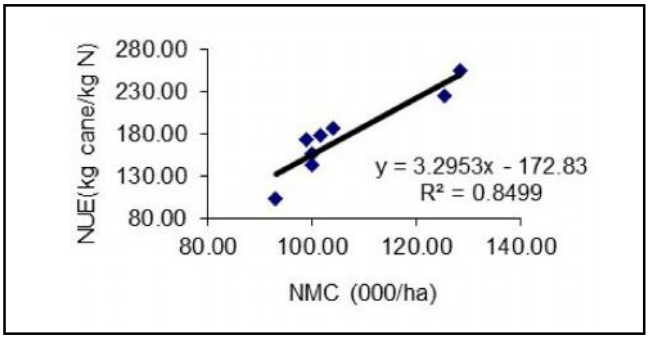

Fig. 2c: NMC *vs* NUE (150 kg N/ha)

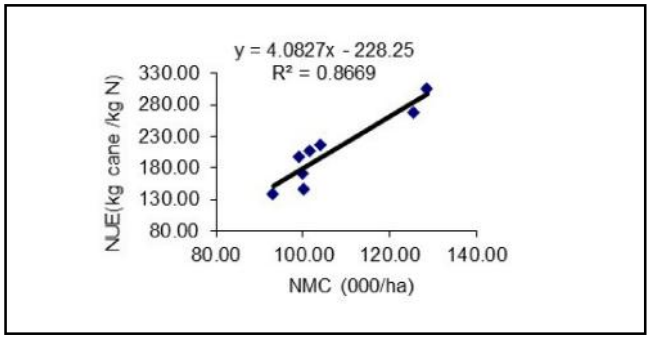

Fig. 2d: NMC *vs* NUE (10 t FYM+150 kg N /ha)

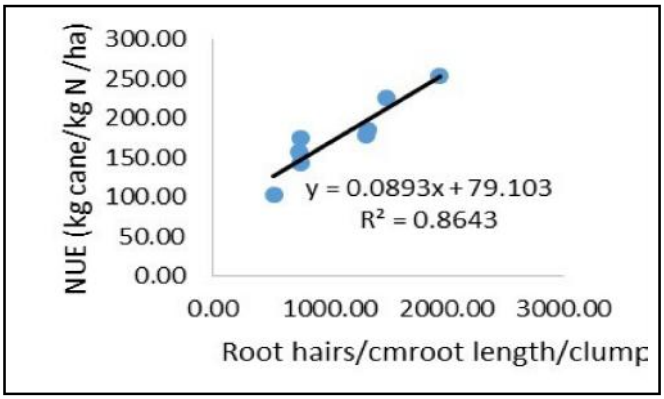

Fig. 3a: Root hairs *vs* NUE (150 kg N/ha)

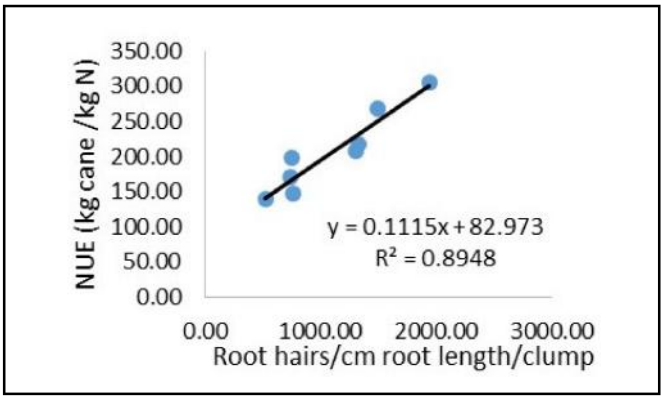

Fig. 3b: Root hairs *vs* NUE (10 t FYM+150 kg N /ha)

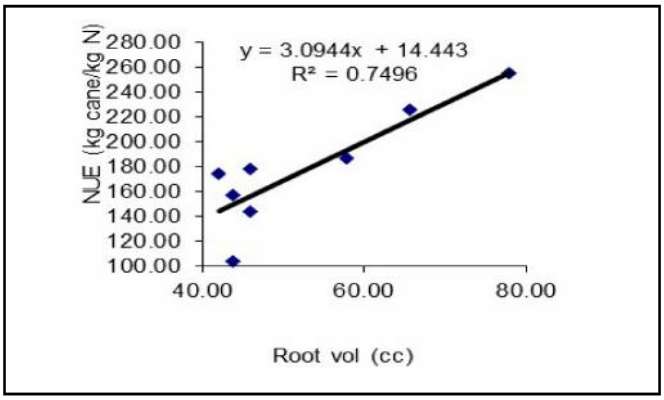

Fig.3c: Root volume *vs.* NUE (150 kg N/ha)

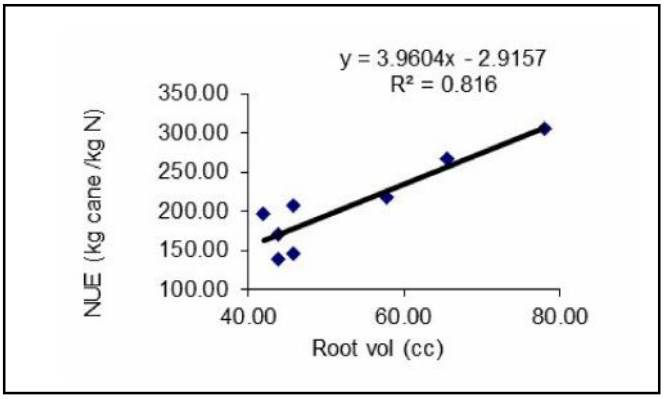

Fig.3d: Root volume *vs.* NUE (10 t FYM+150 kg N /ha)

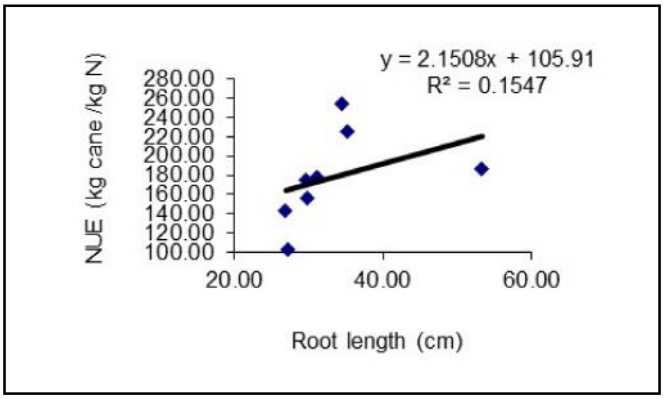

Fig.3e: Root length *vs.* NUE (150 kg N/ha)

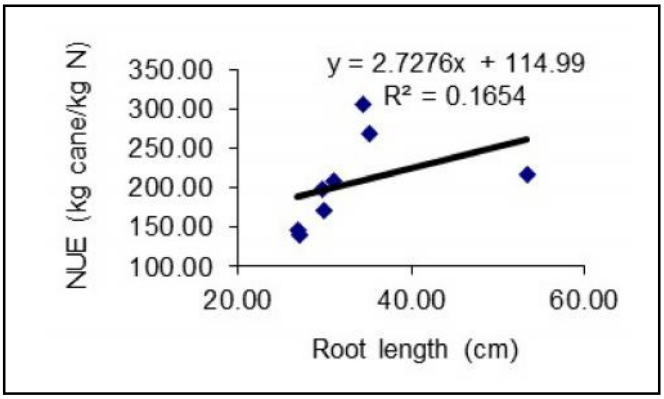

Fig.3f: Root length *vs.* NUE (10 t FYM+150 kg N /ha)

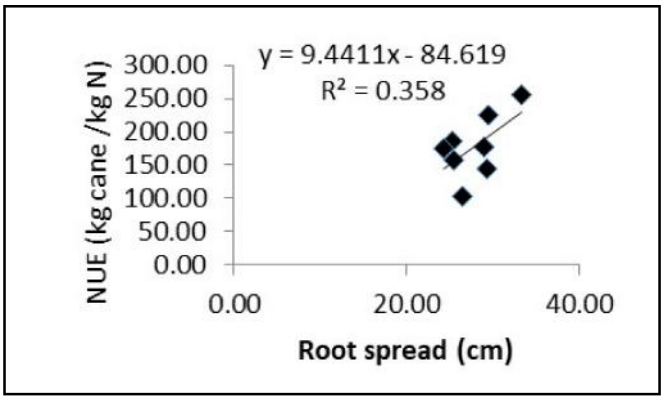

Fig.3g: Root spread *vs.* NUE (150 kg N/ha)

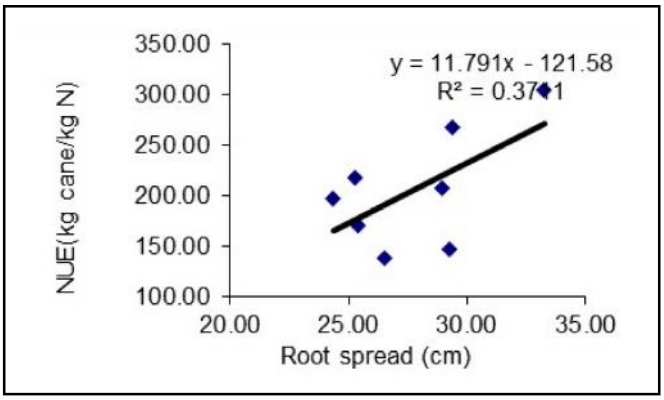

Fig.3h: Root spread *vs.* NUE (10 t FYM+150 kg N /ha)

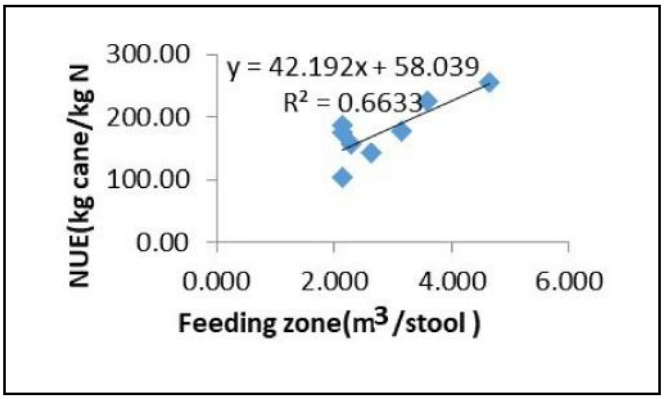

Fig.3i: Root feeding zones. NUE (150 kg N/ha)

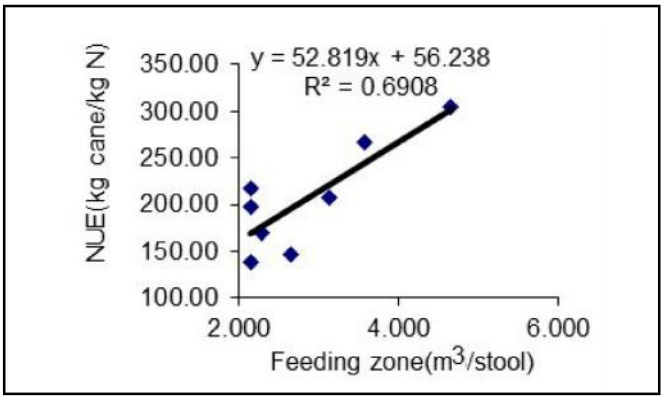

Fig.3j: Root zone *vs.* NUE (10 t FYM+150 kg N /ha)

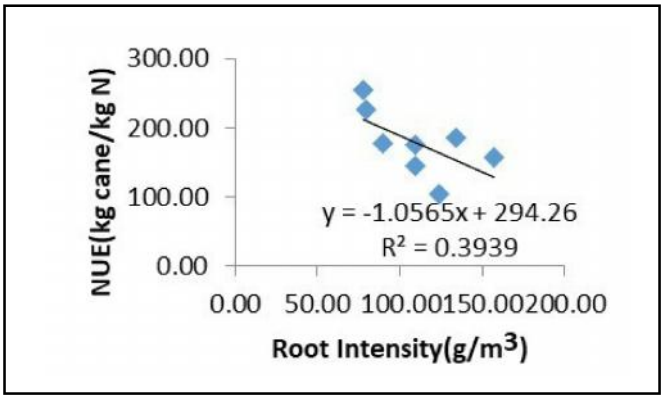

Fig. 3k: Root intensity *vs.* NUE (150 kg N/ha)

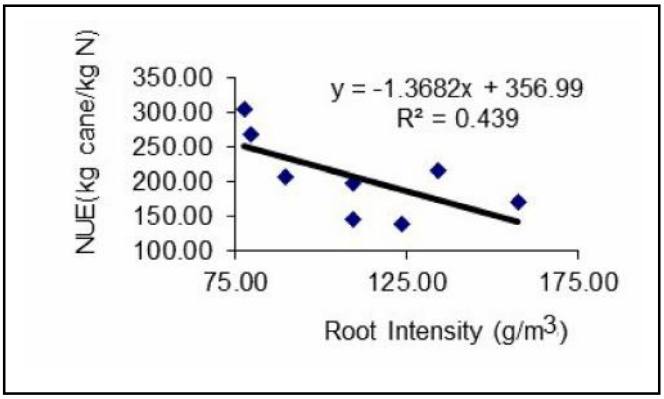

Fig. 3l: Root intensity *vs.* NUE (10 t FYM+150 kg N /ha)

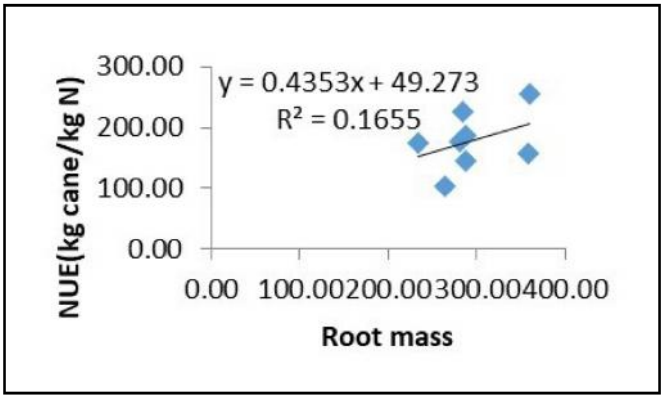

Fig. 3m: Root mass *vs.* NUE (150 kg N/ha)

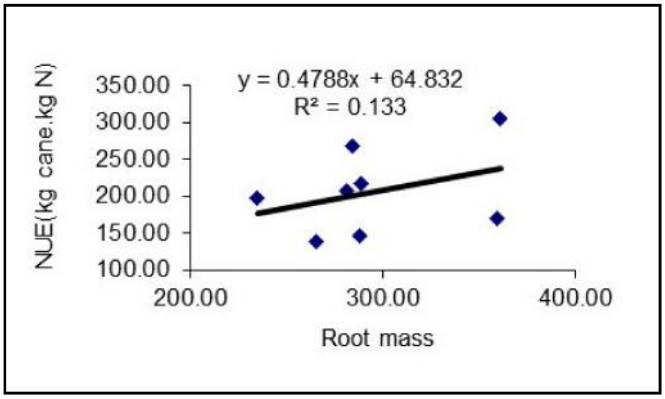

Fig. 3n: Root mass *vs.* NUE (10 t FYM+150 kg N /ha)

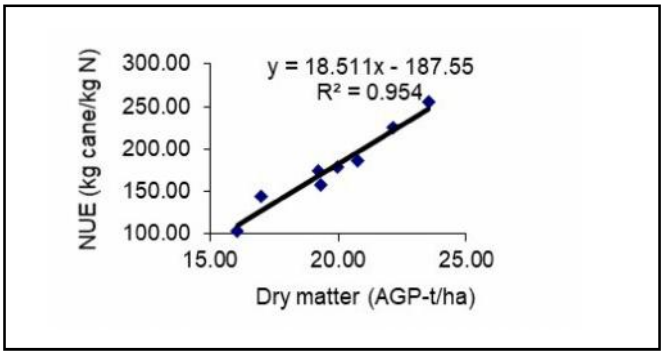

Fig.4a: Dry matter *vs* NUE (150 kg N/ha)

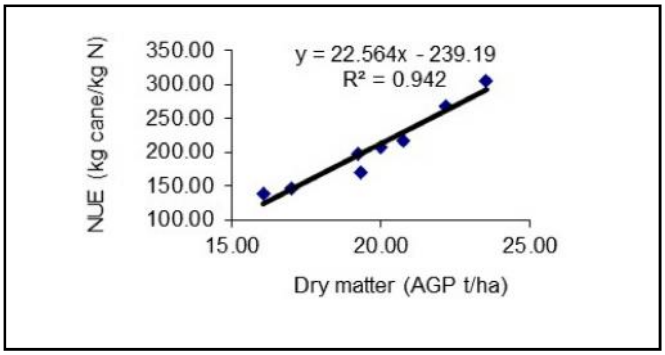

Fig.4b: Dry matter *vs* NUE (10 t FYM+150 kg N /ha)

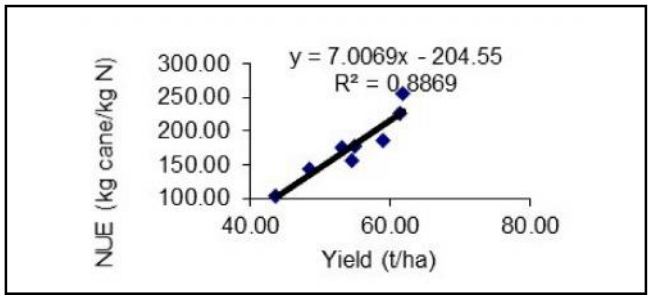

Fig.5a: Yield *vs* NUE (150 kg N/ha)

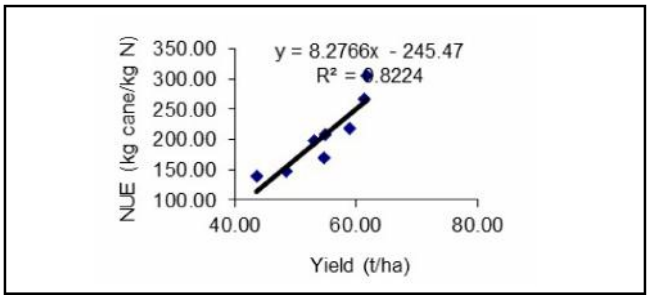

Fig.5b: Yield *vs* NUE (10 t FYM+150 kg N /ha)

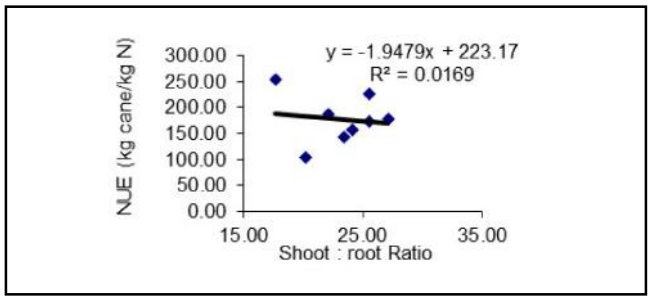

Fig.6a: S: R *vs* NUE (150 kg N/ha)

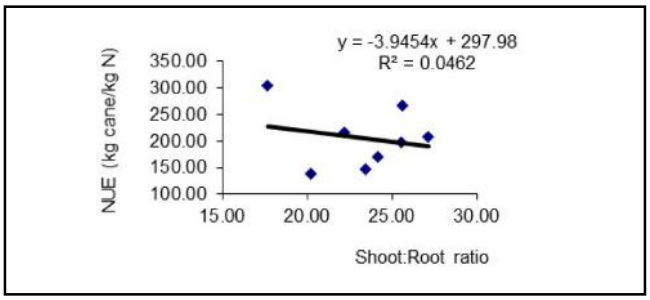

Fig.6b: S: R *vs* NUE (10 t FYM+150 kg N /ha)

Table 4 Photosynthetic rate and stomatal conductance of different genotypes and effect of N levels

Treatment	Photosynthetic rate(u mole/m^2/s)				Stomatal conductance (mill mole/m^2/s)			
Genotype	May	June	July	Aug	May	June	July	Aug
'CoJ 20193'	23.18	24.79	24.13	24.65	230.00	211.05	243.28	267.67
'CoS 99259'	22.69	22.60	23.36	24.21	221.91	208.40	243.65	265.45
'CoS 96275'	25.60	23.42	25.56	24.81	237.99	219.98	272.74	275.33
'CoPant 99214'	23.77	24.45	24.36	25.82	247.86	231.89	260.23	280.65
'CoH 110'	25.37	25.80	28.16	27.50	278.72	247.00	317.86	307.37
'CoH 119'	21.47	24.17	24.43	24.50	204.98	204.53	265.18	273.97
'CoLk 9616'	25.74	25.70	27.88	27.61	272.80	224.79	328.25	312.10
'CoJ 99192'	22.40	23.47	24.35	24.48	229.00	204.59	268.93	269.80
C D (P=0.05)	NS	1.47	2.46	NS	10.81	8.69	22.64	11.76
N levels								
0- Control	21.26	21.35	23.29	23.03	212.37	195.23	254.90	263.69
150 kg N /ha	25.19	25.63	26.11	26.21	260.06	234.38	289.48	293.83
10 t FYM	22.13	22.72	24.04	24.37	218.20	200.71	257.50	266.08
150kg N +10t FYM	26.52	27.50	27.67	28.18	270.99	245.80	298.18	302.57
CD (P=0.05)	3.65	3.23	2.87	2.31	8.63	7.34	18.76	8.34

observations on stomatal conductance, transpiration rate and Leaf Area Index also showed the similar trend. Increase in physiological parameters was positively correlated with increase in NUE at different levels of N nutrition with varying R^2 values of 0.21 to 0.89 (Fig. 7a, b, c, d, e and f).

DISCUSSION

The results of the study showed very large genetic variation for NUE, germination, tillering pattern, dry matter partitioning, root characteristics and crop physiology. Photosynthesis, growth and yield are strongly linked to N availability in grass crops (Ranjith and Meinzer 1997). The increase in NUE of the genotypes under study due to application of FYM in the treatment was due to improvement in soil conditions (Singh *et al*. 2007). The number of root hairs in upper and lower portion of roots may also play an important role in increasing the NUE. However, the variation in germination percentage of the genotypes is only due to genotypic variability (Singh *et al*. 2002).

Tillers are the basis for optimizing the plant density and ultimately contributing to number of millable canes (NMCs). Higher tillering in the genotype 'CoLk 9616' is due to its high

Table 5 Transpiration rate and LAI of different sugarcane genotypes and effect of N levels

Treatment	Transpiration rate (milli moles/m^2/s)				Leaf Area Index			
Genotype	May	June	July	Aug	May	June	July	Aug
'CoJ 20193'	3.29	2.82	2.38	1.77	1.97	2.82	4.30	5.44
'CoS 99259'	2.99	2.67	2.06	1.71	2.10	2.64	3.90	3.88
'CoS 96275'	3.30	2.77	2.50	1.82	2.31	2.88	4.50	5.87
'CoPant 99214'	3.23	2.79	2.61	1.78	2.43	2.75	4.04	5.00
'CoH 110'	3.33	2.92	2.76	2.24	2.96	3.88	5.32	6.44
'CoH 119'	2.96	2.54	2.33	1.59	2.45	2.82	4.30	4.79
'CoLk 9616'	3.33	2.95	2.68	2.04	3.02	3.96	5.22	6.56
'CoJ 99192'	2.79	2.59	2.47	1.58	2.48	2.79	4.18	5.04
C D (P=0.05)	0.25	0.22	0.29	0.35	0.86	0.56	1.16	1.21
N levels								
0- Control	3.01	2.61	2.33	1.65	2.14	2.73	3.79	4.65
150 kg N /ha	3.20	2.83	2.56	1.86	2.69	3.22	4.88	5.90
10 t FYM	3.07	2.67	2.38	1.82	2.21	2.87	4.03	4.84
150kg N +10t FYM	3.32	2.92	2.62	1.93	2.82	3.43	5.17	6.11
CD (P=0.05)	0.22	NS	0.17	0.21	0.53	0.63	0.87	0.93

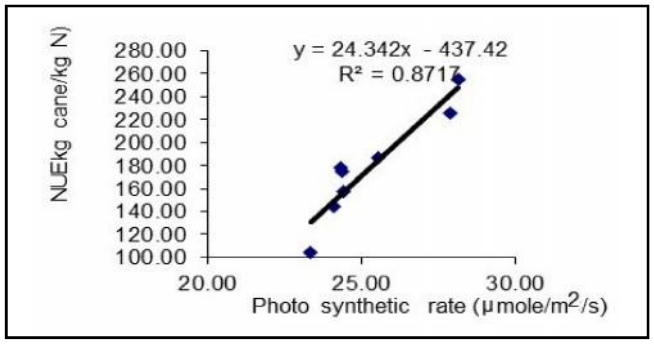

Fig.7a: Photosynthetic rate (PR) *vs* NUE (150 kg N/ha)

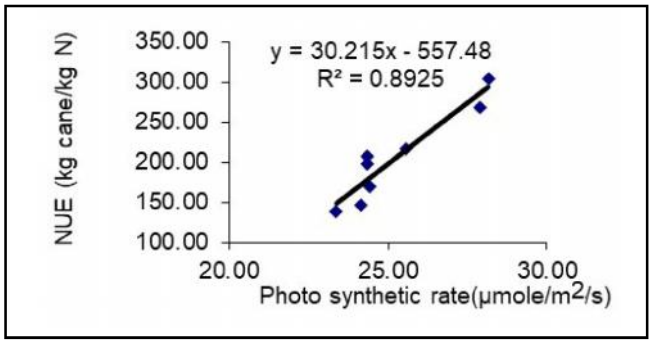

Fig.7b: PR *vs* NUE (10 t FYM+150 kg N /ha)

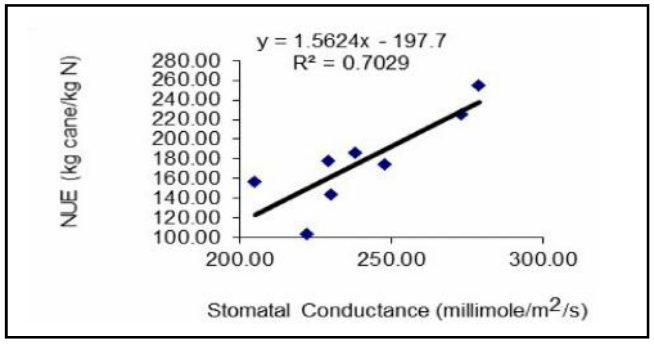

Fig.7c: Stomatal Conductance (SC) *vs* NUE (150 kg N/ha)

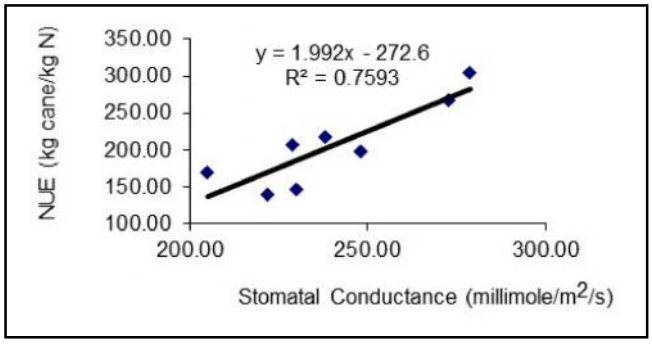

Fig.7d: S C vs NUE (10 t FYM+150 kg N /ha)

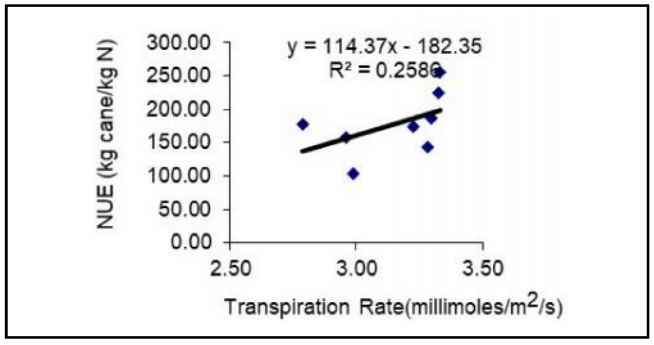

Fig.7e: Transpiration rate (TR) *vs* NUE (150 kg N/ha)

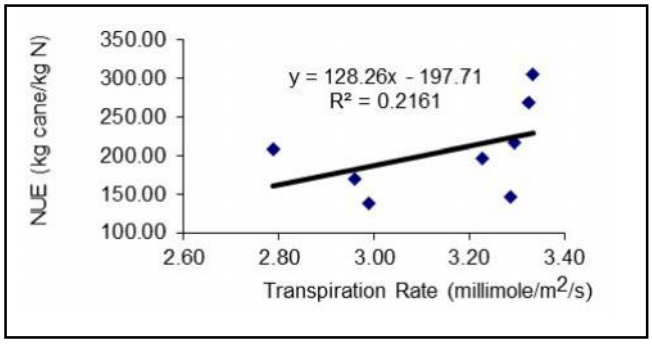

Fig.7f: TR NUE (10 t FYM+150 kg N /ha)

NUE capability which also enhanced the photosynthetic rate, stomatal conductance, transpiration ratio and leaf area index.

In grass crops like sugarcane, the yield is a function of tillering. Tillers in sugarcane are stalk or shoots arising from the base of the plant grouped under tufted grasses (Nickell 1984). In tufted grasses which include sugarcane, the under ground branching is limited and is followed by formation of a number of erect stalks (Shoots), which makes individual plants (Yadav 1993). So the higher number of tiller followed by higher NMC are responsible for targeted yield of the genotype 'CoLk 9616' and 'CoH 110'.

The quality parameters of the genotypes were again a part of varietal character. Sugar yield is a function of CCS% and cane yield. The higher sugar yield of the genotype 'CoH 110' and 'CoS 96275' were due to higher CCS% and cane yield. The increase in dry matter of the genotypes was due to higher nitrogen recovery. The increase in root length may be the result of high apparent N recovery. The root biomass is observed to be the function of plant genotype and N nutrition in the present study.

Sugarcane prefers N in NO_3 form and also takes the NH_4 form. The latter is subject to microbial attack that depletes NH_4-nitrogen. The response of sugarcane to applied N is almost universal and several attempts were made to express this relationship mathematically. The inverse – yield concept, Mitschlich equation, exponential function, square root and second degree polynomial equations were employed to predict N need of sugarcane (Hunsigi 1993). But the quadratic equation seems to predict the N need of cane more satisfactorily.

Yadav *et al*. (1997) demonstrated that the responses and N recovery declined sharply as the N dose increased from 75 to 300 kg/ha to sugarcane grown in subtropical region. The highest response and N recovery were obtained at lower level of N dose (75 kg/ha) and N recovery barely exceeds 30 to 40 %. After application, a part is used by plants, a part remains in the soil, and rest is depleted through gaseous loss and leaching. Applied nitrogen to soil whether cropped or uncropped may be lost through leaching, NH_3 volatilization, nitrification, denitrification, runoff, NH_4 fixation, biological immobilization including the uptake of nitrogen by plants, weeds and microbes.

The key results in this investigation were very large number of biometric characters that revealed variation for nitrogen use efficiency in mid late genotypes of sugarcane. Some biometric characters like tiller population, number of millable canes, above ground part dry matter (AGPDM) and photosynthetic rate were identified as highly responsive for nitrogen use efficiency. NUE measurement across the genotypes also demonstrated a high level of repeatability in relation to different biometric markers with and without organic manure application. The results collectively illustrate a high potential for varieties to alter the NUE. It is important that results obtained relating to impact of varietal biometric markers and N schedules on NUE are interpreted in terms of likely impact on Agronomic Efficiency before its application in breeding programmes. In estimating these impacts, it is important to consider two issues, (i) effect on NUE with fertilizer N application alone and (ii) effect of organic manure modulated biometric parameters on NUE. The nitrigen use efficiency variations among the genotypes also suggested by Elliosha *et al.* (2015). However, use of best available knowledge to guide breeding programme usually leads to enhance NUE in coming future, compared with adopting arbitrary experiences without any prior analysis.

Photosynthesis, growth and yield are strongly linked to N availability particularly in grass crops (Subasinghe and Meinzer 1997). N is required in large amount relative to other nutrient, maximizing photosynthesis and dry matter production. Nitrogen use efficiency based on photosynthesis or dry matter production is widely reported to be higher in C_4 plants (Brown 1978; Schmitt and Edwards 1981). The superior NUE in C_4 species is generally through of their CO_2 concentrating system (Sage *et al*. 1987). The physiological observations like photosynthetic rate, stomatal conductance and transpiration rate of different genotypes are the basis for variation in NUE under the study. Higher NUE of the some genotypes might be associated with maintenance higher plant hydraulic conductance and high water potential (Subasinghe and Meinzer 1997).

The biometric markers identified for higher NUE showed disparity in responses and measured strong relations ($R^2 = 0.7$ to 0.9) with mid late maturing genotypes except for root length, root intensity, shoot: root ratio and transpiration rate. Genotypic differences in nutrient absorption, content and use are known to exist widely in different crops (Batten 1992 and Fageria *et al.* 1988). Tolerance to mineral stress as a genetic trait is usually termed as "nutrient efficiency" (Batten 1992). A tolerant plant may have a lower nutrient requirement per unit time and/ or ability to extract more nutrient from the soil. Only these tolerant genotypes showed higher NUE in present study. Apart from genetic behaviour agro-technologies are also responsible for increasing the NUE (Meyer *et al*. 2007 and Sundara 2011).

CONCLUSION

Biometric markers were identified for nitrogen use efficiency (NUE), productivity and quality of mid-late maturing sugarcane genotypes grown with and without organic manure application under Indian sub-tropics. The genotypes possessing biometric markers such as high tillering with high root volume, more root hairs/cm/clump and broader feeding zone can be tagged for higher nitrogen use efficiency for mid-late maturing sugarcane genotypes. The physiological observations like photosynthetic rate, stomatal conductance and transpiration rate of different genotypes are the basis for variation in NUE under the study. Increase in physiological parameters positively correlated with increase in NUE at different levels of N nutrition.

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Indian Journal of Sugarcane Technology **32(02)**: 79-83, December 2017

Exploring various agro-wastes for mass multiplication and delivery of *Trichoderma harzianum* and its impact on growth enhancement in sugarcane

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ABSTRACT

Trichoderma sp. are cosmopolitan fungi with multifaceted potential as antagonists of various plant pathogens, as growth promoters, producers of hydrolytic enzymes etc. For field level adoption and use of any microbial bio-agent, identifying suitable substrates for their multiplication and delivery in context of the target crop is essential. In the present study, experiments were carried out to screen ten locally available agro-substrates/byproducts (sorghum grains, bagasse, molasses, fallen tree leaves, wheat bran, maize grain, groundnut shell, sugarcane trash, press mud and farmyard manure) for mass multiplication of *T. harzianum*. Out of different substrates tested, sorghum grains and bagasse were observed to be the best substrates for *T. harzianum* multiplication with cfu of 19.0 x 10^{12} spores/ g and 10.4 x 10^{12} spores/ g observed in sorghum grains and bagasse, respectively after 30 days of incubation. The viability of *T. harzianum* was assessed for a period of 6 months on both these substrates. It was observed that the population remained almost stable for first two months of storage after which a decline was observed on both substrates with cfu of 7.5 x 10^{6} recorded in sorghum grains at 150 days of storage. Field experiment was conducted in 2015-16 to evaluate impact of *T. harzianum* application on growth and yield of sugarcane transplanted using settlings raised in polybags and by spaced transplanting technique (STP). Sett treatment with *T. harzianum* spore suspension followed by soil application of *T. harzianum* through farmyard manure at the time of transplanting was found effective in significantly improving cane yield over untreated control in both STP and polybag raised settlings.

Key words: *Trichoderma*, Mass multiplication, Sorghum, STP, Sugarcane

Trichoderma species are ubiquitous fungi which occur both as free living fungi as well as in endophytic association with plants. In addition to their well-established potential as bio-control agents against several diseases, these fungi are also known for their ability to promote plant growth and vigour, solubilisation of micro and macro nutrients in soil, for production of hydrolytic enzymes like cellulase and their capability and competence to grow under adverse conditions (Harman *et al.* 2004; Carvajal *et al.* 2009; Pandya *et al.* 2011; Tripathi *et al.* 2013). These properties have made *Trichoderma* an omnipresent genus able to grow in wider habitats and at high population densities (Chet *et al.* 1997, Chaverri *et al.* 2011).

Sugarcane (*Saccharum* spp. hybrids) is an important cash crop of India, cultivated in almost 5 million hectare area. It is a long duration, vegetatively propagated crop in which after harvesting of main crop, ratoon crops are taken in successive years. In general, a decline in yield of ratoon crops is observed over successive years. Under conventional planting method (three bud setts) the requirement for seed cane is very high (6-8 t/ha). To counter this problem, various modified planting methods like spaced transplanting technique (STP), bud chip, cane node, polybag raised settlings etc. have been developed (Solomon *et al.* 2014). These planting methods reduce seed material requirement providing higher seed multiplication rate. However, high settling mortality after transplanting necessitates frequent gap filling, often resulting in yield loss. Moreover, sugarcane is a long duration, nutrient exhaustive and extracting crop because of which the soils of the Indo-Gangetic plains are becoming nutrient-deficient. In addition, the continuous use of chemical fertilizers often causes apparent deficiency in other micronutrients. In this scenario, application of a potent multifaceted microbe like *Trichoderma*, which has well documented growth promoting and nutrient solubilizing potential may facilitate successful establishment of the settlings in field and also improve cane growth and yield.

For field level adoption and use of any microbial bio-agent, identifying suitable substrates for their multiplication and delivery in context of the target crop is essential (Lumsden and Lewis 1989). Choosing a suitable substrate for mass production of biological control agents not only depends upon potential biomass production at the end of the process and maintaining viability of the bio-agent for longer durations on the substrate (Adekunle *et al.* 2001), but is also impacted by the cost and ease of availability of that substrate. While a number of *Trichoderma* formulations based on inert carriers are presently available commercially, multiplying *Trichoderma* spp. on easily biodegradable and locally available substrates with long shelf-life would be beneficial for field application as such substrates would also act as food source for the applied bio-agent at time of application and help in its establishment

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in the crop ecosystem (Thangavelu *et al.* 2004). Moreover, use of such locally available agro-substrates and by-products as substrate for mass production can be an effective method for the recycling of such residues and is also amenable for small scale production of bio-agents in the vicinity of the target crop itself. As such, the present study was undertaken to screen and identify suitable, low cost, locally available organic substrates for mass multiplication of *T. harzianum* and to further evaluate the efficacy of *T. harzianum* delivery through farmyard manure on sugarcane growth attributes under different planting methods.

MATERIALS AND METHODS

Mass multiplication of T. harzianum on different substrates

A strain of *Trichoderma harzianum* previously isolated from sugarcane field soils was used in the present study. Ten locally available agricultural substrates/ sugarcane residues were screened for mass multiplication of *T. harzianum*. The agro-substrates screened were (i) sorghum grains (ii) sugarcane bagasse (iii) groundnut shells (iv)fallen tree leaves (v) maize grains (vi)press mud (vii) wheat bran (viii) farmyard manure (ix) molasses (x) sugarcane trash. Fifty grams of each material was moistened with distilled water, filled in 500 ml conical flasks and sterilized by autoclaving at 121°C for 20 minutes on two consecutive days. Spore suspension of *T. harzianum* was prepared by flooding seven day old sporulating culture of *T. harzianum* in petri dishes with sterile water followed by filtration through muslin cloth. CFU of the suspension was estimated using a haemocytometer and final concentration of the suspension was adjusted to 10^6 spores/ ml. Each sterilized conical flask was inoculated with 10 ml of *T. harzianum* suspension.Initial moisture content of the flasks was maintained at 50%. The inoculated flasks were incubated at 28±1°C for 30 days with manual shaking on alternate days.The colonization of different substrates by *T. harzianum* after 30 days was estimated by dilution plate technique using *Trichoderma* specific medium (TSM) (Elad *et al.* 1981). Briefly, 1gram of colonized substrate was withdrawn from the flaks and suspended in 9 ml sterile water, shaken well and serial dilutions of 10^{-2} to 10^{-12} were prepared. One ml of each dilution was added to sterile petri plates and molten cooled TSM was poured in the plates with three replications for each dilution. The plates were incubated at 28±1°C for 7 days and the number of *Trichoderma* colonies appearing in each plate was recorded.

Shelf life studies in stored formulation

The two most promising substrates observed in the above study were selected and shelf life of *T. harzianum* on the two selected substrates was assessed upto a period of 180 days under storage at room temperature (15-35°C). *T. harzianum* colonized substrates prepared following the above mentioned protocol were selected for shelf life studies. The viability of *T. harzianum* spores on the two substrates was monitored periodically by counting colony forming units (CFUs) at time of initiation of the study (0 days) and after 30, 60, 90, 120, 150 and 180 days of storage estimated by serial dilution technique using *Trichoderma* specific medium (TSM) as described previously (Elad *et al.* 1981).

Field trial of Trichoderma harzianum under different planting methods

During 2015-16, a field trial was conducted to assess the impact of *T. harzianum* application on germination, growth and yield of sugarcane under spaced transplanting technique (STP) and poly bag raised seedlings. The experiment was conducted using variety 'CoLk 94184'. For raising settlings by STP method, single bud setts of variety 'CoLk 94184' were cut from cane just above the growth ring leaving 9-10 cm of the internode below the bud. These setts were then dipped in *T. harzianum* spore suspension (cfu 10^6 ml^{-1}) for 30 minutes and then dibbled vertically (600-800 setts/ m^2) in the nursery, followed by mulching with trash and pulverized soil. Most of the buds germinated within 3-4 weeks. At time of planting the settlings were carefully removed and the leaf laminae detopped prior to transplanting. For raising settlings in polybags, single bud setts were cut from cane, as described above, dipped in *T. harzianum* spore suspension and dibbled vertically in small polybags (@ 1 sett/ bag) filled with soil. For both STP and polybag raised settlings, control settlings were raised separately without dipping in *T. harzianum* spore suspension.

For field application of *T. harzianum*, *Trichoderma* multiplied culture (TMC) of *T. harzianum* was prepared. For preparing TMC, *T. harzianum* was first multiplied on sterilized sorghum grains for 30 days under laboratory conditions. Fully colonized sorghum grains were mixed with 20 kg of FYM@ 2kg grains/ 20 kg FYM, covered with polythene sheet and kept for 30 days to allow further colonization of FYM by *Trichoderma*. The FYM was watered periodically to maintain sufficient moisture (30%) for better growth of *Trichoderma*. After 30 days the colonized FYM is further mixed with 200 kg of FYM. This prepared TMC was then applied near the roots of settlings at time of transplanting (@ two gm/ settling). For each planting technique, control plots having no *Trichoderma* application were maintained. Data on germination, NMC, sucrose per cent and yield was recorded. Statistical analysis was performed using Statistical Package for Social Scientists (SPSS) software (version 10.0) at $P< 0.05$. Mean comparisons were performed using the least significant difference test (LSD).

RESULTS AND DISCUSSION

Evaluation of different substrates for mass multiplication of T. harzianum

To identify a suitable low cost, easily available substrate for mass multiplication of *Trichoderma*; 10 locally available substrates were evaluated in the present study for growth and colonization by *T. harzianum*. The results on population density (cfu g^{-1}) of *T. harzianum* on different substrates after

30 days incubation revealed considerable variation among the different substrates. Among the 10 substrates, highest population of *T. harzianum* was recorded on sorghum grains (59.6 x 10^5cfu g^{-1}) followed by bagasse (34.0 x 10^5cfu g^{-1}), maize grains (30.0 x 10^5cfu g^{-1}), wheat bran (28.0 x 10^5cfu g^{-1}) and sugarcane trash (25.3 x 10^5cfu g^{-1})(Fig. 1).The remaining five substrates did not support high colonization and multiplication of *Trichoderma* with < 20 x10^5cfu g^{-1} recorded in the remaining substrates after 30 days. Amongst the three sugarcane residues and processing by-products evaluated in

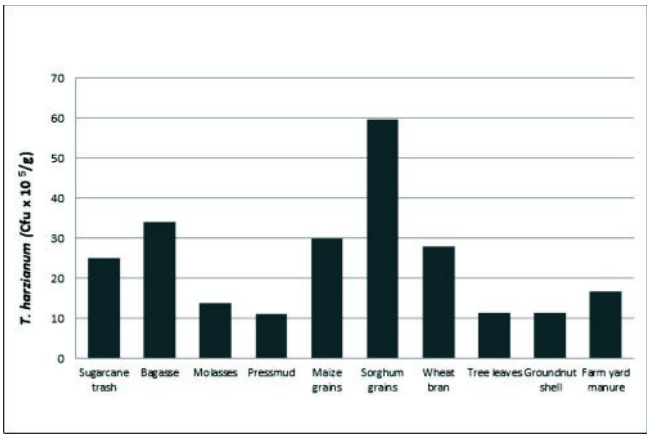

Fig. 1 Population density of *T. harzianum* on different substrates after 30 days.

the study; bagasse was observed to be the most suitable substrate for *Trichoderma* multiplication (34.0x 10^5cfu g^{-1}) followed by sugarcane trash (25.3x 10^5cfu g^{-1}) and molasses (10.3x 10^5cfu g^{-1}) (Table 1). Previous studies have also reported sorghum grains and bagasse as suitable substrates for mass production of *Trichoderma* (Rodriguez *et al.*1999, Rini and Sulochana 2007, Ramanujam *et al*. 2010). Solid state fermentation, which relies on use of various cheap grains/ agro-substrates, is a cheap and easy method for mass multiplication of *Trichoderma* and is highly suitable for small scale production at farmers level itself since it does not require any expensive instruments like bio-fermenters etc. (Ramanujam *et al.*2010). Our study has shown sorghum grains and bagasse to be the most suitable substrates for mass production of *T. harzianum* supporting high biomass (>10^{13} spores g^{-1}in both substrates) within 30 days. Since both these substrates are low cost and easily available, they can easily be exploited for mass multiplication of *Trichoderma*.

Shelf life studies in stored formulation of T. harzianum

Shelf life of *T. harzianum* on the two most promising substrates (sorghum grains and bagasse) was estimated at 30 days interval upto a period of 180 days, at room temperature. It was observed that, on both substrates, there was a decline in population of *T. harzianum* with increase in storage time (Table 2). However, for the first two month (till 60 days of storage), the population was almost stable. At 60 days of storage, population of >1.5 x 10^8cfu g^{-1} was recorded in both substrates. However, beyond 60 days a considerable decline in population was observed with cfu of 18 x 10^6cfu g^{-1} and 22x 10^6cfu g^{-1} recorded in *sorghum* grains and bagasse, respectively at 90 days of storage. Thereafter, the population showed a slight decline only till 150 days of storage on both substrates (CFU of 7.5 x 10^6g^{-1} on sorghum grains and 5.0 x 10^6g^{-1} on bagasse at 150 days). A further considerable decline in *T. harzianum* population was observed in samples drawn at 180 days of storage on both substrates (cfu 6.5 & 9.3 x 10^5 g^{-1} on sorghum grains and bagasse respectively). Singh *et al.* (2007) also observed that during storage the *Trichoderma* population remained constant for first two months of storage followed by a decline, with the rate of decline varying across the substrates.

Field evaluation of T. harzianum under different planting methods

The results of the one year field experiment conducted to evaluate the impact of *T. harzianum* application on sugarcane under two different planting methods revealed that *T. harzianum* application significantly improved cane yield over control in both STP and polybag raised settlings (Table 3). In both methods, *T. harzianum* was applied as sett treatment

Table 1 Population density of *T. harzianum* on four promising substrates after 30 days

Substrate	*T. harzianum* population after 30 days of incubation at different dilution (mean of three replications)							
	10^5 cfu g^{-1}	10^6 cfu g^{-1}	10^7 cfu g^{-1}	10^8 cfu g^{-1}	10^9 cfu g^{-1}	10^{10} cfu g^{-1}	10^{11} cfu g^{-1}	10^{12} cfu g^{-1}
Sugarcane trash	25.3	21.0	16.7	15.7	14.7	13.7	02.7	00.7
Bagasse	34.0	31.0	27.6	17.3	14.3	13.3	12.7	10.4
Molasses	10.3	08.7	05.7	03.7	01.7	00.6	0.00	0.00
Sorghum grains	59.6	49.6	36.0	34.3	30.4	29.0	25.6	19.0

Table 2 Population of *T. harzianum* on sorghum and bagasse at different storage durations

Substrate	Population of *T. harzianum* in colonized substrates at different times of storage (days)						
	0	30	60	90	120	150	180
Sorghum grains	8 x 10^8	2.3 x 10^8	1.8 x 10^8	18 x 10^6	15 x 10^6	7.5 x 10^6	9.3 x 10^5
Bagasse	6 x 10^8	2 x 10^8	1.5 x 10^8	22 x 10^6	13 x 10^6	5 x 10^6	6.5 x 10^5

Table 3 Effect of *Trichoderma* multiplied culture (TMC) application on yield of 'CoLk 94184' under different planting methods

Treatment	STP Method				Polybag Raised Settlings			
	G* (%)	NMC ('000/ha)	Yield (t/ ha)	Sucrose (%)	G (%)	NMC ('000/ha)	Yield (t/ ha)	Sucrose (%)
T. harzianum application	56.0	75.31	51.17	19.47	59.0	72.68	52.10	19.78
Control	53.0	70.37	42.03	18.97	54.0	70.45	44.62	19.56
CD @ 5%	NS	NS	4.51	NS	NS	NS	4.69	NS

*G=Germination

(sett dipped in spore suspension) as well as in field (TMC though FYM). In case of settlings raised by STP with *Trichoderma* treatment, significantly higher yield of 51.17 t/ha was recorded as compared with untreated control (42.03 t/ha). Similarly for polybag raised settlings, cane yield was significantly higher (52.1 t/ha) in *Trichoderma* treatment as compared to its untreated control (44.62 t/ha). In both methods, germination percent, NMC and sucrose % recorded was also higher with *Trichoderma* treatment relative to control, however the difference was non-significant. The potential of *Trichoderma* spp. to promote plant growth directly by production of growth hormones like IAA as well as indirectly by facilitating solubilisation of various micro and macro nutrients in soil and enhancing uptake of nutrients by plants has been well documented in several crops (Carvajal *et al.* 2009, Zhang *et al.* 2013, Toghueo *et al.* 2016). *Trichoderma* application is reported to improve root and shoot development and enhance plant vigour (Chowdappa *et al.* 2013). In the present study also, production of growth promoting hormones and nutrient solubilisation in soil along with imparting resistance to the transplanted settlings against native deleterious microflora may have contributed towards better establishment of settlings and improved growth and yield of transplanted cane.

CONCLUSION

Among the various substrates tested, sorghum grains and bagasse were found most suitable substrates for multiplication of *T. harzianum*. These substrates also supported high population of *Trichoderma* upto 150 days of storage (cfu 5 x 10^6 and 7.5 x $10^6 g^{-1}$). Mass multiplication of *T. harzianum* on sorghum grains followed by its field application through FYM showed significant improvement in cane yield under STP and polybag raised settling methods and can be exploited for ensuring better establishment and growth of cane.

ACKNOWLEDGEMENT

We are grateful to the Director, ICAR-Indian Institute of Sugarcane Research, Lucknow for providing facilities and constant encouragement.

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Indian Journal of Sugarcane Technology **32(02)**: 84-89, December 2017

Molecular diversity and genetic relatedness of some top borer tolerant sugarcane genotypes

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ABSTRACT

SSR markers were used to analyze the molecular diversity and genetic inter-relationship among elite genotypes of sugarcane having tolerance to top borer. A set of 24 primer pairs comprising of di- to penta-nucleotide repeat motifs and complex repeats was used to amplify the DNA. Three primer pairs showed high PIC index indicating their suitability to study molecular polymorphism and genetic diversity. A new term 'NPIC' is coined here to give a more comprehensive evaluation of a SSR marker. The grouping pattern of ten genotypes based on their similarity coefficients suggested the existence of sufficient genetic diversity among some of them. A large degree of SSR polymorphism was found and genotypes under study; more than 76% of the markers being polymorphic. The level of polymorphism indicated that distinction between any two genotypes was possible with appropriate SSR primer pair.

Key words: SSR polymorphism, NPIC, *Saccharum*, homology tree

Sugarcane (*Saccharum* spp. hybrid) is one of the most important agricultural crops of the world which is cultivated in both tropical and sub-tropical countries and accounts for more than 70% sugar production worldwide. It belongs to the genus *Saccharum*, family *Poaceae* and subfamily *Andropogoneae*. Cultivated sugarcane is mainly derived from two species, *S. officinarum* and *S. Spontaneum* (Roach 1972). It is genetically the most complex crop species, and offers tremendous challenges to plant breeders with respect to its genetic improvement. The production and productivity of sugarcane in India depends on various biotic and abiotic factors. Among the biotic factors, insect pests continue to be a major threat for cane production. The top borer (*Scirpophaga excerptalis* Walker) is a major pest of sugarcane in sub-tropical India, which causes death of up to 50 per cent of canes along with approximately 60 per cent reduction in yield and an average 3.15% reduction in sucrose (Mukunthan1990). Limited genetic base is apparently the major bottleneck in inculcating tolerance towards biotic and abiotic stresses, because most commercial sugarcane cultivars in the world today are derived from the crosses made with a few clones used during initial interspecific hybridization phases. *Erianthus arundinaceus* (Retz.) Jeswiet, a close relative of sugarcane (*Saccharum officinarum* L.) has great potential as a germplasm source and, gene introgression from it has always been the focus of inter-generic sugarcane breeding programmes for insect pests tolerance, disease resistance and abiotic stress tolerance (Nair *et al.* 2006). Efforts to utilize *E. arundinaceus* in sugarcane breeding programmes world over have resulted in introgression of genes for cold tolerance, red rot resistance and have surpassed most of the existing types of plant vigour (Sreenivasan and Sreenivasan 2000). Hybridization of *Erianthus* with sugarcane resulted in introgression of genes for cold tolerance and red rot resistance (Ram *et al.* 2001). Introgressive hybridization activities using this approach at ICAR-IISR, Lucknow have produced some excellent top borer tolerant genetic stocks which have been sent to National Hybridization Garden, SBI, Coimbatore. These genetic stocks may serve as good parental material for breeding towards top borer tolerance. Complex genetic nature of sugarcane pretences several challenges to sugarcane breeders. Comprehensive information of genetic diversity existing in the gene pool would facilitate more efficient selection of parental genotypes. Molecular markers offer several advantages over conventional methods for characterization of diversity within parental genotypes, especially for disease and pest resistance where precise phenotyping is difficult.

Among the molecular markers, SSR markers have been the most efficient and are widely used in germplasm characterization (Cordeiro *et al.* 2003), varietal testing (Pan 2006), association mapping (Racedo *et al.* 2016), linkage map construction (Andru *et al.* 2011; Oliveira *et al.* 2007), and diversity analysis (Cordeiro *et al*. 2003; Kharate *et al.* 2016; Pinto *et al.* 2006; Singh *et al.* 2013; Srivastava *et al.* 2005a, 2011). Therefore, an attempt has been made in this study to use the SSR markers for analyzing molecular diversity and genetic inter-relationship among elite genotypes of sugarcane having tolerance to top borer.

MATERIALS AND METHODS

Plant material

Ten elite genotypes of sugarcane developed at ICAR-IISR, Lucknow ('LG 07690', 'LG 07675', 'LG 06618', 'LG 07684', 'LG

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07692', 'LG 07650', 'CoLk 07201' 'LG 07680', 'LG 05609' and 'LG 05610') showing different levels of top borer tolerance were selected for molecular genetic diversity analysis and assessment of their inter-relationship.

DNA isolation and PCR amplification

Total genomic DNA was extracted from fresh leaf tissues of field grown plants using modified CTAB procedure (Srivastava and Gupta 2001). The DNA was purified, quantified and stored at -20°C. PCR amplification was performed on thermal cycler PTC 200 (Peltier thermal cycler, MJ research Pvt. Ltd., USA). The reactions were carried out in 20µl final volume of the reaction mix, containing 20 ng template DNA, 0.5 Unit Taq polymerase, 2 µl of 10 X PCR buffer, 2 µl of 25 mM $MgCl_2$, 1.6 µl of 10 mM dNTPs and 4.0 pmoles each of the forward and reverse primers. The PCR conditions were as follows: an initial step of denaturation at 94°C for 3 minutes, thirty five cycles of denaturation for 45 sec at 94°C, annealing for 30 seconds at 54-56°C (depending upon annealing temperature of the primers) followed by 30 sec at 72°C and a final elongation step at 72°C for 10 minutes. A set of 24 primer pairs comprising of di- to penta-nucleotide repeat motifs and complex repeats were used as primers to amplify the DNA (Table 1).

Electrophoresis and gel documentation

The PCR products were stored at 4°C before loading. Ten µl of PCR products was mixed with 2 µl gel loading buffer and loaded in 3% agarose gel containing 1 µg/ml ethidium bromide. The gel was run in 1X TAE buffer in SubCell GT electrophoresis unit (BioRad, USA). Electrophoretic separation was performed at 70 V for 45 min. A 50 bp DNA ladder (Fermentas, Gene Ruler) was used as molecular weight marker. The gels were photographed under UV light, using an AlphaImager™ 1220 (Alpha Innotech Corporation, San Leandro, USA) Gel Documentation System.

Data scoring, processing, similarity index and homology tree

The size of the amplified fragments was calculated by comparison with the 50 bp DNA ladder using the software AlphaImager EC. The bands were arranged in decreasing order of molecular weight for each primer. Each DNA fragment generated was treated as a separate character and scored as a discrete variable. The complete amplification profile of all SSR alleles for all the genotypes was recorded into an arbitrary sequence of "1" or "0" (present or absent) and accordingly a rectangular binary data matrix was obtained which was used for further analysis using the numerical taxonomy software NTSYS-pc version 2.1 (Rohlf 2000) that calculated the pair-wise similarity coefficient matrix for all the markers by simple matching similarity algorithm and produced a homology tree based on the UPGMA (Un-weighted Pair Group Method with Arithmetic Mean clustering) method (Sneath and Sokal 1973) following the SAHN (Sequential Agglomerative Hierarchical Nested) cluster analysis module.

Primer efficiency parameters

The polymorphism information content (PIC) was calculated for each locus according to Anderson *et al.* (1993) as $PIC = 1-\Sigma xi^2$ where, xi is the relative frequency of the i^{th} allele of the SSR loci. PIC provides an estimate of the discriminating power of a locus by taking into account the number of alleles generated by each reaction unit and their frequency distribution in the population. Markers were classified as informative when PIC was $e \geq 0.5$.

Effective Multiplex Ratio (EMR) for an individual primer was obtained by the formula; $EMR = n\beta$, where β = percent of polymorphic markers and n = number of bands per reaction unit.

The marker index (MI) to characterize the ability of each primer to detect polymorphic loci among the genotypes was calculated for all the primers as the product of two functions that is PIC and EMR, as described by Prevost and Wilkinson (1999).

RESULTS AND DISCUSSION

Out of twenty four SSR primers used in this study to analyze the genetic diversity among the ten elite sugarcane genotypes, twenty two primer pairs provided distinct reliable band profiles. Seventeen out of these twenty-two primers showing complete parsimony were very useful for diversity analysis, five primers showed monomorphism and two did not show any amplification.

A total of 437 bands were generated from all the primers across all the genotypes with an average number of 43.7 bands/ genotype of which76.58 % bands were polymorphic. Total number of bands amplified in all genotypes for each primer ranged from 9-50, with an average of 19.86 bands/primer. The molecular weight of these bands ranged from 98-862 bp, based on which these bands were grouped in 67 alleles of which 20 alleles were monomorphic. Composite electrophoregram of the amplified alleles from 22 SSR markers in ten genotypes is shown in Figure 2, where the X-axis shows all amplified SSR alleles in this study and the Y-axis shows the presence of a particular allele in ten genotypes. For molecular diversity purpose, primers showing polymorphism were considered (Table 1). Thus, a total of 327 amplicons from seventeen polymorphic primers with an average of 19.24 amplicons/ primer were taken into consideration. The molecular weight of these 327 amplicons ranged from 98 to 796 bp.

Distribution of alleles and primer efficiency in studied genotypes

To test the general utility of these SSR markers, we calculated the number of alleles and PIC value for each individual SSR marker (Table 1). The total number of alleles produced by any single SSR primer varied from as few as 1 (in 'SS08-1', 'SS 08-13' and 'SS 08-16') and 2 (in 'SS08-2', 'SS 08-3', 'SS 08-4', 'SS 08-10', 'SS08-11', 'SS08-15', 'SS08-21' and

Table 1 Details of microsatellite markers showing polymorphism and their parameters*

Sl. No.	Name of Primer	Repeat motifs	Tm (°C)	Size range (bp)	TA	PA	PIC	EMR	MI
1	SS08-2	$(CACCG)_4$	54	194-571	2	1	0.50	0.5	0.25
2	SS08-5	$(GTTTG)_4$	54	300-633	3	3	0.61	3.0	1.836
3	SS08-6	$(ATGG)_5$	54	103-467	3	2	0.66	1.33	0.88
4	SS08-7	$(CT)_{10}$	54	117-350	4	4	0.64	4.0	2.54
5	SS08-8	$(GT)_{10}$	54	139-562	7	7	0.81	7.0	5.64
6	SS08-9	$(TC)_{16}(TG)_{38}$	56	117-647	4	4	0.60	4.0	2.38
7	SS08-10	$(TGT)_{11}$	55	205-369	2	1	0.50	0.5	0.25
8	SS08-11	$(CTC)_7$	55	131-321	2	1	0.48	0.5	0.24
9	SS08-12	$(CA)_{10}$	54	121-312	3	2	0.61	1.33	0.81
10	SS08-15	$(ACGT)_6$	54	98-174	2	1	0.50	0.5	0.24
11	SS08-16	$(CTC)_5(CT)_6CCGAT(CCT)_5$	54	289	1	1	0.19	1.0	0.19
12	SS08-18	$(TTC)_{52}$	54	250-692	6	6	0.80	6.0	4.66
13	SS08-20	$(ACA)_{36}$	55	200-700	5	5	0.76	5.0	3.82
14	SS08-21	$(TTC)_{26}$	54	145-230	2	2	0.42	2.0	0.84
15	SS08-22	$(GGGAG)_9$	54	105-210	2	2	0.50	2.0	1.00
16	SS08-23	$(CT)_6ATATAT(A)_{85}$	55	180-610	4	3	0.69	2.25	1.55
17	SS08-24	$(GT)_{39}$	55	217-796	4	2	0.75	1.00	0.75
	Range			98-700	1-7	1-7	0.19-0.81	0.5-7.0	0.19-5.64

*Tm=Melting temperature, TA=Total number of alleles, PA=Number of polymorphic alleles, PIC=Polymorphic Information Content, EMR=Effective Multiplex Ratio, MI=Marker Index

'SS08-22') to as many as 7 (in 'SS08-8'), based on which they were grouped into 67 alleles of distinct molecular weight, that ranged from 1 to 7 alleles per primer with an average of 3.05 alleles/primer. Multiple alleles generated by the SSR primers in sugarcane are due to its polyploid nature and large genome size. You *et al.* (2016) and Singh *et al.* (2011) reported more than 10 amplicons per primer. Markers, namely 'SS08-8', 'SS08-18' and 'SS08-20' showed highly polymorphic behaviour by producing 5 or more alleles that were hundred per cent polymorphic. The other markers, namely 'SS08-5', 'SS08-7'and 'SS08-9', were also completely polymorphic but produced fewer alleles (only 3-4 per primer). Remaining markers were less polymorphic and produced 1 to 4 alleles/primer only.

The Polymorphic Information Content (PIC) index ranged from 0.19-0.81 (Table 1) with a mean value of 0.59. Ten out of seventeen primers showed PIC value of more than 0.5 (Table 1). Higher PIC value of these primers meant a lower allele frequency indicating that the particular SSR allele existed in fewer genotypes only hence is suitable for diversity analysis. Effective Multiplex Ratio (EMR) of the primers ranged from 0.5-7.0 with a mean value of 2.47 (Table 1). Marker Index (MI) ranged from 0.19-5.64 with a mean value of 1.64. Overall, the highest value of PIC index (0.81), EMR (7.0) and MI (5.64) were obtained for primer 'SS 08-8' (Table 1). High PIC index were also obtained for the primers 'SS 08-18' (0.80) and 'SS 08-20' (0.76), thus proving the suitability of these three primers to study molecular polymorphism and genetic diversity. High PIC values for SSR markers have been obtained by earlier sugarcane researchers also (Pinto *et al.* 2006; Cordeiro *et al.* 2003; Duarte Filho *et al.* 2010; Liu *et al.* 2011; Kharate *et al.* 2016). They have also suggested the suitability of SSR markers for diversity analysis in sugarcane, on the basis of their high PIC values. EMR and MI have also been used to evaluate the discriminatory power of molecular marker systems in some plant species like wheat (ISSR, EMR = 12, MI = 3.36), apricot (ISSR, EMR = 4.8, MI = 3.74), (Abdollah *et al.* 2015) and sugarcane (SSR, EMR = 2.33, MI = 2.02), (Kharate *et al.* 2016).

Since the number of alleles and PIC values, both may contribute to the unique molecular identity of any sugarcane genotype, a new term NPIC is coined here that equals to the product of total number of alleles (N) and PIC value, to give a more comprehensive evaluation of a SSR marker. In this study, one SSR marker, namely 'SS 08-8', had the highest NPIC value (> 5), and two SSR markers *viz.*, 'SS 08-18' and 'SS 08-20' had high NPIC values of >3 and up to 5 in comparison to NPIC values of 3 or less from the remaining SSR markers (>1 to 3 and up to 1 in 7 SSR markers each). This indicated that the three SSR markers *viz.* 'SS 08-8', 'SS 08-18' and 'SS 08-20' had produced more genotyping information for the ten genotypes and should be more useful in identifying sugarcane clones in general.

Genetic relatedness and homology analysis among the ten genotypes

The 1,0 binary matrix obtained from presence *vs*. absence data of various alleles subjected to genetic similarity co-

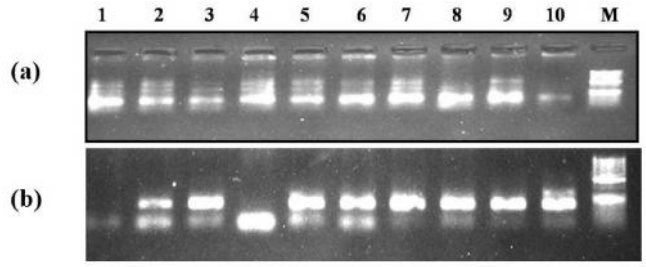

Fig. 1. SSR amplification profile of sugarcane genotypes using primers (a) SS08-6 and (b) SS08-7. 1-10 = sugarcane genotypes, M= 50bp Gene Ruler ladder

efficient analysis (SM similarity quotients) using NTSYSpc (Numerical Taxonomy System Biostatistics, version 2.1) to estimate genetic relatedness among varieties gave a pairwise similarity matrix (Table 2). The SM coefficients ranged from 58.21% ('LG 07675' and 'LG 05610') to 86.57% ('LG 07675' and 'LG 07684'). The overall mean value is 74.03%.

The UPGMA based cluster analysis grouped these genotypes in 2 main clusters, indicating a clear pattern of division among them. There were two groups of genotypes (Fig. 3) having 6 ('LG 07690', 'LG 07675', 'LG 06618', 'LG 07684', 'LG 07692', 'LG 07650') and 3 ('LG 07680', 'LG 05609' and variety 'CoLk 07201') genotypes each. These two groups joined at a homology level of 73% and clustered closely, whereas, the genotype 'LG 05610' did not join any group. The closest relationship existed between 'LG 07675' and 'LG 07684' showing a genetic similarity of 87%.

SSR markers have been used in past also to assess diversity or to find out association with sugarcane borers. Da Silva *et al.* (2005) used microsatellites to identify markers showing association with stem borer susceptibility, but he could observe no significant association between SSR markers and internode damage. However, they identified some informative markers developed from sugarcane disease and insect resistance genes and one of these markers showed a possible association with stem borer susceptibility. Selvi *et al.* (2008) characterized the genetic diversity among sugarcane cultivars with different levels of resistance to top borer and derived associations between top borer resistance/susceptibility and, RAPD and SSR markers. In their report, the genetic similarity values ranged from 55.8% to 83.4% with a mean genetic similarity of 68.3% (Selvi *et al.* 2008). In present study also, the similarity coefficients ranged from 58.21% to 86.57%. The overall high genetic similarity indicates the still narrow genetic base of these sugarcane cultivars and reiterates the need of further genetic base broadening. High similarity coefficients or low level of genetic distance among sugarcane varieties correspond to previous studies (Harvey and Botha, 1996; Oropeza and García, 1997; Srivastava and Gupta, 2006; Srivastava *et al*., 2005a; 2005b; Ubayasena and Perera, 1999). The grouping pattern of studied genotypes suggests that sufficient genetic diversity could be attained among some of them with the help of selected SSR markers and there is a large degree of SSR polymorphism within the genotypes under study; more than 76% of the markers being polymorphic. Srivastava and Gupta (2008) also generated substantial polymorphisms among elite sugarcane varieties with high genetic proximity using selected ISSR primers. The level of polymorphism in the present study indicates that distinction between any two varieties is possible with appropriate SSR primer pair. This also supports the use of SSR markers, as an excellent tool for diversity analysis and loci mapping in sugarcane. It would be beneficial to explore their potentiality in varietal improvement programmes.

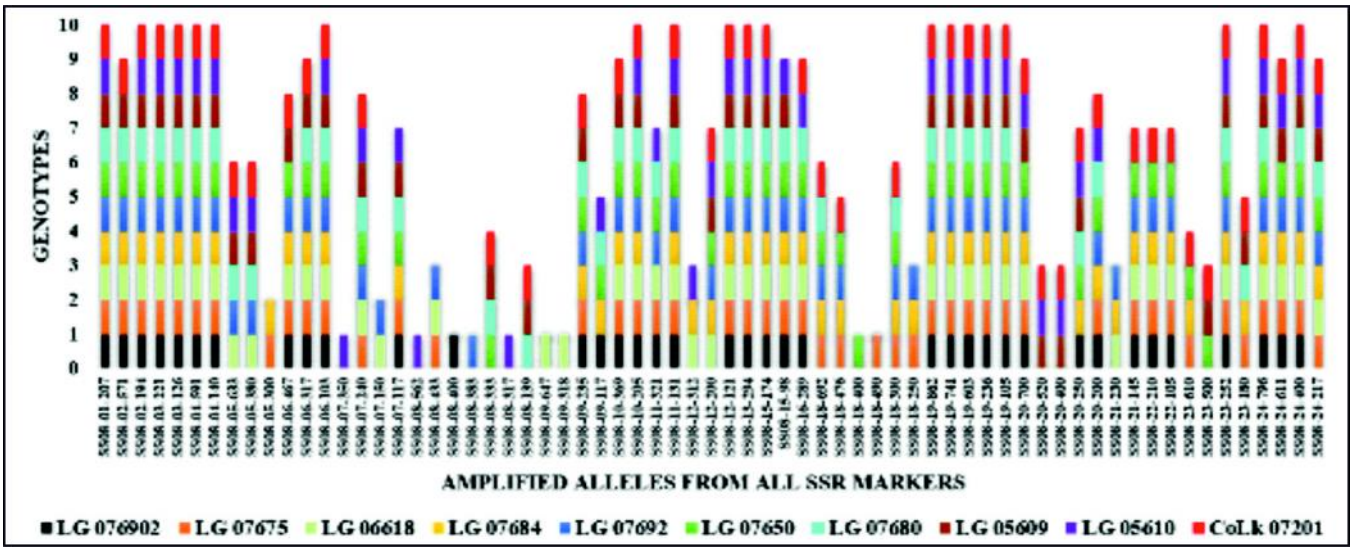

Fig. 2. Composite electrophoregram of amplified alleles from all SSR markers in ten genotypes

Table 2 Pair-wise SM similarity coefficient matrix of ten elite sugarcane genotypes.

	'LG 07690'	'LG 07675'	'LG 06618'	'LG 07684'	'LG 07692'	'LG 07650'	'LG 07680'	'LG 05609'	'LG 05610'	'CoLk 07201'
'LG 07690'	100.00									
'LG 07675'	79.10	100.00								
'LG 06618'	74.63	71.64	100.00							
'LG 07684'	80.60	86.57	70.15	100.00						
'LG 07692'	74.63	83.58	85.07	79.10	100.00					
'LG 07650'	83.58	83.58	70.15	85.07	79.1	100.00				
'LG 07680'	76.12	73.13	65.67	71.64	71.64	77.61	100.00			
'LG 05609'	71.64	65.67	70.15	67.16	67.16	73.13	80.6	100.00		
'LG 05610'	70.15	58.21	68.66	62.69	62.69	65.67	73.13	74.63	100.00	
'CoLk 07201'	70.15	76.12	68.66	77.61	77.61	83.58	76.12	83.58	64.18	100.00

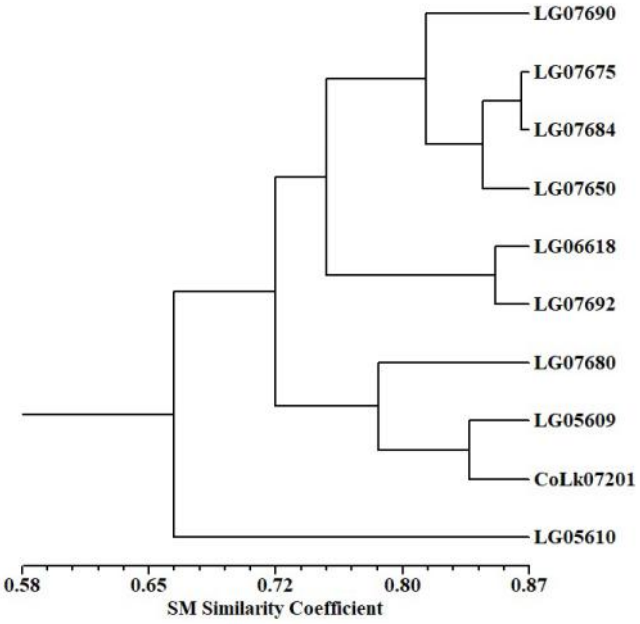

Fig. 3. A homology tree of ten sugarcane genotypes produced by the NTSYSpc (v2.1) software based on SM similarity coefficients.

ACKNOWLEDGEMENT

The first author is grateful to the Director, ICAR-IISR, Lucknow for providing the facilities to carry out this research work.

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Indian Journal of Sugarcane Technology **32(02)**: 90-94, December 2017

Effect of seaweed sap application on growth, yield, juice quality and economics of sugarcane in Rajasthan

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ABSTRACT

A field experiment was conducted during 2013 to 2015 at ARS, Kota to evaluate effective doses of two seaweed sap source, *viz*. *Kappaphycus alvarezii* (K-sap) and *Gracilaria edulis* (G-sap) for increasing the productivity and improving juice quality of sugarcane. The experiment consisted of 10 different seaweed sap concentrations with recommended dose of fertilizers (RDF) *viz.*, T_1:2.5% K-sap + RDF (200:60:40 kg N P_2O_5 K_2O/ha), T_2:5% K-sap + RDF, T_3:7.5% K-sap +RDF, T_4:10% K-sap + RDF, T_5: 2.5% G-sap + RDF, T_6:5% G-sap + RDF, T_7:7.5% G-sap + RDF, T_8:10% G-sap + RDF, T_9: RDF + water spray and T_{10}: 6.25% K-sap + 50% RDF to plant crop were laid out in randomized block design with three replications. Among the treatment combinations of seaweed sap concentrations, application of 10% G-sap + RDF recorded significantly higher germination percent at 40 DAP (52.10 and 43.60), significantly higher number of tillers at 90 DAP (62.93 and 47.78 thousand/ha) and 150 DAP (115.31 and 82.40 thousand/ha), cane length (308.67 and 265.4 cm), number of millable canes (87.39 and 73.80 thousand/ha), cane diameter (3.02 and 2.51 cm), single cane weight (830 and 760 g), cane yield (92.03 and 78.07 t/ha), sucrose% (18.59 and 17.66%), commercial cane sugar yield (11.82 and 9.49 t/ha) over 6.25% K-sap + 50% RDF, RDF + water spray, followed by 10% K-sap + RDF treatment, being on par with rest of treatments during both the years. Application of 10% G-sap + RDF recorded significantly higher gross return (₹182007/ha), net return (₹85360/ha) and B: C ratio (1.88) over 6.25% K-sap + 50% RDF and at par with rest of treatments on pooled mean basis, whereas, °Brix at harvest (21.13 and 19.55%) was found significantly superior by application of 7.5% G-sap + RDF over 6.25% K-sap + 50% RDF only, while sucrose (18.71 %) at harvest was recorded significantly higher through application of 7.5% K-sap + RDF over 6.25% K-sap + 50% RDF, 2.5% K-sap + RDF and 5% K-sap + RDF and 2.5% G-sap + RDF and at par with rest of the treatments during 2013-14. Thus, the results showed that the application of 10% G-sap + RDF (200:60:40 kg N P_2O_5 K_2O/ha) may be recommended for obtaining higher cane yield, net return and quality of sugarcane in spring season planted crop.

Key words: Economics, Fertilizer, Seaweed sap, Sucrose, Sugarcane yield

Sugarcane (*Saccharum officinarum*) is a long-duration nutrient exhaustive crop grown in India over an area of 5.04 million ha to meet country's total sugar requirement with an average productivity of 71.67 tons per hectare (FAO 2015). The average productivity of sugarcane in the state has been around 72.10 t/ha (Anonymous 2016). The development of modern agricultural technologies, continuous use of heavy doses of fertilizers and plant protection chemicals potentially impaired the soil microbial activity, leading to poor soil health (Singh *et al*. 2007). Low sugar recovery as well as cane production is governed by various factors at the farmers' field in Rajasthan, out of which, imbalance use of fertilizers and the lack of organic matter application had led to depletion of soil fertility and posing serious threat to the long-term productivity. The yield of sugarcane has reached a plateau due to decline in factor productivity. The loss in organic matter is the root cause for decline in factor productivity. The deterioration in soil health and crop productivity is associated with decline in soil organic carbon under intensive sugarcane farming system. Restoration of soil organic matter is, thus needed for improving productivity through correction of essential macro and micronutrients deficiencies and improvement in soil health. To stop continuous decline in soil fertility, it is important to use organic manure in combination with chemical fertilizers to meet adequately the nutritional requirements of sugarcane crop (Nagaraju *et al*. 2000).

Seaweeds, a natural source of nutrients, are of great importance to substitute the chemical fertilizers. Seaweeds are the macroscopic marine algae, found to the bottom of relatively shallow coastal waters. They grow in the intertidal, shallow and deep sea areas up to 180 m depth and also in estuaries and backwaters on the solid substrate such as rocks, dead corals and pebbles. Seaweeds have been used as green manure, cattle feed, food for human consumption and as a source of phycocolloids such as sugar, alginic acid and carrageenan. The liquid extracts obtained from seaweeds popularly known as SLF/LSF have gained importance in recent years as foliar sprays for several crops because the extract contains not only nitrogen, phosphorus and potash but also contain ample amount of trace elements like Zn, Mn, Fe, *etc.*, metabolites,

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growth-promoting hormones, *i.e.* auxins (IAA, IBA), cytokinins, vitamins and amino acids. These seaweed extract application have been found beneficial to crop plants, as it increased the crop yield, delay of fruit senescence, improved overall plant vigour, and quality and to improve ability to withstand adverse environmental conditions (Featonby and Van Staden 1983). In addition, the carbohydrates and other organic matter present in seaweeds alter the nature of soil and improve its moisture retaining capacity of soil. So, utilization of seaweeds and their extract will be useful for achieving higher agricultural production. Kavitha *et al*. (2008) and Pramanick *et al*. (2014) reported significant increase in yield of crops with foliar application of seaweed extracts. Hence this study was conducted to evaluate the application of different concentrations of seaweed extract for sugarcane in spring season on clay loam soil of south eastern Rajasthan.

MATERIALS AND METHODS

A field experiment was conducted on clay loam soil during the spring seasons for 2 years from 2013-14 to 2014-15 at Agricultural Research Station, Kota, Rajasthan to study the response of seaweed sap and inorganic fertilization for increasing the productivity and quality of sugarcane. The research station is located between 25 13 N latitude and 75 25 E longitudes at an altitude of 258 m above MSL. The average annual rainfall received during the crop seasons was 1132.3 mm and mean maximum and minimum temperature were 46°C and 19.8 °C, respectively. The experimental soil was clay loam in texture with a pH of 8.20, EC 0.42 ds/m, medium in organic carbon (0.52%), available nitrogen (325 kg/ha), P_2O_5 (22.4 kg/ha) and high in K_2O (288 kg/ha).The initial soil had bulk density 1.40 Mg with ten different seaweed sap concentrations of 2 source *viz*. *Kappaphycus alvarezii* (K-sap) and *Gracilaria edulis* (G-sap) along with recommended dose of fertilizer (RDF) *viz*.,T_1: 2.5% K-sap + RDF (200:60:40 kg $NP_2O_5K_2O$/ha), T_2: 5% K-sap + RDF, T_3: 7.5% K-sap + RDF, T_4: 10% K-sap + RDF, T_5: 2.5% G-sap + RDF, T_6: 5% G-sap + RDF, T_7: 7.5% G-sap + RDF ,T_8: 10% G-sap + RDF, T_9: RDF + water spray and T_{10}: 6.25% K-sap + 50% RDF was conducted in randomized block design with three replications. Recommended dose of 200:60:40 kg N : P_2O_5 : K_2O/ha for sugarcane was applied as per treatments. K-sap and G-sap were obtained from the Central Salt and Marine Chemicals Research Institute, Bhavnagar, Gujarat, India. The nutrients NPK were applied through urea, di-ammonium phosphate (DAP) and muriate of potash (MOP) fertilizers, respectively. Uniformly farmyard manure containing 0.5, 0.2 and 0.4% of N, P and K was incorporated and mixed well in the soil 15 days prior to planting of the cane setts. Zinc sulphate 25 kg/ha applied at the time of planting. Full P_2O_5 and K_2O, and ¼ of N was applied as basal and remaining N was top-dressed in 3 equal splits at early tillering stage (40 DAP), late tillering stage (80 DAP) and earthing up i.e. on onset of monsoon (120 DAP). Sugarcane variety 'CoPK 05191' was planted at 75 cm row distance, keeping three budded four setts per meter row length in the first week of March during 2013-14 and 2014-15, respectively. Cane setts were treated with required concentration of seaweed sap just before planting for 30 minute. Three spray of seaweed sap as per treatment were applied each at early tillering stage (40 DAP), late tillering stage (80 DAP) and grand growth period (170 DAP) with the help of a knapsack sprayer fitted with holocone nozzle with a spray volume of 600 liters/ha. All the recommended agronomic practices were done throughout the crop season. Plot size for each treatment was 6.0 m x 4.5 m =27.0 m^2. Growth, yield attributes, cane yield and quality parameter were statistically analyzed. The economics was worked out based on two years pooled plant crop cane yield data and considering prevailing market rates of input and output.

RESULTS AND DISCUSSION

Growth and yield attributes

Germination percent and growth parameters were significantly influenced by application of seaweed sap of K and G at different growth stages of sugarcane. Germination (52.10%) at 40 DAP was recorded highest under application of 10% G-sap + RDF during 2013-14 which was significantly superior over rest of the treatments and remained at par with 10% K-sap + RDF and 7.5% G-sap + RDF. Tiller population at 90 (62.93 and 47.78 thousand/ha) and 150 (115.31 and 82.40 thousand/ha) DAP stages of crop growth recorded maximum under application of 10% G-sap + RDF which was significantly higher over 6.25% K-sap + 50% RDF, RDF + water spray, 2.5% K-sap + RDF and 5% K-sap + RDF and at par with remaining of the treatments during 2013-14 and 2014-15, respectively. Early development of millable canes with uniform maturity in higher concentration of G-sap resulted in higher brix value and increase in sucrose per cent and thus improved sugar yield (Jha *et al*. 2015). The higher cane length (308.67 and 265.4 cm) at harvest was recorded under application of 10% G-sap + RDF and remained at par with 10% K-sap + RDF, 5% G-sap + RDF and 7.5% G-sap + RDF and significantly superior over rest of the treatments during both the years. Whereas, application of G and K-sap concentrations 10% along with RDF were also found equal effective for increasing the cane length over lower concentrations 2.5 and 5% of both sap along with RDF. The differences between K and G-sap at lower concentrations were non-significant in respect to cane length. However, application of G-sap showed markedly superiority over K-sap on cane length. Sugarcane sprayed with G-sap produced higher cane length and cane diameter than K-sap owing to comparatively better concentration of hormone, as reported by Singh *et al*. (2015).

Application of 10% G-sap + RDF recorded significantly higher number of millable canes (87.39 thousand/ha) over rest of the treatments except 10% K-sap + RDF, 7.5% G-sap + RDF, 5% G-sap + RDF and 7.5% K-sap + RDF which were

found statistically at par. Percent increase was registered to the tune of 11.51,12.91,13.16, 16.66 and 28.51% over 5% K-sap + RDF, 2.5% K-sap + RDF, 2.5% G-sap + RDF, RDF + water spray and 6.25% K-sap + 50% RDF, respectively in 2013-14 (Table 1). Application of 10% G-sap + RDF recorded significantly higher cane diameter (3.02 and 2.51cm) and single cane weight (830 and 760 g) over water spray + RDF and 6.25% K-sap + 50% RDF and the rest of the treatments were found statistically at par with each other during both the year. Increasing spray concentration increased the productive tillers, cane diameter and cane weight up to 10% and thereafter it decreased which might be owing to salt index of the seaweed sap at higher concentration, as reported by Beckett and Van Staden (1990).

Cane yield and juice quality

Application of G-sap significantly improved the cane juice quality parameters (Table 2). The highest brix (21.13%) was recorded under the application of 7.5% G-sap along with RDF which was significantly superior over 6.25% K-sap + 50% RDF only and all other concentrations of K and G-sap were found statistically at par with each other during 2013-14, while during 2014-15 highest brix (20.20%) was recorded under 10% G-sap + RDF which was statistically at par with all the treatments except 6.25% K-sap + 50% RDF. Whereas, the highest sucrose (18.71%) at harvest was recorded under the application of 7.5% K-sap + RDF which was significantly superior over 6.25% K-sap + 50% RDF, 2.5% K-sap + RDF and 5% K-sap + RDF and 2.5% G-sap + RDF and at par with rest of the treatments during 2013-14, while during 2014-15 highest sucrose (17.66%) at harvest recorded in sprayed of 10% G-sap + RDF which was significantly superior over 6.25% K-sap+ 50% RDF and 2.5% K-sap +100% RDF and found statistically at par with 5% K and G-sap + RDF,10% K-sap + RDF and RDF + water spray. These findings are in conformity with the work of Beckett and Van Staden (1990), Kavitha *et al.* (2008) and Pramanick *et al*. (2014).

Perusal of cane yield data (Table 2) revealed that application of 10% G-sap + RDF recorded the highest cane yield (92.03 and 78.07 t/ha) and CCS yield (11.82 and 9.49 t/ha) which was found significantly superior over 6.25% K-sap + 50% RDF, RDF + water spray and 2.5% K-sap + RDF and statistically at par with all the treatments during 2013-14, while during 2014-15 it was found statistically at par with all the treatments except 6.25% K-sap + 50% RDF. Application of G and K-sap resulted in maximum and significantly higher cane yield and increased with each increment in sap concentrations up to 10%, as reported by Singh *et al*. 2015. However, the difference in cane yield and CCS yield at concentrations of K and G-sap (5, 7.5 and 10%) were found statistically at par with each other. Whereas, commercial cane sugar was recorded significantly higher under application of 7.5% K-sap + RDF (12.99%) over 6.25% K-sap + 50% RDF,2.5% K-sap + RDF and 5% K-sap + RDF but remained statistically at par with rest of the treatments during 2013-14 whereas, during 2014-15 significantly higher commercial cane sugar was recorded in 10% G-sap + RDF (12.15%) which was statistically at par with all the treatments except 6.25% K-sap + 50% RDF. This confirms the findings of Pramanick *et al.* (2014) and Singh *et al*. (2015).

Economics

Application of 10% G-sap + RDF (200:60:40 kg $NP_2O_5K_2O$/ha) to plant crop recorded significantly higher

Table 1 Effect of seaweeds sap on germination and yield parameter of sugarcane during 2013-14 and 2014-15

Treatment	Germination at 40 DAP (%)		Tiller population (thousand/ha)				Cane length at harvest (cm)		NMC (thousand/ha)		Cane diameter (cm)	
			90 DAP		150 DAP							
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
2.5% K-sap + RDF	40.10	40.20	55.70	45.10	104.23	78.70	279.53	255.70	77.40	70.50	2.55	2.39
5% K-sap + RDF	43.30	40.27	56.67	45.25	107.34	79.40	279.55	257.40	78.37	70.80	2.61	2.37
7.5% K-sap + RDF	45.13	41.30	59.67	46.70	110.23	79.70	288.07	256.80	80.38	71.30	2.64	2.42
10% K-sap + RDF	49.40	43.10	60.50	46.90	112.57	80.00	295.60	260.40	84.72	72.50	2.79	2.43
2.5% G-sap + RDF	45.60	40.70	56.08	45.70	108.29	79.00	283.83	257.00	77.23	71.40	2.57	2.42
5% G-sap + RDF	48.07	41.50	59.60	46.00	110.32	79.80	290.20	260.10	80.40	71.70	2.64	2.43
7.5% G-sap + RDF	48.00	42.00	60.44	47.20	111.85	80.70	294.98	261.70	81.40	72.30	2.71	2.45
10% G-sap + RDF	52.10	43.60	62.93	47.78	115.31	82.40	308.67	265.40	87.39	73.80	3.02	2.51
RDF + water spray	35.27	42.50	52.00	45.30	102.83	80.00	270.00	257.30	74.91	70.30	2.39	2.45
6.25% K-sap + 50% RDF	33.57	40.00	45.30	42.40	80.00	73.20	243.33	230.40	68.00	62.80	2.07	2.05
SEm ±	1.60	2.60	1.90	1.82	4.10	2.92	6.80	6.63	3.00	2.50	0.16	0.14
CD (P=0.05)	4.80	NS	5.60	5.35	12.20	8.60	20.20	19.70	9.00	7.40	0.48	0.43

RDF: Recommended dose of fertilizer; NMC: Number of millable canes

Table 2 Effect of seaweeds sap on single cane weight, quality, CCS and cane yield of sugarcane during 2013-14 and 2014-15

Treatment	Single cane weight (g)		Cane yield (t/ha)		Brix % at harvest		Sucrose % at harvest		CCS (%)		CCS (t/ha)	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
2.5% K-sap + RDF	800	725	86.30	75.47	20.40	19.40	17.86	16.83	12.30	11.54	10.61	8.70
5% K-sap + RDF	810	730	86.60	75.80	20.50	19.60	17.97	17.04	12.38	11.69	10.72	8.86
7.5% K-sap + RDF	815	730	88.60	76.80	21.00	19.50	18.71	16.94	12.99	11.62	11.51	8.92
10% K-sap + RDF	820	740	88.63	77.07	21.00	20.00	18.49	17.45	12.75	11.99	11.30	9.24
2.5% G-sap + RDF	810	730	87.00	75.80	20.60	19.50	18.06	16.94	12.45	11.62	10.83	8.81
5% G-sap + RDF	810	745	86.88	76.87	20.80	19.60	18.29	17.04	12.61	11.69	10.96	8.99
7.5% G Sap + RDF	825	750	88.68	77.00	21.13	19.55	18.62	16.99	12.86	11.66	11.45	8.97
10% G-sap + RDF	830	760	92.03	78.07	21.10	20.20	18.59	17.66	12.84	12.15	11.82	9.49
RDF + water spray	790	745	84.22	77.50	20.70	19.70	18.18	17.14	12.54	11.76	10.73	9.12
6.25% K-sap + 50% RDF	780	705	75.40	67.20	19.50	18.60	17.54	16.00	12.06	10.92	9.46	7.34
SEm ±	13.79	12.50	1.90	1.70	0.30	0.27	0.20	0.22	0.20	0.30	0.30	0.40
CD (P=0.05)	40.00	37.00	5.70	5.20	0.90	0.80	0.60	0.65	0.50	0.90	0.80	1.20

CCS: Commercial cane sugar

Table 3 Economics of sugarcane under different treatments of seaweeds sap (pooled mean data of 2013-14 and 2014-15)

Treatment	Cost of cultivation (₹/ha)	Gross return (₹/ha)	Net return (₹/ha)	B:C ratio
2.5% K-sap + RDF	94960	173104	78144	1.82
5% K-sap + RDF	95530	173770	78240	1.82
7.5% K-sap + RDF	96080	176980	80900	1.84
10% K-sap + RDF	96650	177300	80650	1.83
2.5% G-sap + RDF	94960	174200	79240	1.83
5% G-sap + RDF	95530	175200	79670	1.83
7.5% G-sap + RDF	96090	177280	81190	1.84
10% G-sap + RDF	96650	182007	85360	1.88
RDF + water spray	94400	173040	78640	1.83
6.25% K-sap + 50% RDF	92310	152580	60270	1.65
SEm ±	-	3430	3380	0.03
CD (P=0.05)	-	9850	9700	0.09

*Common cost of cultivation ₹93,500/ha, K-sap & G-sap cost ₹15/lit and Cane selling price ₹2140/ ton

gross return (₹182007/ha), net return (₹85360/ha) and B:C ratio (1.88) over 6.25% K-sap + 50% RDF and at par with rest of treatments on pooled mean basis (Table 3). The net monetary advantage to the tune of ₹25088/ha was fetched by application of 10% G-sap + 100% RDF over 6.25% K-sap + 50% RDF. Net return and B:C ratio of sugarcane increased with increasing level of G-sap concentration up to 10% than K-sap. Sugarcane sprayed with G-sap gave higher net return and benefit: cost ratio than K-sap due to lower cost and higher cane yield. This confirms the findings of Pramanick *et al.* (2014).

Maximum production cost of cane (₹96650/ha) recorded with the application of 10% G-sap + RDF and 10% K-sap + RDF owing to use of higher dose of seaweed sap, whereas minimum production cost (₹92310/ha) recorded in application of 6.25% K-sap+50% RDF (100:30:20 kg $NP_2O_5K_2O$/ha) treatment. The lowest net return of ₹60272/ha and B:C ratio of 1.65 was obtained with the application of 6.25% K-sap + 50% RDF in plant crop due to lower cane yield. The results confirm the findings of Meena *et al.* (2015) and Singh *et al.* (2015).

CONCLUSION

Thus, it was concluded that foliar spray of 10% G-sap along with 100% RDF (200:60:40 kg $NP_2O_5K_2O$/ha) to cane plant crop is productive and remunerative, as it gave higher cane yield and B: C ratio.

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Indian Journal of Sugarcane Technology **32(02)**: 95-97, December 2017

Genetic variation for anthocyanin content in sugarcane genotypes during winter season

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ABSTRACT

Ten varieties of sugarcane *viz.*, 'CoLk 8102', 'CoLk 8001', 'CoS 767', 'CoLk 9606', 'CoLk 9617', 'CoS 95255', 'BO 91', 'CoJ 64', 'Co 1148' and 'CoS 97624' planted under field conditions were evaluated for anthocyanin content during winter season. The accumulation of anthocyanin increased significantly with decrease in atmospheric temperature irrespective of genotypes tested; highest increase was observed in the month of January. Among the genotypes studied, maximum anthocyanin content was obtained in variety 'Co 1148' (2.755 µg per 100 cm^2) and minimum (0.433 µg per 100 cm^2) in variety 'CoS 767'. Higher accumulation of anthocyanin may protect sugarcane plants from oxidative damage during winter season and imparting resistance to cold stress.

Key words: Sugarcane, Low temperature stress, Anthocyanin.

Anthocyanins have been implicated in tolerance to stresses like drought, UV-B, and heavy metals, as well as resistance to herbivores and pathogens (Krupa *et al.* 1996; Chalker-Scott 2002; Close and Beadle 2003). Anthocyanins also mitigate photo-oxidative injury in leaves by efficiently scavenging free radicals and reactive oxygen species. Anthocyanins diminish the oxidative load in a leaf by filtering out yellow-green light, since the majority of reactive oxygen in plant cells is derived from the excitation of chlorophyll. In *Arabidopsis*, strong light and low temperatures caused more lipid peroxidation in anthocyanin-deficient mutants as compared to wild-type plants (Harvaux and Kloppstech 2001). Anthocyanins are associated with enhanced resistance to chilling and freezing, heavy metal contamination, desiccation, and to wounding (Close and Beadle 2003; Christie *et al.* 1994; McKown *et al.*1996; Nozzolillo *et al.* 2002; Oberbaueri and Starr 2002; Solecka and Kacperska 2003; Steyn *et al.* 2002). Chalker-Scott (2002) reported a generalized role of anthocyanins as osmoregulators in plant cells, since most types of suboptimal environments induce water stress, either directly or indirectly. Anthocyanins are also considered as stress indicators due to their involvement in the response to many stresses including high light and low temperature (Dong *et al.* 2018; Schulz *et al.* 2016). In subtropical India, low temperature stress serves as a severe constraint, limiting the productivity of sugarcane through bud injury, poor stubble sprouting and deterioration in juice quality (Singh 1987, Jain *et al.* 2007). Development of cold tolerant varieties is the only solution to save the crop from low temperature stress. Present investigation was aimed to study the genetic variability in anthocyanin accumulation potential of ten sugarcane genotypes during winter season as an indicator to impart resistance to cold stress and tolerate oxidative damage during winter season.

MATERIALS AND METHODS

Studies were made using ten sugarcane genotypes (Table 1) planted in the spring season at ICAR- Indian Institute of Sugarcane Research, Lucknow farm in a randomized block design with three replications. Each genotype was raised in three rows of 10 m length with a spacing of 75 cm between rows. Basal doses of N P K (180:80:80) were applied. LTM (Last transverse mark) leaves were used to determine anthocyanin content during winter season in the month of December and January. Anthocyanin content was determined in fresh leaves of ten sugarcane genotypes *viz.*, 'CoLk 8102',

Table 1 List of sugarcane genotypes (*Saccharum* spp. hybrids) used in the present study.

S. No.	Sugarcane genotype	Agronomic traits*
1	'CoLk 8102'	MLM, MS, MRRR
2	'CoLk 8001'	EM, HS, MRRR
3	'CoS 767'	MLM, HS, FT
4	'CoLk 9606'	MLM, HS, MRRR
5	'CoLk 9617'	MLM, MS, RRS
6	'CoS 95255'	EM, MS, MRRR
7	'BO 91'	MLM, MS, FT
8	'CoJ 64'	EM, HS, RRS
9	'Co 1148'	MLM, HS, HTV
10	'CoS 97624'	MLM, MS, RRR

*EM Early maturing, MLM-Mid-late maturing, HS = High sugar, MS = Medium sugar, FT = Flood tolerant, RRR = Red-rot resistant, MRRR = Moderate red-rot resistant, RRS = Red-rot susceptible, HTV = High tillering variety.

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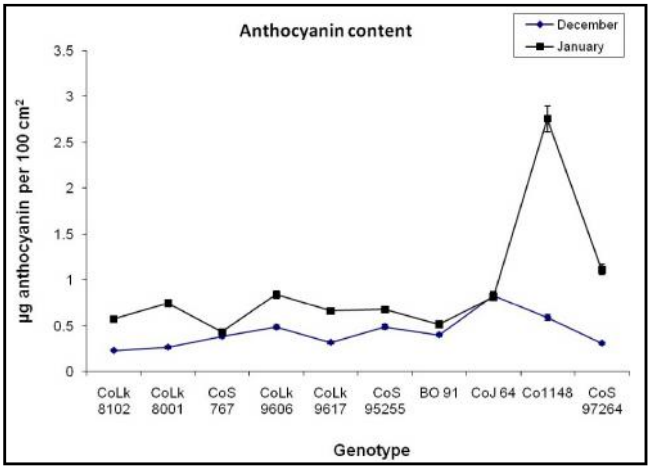
'CoLk 8001', 'CoS 767', 'CoLk 9606', 'CoLk 9617', 'CoS 95255', 'BO 91', 'CoJ 64', 'Co 1148' and 'CoS 97624' by the method of Mancinelli *et al*. (1991). Fresh leaf discs (10) were fixed in 5 ml 1% HCl in methanol and kept at 4°C for 48 hr. After 48 hr, absorbance of color solution was measured at 533 nm and 657 nm wavelength using UV-VIS spectrophotometer. The concentration of anthocyanin was calculated using formula as: [A533-(0.25 x A657)]. The data presented are the mean values of three replications and analyzed statistically for ± SE (standard error).

RESULTS AND DISCUSSSION

Anthocyanin concentration in sugarcane leaves showed considerable difference at different time intervals during winter season. Decreasing the atmospheric temperature led to an accumulation of anthocyanin in all the tested genotypes, but the level of accumulation varied among different genotypes. Anthocyanin content was in the range of 0.229 (in 'CoLk 8102') to 0.831 µg per 100 cm^2 (in 'CoJ 64') in December and 0.433 (in 'CoS 767') to 2.755 µg per 100 cm^2 ('Co 1148') in the month of January (Figure 1). Anthocyanin content was found to be highest in low temperature tolerant genotype, 'Co 1148' in the last week of January. It may be stated that the tolerant genotype 'Co 1148' may be associated with an accumulation of anthocyanin which increased the ability for survival under very low temperature conditions, while in genotypes 'CoJ 64' and 'CoS 767', the difference in anthocyanin content was almost negligible at different time intervals. The significance of anthocyanin accumulation in response to low temperature stress has been reported in crops other than sugarcane by several workers (Gloud *et al.* 2000; Steyn *et al.* 2002; McKnown *et al.* 1996). Anthocyanins are excellent scavengers of free radicals under low temperature condition (Close and Beadle 2003). Jain *et al.* (2007) earlier observed significant reduction in chlorophyll and carotenoids contents in sugarcane genotypes during winter season. In present study, higher increase in anthocyanin content in the month of January may be due to continuous low temperature for longer period.



Vertical bars represent ±SE

Fig. 1. Anthocyanin content of sugarcane genotypes in the months of December and January.

The findings obtained indicated a higher accumulation of anthocyanin content in sugarcane leaves in the month January as compared to December. Among all the tested genotypes, the genotype 'Co 1148' showed highest anthocyanin content which indicates that it is suitable as breeding material for developing sugarcane genotypes tolerant to cold stress which is one of the limiting factors in sugarcane production.

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INDIAN JOURNAL OF SUGARCANE TECHNOLOGY

Statements about Ownership and Other Particulars

Place of Publication	:	Lucknow
Periodicity of Publication	:	Half Yearly (June and December)
Publisher's Name	:	Dr. Sanjeev Kumar
Nationality and Address		Indian Hony. Secretary, The Association of Sugarcane Technologists of India, ICAR-Indian Institute of Sugarcane Research, Dilkusha P.O., Lucknow – 226002 India
Chief Editor's Name	:	Dr. Sangeeta Srivastava
Nationality and Address	:	Indian ICAR-Indian Institute of Sugarcane Research, Dilkusha P.O., Lucknow – 226002 India
Printer's Name and Address	:	Stuti Enterprises 326, Subhash Mohal, Sadar Lucknow - 226 002 India
Owner's Name and Address	:	The Association of Sugarcane Technologists of India, ICAR-Indian Institute of Sugarcane Research, Dilkusha P.O., Lucknow – 226002 India

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INDIAN JOURNAL OF SUGARCANE TECHNOLOGY

ISSN 0970-3233 Issue: Volume 32 No.2 December 2017

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