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Role of planting dates on production, juice quality, physiology and antioxidant enzymes of Sugarcane bud nodes under semi-arid conditions

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Abstract

Late plantation through conventional methods significantly decreased sugarcane production and juice quality due to high temperature and short period for growth. These causes lead to restricted physiological activities that ultimately resulted in the form of lower cane production. Timely planted sugarcane propagated through bud nodes improved sugarcane production; however, limited literature is available on planting dates of sugarcane especially bud nodes in improving sugarcane production. Sugarcane bud nodes were scooped with Bud chips/Bud node Scooping Machine (Intellectual Property Organization Karachi Pakistan; Patent Number 143641). The study was conducted during continuous years 2018 and 2019, to investigate the role of planting dates in improving production, juice quality, physiology and antioxidant enzymes growth of sugarcane propagated through bud nodes. Four planting dates, P_1 = 20th February, P_2 = 10th March, P_3 = 30th March and P_4 = 20th April were included in the study. Planting at 10th March significantly improved production, juice quality, physiological traits and antioxidant enzymes compared to 20th February, 30th March and 20th April plantation. During 2018 and 2019, 10th March led to 83 and 80% higher sprouting establishment, due to a significant increase in bud nodes led to increased number of millable canes (42.10 and 45.71%), cane height (31.66 and 23.57%), cane diameter (22.97 and 23.98%), cane yield (58.01 and 58.56%) at the harvest stage. Significant sprouting establishment increased the juice quality traits compared to 20th April sugarcane bud nodes planting. The March 10th planting increased total chlorophyll (10.02 and 10.37%), carotenoids (7.48 and 8.83%), total soluble sugars (2.88 and 2.89%), reducing sugars (2.52% and 2.81%), proline (1.78 and 2.16%), glycine betaine (4.90 and 4.45%). These significant changes were due to timely response of antioxidant enzymes in vivo. In Crux, 10th March planting date is recommended for higher yield and productivity.

Keywords: Antioxidant enzymes, Juice quality, Planting dates, Sprouting establishment, Sugarcane bud nodes

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Introduction

Sugarcane is planted in over 110 countries around the world. Sugarcane is a profitable crop in Pakistan and has a significant impact on its economy (Rasool & Arslan, 2019; Ali et al., 2021). Pakistan is fourth in terms of land and production, but it ranks 27th in terms of yield (Rahman, 2025). During 2021-22, sugarcane contributions in agricultural expansion of value and GDP are 3.7% and 0.8% respectively. However, its production in 2021-22 was significantly increased as compared to 2020-21 from 81.0 million tonnes to 88.6 million tonnes. The lower production in 2020-21 compared to 2021-22 was due to increase in area under sugarcane i.e. 1.16 million hectares to 1.26 million hectares (Govt. of Pakistan, 2021-22). Farming community in Pakistan heavily dependents on sugarcane production for their source of earning. Sugarcane is highly labor dependent crop for its different unit operations from planting to harvest. However, labor shortage at operations like planting, weeding, and harvesting, resulted in significant crop biomass losses. Economic analysis of sugarcane cultivation process indicates that cost share of different unit operations is 21% in root and seed bed preparation, 16% in setts planting, 10% in weeds control, 10% in nutrients application, 8% in irrigation management and 35% in crop harvesting, de-trashing and loading (Dharmawardene, 2006). The bumper sugarcane crop is heavily dependent on its per acre yield. Therefore, good quality seed, and its planting could increase the profit margin of the farmers.

In Pakistan, sugarcane is planted through stalk cuttings or setts due to the tremendous variability in the seed population (Rehman et al., 2022). Normally the per hectare used planting materials account for 10% of all sugarcane yield (Kumar & Kumar, 2020). There are several issues associated through the utilization of this massive amount of planting materials, including transport, management, more change disease or insect infestation and storage (Rehman et al., 2021, Rehman et al., 2022). To reduce planting materials cost and provision of healthy seed cane for planting, there are buds of sugarcane commonly called bud nodes/bud chip (Kumar & Kumar, 2020). These bud nodes/bud chips have characteristics of less massive, easily shifted from one place to another and economical. Therefore, by using bud nodes/bud chip technology approximately 80% of the seed material by weight can be saved (Kumar, 2020, Kumar et al., 2024). These bud nodes are suitable for commercial planting and have the ability to perform very well where conditions for cultivation are favorable (Jain, 2011; Kumar & Kumar, 2020; Rehman et al., 2022). Manual bud scooper is available for preliminary research; however, it is not feasible on a commercial basis. So, there was an urgent need to change traditional sugarcane production technology to mechanization to overcome these problems. Nowadays, a greater number of farmers showed their interest and adopting this bud nodes/bud chip technology in sugarcane planting as compared to the traditional planting method. Bud nodes/bud chip technique needs fewer seeds, lower water, and better land usage to gain maximum production (Biksham, 2011). Moreover, a little portion of tissue and even one root primordial are sufficient to improve the sprouting process (Loganandhan et al., 2013).

Sugarcane planting times are autumn and spring in Pakistan, having 420 and 360 days of growth duration. Generally, sugarcane planting date is the most variable factor that may influence crop productivity quality (Hoy et al., 2006). Furthermore, delays in planting affected the crop sprouting establishment and tillers number, which finally resulted in reduced number of millable canes (Rana et al., 2023). However, in March planted sugarcane resulted in higher cane yield due to prolonged period of growth duration. Moreover, planting date between 3rd week of February and 1st week of March are considered suitable for cane yield and its components due to favorable climatic conditions for its initial growth compared to the May plantation that having unsuitable climatic conditions (40-43°C) at sprouting stage (Okaz et al., 2011). In addition, the elevated temperature could reduce rate of CO_2 intake for photosynthesis that significantly decreased the photosynthetic and dry matter accumulation leading to lower number of tillers per plant. Furthermore, elevated temperature forces negative effect on assimilates synchronism and utilization among tillers that reduced number of tillers (Bita & Gerats, 2013). Physiologically, elevated temperatures reduced the moisture availability, lowering the rate of leaf development and shorter the internodal length at tillering phase (Bonnett et al., 2006).

The elevated temperature causes quick accumulation of abscisic acid and oxygen species that are reactive (Bonnett et al., 2006). As a result of high temperature, oxidizable oxygen produced caused membrane injury, dreadful conditions of protein, enzyme deactivation, deoxyribonucleic acid thread interference and deficiency of water (Maestri et al., 2002). Above disturbance due to excess of reactive oxygen leads to damage biomolecules of cells that restricted crop developmental and physiological traits (Imlay, 2003). Malondialdehyde is utilized for measurement of lipid peroxidation damage in high temperatures (Lokhande et al., 2011). The destructive nature of plant cells manages reactive oxygen species by producing lipid antioxidants, water reductants and antioxidant enzymes production (Radhika Desikan et al., 2004). Lack of research creates a need for comprehensive studies that not only assess the effects of temperatures on sugarcane growth and production propagated through bud nodes but also identify the most effective planting strategies under varying thermal and climatic conditions, particularly shifting environments of Pakistan. Moreover, no research is available on the consequences of different planting dates in the sugarcane propagated through bud nodes. This study hypothesizes that planting sugarcane via bud nodes during the optimal early spring planting dates window will lead to improved sprout establishment, increased tiller numbers and superior juice quality. Two-year consecutive field experiments were conducted with aim 1) to compare planting dates for production, physiology, juice quality and antioxidant enzymes 2) to identify the suitable planting date of sugarcane production propagated through bud nodes.

Materials and Methods

A two-year research trial was conducted at the College of Agriculture, University of Sargodha, Sargodha 32.08°N, 72.67°E, and 193 m height, during the sugarcane growing season of 2018 and 2019. Hydrometer methods were utilised for soil textural determination, which was sandy clay loam and was suited for the Hafizabad soil series (fine-loamy, mixed, hyperthermic, Typic Calciargids) (Khan, 1986). In research trial, water absorbency and retention were sustained at 33% and 70%, respectively. Dry soil was pulverized at size of 2 mm before the trial, adopting the methodology of (Ryan et al., 2001). Soil physio-chemical examination is given in Table 1. Sargodha's climate is subtropical to semiarid, with an annual rainfall of 400±5 mm. Monsoon spells began in July and finished in September, receiving 70% of rain. In the winter, the average lowest temperature was around 10 °C (Fig. 1).

Table 1 Physio-chemical characteristics of experimental site at College of Agriculture, University of Sargodha, Sargodha, Pakistan

Soil characteristics	2018			2019		
	0-15 cm	15-30 cm	Mean	0-15 cm	15-30 cm	Mean
pH of soil	7.8	7.5	7.7	7.7	7.6	7.7
EC (dS m^{-1})	2.19	2.08	2.13	2.22	2.09	2.15
Organic matter (%)	0.72	0.58	0.70	0.73	0.60	0.67
Total Nitrogen (mg kg^{-1})	4.12	3.95	4.03	4.16	3.96	4.06
Available Phosphorus (mg kg^{-1})	6.59	6.32	6.45	6.60	5.43	6.01
Available Potassium (mg kg^{-1})	265	247	256	268	255	261
Texture	Sandy clay loam			Sandy clay loam		

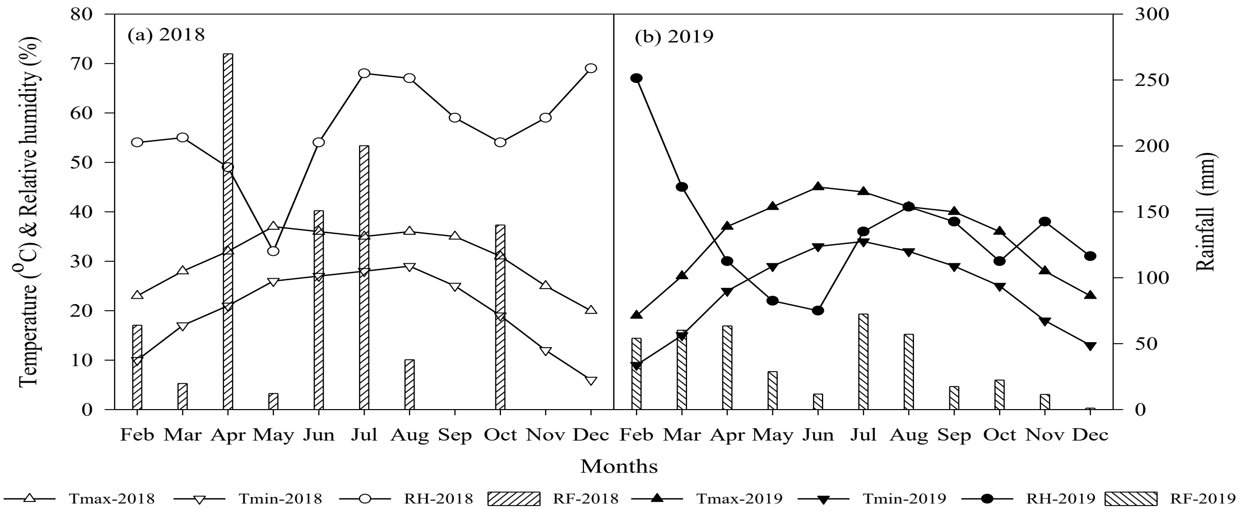

Fig. 1 Weather conditions exist throughout study at College of Agriculture, University of Sargodha, Sargodha, Pakistan in 2018 and 2019

Study layout and treatments

Field treatments were arranged in a randomized complete block design with 04 replicates making net plot size of 9 m × 36 m. Sugarcane (*cv*. CPF-237) bud nodes were planted at 400 kg per ha keeping row-to-row distance of 75 cm and a plant-to-plant distance of 45 cm for manual planting. The main objective was to assess the role of planting dates of sugarcane bud nodes *viz*., P_1 = 20th February, P_2 = 10th March, P_3 = 30th March and P_4 = 20th April on production, juice quality, physiology and antioxidant defense system in semi-arid condition.

Bud nodes were extracted from eight months old cane *cv*. CPF-237 having inter-nodal length of 15-18 cm. At the time of planting, bud nodes were soaked for 3 to 4 minutes in Thiophanate methyl 1.5 g per liter of water. Then sugarcane bud nodes dried under shade. Sugarcane bud nodes were taken by working on Bud chip/Bud node Scooping Machine (Fig. 2; Patent Number 143641) of Sugarcane engineered and made-up by the Department of Agronomy, College of Agriculture, University of Sargodha, Sargodha, Pakistan and the Department of Agronomy, Bahauddin Zakariya University, Multan, Pakistan.

Preparation of the bud nodes

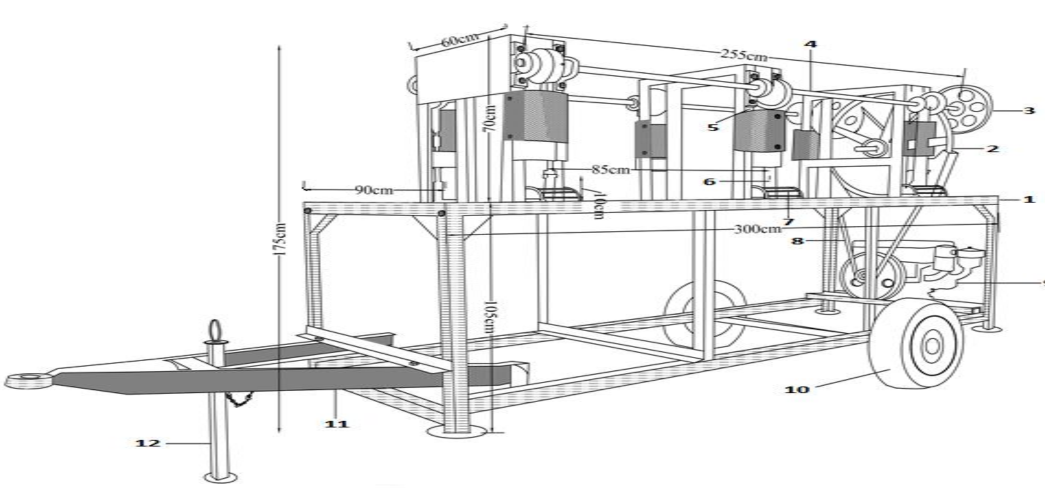

Fig. 2 Bud chip/bud node scooping machine (Basic parts; 1- Main frame; 2- Large pulley; 3-Small pulley; 4- Shaft/beam; 5- Cam; 6- Cutter; 7- Protecting cover; 8- Belt; 9- Peter engine; 10- Wheel; 11- V-shaped hook; 12- Stand) (Patent submitted; Intellectual Property Organization Karachi Pakistan; Electronic Publication of Patents Journal under The Patents (Amendments) Act, 2016; (Patent Number 143641)

Crop management

Overall, 80 bud nodes were physically planted in each line at 3.8 cm depth. on respective dates of 20th February, 10th March, 30th March and 20th April 2018 and 2019. The soil of experimental units was tested and maintained up to 70% by weight of soil water on regularly. The recommended N: P: K dosages 120: 70: 50 kg ha^{-1} were mixed into the field. Nitrogen was utilized in three splits, with 1/4th put into the soil before planting, coupled with total phosphorus and potassium fertilizers. At cultural practice of earthing up, the rest of nitrogen amount was fertilized in three portions. Bud nodes were grown until physiological maturity than study was stopped, 2018 and 2019. The entire plant was harvested, detopped, detrashed, and tied by hand to produce bundles to record cane yield by floor weighing balance for each study unit. During the research period, appropriate agronomic procedures were implemented, and crops were kept pest-free by using pesticide.

Data collection

Yield and yield traits

Plants were tagged in each research plot and examined on a regular basis for calculating sprouting establishment percentage by using following formula:

$$SP = \frac{Sprouted\ buds}{Total\ buds\ planted} \times 100$$

The number of mill-able canes were noted from 1 m^2 in each experimental area at maturity. Cane length (cm) was measured at maturity using a meter rod on 10 randomly selected cane from every experimental unit and then averaged. Cane diameter (cm) was measured using a Vernier caliper (Truper, CALDI-6MP) from top, middle and bottom of the plants and averaged. The stripped yield was measured by cutting the cane. Each experimental unit was cut, removed trash, weighed on floor balance and converted into Mg per ha.

Juice quality traits

Chemical analysis of sugarcane juice was noted at harvest. Brix (%) defined total soluble solids, pol (%) defined percentage of sucrose and purity (%) defined the ratio of sucrose. Three samples were chosen at random and processed to juice extraction using a cane crusher having 70% juice extraction ability at the Sugarcane Technology Laboratory in Faisalabad. Brix (%) was measured in a glass cylinder of cane juice using a Brix Hydrometer. The solution was purified by filtering, and the filtrate was put into a 200 mm Polarimeter tube for the measurement of juice quality traits using Chen & Chou (1993) technique.

Analysis of chlorophyll and carotenoid

For total chlorophyll and carotenoid, leaf having 200 mg FW was crushed in acetone (80% v/v) mortar. At the completion of extraction, the extracts were passed through filter paper then adjusted volume up to 10 mL with acetone. Sample was passed through a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Japan) and absorbance was noted at 664, 647, and 470 nm according to Lichtenthaler (1987).

Analysis of total soluble sugars

Watanabe et al. (2000) method was used to determine total soluble sugars. This was carried out by mixing a 200 mg sample in 10 mL of 80% v/v ethanol at 6000 rpm for 10 minutes at 4°C. Tubes were heated at 100°C for 10 minutes after adding 1 mL of supernatant to 3 mL of newly made Anthrone reagent. It was immediately refrigerated with ice to halt the reaction, and the absorbance at 620 nm was then passed. The total soluble sugars (mg g^{-1} FW) were calculated by means of glucose as regular.

Analysis of reducing sugars

Standardize the 100 mg sample in ethanol (80%). The sample was homogenized at 6000 rpm for 10 minutes at 4°C to prepare a homogeneous sample, and final product was combined with same amount of 3, 5-dinitro-salicylic acid (DNSA) reagent. As an alternative to the supernatant, distilled water was utilized to prepare the blank sample. After vortexing the reactants, glass tubes were put in hot water for 10 minutes then cooled on ice. The absorption was determined at 540 nm and reducing sugars (mg g^{-1} FW) were found by using a standard graph with glucose.

Analysis of proline

Bates et al. (1973) methodology was applied to estimate the proline contents. A 200 mg leaves sample was mixed in liquid sulfosalicylic acid (3%). Then filtrate material was then carried through a reaction with an equal amount of acid ninhydrin and acetic acid at 100°C for 1-hour and ice bath was used to stop reactions. Reaction solution containing 4 mL solvent was vigorously blended with a stirrer for 15 seconds. Solvent mixture having chromophore was removed and heated at normal condition. Transmission density measured at 520 nm by means of solvent as a blank. L-proline is applied to determine proline content (mg g^{-1} FW).

Analysis of glycine betaine

By using the Grieve & Grattan (1983) procedure, glycine betaine was measured. Specifically, 3 mL of purified water was agitated with 200 mg of fine material for 16 hours at 25°C after 200 mg of leaves were mashed in liquid nitrogen. Following that, samples were filtered and diluted with 2N H_2SO_4 (1:1). 500 mL of the diluted sample was collected, chilled in an ice bath for an hour, and blended with 200 mL of the potassium iodide-iodine mixture. Following the conclusion of the 16-hour development period at 4°C, the glass tubes were centrifuged at 10,000 rpm for 15 minutes at 0°C. Crystal of per-iodide developed during the centrifugation process were dissolved by adding 9 mL of 1, 2-dichloromethane within 2 hours, and transmission density was recorded at 365 nm. From the standard graph, the glycine betaine content (mg g^{-1} FW) was calculated.

Analysis of lipid peroxidation

Lipid breakdown was determined using the approach developed by Heath & Packer (1968) method after slight amendments to determine the levels of Thio barbituric acid reactive compounds (TBARS). 400 mg of leaf sample was crushed for this purpose in pre-chilled liquid nitrogen, and 10 mL of trichloro acetic acid-TCA was mixed into the sample to make it homogenous. The extract was homogenized for 10 minutes at 10,000 rpm. After homogenization, an equivalent amount of 2-thiobarbituric acid was mixed than mixture heated at 100°C for 30 minutes. Sample was rapidly placed in an ice bath to cease the reaction. After centrifuging at 5000 rpm for 60 seconds, absorbance at 532 nm using TBA in TCA as a blank was measured. The density value reported at 600 nm was deducted to precise the non-precise turbidity. Malondialdehyde was measured applying an action constant ($\mathcal{E}$ = 155 mM^{-1} cm^{-1}) and showed as mol TBARS g^{-1} FW.

Antioxidant enzyme tests

Extraction

Fresh leaf samples weighing 200 mg were used for enzyme examination. The enzyme extraction process took place entirely at 4°C. The extract was blended in a 3 mL refrigerator containing 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA and polyvinyl pyrrolidone (PVP 1%). The well blended material spun up for 20 minutes at 15,000 rpm at 4°C. For determining antioxidant enzyme tests, the remaining fluid was employed to extract basic enzymes. Every enzyme test was carried out at room temperature, and a spectrophotometer was employed to record enzymes' activities.

Superoxide dismutase (SOD) test

Superoxide dismutase movement was checked in relation to suppression in photochemical decline of NBT-nitro blue tetrazolium (Beyer & Fridovich, 1987). Solution having volume of 3 mL containing 2.2 mM riboflavin, 14.3 mM methionine, 82.5 mM NBT, and 50 mM phosphate buffer (pH 7.8). NBT reduction that was computed in reaction solution with all constituents except enzyme was boosted by free radicals. Reaction was conducted by placing the test tubes below 30 cm of six fluorescent tubes having 15W of each for 30 minutes then switched off the light to stop reactions. After that 100 mL of enzyme and all reactants were extracted and raised in dark blank. By tracking the shift in absorbance at 560 nm, the NBT decrease was estimated. Dark blank reading was utilized for calculation of enzyme units. Enzyme movement was showed as unit mg^{-1} protein.

Catalase (CAT) test

Catalase test based on the breakdown of H_2O_2 as explained by Cakmak & Marschner (1992) using few changes. For this purpose, 1 mL of reaction solution has 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2. Enzyme solution of 50 ml was added to begin the reactions. Movement was checked by measuring the lower value of absorbance at 240 nm ($\mathcal{E}$ = 36 mM^{-1} cm^{-1}), every 15 seconds for two minutes. The enzyme reaction was calculated by using slope of assay (DA), which revealed itself as mKat mg^{-1} protein.

Ascorbate peroxidase (APX) test

Ascorbate peroxidase was found through using the methodology of Nakano & Asada (1981). A 2 mL reaction liquid with 100 mL of enzyme mixture, 50 mM buffered sodium phosphate (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate, and 1 mM H_2O_2 is required for activity. Minimum transmission density at 290 nm ($\mathcal{E}$ = 2.8 mM^{-1} cm^{-1}) was used to track the reaction of ascorbate that was reliant on hydrogen peroxide. APX action was shown in terms of mKat mg^{-1} protein.

Statistical method

Study treatments were examined by applying randomized complete block design. SAS program (Edition 9.1; USA) (SAS Institute, 2008) was applied for data calculation (ANOVA) and averages were distinguished by using Least Significant Difference test at 0.05 level of significance (Steel, 1997). Regression equations were calculated through (Minitab, 2000) (Edition 12, USA).

Results

Growth and yield parameters

Planting dates had dominant role in sugarcane growth and yield parameters (Table 2). In the 2018 and 2019 growing season, 10th March had significantly higher sprouting establishment over others (Table 2). During 2018, 10th March had 35 and 19% higher sprouting establishment than on 20th April and 30th March. Sprouting establishments of 10th March and 15th February was at par with each other. Similarly, sprouting establishment of 10th March was 35% and 22.5% higher than on 20th April and 30th March planting date during the 2019 growing season. In 2018, 10th March had 42% and 26.3% higher millable cane m^{-2} than on 20th April and 30th March. Millable cane m^{-2} of 10th March and 15th February was at par with each other. Similarly, millable cane m^{-2} of 10th March was 46% higher than on 20th April planting date during the 2019 growing season. Moreover, millable cane m^{-2} of 30th

March and 20th February planting were at par with each other. In 2018 to 2019, the 10th of March produced 32, 26 and 18% taller cane than on 20th April, 30th March and 20th February, respectively. However, the 20th February produced the 17 and 10.5% taller cane height than on 20th April and 30th March (Table 2). In both seasons, 10th March had the higher cane diameter of sugarcane compared with 20th April, 30th March and 20th February (Table 2). In the 2018 season, sugarcane yield was significantly higher (70.5 Mg ha^{-1}) on 10th March as compared with 20th April, 30th March and 20th February. The 10th of March gave 58 to 18% higher cane yields than 20th April, 30th March and 20th February. Similarly, sugarcane yield was significantly higher (68.3 Mg ha^{-1}) on 10th March compared with 20th April, 30th March and 20th February during 2019 growing season. Similarly, the highest total cane yields (70.5 and 68.3 Mg ha^{-1}) were attained on 10th March over other treatments (Table 2). Cane production during 2019 was 3% lower than 2018 growing season due to higher maximum and minimum temperature and 58% lower rainfall during growth stages (Table 1). The regression analysis (Table 6) showed significant ($p \leq 0.05$) result of planting dates on sugarcane propagated through bud nodes growth and yield. Moreover, late planting reduced growth and yield parameters gradually.

Juice quality parameters

Planting dates had significant effects on juice quality parameters (Table 3). The brix % significantly decreased over time under all planting dates treatments except 10th March. In the 2018 to 2019 growing season, the brix % under 20th April decreased by 1.2, 2.2 and 3.3% as compared to that of 30th March, 20th February and 10th March. Similar effects of planting dates on brix % were also observed in the 2019 growing season. In both growing seasons, pol % juice was highest on 10th March, and lowest on 20th April (Table 3). In 2018 to 2019, the 10th March had 7% higher pol % juice than on 20th April. Moreover, the 10th of March had 4.2% more pol % cane in both growing seasons than on 20th April. In the 2018 to 2019 growing season, purity was significantly higher on 10th March than other planting dates treatments. Regression analysis depicted that planting dates had significant ($p \leq 0.05$) result on the cane juice quality parameters of sugarcane (Table 6).

Table 2 Role of planting dates of sugarcane propagated through bud nodes on sprouting establishment, millable canes, cane height, cane diameter and cane yield during 2018 and 2019

Treatments	Sprouting establishment (%)		Millable canes (m^{-2})		Cane height (cm)		Cane diameter (cm)		Cane yield (Mg ha^{-1})	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
P_1= 20th February	76 a	72 a	30 ab	27 b	210.33 b	192.80 b	2.61 b	2.59 b	58.1 b	57.4 b
P_2= 10th March	83 a	80 a	38 a	35 a	255.58 a	212.40 a	2.96 a	2.96 a	70.5 a	68.3 a
P_3= 30th March	67 b	62 b	28 b	24 bc	188.36 c	178.52 c	2.45 c	2.40 c	47.4 c	45.1 c
P_4 = 20th April	54 c	52 c	22 b	19c	174.65 d	162.32 d	2.28 d	2.25 d	29.6 d	28.3 d
SE (M)	4.082	3.316	3.488	3.266	3.266	3.266	0.040	0.037	1.633	2.449
$LSD_{0.05}$	9.414	7.648	8.043	7.531	7.531	7.531	0.094	0.085	3.765	5.648

Four treatments (P_1 = 20th February planting, P_2 = 10th March planting, P_3 = 30th March planting, P_4 = 20th April planting; SE (M) = Standard error of mean; n = 04; LSD = Least significant difference). Average value of the columns with the same letter did not vary significantly at $p \leq 0.05$

Table 3 Role of planting dates of sugarcane propagated through bud nodes on brix (%), pol % juice, pol % cane and purity (%) during 2018 and 2019

Treatments	Brix (%)		Pol % juice		Pol % cane		Purity (%)	
	2018	2019	2018	2019	2018	2019	2018	2019
P_1 = 20th February	20.29 b	20.28 b	19.19 b	19.18 b	16.01 b	16.01 b	87.12 b	87.10 a
P_2 = 10th March	20.52 a	20.52 a	19.85 a	19.85 a	16.25 a	16.23 a	87.52 a	87.51 a
P_3 = 30th March	20.10 c	20.11 c	18.73 c	18.75 c	15.85 c	15.84 c	86.25 c	86.24 a
P_4 = 20th April	19.84 d	19.85 d	18.52 d	18.52 d	15.57 d	15.55 d	85.47 d	85.47 a
SE (M)	0.034	0.024	0.016	0.016	0.024	0.016	0.032	2.309
$LSD_{0.05}$	0.080	0.056	0.037	0.037	0.056	0.037	0.075	5.325

Four treatments (P_1 = 20th February planting, P_2 = 10th March planting, P_3 = 30th March planting, P_4 = 20th April planting; SE (M) = Standard error of mean; n = 04; LSD = Least significant difference). Average value of the columns with the same letter did not vary significantly at $p \leq 0.05$

Table 4 Role of planting dates of sugarcane propagated through bud nodes on total chlorophyll, carotenoids content, total soluble sugars, reducing sugars, proline and glycine betaine during 2018 and 2019

Treatments	Total chlorophyll (μg g^{-1} FW)		Carotenoids content (μg g^{-1} FW)		Total soluble sugars (mg g^{-1} FW)		Reducing sugars (mg g^{-1} FW)		Proline (μg g^{-1} FW)		Glycine betaine (μg g^{-1} FW)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
P_1 = 20th February	1051.23b	1048.14b	176.21b	174.23b	4.12ab	4.10ab	3.55a	3.54ab	7.82ab	7.80ab	246.12b	244.48ab
P_2 = 10th March	1121.74a	1118.24a	182.52a	181.48a	4.16a	4.15a	3.56a	3.55a	7.86a	7.85a	249.71a	247.42a
P_3 = 30th March	1012.32c	1008.21c	170.25c	167.15c	4.09ab	4.08bc	3.50a	3.48bc	7.78bc	7.75b	241.52c	240.48bc
P_4 = 20th April	1008.47d	1002.25d	168.85c	165.45c	4.04b	4.03c	3.47a	3.45c	7.72c	7.68c	237.45d	236.40c
SE (M)	1.633	2.449	0.816	1.633	0.040	0.028	0.040	0.028	0.033	0.028	0.816	2.160
$LSD_{0.05}$	3.765	5.648	1.882	3.765	0.094	0.066	0.094	0.064	0.077	0.065	1.882	4.981

SE (M) = Standard error of mean; n = 04; LSD = Least significant difference). Average value of the columns with the same letter did not vary significantly at $p \leq 0.05$.

Table 5 Role of planting dates of sugarcane propagated through bud nodes on MDA content (μmol g^{-1} FW), SOD activity (U mg^{-1} protein), CAT activity (mKat mg^{-1} protein) and APX activity (μKat mg^{-1} protein) during 2018 and 2019

Treatments	MDA content (μmol g^{-1} FW)		SOD activity (U mg^{-1} protein)		CAT activity (mKat mg^{-1} protein)		APX activity (μKat mg^{-1} protein)	
	2018	2019	2018	2019	2018	2019	2018	2019
P_1 = 20th February	10.02 b	10.01 b	50.12 b	50.08 b	1.63 a	1.62 a	5.05 a	5.03 a
P_2 = 10th March	10.12 a	10.10 a	50.37 a	50.36 a	1.65 a	1.64 a	5.08 a	5.07 a
P_3 = 30th March	9.85 c	9.84 c	49.45 c	49.40 c	1.57 b	1.55 b	4.98 b	4.96 b
P_4 = 20th April	9.72 d	9.70 d	49.02 d	48.92 d	1.52 c	1.50 b	4.95 b	4.93 b
SE (M)	0.024	0.025	0.023	0.033	0.016	0.023	0.020	0.027
$LSD_{0.05}$	0.056	0.059	0.054	0.076	0.377	0.054	0.048	0.064

Table 6 Regression analysis of various studied sugarcane propagated through bud nodes parameters 2018 and 2019

Parameters	A		B		R^2		Probability	
	2018	2019	2018	2019	2018	2019	2018	2019
Sprouting establishment %	86.0±10.20	90.5±10.01	-7.8±3.72	-8.2±3.65	0.68	0.71	0.17	0.15
Millable cane m^{-2}	35.0±7.42	38.0±7.40	-3.5±2.71	-3.4±2.70	0.45	0.44	0.32	0.33
Cane height (cm)	217.8±20.74	250.7±41.04	-12.5±7.57	-17.4±14.98	0.57	0.40	0.23	0.36
Cane diameter (cm)	2.9±0.34	2.9±0.32	-0.1±0.12	-0.1±0.11	0.44	0.44	0.33	0.33
Cane yield (Mg ha^{-1})	77.4±14.33	78.5±15.27	-11.1±5.23	-10.8±5.57	0.69	0.65	0.17	0.19
Brix (%)	20.6±0.26	20.6±0.26	-0.1±0.09	-0.1±0.09	0.60	0.62	0.22	0.20
Pol % juice	19.8±0.64	19.8±0.64	-0.3±0.23	-0.3±0.23	0.46	0.47	0.32	0.31
Pol % cane	16.4±0.26	16.4±0.26	-0.1±0.09	-0.1±0.09	0.60	0.63	0.22	0.20
Purity (%)	88.1±0.66	88.1±0.66	-0.6±0.24	-0.6±0.24	0.76	0.76	0.12	0.13
Total chlorophyll (μg g^{-1} FW)	1107.9±63.97	1106.1±64.13	-23.7±23.36	-24.7±23.41	0.34	0.35	0.41	0.40
Carotenoids content (μg g^{-1} FW)	183.0±6.60	182.2±7.67	-3.4±2.41	-4.0±2.80	0.50	0.51	0.29	0.28
Total soluble sugars (mg g^{-1} FW)	4.2±0.04	4.1±0.04	-0.0±0.01	-0.0±0.02	0.62	0.54	0.20	0.26
Reducing sugars (mg g^{-1} FW)	3.6±0.02	3.5±0.02	-0.0±0.01	-0.0±0.01	0.83	0.83	0.09	0.08
Proline (μg g^{-1} FW)	7.9±0.04	7.8±0.06	-0.0±0.02	-0.0±0.02	0.66	0.67	0.18	0.18
Glycine betaine (μg g^{-1} FW)	252.3±4.52	249.9±3.91	-3.4±1.65	-3.1±1.42	0.68	0.70	0.17	0.16
MDA content (μmol g^{-1} FW)	10.2±0.14	10.2±0.13	-0.1±0.05	-0.1±0.04	0.72	0.75	0.15	0.13
SOD activity (U mg^{-1} protein)	50.8±0.43	50.8±046	-0.4±0.15	-0.4±0.17	0.77	0.77	0.12	0.12
CAT activity (mKat mg^{-1} protein)	1.7±0.03	1.6±0.04	-0.0±0.014	-0.0±0.01	0.80	0.81	0.10	0.09
APX activity (μKat mg^{-1} protein)	5.1±0.04	5.1±0.05	-0.0±0.01	-0.0±0.02	0.73	0.66	0.14	0.18

Physiological characteristics

Planting dates effect on physiological characteristics of sugarcane propagated through bud nodes were significant statistically in both years 2018 and 2019 as depicted in Table 4. In the 2018 growing season, 10th March was 10.1, 9.8 and 6.3 higher total chlorophyll than on 20th April, 30th March and 20th February, respectively. Similar effects of planting dates on total chlorophyll were also observed in the growing season of 2019. During 2018, 10th March had 7.5, 6.7 and 3.5% higher carotenoids content than on 20th April, 30th March and 20th February, respectively. Carotenoids content of 20th April and 30th March were at par with each other. Similarly, carotenoids content of 10th March was 8.7, 7.8 and 4% higher than on 20th April, 30th March and 20th February planting date during the 2019 growing season (Table 4). In both growing seasons, total soluble sugars were higher on 10th March, and lower on 20th April (Table 4). During 2018 to 2019, 10th March had 2.8% higher total soluble sugars than on 20th April. Total soluble sugars of 10th March, 15th February and 30th March were statistically at par with each other in 2018 while 10th March and 20th February were at par in 2019. In 2018, planting dates had a non-significant effect on reducing sugars while in 2019, 10th March had 2.8% higher reducing sugars than on 20th April. Moreover, the 10th March had 1.8% more proline in both growing seasons than on 20th April (Table 4). In the 2018 to 2019 growing season, glycine betaine was significantly higher on 10th March than other planting dates treatments. In 2018, 10th March had 4.9% and 3.3% higher glycine betaine than on 20th April and 30th March. Similarly, glycine betaine of 10th March was 4.5% higher than on 20th April planting date during the 2019 growing season. Moreover, glycine betaine of 10th March and 20th February and 30th March and 20th April were at par with each other. Analyzing the regression results (Table 6) indicated that the physiological parameters of sugarcane propagated through bud nodes were reduced by the late planting.

Antioxidant metabolism

The effect of planting dates on antioxidant enzymes of sugarcane propagated through bud nodes were significant in both years 2018 and 2019 as depicted in Table 5. In the 2018 growing season, 10th March was 4, 2.6 and 1% higher MDA than on 20th April, 30th March and 20th February, respectively. Similar effects of planting dates on total chlorophyll were also observed in the 2019 growing season (Table 5). In the 2018 to 2019 growing season, higher SOD activity was observed on 10th March (by 2.7%) planting plants over the 20th April. In the 2018 to 2019 growing season, 10th March and 20th February had significantly higher CAT activity over others (Table 5). During 2018, 10th March had 7.9 and 4.8% higher CAT than on 20th April and 30th March. Similarly, CAT activity of 10th March was 8.5% and 5.5% higher than on 20th April and 30th March planting date during the 2019 growing season. During both years, CAT activity of 10th March and 15th February was at par with each other. During 2018 to 2019 growing season, 10th March and 20th February had significantly higher APX activity over others (Table 5). During 2018, 10th March and 20th February had 2.6% higher APX than on 20th April and 30th March. Similarly, the same trend was recorded during 2019. During 2018 to 2019 growing season, APX activity of 10th March and 15th February and 30th March and 20th April were at par with each other. Regression analysis depicted that planting dates had statistically ($p \leq 0.05$) improved the antioxidant metabolism of sugarcane (Table 6).

Discussion

A two-year field study was planned to know the effect of planting dates having an impact on sugarcane bud nodes technique through a change in the yield, juice quality, physiology, and enzymes activity. Planting dates 10th March resulted significant effects on sprouting establishment, millable cane, height, diameter, and yield. Our outcomes corroborate with the results of (Rana et al., 2001; Rehman et al., 2022), noticed major differences in production of cane at different planting dates and concluded that timely planting produced maximum growth and yield traits, while the least growth and development of sugarcane was obtained in late sowing. The reason behind low sprouting establishment on 20th April was due to maximum and minimum temperatures that resulted in mortality in eyes of bud nodes Singh et al. (2014) in which higher temperatures deteriorate the capability of eyes of bud nodes. Higher millable cane m^{-2} was noted in treatment in which bud nodes were sown on 10th March. This was due to survival of seedlings in this treatment (Ghaffar et al., 2012). The lower number of millable canes could be due to higher temperatures in May. Results in Table 2 illustrate that cane height and diameter were mainly affected by planting dates during both growing seasons. Planting dates of 10th March contributed maximum cane yield compared with planting sugarcane on 20th April. High temperature restricts the tillering ability of plants (Rana et al., 2023). Higher cane per m significantly improved the stripped yield and it is considered a vital attribute of sugarcane yield (Loganandhan et al., 2013).

The present study proved a strong relationship between the planting dates and yield traits. The possible reason for lower millable cane, cane height and diameter on 20th April due to high temperature that might lead to lower production of millable cane, cane height and diameter. Late planting reduced the plant height due to high temperature which supported the results of earlier studies that maximum temperature reduced cell division and elongation rate by disturbing cellular and photosynthetic function. Similar outcomes were concluded by Rana et al. (2023), late planting could reduction yield traits like millable cane, cane height and diameter. Our findings supported the results of El-Gergawy et al. (2000) that delay in planting showed significant reduced in yield traits. Other authors also reported similar findings, might be due to early sprouting establishment that reduced the chances of high and low temperature shocks. Higher cane yield (70.5 and 68.3 Mg ha^{-1}) was achieved where bud nodes seedling planted on 10th March and did not restore on 20th April due to increase in temperature as stated by Jain et al. (2014). Higher cane production in timely planting (10th March) might be increased in rate of photosynthesis due to maximum leaf growth and thereby improved cane yield. The above findings were in line with Munsif et al. (2018), they reported higher cane yield in timely planting.

Our results showed minimum cane yield on 30th March and 20th April planting dates. The potential cause for minimum yield in late planting might be reduction in sprouting establishment, millable cane m^{-2}, cane height and diameter. Our findings confirmed the conclusions of Balole (2003), who concluded that maximum cane production in timely planting was linked with maximum growing time from emergence to tillering and stem elongation stage; and increased millable cane and height. Maximum cane yield in timely planting was probably increased in root length of cane, which facilitated the newly germinated bud to start growth than delayed planted. Significant improvement in yield traits at appropriate planting time might be the existence of suitable environment for growth than delay planting (Shahzad et al., 2002). The capability of the timely planted cane to capture photo active light and produce photosynthate (Andrade et al., 2002). Jintrawet et al. (2000) published that timely plantation produced significant production. The variation in sugarcane yield was characteristic to change in sugarcane height achieved during growth duration in different planting dates (Mohamed & El-Taib, 2007; Okaz et al., 2011).

As experimental results proved the beneficial effect of timely sowing (10th March) on juice quality traits have been discussed in detail in the results section. Our study encouraged the conclusion of Almodares & Darany (2006) that significantly maximum brix, pol percentage and purity was noted in timely plantings. The required output of timely planting on juice quality might be credited to the favorable environment during both years 2018 and 2019 (Sanghera et al., 2015). Longer time duration enables the sugarcane to produce higher photosynthate in early planting than late planting after 10th March. Our results supported the results of Balole (2003) only in case of cane yield that timely planting improved the cane production through improvement in millable cane and stem height, but lowered juice quality, therefore a negative relationship exists between cane yield and juice quality. However, our study findings corroborate the results of several research that there is an encouraging association existing between cane yield and juice quality. Both stalk yields and brix value were higher on 10th March planting than the late planting. Significantly lower juice quality traits were recorded in late planting which might be short growing season that leads to the higher ratio of young internodes than old internodes, resulting in the form of lower sucrose content in the whole stalks, ultimately the purity of the juice lowered than in a timely planting. Our study strengthens the outcomes of Inman-Bamber et al. (2002), they concluded that sucrose concentration was associated with ratio of young internodes that had lower sucrose concentration while older internodes had higher sucrose concentration. Furthermore, Lingle et al. (2009) concluded that late planting, developed short internodes, had lower sucrose contents due to short growth period. Publicize significantly higher juice quality traits were recorded in timely planting (Kabiraj et al., 2007; Rahman et al., 2010).

Both years' experimental results proved that 10th March bud nodes planting contributed to a significantly higher physiological traits compared to the 30th of March and 20th April. However, to the best of our knowledge, there is not much research reported yet to find the influence of planting dates on sugarcane physiological traits. Based on our study during 2018 and 2019, significantly higher physiological traits were recorded on 10th March compared to 30th March and 20th April however, higher protein contents were recorded on 20th April. Lower physiological traits in late planting might be short growing seasons, higher mean maximum and minimum temperatures, and lower humidity that led to a higher ratio of young internodes than old internodes, resulting in the form of lower physiological traits in the whole stalks than in a timely planting. Furthermore, lower physiological traits on 20th April could be delayed in planting due to high temperature and lower humidity which could consequently produce less plant growth and development. Significantly higher total chlorophyll contents due to improved light interception and utilization which leads to higher cane yield.

Our results encouraged by the study of Zhang et al. (2009), they concluded that higher total chlorophyll content improves photosynthetic efficiency which leads to higher cane yield. Positive correlations have been reported between total chlorophyll contents with stomatal opening and production. Parry et al. (2011) also concluded the same findings that enhanced stomatal conductance and chlorophyll contents increased the cane yield. Delayed planting (after 10th March) crop faced environmental stress at initial stage which influence the leaf chlorophyll and carotenoids contents (Paknejad et al., 2007). Furthermore, da Silva et al. (2006) found the same findings and concluded that environmental stress in form of high temperatures and moisture stress caused a decline in sugarcane leaf carotenoids contents. Late planting crops have lower chlorophyll and carotenoids content due to high temperature and moisture deficit at initial growth stages and high transpiration rate. Study results were in line with the conclusion of Daneshmand et al. (2010), stomatal conductance and chlorophyll content decreased as the moisture content of the soil was decreased. Timely planting (10th March) crop had higher concentration of proline contents that might be faced with higher temperature and moisture stress in the later growth stages which produced higher proline contents to cope with the stress.

Our results supported by the findings of Verbruggen and Hermans (2008) reported that proline is organic osmolytes that stores in crop plants against stresses. Moreover, Jaleel et al. (2008) concluded that higher proline contents in stressed plants can be a continuously supplying energy, by playing on the osmotic adjustment, supporting osmatic adjustment in cells organelles. Significantly higher glycine betaine was recorded in timely planting sugarcane that reduced the high temperature and moisture stress through activation of antioxidant enzymes. MIR & Khan (2017) reported the same results that glycine betaine has an energetic role in encouragement of the antioxidant system. Similar results were recorded that glycine betaine has the capability to widely enhance the performance and application of antioxidant enzymes (Rasheed et al., 2018). Additionally, it was observed that glycine betaine helps in enhancing chlorophyll and photosynthetic rate (Farooq et al., 2016). It is concluded that glycine betaine and proline are produced in plants in response to higher temperatures. Several researchers reported a positive link between increase of glycine betaine and proline in plants during stress (Ashraf & Foolad, 2005).

Present study demonstrates that the highest activity of the enzymes was found on 10th March planting. It has been concluded in several studies that improvement in antioxidant enzymes produced as a result to unfavorable environment such as higher temperature and drought and can be responsible for modifications in metabolism (Cavalcanti et al., 2004; Willadino et al., 2011). Meanwhile accumulation glycine betaine on 10th March planting (Table 4) initiates the antioxidant enzymes like MDA, SOD, CAT and APX, which finally defends plant from oxidative destruction (Rasheed et al. 2018). Moreover, lower concentrations of antioxidant enzymes on 30th March and 20th April planting of sugarcane from external moisture deficit and high temperature in the initial growing months. Our results correspond to the conclusion of Inman-Bamber & Smith (2005) that insufficient external moisture and high temperature caused weak initial shoot development in sugarcane. The oxidative harm was recorded in forms of MDA and membrane destruction was comparatively more in leaves due to direct exposure to environment. This could therefore be attributed to dehydration. Enzymes action response in many ways against stress: it can improve, decline, or continue unaffected (Zgallaï et al., 2006). Lower rainfall resulted in lower moisture contents in soil that caused water shortage in soil profile. These results lead to reduce cane juice (Qudsieh et al., 2002). Shehab et al. (2010) concluded that two-times more enzyme action is a strong signal that any stress in fact directed to oxidative stress.

Conclusion

The two-year field experiment demonstrated that planting sugarcane propagated through bud nodes on March 10th provides a significant protective effect, ensuring distinct agronomic advantages across key growth and production parameters. This specific planting date successfully maximizes millable cane yield and substantially improves sugar juice quality at harvest. Physiologically, the March 10th planting date accelerates physiological development within the crop's vegetative duration by mitigating climate-induced oxidative stress. It enhances the intrinsic antioxidant functions of the bud nodes, triggering more robust enzyme actions that shield plant cells from thermal degradation and lipid peroxidation. Based on these findings, it is highly recommended to adopt the March 10th sowing timeframe for sugarcane propagated through bud nodes to exploit its strong potential for maximizing regional yield and farmer profitability under the specific agro-ecological conditions of Sargodha, Pakistan.

Conflict of Interest The authors declare that they have no conflict of interest.

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