Genetic variability of sugarcane genotypes for red rot disease

M.S. Iqbal[1], B. Tabassum[1], M.F. Awan[1, 2], M. Tariq[1], Q. Ali[1, 3] and I.A. Nasir[1*]

[1]Centre of Excellence in Molecular Biology, University of the Punjab Lahore, Pakistan
[2]University of Management and Technology Sialkot, Pakistan
[3]Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan

Corresponding author: I.A. Nasir
E-mail: dr.idrees@gmail.com
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ABSTRACT. Sugarcane is a vital crop grown for sugar and sugary products. The importance of sugarcane has been increased greatly as many countries have started the biofuel production using sugarcane as a source. This crop suffers many diseases caused by bacteria, fungi, and viruses. Red rot is one of the major diseases which limit the yield every year. We have screened six sugarcane genotypes for cane girth, height, sugar recovery, and red rot fungus resistance. All these genotypes are commonly adopted for cultivation locally. The screening was done using manually infecting the cane stalks with fungus inoculum and was graded for their response to the pathogen. The six genotypes (3 resistant and 3 susceptible) showed that there is a strong relationship between the sugar content in cane stalk and fungus propagation i.e., more sugar recovery rate helps the fungus to grow at a faster rate. Fungus usually grows much rapidly towards the aerial parts of the plant as compared to the roots, from the point of infection. Higher girth values also show a strong correlation with disease progression. We have also noted that in resistant cultivars the nodes are much more efficient barriers to let the infection progress to the next internode as compared to susceptible cultivars. The productivity level of sugarcane can be improved by identifying the red rot resistance genes in resistant and susceptible cultivars and use these genes to produce more productive varieties. The coefficient of variation was found lower for all traits which indicated that there was consistency among the results. The average cane height was recorded as 2.48789 ± 0.1185 m, cane weight 9.5033 ± 0.1246 kg, cane girth 2.1522 ± 0.0179 cm and sugar recovery 8.3572 ± 0.0758%. There was a strong and positive correlation of sugar recovery with cane girth, average cane

weight and sugar recovery percentage The regression analysis indicated that the cane height showed a negative impact on sugar recovery, while cane weight showed the positive effect to increase sugar recovery. The cane girth showed higher and positive effects to improve the sugar recovery percentage. The selection of crop plant genotypes may be made depending upon the positive and strong correlation of the response variable with independent variables.

Keywords: Sugarcane; Biofuel; Red Rot; Pathogen; Correlation; Regression

INTRODUCTION

The major producer of sugarcane in Brazil followed by Asian countries including China, India, and Pakistan who contributed most of the sugarcane production in the world (Qureshi and Afghan 2005). Pakistan contributed 4% in the production of sugarcane and grows sugarcane as a major cash crop throughout the country for its sugar and raw materials used in the board and paper industry (Figure 1) (Ali et al. 2013). Sugarcane contributes 0.6% in the GDP of the country. The average production of sugarcane was 1132 thousand hectares with 65475 thousand tons of production of sugar (Anonymous, 2015-16). The average yield of Pakistan is low as compared to the other sugarcane growing countries (Bastiaanssen and Ali 2003). The low sugarcane yield is mainly due to various reasons, like non-availability of high yielding varieties and genotypes, improper agronomic/cultural practices, drought, climate barriers, salinity, weeds, uncertified seed, late harvesting and sowing, diseases, insect pests, poor rationing and imbalanced nutrition (Qamar et al., 2017; Faqir et al. 2010, Khaliq et al. 2005). Sugarcane (Saccharum officinarum L.) belongs to the genus Saccharum from family Poaceae. It is one of the most imperative agro-industrial crops cultivated throughout the world. S. officinarum is a monocotyledonous crop that can reproduce through asexual as well as sexual means of reproduction (D'Hont et al. 1998, van der Pol et al. 2015). The sugarcane genome is highly complex with up to octaploid (chromosome number ranged from 80 to 120) (D'Hont et al. 1998, Joyce et al. 2010, van der Pol et al. 2015). Sugarcane is mostly reproduced through cloning as it has very high polysomatic and heterozygosity in the genome. It has also a large number of byproducts besides sugar i.e., paper, furfural, alcohol, dextran, chipboard, chemicals, confectionery, paints, beverages, plastics, fiber, synthetics, detergents, insecticides and pharmaceutical products (Awais et al., 2017; Qamar et al., 2015; Nasirpour et al. 2014, van der Pol et al. 2015).

Various fungi, bacteria, nematodes and virus species decrease the production level of sugarcane crops. However, the subcontinent is majorly affected by the Red rot every year. The red rot of sugarcane was first reported in 1883 in the Indo-Pakistan region in 1906 (Butler 1906). A detailed description of this disease was published by (Edgerton 1910). Red Rot is mainly caused by a fungus: *Colletotrichum falcatum* also called *Glomerella tucumanesis*. The fungus is prevalent in almost all sugarcane growing countries of the world. The susceptible varieties show considerable losses due to secondary infection, intensive cultivation, and poor management practices. Yield losses due to red rot are around 18-31% in planted crops (Sharma and Tamta 2015) and 52-73 % in ratoon crops (Viswanathan and Rao 2011). The most satisfactory and economical method to control the disease is the use of resistant varieties. The evaluation of resistant germplasm of sugarcane is currently playing a leading role in the development of resistant varieties through breeding (Awais et al., 2019; Begum et al. 2008). This study is conducted to assess the genetic variability among sugarcane varieties for morphological and physiological traits and red rot fungus resistance capacity. The screening of higher-yielding and good quality sugar varieties was also kept in consideration to screen improved verities for better yield.

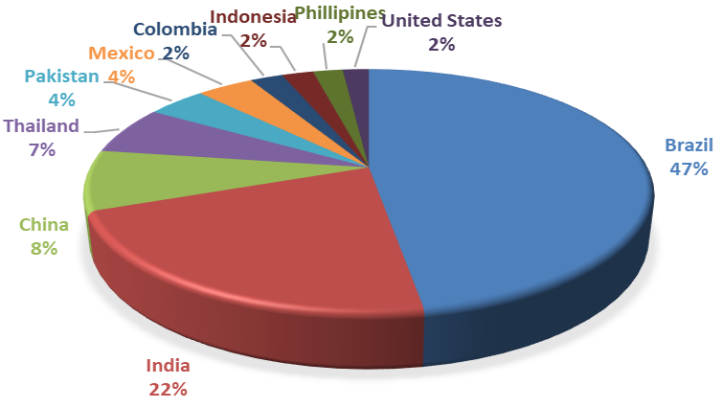

Figure 1. Top 10 Sugar Producing countries in the world.

RESEARCH METHODOLOGY

Plant material and growth environment

Six commercial sugarcane genotypes were obtained from AARI (Ayub Agricultural Research Institute Jhang Rd, Faisalabad, Punjab) where these cultivars have been developed and maintained by different breeding programs (Khan et al. 2011). Among these obtained cultivars, we divided them into two groups, the 1st group with three highly susceptible to red rot disease and 2nd group with three resistant varieties. The behavior was also proven based on two years of field observations at CEMB, Lahore and data were collected. The red rot response of these cane varieties was noted for 2 seasons. The germplasm was sown in replications followed under a complete randomized block design. The data was recorded for following morphological traits, viz., plant height, number of internodes, leaf length leaf width leaf area, sugar recovery, stem diameter. The data were collected and statistically analyzed for analysis of variance (Steel et al., 1997) to access the significance of results.

Fugal suspension and inoculation

Media preparation: Red rot fungus isolated and identified by Seed Biotech Lab was used for the infection. The pathogen was grown on the PDA agar and broth for the infection purpose. Mainly this pathogen was obtained from AARI Faisalabad where they have designated this type as the major red rot strain infecting all the varieties in most of the parts of the country. This strain is the default type of red rot while varieties are tested for varietal development.

PDA agar: 3.9 g of PDA (TM334, Titan Biotech, India) in 100 ml of autoclaved distilled water and Petri plates were prepared.

PDA broth: Inoculum from the freshly prepared plate was used to grow the fungus in PDA broth (2.4 grams of Fluka P6685-250G) was dissolved in 100 ml of autoclaved distilled water). The culture was left for 2 days on 30°C shaker.

Spore suspension: Petri plate growth was used to make the fungus suspension. 15 ml injection water was poured on the fungus growth in the plate. An upper layer having spores and mycelia were dissolved in this water using a glass slide. This mixture was passed through autoclaved mousseline cloth. The suspension was collected in a 50 ml tube.

Spore count: Spore count was done by using hemocytometer 200 ml of the suspension was poured on each side channel of hemocytometer and spores were counted as per specified formula. The suspension was diluted to get the 104 spores/ml. *Colletotrichum falcatum* was grown in PDA (39 g/L) plates at 30°C. Fungal spores were harvested by treating the plate with 10 mL of sterile water. Spore count was performed using a hemocytometer. Spore suspensions were diluted to 10^5 spores mL^{-1} to be used for inoculating sugarcane plants. Sugarcane plants were inoculated with 1 mL of the culture prepared were further studied for the progression of infection in each cultivar

and response was visualized by horizontally cutting the inoculated stems. The response was measured according to the standard protocol for this purpose (Khan et al. 2011).

Fungal infection: Susceptible and resistant to red rot varieties of Sugarcane were infected and infection progress was noted from day-2 today-12 by cutting the stems of infected plants. The fungus was inoculated by boring a hole in sugarcane stem about 12-14 mm deep using inoculator. Slanting bore was made on the 2nd internode from the bottom of the stem and 3-4 drops of fungus culture (water dissolved) were injected in the hole. The hole was closed again with the same piece and the wound was covered with parafilm. Inoculated sugarcanes were longitudinally cut to observe the fungal infection after every day until day 10.

Cut analysis: Red rot disease progress in the sugarcane stem was assessed after inoculation with C. falcatum by splitting open canes and observing the symptoms like lesion width, cane drying, and transgression of lesion across the nodes and prominence of white spots in the stalks at different intervals. Disease intensity was scored on the 0–9 scale (Srinivasan and Bhat 1961) (Hassan et al. 2010). The data was recorded for various sugarcane traits and analyzed for analysis of variance by using analysis of variance technique (Steel et al., 1997) through SPSS 23.1 version.

RESULTS AND DISCUSSION

Sugarcane genotypes were planted according to a randomized field plan (Figures 2-4) and data was noted for 2 seasons. All six sugarcane genotypes were showed significant variation in the morphological characteristics and red rot infection response. We noted down the data for Cane weight, Cane height, Cane Girth, Sugar Recovery, and red rot response. We correlated these recorded characteristics using statistical tools. The Figure 4 indicated that the sugarcane stem with red rot disease effects, the resistant genotype showed that there was less amount of sugar contents and the stem was having compact structure which provided less chances to red rot for growth while the susceptible genotype indicated that the stem was not hard or much compact, due to the loose structure of stem attack of red rot was higher in susceptible genotypes. The results from Figure 5 indicated the growth of fungus for inoculation on sugarcane plants in filed. The results from Figure 5 indicated that the effects of sugarcane red rot were started on leaves after 12 days of inoculation of fungus. The susceptible genotypes showed leaf strikes as well as stem after attack of fungus.

Figure 1. Sugarcane field plan.

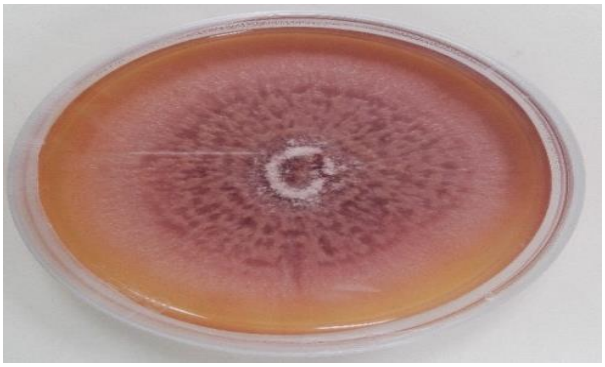

Figure 2. Red rot fungus growth after 5 days.

Figure 3. Red rot fungus growth in sugarcane stem after 12 days of infection in a humid environment.

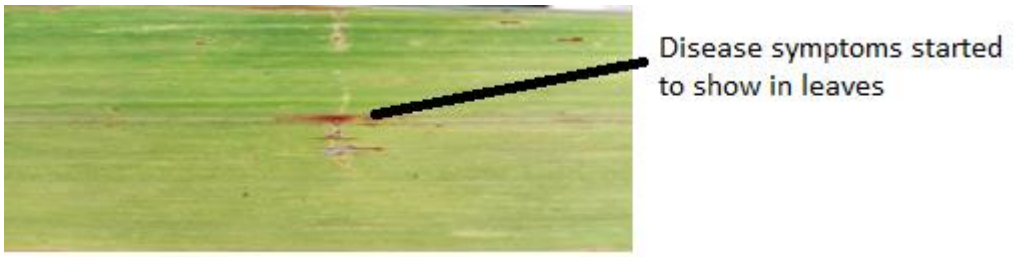

Figure 4. Fungus infection reached in the leaves of susceptible variety after 12 days.

Genetic diversity is indispensable in a sustainable breeding program. A clear understanding of the diversity level held in the germplasm is fundamental to the development of breeding programs and for efficient management of the gene pool. Sugarcane breeders have traditionally relied on agro-morphological traits and pedigree information for planning their crosses. We have various red rot resistant varieties as a result of the efforts from breeders. Recently various methods have been developed to analyze the genetic differences between resistant and susceptible verities. Different varieties of sugarcane highly resistant to red rot are being cultivated in different parts of the world. Sugarcane diversity has been studied using ribosomal DNA (Glaszmann et al. 1990), random fragment length polymorphism (Coto et al. 2002), simple sequence repeat (Cordeiro et al. 2000), amplified fragment length polymorphism (Butterfield et al. 2001) and random amplified polymorphic DNA (Alvi et al. 2008).

These studies have provided an understanding of the complex genetic structure of sugarcane. Furthermore, most of the genetic diversity found in modern sugarcane has been attributed to *S. spontaneum*. Another solution to the problem is to use fungicides, but it is not a wise option. This approach poses some serious threats to human health and causes environmental pollution, so there is a great need to produce fungicide-free red rot management strategies utilizing biological techniques (Khan et al. 2011) (Wu et al. 2013) the molecular basis of sugarcane

resistance to Sporisorium scitaminea (fungus) by utilizing high throughput sequencing. (Vicentini et al. 2015) used RNA-Seq to analyze the differential gene expression of developing internodes of two sugarcane genotypes varied for lignin production. This study revealed a set of more than 2,000 transcripts that showed differential expression between the contrasting genotypes. The ever-growing demand for food and others (Holl 1975).

GM crops have been playing a role in food insecurities, disease-resistant crops; increase the shelf life of food products, better performance under environmental stress to meet the needs of a growing population of the world. GM crops also are playing a role in food quality along with social and economic factors of the people related agribusiness (Qaim and Kouser 2013). Like many other crops' sugarcane is also genetically modified to induce resistance against yield-limiting pathogens in different parts of the world. Diatraea saccharalis (Braga et al. 2003), Eoreuma lofting (Legaspi and Mirkov 2000), sugarcane mosaic virus (SCMV) (Guo et al. 2015) are a few examples of such studies. Despite all the challenges like the big polyploid genome, less survival rate in transformation, transgene inefficiency and difficult gene editing, sugarcane is one of the key choices among researchers because of sucrose and biofuel products (Mohan 2016).

It was persuaded from results given in Table 1 that there were significant differences among the genotypes for all studied parameters. The coefficient of variation was found lower for all traits which indicated that there was consistency among the results. The results showed reliability to proceed for the selection of genotypes against studied traits. The average cane height was recorded as 2.48789 ± 0.1185 m, cane weight 9.5033 ± 0.1246 kg, cane girth 2.1522 ± 0.0179 cm and sugar recovery 8.3572 ± 0.0758%. Correlation analysis provides the opportunity for researchers to identify the traits which have positive and strong association among each other.

The selection of crop plant genotypes may be made depending upon the positive and strong correlation of the response variable with independent variables (Ali et al., 2107). From our results, it was found that there was a strong and positive correlation of sugar recovery with cane girth, average cane weight and sugar recovery percentage (Table 2). The results indicated that the higher cane weight and girth caused increase sugar recovery while under the red rot disease of sugarcane. The recovery of sugar is also directly related to disease attack. The regression analysis was performed to calculate the contribution of independent variables on sugar recovery percentage (Ali et al., 2014). The coefficient of determination was found higher 93.34% which indicated that the selection on the basis of sugar recovery may be helpful to improve sugarcane production (Table 3, Figures 6 and 7). The regression equation was predicted as given below which indicated that the cane height showed a negative impact on sugar recovery, while cane weight showed the positive effect to increase sugar recovery (Ali et al., 2016). The cane girth showed higher and positive effects to improve the sugar recovery percentage.

Y = -9.2871 – 0.5907(Cane height) + 0.5554 (Cane weight) + 6.4260(Cane Girth). The results from correlation and regression analysis revealed that the selection of sugarcane genotypes may be fruitful to improve sugar recovery under the attack of red rot disease of sugarcane.

Table 1. Analysis of variance for studied traits of sugarcane

Sources	DF	Average Cane height	Average cane weight	Cane Girth	Sugar Recovery
Replication	2	0.08202ns	0.05312ns	0.00054ns	0.0074ns
Varieties	5	0.11016*	4.43065*	0.15241*	14.4168*
Error	10	0.04216	0.04657	0.00096	0.0172
Coefficient of variation		8.28	2.27	1.44	1.57
Grand Mean		2.48789	9.5033	2.1522	8.3572

Standard Error	0.1185	0.1246	0.0179	0.0758

*= Significant at 5% probability level, ns = non-significant

Table 2. Correlation among traits of sugarcane.

Variables	Coefficients	Standard Error	t Stat	Partial R^2	Lower 95%	Upper 95%
Cane height	-0.59068	0.810891	-0.72843	47.8356	-2.32987	1.148507
Cane weight	0.555412	0.407016	1.364593	19.3914	-0.31755	1.428375
Cane Girth	6.426042	2.193225	2.929952	1.0973	1.722044	11.13004

Y = -9.2871, Multiple R^2 = 0.9334, R^2 = 0.8720, Adjust R^2 = 0.8446, Standard Error = 0.8128

Table 3. Regression analysis for Sugarcane recovery.

Traits	Cane Height	Cane weight	Cane Girth
Cane weight	0.3385		
Cane Girth	0.3213	0.9067*	
Sugar Recovery	0.2448	0.8888*	0.92304*

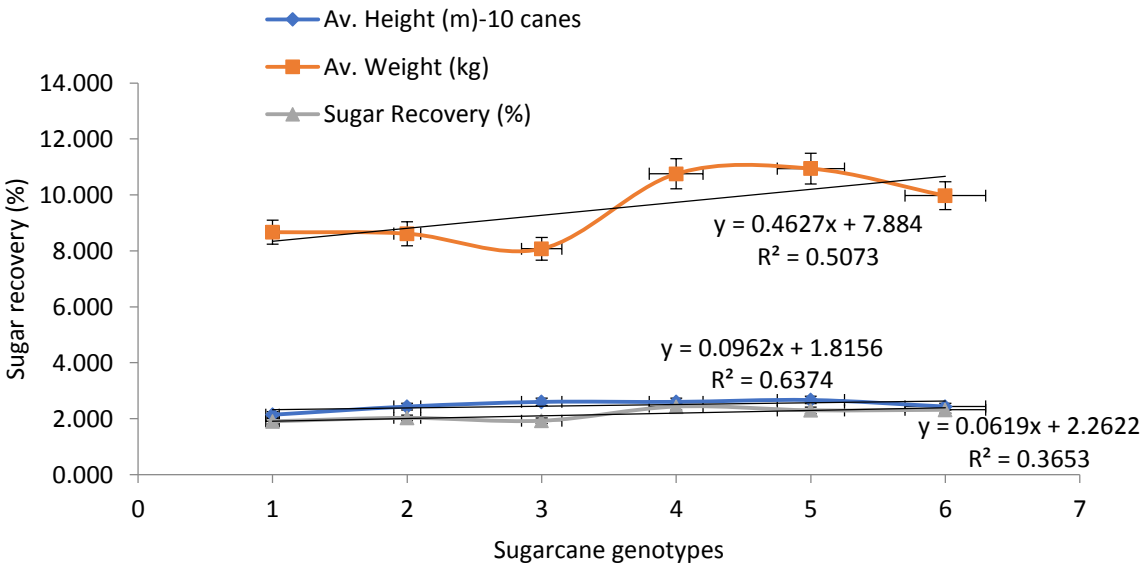

Figure 5. Regression graph for average cane height, average cane weight vs. sugarcane recovery

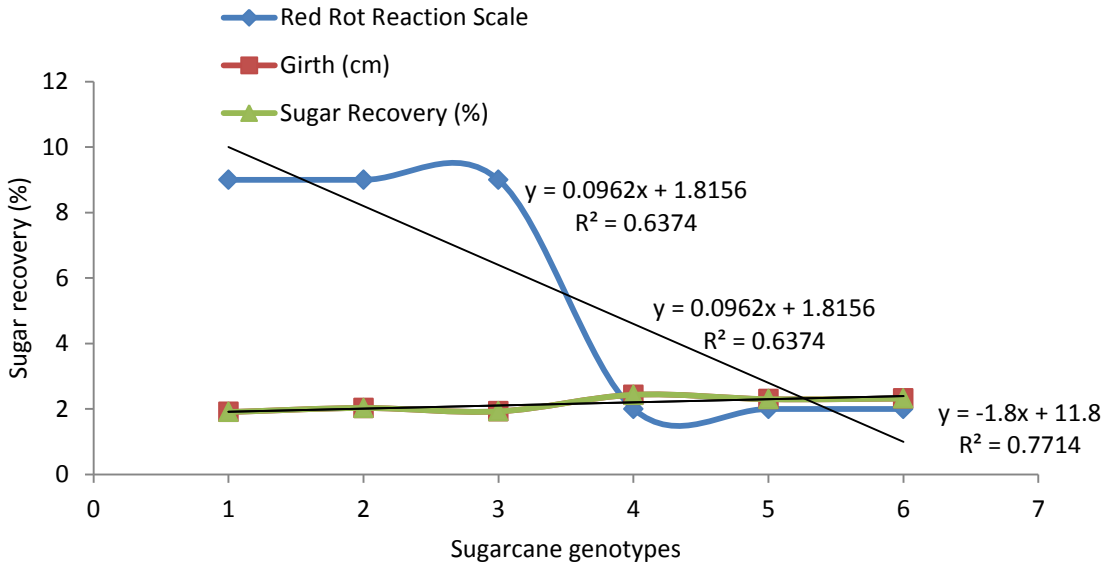

Figure 6. Regression graph for cane girth, red rot reaction scale vs. sugarcane recovery

CONCLUSION

This study concludes that the red rot fungus grows more rapidly in sugar-rich stalks/genotypes as compared to varieties having low sugar recovery. Higher girth values also support the red rot growth inside the stem.

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