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COMPARATIVE ANALYSIS OF SUGARCANE GENOTYPES FOR POST-HARVEST DETERIORATION UNDER NATURAL CONDITIONS

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Abstract

Post-harvest sugarcane deterioration is a vexing and worrisome problem of the sugar industry as such losses diminish the economic returns of the farmers as well as the mills. This study was initiated to investigate the post-harvest differences among three sugarcane genotypes (1026-P7, NIA-S3, and 1254) and one check variety (Thatta-10) subjected to staling for a period of seven days. Post-harvest changes were determined for cane weight, sucrose, brix, dextran, bacterial density, and juice yields. All of the genotypes evaluated in this study showed decline in cane weight on staling. NIA-S3 exhibited minimum rate of decline, whereas highest rate of cane weight reduction was observed in Thatta-10. Final cane weight losses of 1.85%, 2.05%, 2.054% and 2.16% on day 7 were recorded for NIA-S3, 1026-P7, 1254, and Thatta-10, respectively. A gradual drop in sucrose percentage was seen. 1254 showed lowest rate of decline for sucrose, whereas highest rate of sucrose losses was observed in 1026-P7 which was recorded 8.81% sucrose on day 7 against 14.46% on day 1. Contrarily, brix percentage of the clones was seen to increase over time. Thatta-10 showed minimal brix% values and the lowest rate of rise in this parameter. On the other hand, highest values were recorded for 1254 as its brix% was increased from 19.66 to 25.13%. Furthermore, manifold increase in dextran was observed in all the genotypes. 1026-P7 showed maximum progression in dextran levels ultimately showing dextran contents of 1316 ppm on day 7. The least rate of dextran formation was noticed for Thatta-10. Bacterial density was also estimated to rise in cane juice for initial days of staling, while pH of the cane juice lessened continuously over time. The juice yields per unit weight were observed to reduce at highest rate for 1026-P7 as its juice quantity was decreased by 51.29% during the study period. In this study, NIA-S3 showed minimum cane weight losses. Moreover, 1254 and NIA-S3 relatively maintained their sugar contents over time. These clones can be optimal candidates for cultivation in areas distant from the vicinity of sugar mills. Growing such post-harvest losses resistant genotypes would be extremely important to farmers as well as the sugar industry to minimize their economic damages from cut-to-crush delays.

Key words: Sugarcane; Staling; Sucrose; Brix; Dextran.

Introduction

The time lag between the harvesting of sugarcane and its processing has always been an area of concern for sugar mills. Economic benefits of this industry depend largely upon the recovery of sucrose from sugarcane milling (Khan *et al*., 2019). However, sugar contents of the crop deteriorate as the time between harvesting-to-milling prolongs. Once the sugarcane is harvested, the enzymatic mechanisms in the stalks are no more balanced through feedback regulations, photosynthesis stops, and the microbial activities are enhanced (Singh *et al.*, 2008). Moreover, sucrose inversion and formation of polysaccharides take place, while ethanol and organic acids aggravate during the staling period (Solomon, 2009). These factors contribute towards degrading the sugar contents of the crop, ultimately decrementing commercial gains of the mills. Hence, huge economic outcomes of sugarcane staling are vexing and worrisome problem of the cane industry.

Loss of recoverable sugar from sugarcane as a result of post-harvest degradation is a common issue of sugar industry all around the world (Solomon, 2009; Srivastava *et al.*, 2009). Sugarcane deterioration not only results in economic losses to mills but to farmers as well. The sugarcane weight reduces as the cane dries out leading to lower payments. Moreover, in some countries, the payments are made on the basis of recoverable sugar which is directly dependent upon the staling status of the sugarcane (Zhao *et al.*, 2012). Cane quality degradation is directly related with the time between harvesting and milling. In spite of various improvements in harvesting practices, delivering the cane in short time remains a challenge. In many of the cane growing countries, this lag time ranges between 3-10 days (Solomon, 2009). Major factors which affect the cane deterioration include ambient temperature, sugarcane genotype, and the humidity.

Accretion of unwanted forms of carbohydrates, phenols, and alcohols in high concentrations as post-harvest changes also affect the milling process. Accumulation of these contaminants is carried-out by the microbial communities mainly *Saccharomyces*, *Lactobacillus*, and *Leuconostoc* (Solomon, 2009). Dextran and starch are the main polysaccharides which are formed as a result of such changes. These polysaccharides enhance the viscosity of the juice, whereas monosaccharides, on the other hand, hinder the sugar crystallization during sugarcane milling (Ramos & Ravelo, 2009; Hector *et al.*, 2015). Acidic pH and augmentation of invertase enzyme activities in cane juice impact the factory's efficiency and diminish the ultimate product quality resulting in coloured sugar (Singh *et al.*, 2009; Datir & Joshi, 2015).

Dextran is one of the most important factors involved in sucrose losses during cane deterioration. Various approaches have been tested to minimize the microbial degradation of sugarcane and dextran formation. These include the shortening of lag time between harvest and crushing, and the use of various disinfectants (Solomon *et al.*, 2006; Solomon *et al.*, 2007). Nevertheless, both of the approaches have limitations because it is not always possible to keep the

transportation and crushing aligned, or to ensure disinfectant-based treatment of huge sugarcane materials due to high costs. Therefore, sucrose losses remain a major bottleneck of the sugarcane industry.

Pakistan is the fifth largest cane grower of the world with a total production of 73 million tons of sugarcane on an area of 1.2 million hectares (Anon., 2017; Anon., 2017; Seema *et al*., 2017). Sugarcane sector makes the second largest industry of the country, and it is a source of earning for a large segment of the population engaged in cane farming, and the technical and non-technical personnel involved in the sugar industry. Moreover, sugar industry is also source of other important products including ethanol and energy (Khan *et al*., 2017a, b; Ahmad *et al*., 2019; Khan & Khan, 2019; Raza *et al*., 2019; Haq *et al*., 2020). Despite such importance of the cane crop, studies have not been conducted on main varieties being grown in the country in context of sucrose deterioration. Such comparative studies can shed light on differences among sugarcane genotypes for possible role in mitigating this dilemma through simplest approach possible *viz*. breeding for high storage potential (Verma *et al*., 2012; Datir & Joshi, 2015).

Apart from sugar production, the sugarcane juice is a popular beverage in many countries including Pakistan. The use of cane juice as a sweet thirst-quenching beverage is also reliant on changes in its quality over time. In recent past, there has been considerable progress in researchers' and start-ups' focus on enhancing the shelf-life of sugarcane juice to explore the possibilities of making this popular beverage portable which primarily depends on the preservation of the quality parameters of the juice (Yusof *et al.*, 2000).

This study was initiated to investigate the changes in cane quality during sugarcane staling over a period of seven days. The experiment determined the physicochemical and microbial changes in sugarcane as well as stored juice, over time. The study compared different sugarcane genotypes regarding sucrose changes and juice quality-related characteristics to help in determining the dependence of pace of deterioration on genotypic material. Moreover, this study also explored the pH and microbial growth changes in sugarcane juice stored at freezing temperature.

Materials and Methods

Four local promising genotypes were evaluated in this study which included three new lines (1026-P7, NIA-S3, and 1254), and one check (Thatta-10). The plant material was grown at the experimental farm of the Nuclear Institute of Agriculture (NIA) Tandojam, Pakistan for 13-months. The plant population was grown using Randomized Complete Block Design (RCBD), following recommended agronomic practices (Malik, 2010). All the plant population was harvested on the same day, however; analysis was done over a period of seven days storing the cane in ambient natural conditions. Three plants were removed from the storage and their parameters were recorded at each day of analysis. Height, cane weight, tillering potential, and internode parameters were recorded at harvesting. Fiber% was estimated by determining the difference between fresh and dried cane weights. Moreover, juice yield was quantified while crushing the sugarcane samples.

Post-harvest losses in cane weight: Samples were weighed each day for determining the changes in cane weight for every genotype. Cane weight was recorded for each sample immediately after the harvest and before the crushing at each interval. The weight variations were later converted into percent changes.

Post-harvest losses of sucrose: Sucrose% of the genotypes was determined using polarimeter. Changes in each plant were recorded over the whole study period.

Changes in brix%: Changes in brix% were determined using hand-held refractometer employing the method of Ranganna (1977).

Post-harvest changes in dextran: Post-harvest changes in dextran contents of the evaluated clones were estimated using the polarimetric method as reported by Bukhari *et al*., (2015).

Post-harvest changes in pH: The sugarcane juice extracted from every plant was subjected to pH analysis each day. Moreover, pH changes in stored juice were determined by keeping the sugarcane juice at 0°C for seven days and recording the said parameter each day using Jenway 3510 pH meter.

Estimation of bacterial density: Bacterial growth was estimated for freshly crushed cane, as well as for juice stored at low temperature using spectrophotometer. Method of Sutton (2006) was used for this purpose. The sugarcane juice of the genotypes was diluted to a ratio of 1:100 in order to get the OD600 values in an interpretable range.

Losses in juice yield: Juice quantity was recorded at the time of crushing for every sampling interval. The changes in cane juice quantity were then converted into losses per kg of the cane as the total juice yield was directly dependent on the weight of the cane crushed.

Statistical analysis: Data were statistically analysed for analysis of variance using Windows operated program Statistix 8.1.

Results and Discussion

Agronomic parameters of the genotypes: The statistical analysis of the crop's agrocnomic parameters has been shown in Table 1. While the data for cane height, weight, tillering potential, and number of internodes are presented in Table 2. The genotype 1026-P7 was seen to be the highest yielding clone regarding cane and juice yield (88.33 t ha^{-1} and 3266.67 ml, respectively). High values for related agronomic parameters like height, girth,

weight and no. of tillers were also recorded in this genotype. Thatta-10 also followed 1026-P7 in cane yield. However, both of these clones showed lower sucrose%, the most important parameter of interest for sugar mills. An optimal genotype encouraged for cultivation and anticipated to be accepted by the sugar mills needs to have a good blend of sucrose% and cane yield (Khan *et al*., 2018; Malik & Tabassum, 2018; Khonghintaisong *et al*., 2020). Therefore, in spite of comparatively lower cane yield, the genotypes 1254, and NIA-S3 are of great interest because of their higher sucrose yields which eventually determine the economic returns of the sugarcane sector (Tabassum, 2018).

Post-harvest losses of cane weight: All of the genotypes showed a decline in cane weight over staling (Fig. 1). Reduction in cane weight was directly related to storage period. The cane weight losses ranged from 0.28% in NIA-S3 on day 1 to a maximum decline of 2.16% in Thatta-10 on day 7. Cane weight determines the ultimate cane yield of the crop; therefore, it leads to economic consequences to farmers (Khan *et al*., 2017c). Final cane weight losses of 1.85%, 2.05%, 2.054%, and 2.016% were recorded for NIA-S3, 1026-P7, 1254, and Thatta-10, respectively. It was seen that NIA-S3 showed the slowest pace of decline whereas the cane weight was observed to decrease at the greatest rate for Thatta-10. Similar findings of cane weight staling and the variations among varietal tolerance against such decline have been reported by earlier researchers as well (Singh & Solomon, 2003; Rajeswari *et al*., 2009).

Post-harvest losses of sucrose: Sucrose is the most important parameter of sugarcane crop with respect to sugar mills' perspective. The gradual decrement in sucrose of the evaluated genotypes was observed over the whole staling period in this study. However, genotype 1254 showed excellent response against sucrose deterioration. The sucrose% age values were not only highest for this clone during all seven days, but it also showed minimal rate of reduction for this parameter (Fig. 2). Its sucrose% values ranged from 17.37% to 15.84% over the period of the study. Moreover, NIA-S3 also showed good tolerance against decrease in sucrose% with a variation of 16.22% on day 1 to 12.61% on day 7. The highest rate of sucrose decline was observed in 1026-P7 which was recorded a sucrose% of 8.81% on day 7 against 14.46% on day 1. The results of our study agreed to previous studies which unanimously reported that the cane degradation over storage time led to loses of sugar contents (Singh & Solomon, 2003; Solomon *et al.*, 2006; Solomon, 2009; Datir & Joshi, 2015). Such decline in sugar contents is primarily attributed to the enzymatic and microbial actions which convert sucrose into reducing sugars. As soon as the sugarcane is harvested, its enzymes are no more regulated through feedback mechanisms and the activities of acid and hydrolytic enzymes, like natural invertase, enhance (Verma *et al.*, 2012). Two products are generally yielded from such conversions, one being reducing sugars and the other one dextran (Suman *et al.*, 2000). Yusof *et al*., (2000) also reported a similar decline in sugar contents on cane staling.

Post-harvest changes in brix%: In general, a trend of increase in brix% was observed for all the genotypes under evaluation (Fig. 3). Thatta-10 showed minimal brix% values as well as the lowest rate of rise in brix% over the period of seven days. It was seen that all of the clones maintained their rank in terms of brix% when compared with other clones for the same trait. Highest values were recorded for 1254 as its brix% increased from 19.66 to 25.13% over the study period. This rate of change was followed by NIA-S3 (19.16 to 23.66% brix% on day 1 to day 7). The observations of an increase in brix% have earlier been reported by Datir & Joshi (2015). Moreover, Yusof *et al*., (2000) have also proposed similar rise in brix% values for a period of 15 days, even at lower storage temperature. Other reports agreeing to the results include the study of Eggleston (2002).

Table 1. Analysis of variance (mean squares) for various traits of sugarcane genotypes.

Genotypes	df	Height (cm)	Girth (cm)	Cane weight (kg)	No. of tillers per plant	No. of internodes	Internode length (cm)	Fiber %	Cane juice (ml)	Cane yield (t ha^{-1})
Rep	3	705.51	0.012	0.003	0.56	7.38	26.64	4.85	36633	0.33
Genotypes	3	8589.23	0.10	8.60	0.36	16.51	48.27	10.50	2497808	859.22
Error	9	355.82	0.018	0.33	0.53	4.15	1.85	0.84	32467	32.56
Total	15									
CV		8.85	5.77	8.80	14.20	8.61	12.01	7.03	9.45	8.80

Analysis of variance (ANOVA) was performed using $p<0.05$ through Tukey's LSD test

Table 2. Comparative performance of sugarcane genotypes.

Genotype	Height (cm)	Girth (cm)	Cane weight (kg)	No. of tillers per plant	No. of internodes	Internode length (cm)	Fiber %	Cane juice (ml)	Cane yield (t ha^{-1})
1026 - P7	281.33 a	2.43 a	8.83 a	5.17 a	24.92 ab	16.21 a	14.51 a	3266.67 a	88.33 a
NIA - S3	192.58 b	2.33 a	5.33 c	4.67 a	21.92 b	11.33 b	12.58 b	1543.33 b	53.33 c
1254	178.58 b	2.37 a	5.17 c	5.25 a	21.92 b	8.46 c	11.46 b	1496.67 b	51.67 c
Thatta-10	199.92 b	2.07 b	6.60 b	5.33 a	25.83 a	9.29 bc	14.38 a	1316.67 b	66.00 b
Tukey's LSD	9.43	0.21	1.14	1.16	3.26	2.18	1.46	104.03	0.33
S.E.M	9.43	0.06	0.33	0.36	1.02	0.68	0.65	359.99	1.14

Values are means of four replications. Parameters were recorded on harvesting the cane genotypes after thirteen months of planting. Means followed by different letters are significantly different ($p<0.05$)

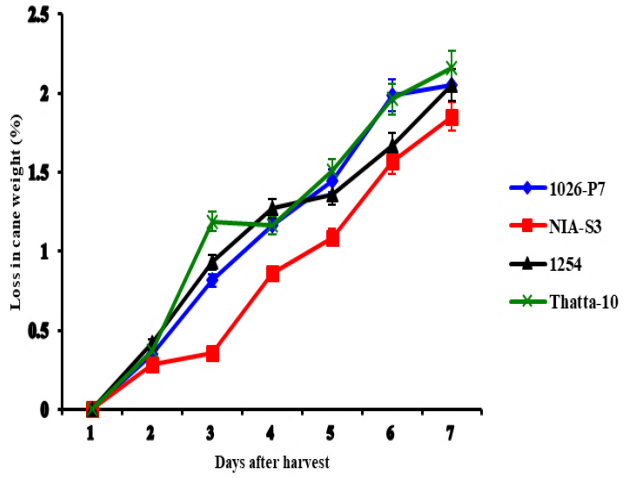

Fig. 1. Post harvest losses in weight of sugarcane genotypes after one to seven days of staling. NIA-S3 was observed to show least decline in cane weight as compared to the other genotypes in the study.

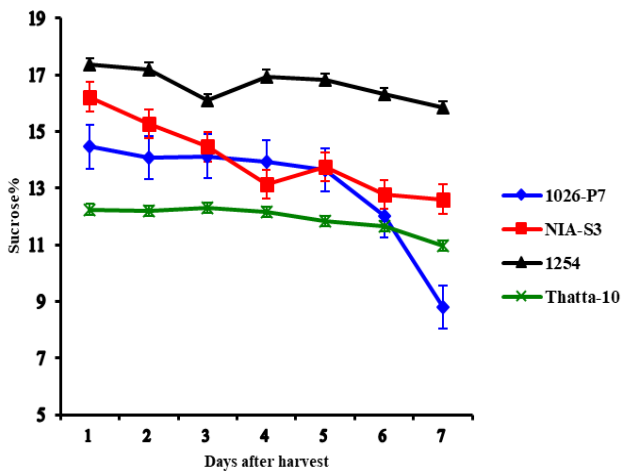

Fig. 2. Post harvest decline in sucrose contents of four sugarcane genotypes. Observations were recorded after one to seven days of staling. All of the genotypes showed reduction in sucrose% over time.

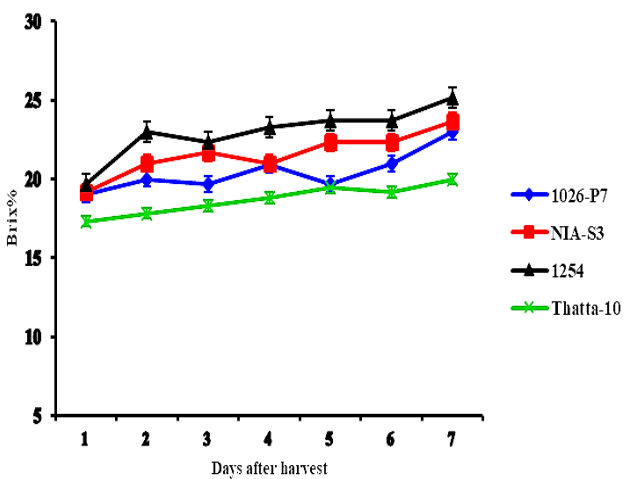

Fig. 3. Post harvest effects on brix% of four sugarcane genotypes. Observations were recorded after one to seven days of staling. Generally, all of the genotypes showed increase in brix% over time.

Post-harvest changes in dextran: Engenderment of dextran is one of the main causes of sucrose losses and cane deterioration. Moreover, it affects the processing of sugar as well. Additionally, the presence of dextran not only impacts sugar recovery negatively, but it also influences the overall quality of the sugar produced at the mills – resulting in export barriers (Solomon, 2009). Manifold increase in dextran was observed in all the genotypes over time (Fig. 4). The highest rate of dextran formation was observed in 1026-P7 which showed an ultimate dextran quantity of 1316 ppm. NIA-S3 and 1254 showed a relatively lower rise in dextran contents (16-779.7 and 12-726.36 ppm, respectively). However, the least rate of dextran formation was observed in Thatta-10. The results agreed with the reports of Solomon *et al*., (2006), Singh *et al*., (2009) and Singh & Solomon (2003). Dextran causes an increase in cane juice viscosity which hinders the crystal formation in the cane milling process diminishing the overall process efficiency and quality (Singh *et al.*, 2009). Developed countries penalize low-quality sugar having high amounts of dextran; therefore, this parameter is extremely important to sugar-exporting countries.

Post-harvest changes in pH: An increase in acidity of the sugarcane juice was observed with the passage of time (Fig. 5). A general trend of decline in pH values was seen in all sugarcane genotypes. The pH values were observed to decrease rapidly during day 1 and 2, after which the values maintained until day 5, followed by a rapid decline until day 7. The pH values of NIA-S3 were observed to remain highest till day 6. Thatta-10 showed the least pH value of 4.34 on day 7. These results agreed to the study of Yusof *et al*., (2000). Moreover, Bhupinder *et al*., (1991) have also suggested an increase in acidity during staling period. The development of acidity is attributed to lactic and acetic acid engenderment in harvested sugarcane (Yusof *et al.*, 2000).

Estimation of bacterial density: OD600 values were recorded for sugarcane genotypes for the whole study period (Fig. 6). The OD600 values give an estimation of probable bacterial intensity in the juice. It was observed that the OD600 values increased rapidly for the initial four days after which the values started declining. The sugarcane genotype 1026-P7 was observed to have the highest OD600 values since very start i.e. day 1, and its rate of increase was also maximum. It showed a maximum OD600 value of 0.62 on day 4. NIA-S3, as well as 1254, also showed highest records on day 4. However, peak OD600 values for Thatta-10 (0.41) were seen on day 5. Bacteria from genera like *Lactobacillus*, *Streptococcus*, and *Leuconostoc* are reported to be involved in causing cane juice deterioration. OD600 values are directly related to bacterial population and such increase in OD600 suggested a rise in the bacterial population in the sugarcane juice. Yusof *et al*., (2000), Eggleston (2002), and Solomon *et al*., (2006) also recorded enhanced bacterial growth in relation to sugarcane staling.

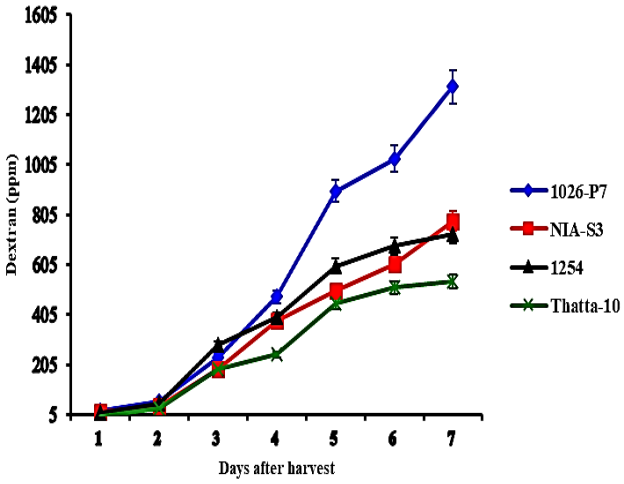

Fig. 4. Post harvest increase in dextran on staling of sugarcane genotypes. Observations were recorded after one to seven days after harvesting. 1026-P7 showed a rapid increase in dextran contents.

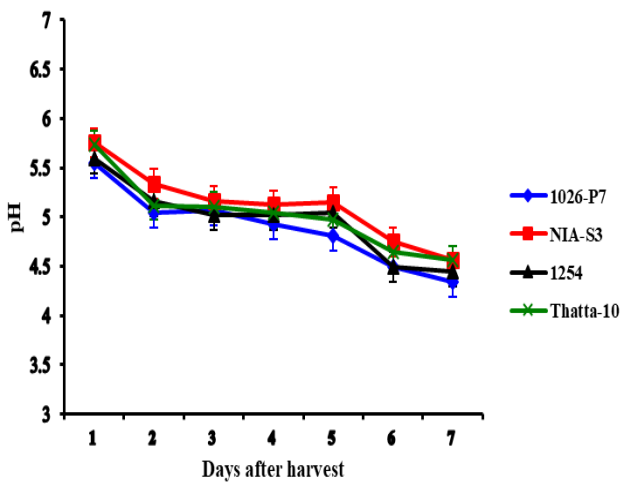

Fig. 5. Post harvest effects on pH of sugarcane juice of four genotypes. Observations were recorded after one to seven days of staling. A decline in juice pH was observed over time. NIA-S3 showed comparatively less drop in pH.

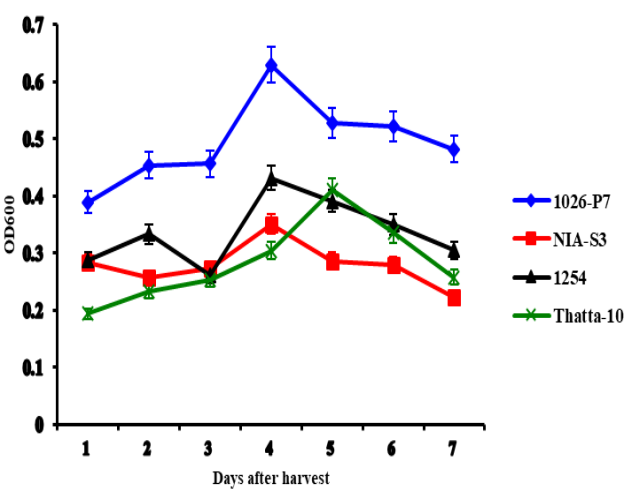

Fig. 6. Post harvest changes in microbial count of sugarcane juice of four genotypes. Observations were recorded after one to seven days post-harvesting. 1026-P7 showed a rapid increase in microbial count.

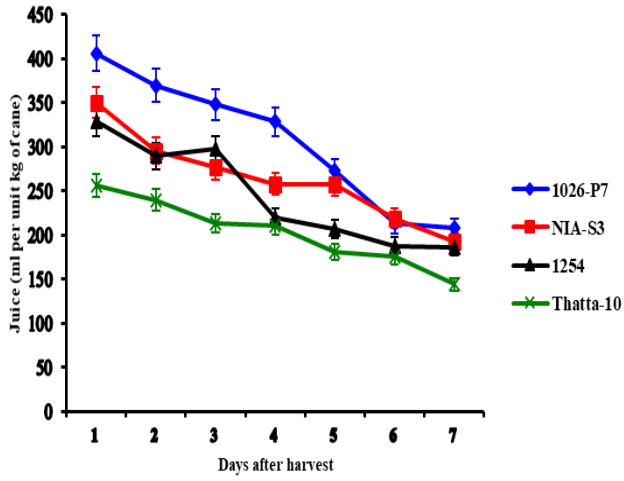

Fig. 7. Post harvest losses in juice yield on staling of sugarcane genotypes. Observations were recorded after one to seven days after harvesting. Thatta-10 was observed to have least juice yields during all seven days.

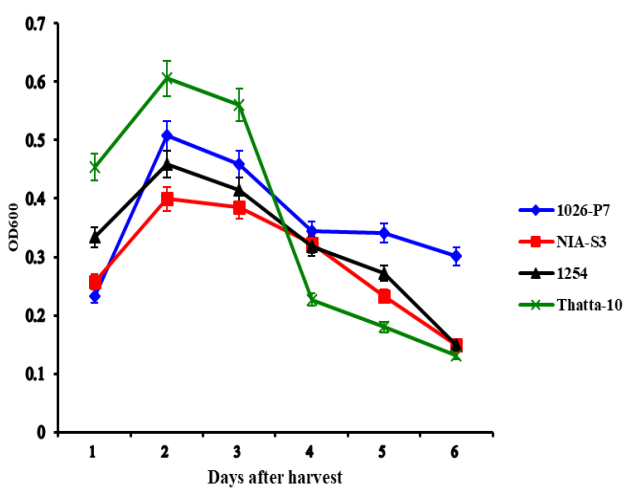

Fig. 8. Changes in OD600 of stored sugarcane juice. Observations were recorded over a period of six days. OD600 rapidly increased during initial days of storage; however, it decreased over time after an initial rise.

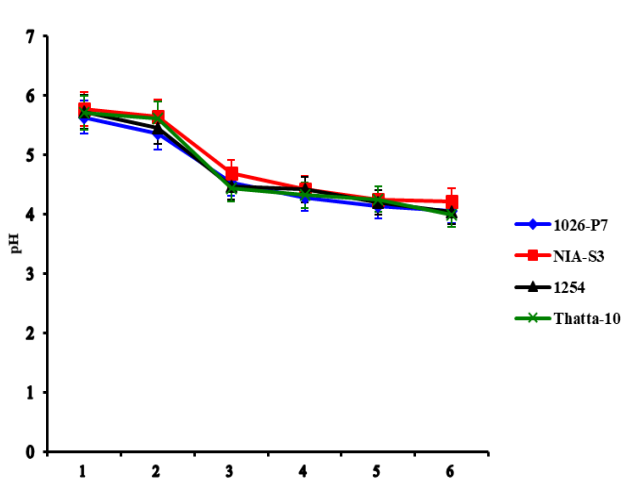

Fig. 9. Changes in pH of stored sugarcane juice. Observations were recorded over a period of six days. pH of the juice was seen to turn acidic over time.

Post-harvest losses in juice yield: The juice yields from evaluated genotypes were forward to decline with the increase of storage were (Fig. 7). Juice yields were observed to decline at the highest rate for 1026-P7 as the juice quantity per unit weight basis was decreased by as high as 51.29% for this clone. Juice changes in Thatta-10 were seen to be lowest; however, the total juice yields of this variety were also nethermost against all other evaluated genotypes. In parallel to our observations, Yusof *et al*., (2000) also reported a similar decline in juice yields of the sugarcane clones. Such decrease in juice yields is extremely important for sugar industry as well as the sugar juice sellers as the time lag between the harvest and juice selling is quite high. Genotypes having a high rate of juice decline can have economic consequences to this market.

Changes in stored sugarcane juice: Changes in stored sugarcane juice were also analyzed keeping in view the use of sugarcane juice as a thirst-quenching drink. OD600 and pH changes were recorded which gave an insight into possible bacterial contamination and acidity development in the juice – essentially important regarding food chemistry aspects of the juice. pH of the juice continuously reduced over time, whereas OD600 readings showed an initial increase and then a rapid decrease in values (as presented in Figs. 8 and 9). Sugarcane genotype Thatta-10 was seen to have the highest microbial communities until the first three days of juice storage. Both of the changes indicated deterioration of sugarcane juice over time. The drop in pH values suggests an increase in lactic and acetic acid formed because of the bacterial activities colonizing the juice (Bhupinder *et al.*, 1991; Yusof *et al.*, 2000; Solomon, 2009).

This study showed a rapid decline of cane quality parameters over staling. Principally, the sugarcane harvesting should only be done in coordination with the mills in the vicinity so that economic losses of the farmer's as well as mills could be kept at a minimum. It is evident that all deterioration aspects are directly related to the time lag between harvest and cane processing; therefore, inordinate delays between crushing and harvesting must be avoided.

There are numerous intrinsic as well as extrinsic factors which aggravate biodeterioration of sugarcane. Two main players of cane deterioration are the invertase enzymes of sugarcane, and the microbial communities inhabiting the staled cane (Solomon, 2009). As soon as sugarcane is harvested, the enzymatic activities are no more controlled through feedback mechanisms. The invertase enzymes show high activity after the harvest producing lactic and acetic acid, ethanol, and polysaccharides (especially Dextran). Moreover, bacterial activities, especially of those belonging to *Leuconostoc* spp. also contribute to cane deterioration. These bacteria are facultative anaerobes, and they rapidly colonize the sugarcane which is stored in piles and have poor ventilation. Exogenous dextransucrase and invertase enzymes enhance the sugarcane deterioration process leading to cane tonnage and sugar losses (Suman *et al.*, 2000).

Cane deterioration detriments farmers' as well as mills' economic returns. The loss of cane tonnage leads to lower payments to farmers. Whereas, loss of recoverable sugar leads to economic damages to the industry (Solomon *et al.*, 2003). Moreover, dextran formation in deteriorated cane intricate the milling process and hinders the production of fine sugar crystals. Many factors such as cane variety, storage temperature, and humidity influence the sugarcane deterioration. However, sugarcane genotype is the biggest dictating factor in this regard (Singh & Solomon, 2003; Datir & Joshi, 2015). Therefore, cane logistics, as well as cultivation of varieties of interest in particular areas must be organized on the basis of probable post-harvest losses profiles (Singh *et al.*, 2009).

In this study, NIA-S3 showed the lowest pace of cane tonnage losses; moreover, its sucrose contents were also good and the speed of decrease was lower than the check variety Thatta-10. Another potential clone in this regard, 1254, having highest values for sucrose contents showed minimal sugar losses although its weight losses were comparable to other genotypes in the study. On the other hand, 1026-P7 showed excellent agronomic parameters; however, its sucrose losses were high. Sugar contents of the check variety were extremely low, while its cane tonnage losses were also high.

The cut to crush delay is always apparent and it is extremely difficult to rule it out completely. Therefore, varieties showing a high rate of deterioration must be grown only closer to the mills' vicinity and their harvesting should always be done in coordination with the mills. Areas at distant locations should avoid such varieties and instead opt for storage tolerance genotypes in order to minimize such losses during transportation and storage before milling. The study indicated that NIA-S3 and 1254 were potential clones for such locations. Moreover, high recovery of these varieties is also suitable to the sugar industry.

Conclusion

It is concluded from the study that post-harvest deterioration of sugarcane is largely dependent upon the subject sugarcane variety. In this study, NIA-S3 showed minimum cane weight losses. Moreover, NIA-S3 and 1254 were also observed to have the least losses in sugar contents. Therefore, these clones can be optimal candidates for cultivation in areas which are not located in the vicinity of the sugar mills.

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