ASSESSING CURRENT SITUATION AND FUTURE PROSPECTS OF SUGARCANE RATOONING ABILITY IN PAKISTAN

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ABSTRACT

Sugarcane is a significant cash crop and it can be subjected to rationing for several years. The advantages of ratooning include quality improvement, efficiency enhancement, and reduced costs and energy use. The genotype, environment, cultivation management, and harvesting technology affect the productivity and longevity of ratoon cane, with the genetic basis being the most critical factor. However, the majority of research has been focused on only limited genotypes, and a few studies have evaluated up to 100 sugarcane germplasm resources. They mainly focus on the comparison among different genotypes or among plant cane, different selection strategies for the first and second ratoon crops, together with screening indicators for the selection of stronger ratooning ability. In this paper, previous studies are reviewed in order to analyse the importance of sugarcane ratooning, the indicative traits used to evaluate ratooning ability, the major factors influencing the productivity and longevity of ratooning, the genetic basis of variation in ratooning ability, and the underlying mechanisms. Furthermore, the shortcomings of the existing research on sugarcane ratooning are highlighted. We then discuss the focus of future ratoon sugarcane research and the technical methods that will shorten the selection cycle and increase the genetic gain of ratooning ability, particularly the development of linked markers. This review is expected to provide a reference for understanding the mechanisms underlying the formation of ratooning ability and for breeding sugarcane varieties with a strong ratooning ability.

Keywords: Sugarcane ratooning ability, Trait markers and breeding.

INTRODUCTION

Sugarcane (Saccharum spp. hybrids) is an important sugar crop that can be subjected to ratooning over multiple years. from sugarcane Sucrose accounts for 86% of the world's (OECD-FAO, 2020) and 98% of Pakistan's total (Planning sugar output Commission of Pakistan, 2020). In Pakistan, approximately 60-70% sugar production costs are spent on sugarcane procurement. Compared with newly planted sugarcane, i.e., plant cane, ratoon cane has multiple advantages including faster leaf spreading, more plant growth, earlier rapid strike maturity and reduced production costs due

savings on seed cane, field preparation, fertilizers, planting and early field management.

harvesting of cane, After fresh stalks consumed as soon as possible because immediate processing performed to minimize the conversion of sucrose into reducing sugars within the sugarcane stem to increase the sugar output. Sugarcane originated from the tropics and requires a warm climate, and only when the temperature is above 20 °C effective can the mean accumulated temperature meet the requirement for sugarcane normal growth and development. It is suggested from previous studies that the non-optimum germination or sprouting temperatures, too low or too high, may be a factor for yield decline in ratoon cane (Gascho *et al*, 1973).

Compared with plant cane, plants ratoon have an established and strong root representing system, the unique skeleton of carbon and energy source for the development initial plant (Pissolato et al, 2021). The root which system, is essential for regrowth sugarcane and the ratoon vigour of each cycle, can be used for water transport to leaves during the period of photosynthesis. in which photosynthetic products are accumulated and in turn

rapid promote a leaf expansion and plant growth during the early growing stage. Therefore, ratoon plants have more effective accumulated temperature and effective growth longer resulting period, in more sugar accumulation and earlier technical maturity.

In contrast, newly planted sugarcane plants firstly need especially grow roots, permanent roots. which requires a relatively longer period and a higher effective accumulated temperature. As a result, the newly planted sugarcane does not use light and thermal sources efficiently at this stage. Therefore, from the perspective of energy utilization, ratoon sugarcane a significant energysaving characteristic.

According published to reports, ratoon sugarcane only 89,040,000 requires calories per ton of sugarcane production. while newly planted sugarcane requires 204,550,000 calories per ton (Sehtiya and Dendsay, 1992), suggesting that plant cane uses 2.3 times more calories than ratoon cane. This opinion is supported bγ (Hunsigi and Krishna, 1998), who believed that an irrigated ratoon crop requires only 295 days for its maturity compared with 482 days in plant cane.

Ratoon cane is very important in sugarcane production. After the harvest of sugarcane, the underground portion of the gives strikes rise to a succeeding crop, which is ratoon crop. known as Sugarcane ratooning is а planting system that generally adopted by each sugarcane-producing country. However, the number of ratoons varies from 1-8 (Singh and Rathore, 2015). The proportion of the ratoon cane is generally around 50% of the cultivated area, and can even reach 75% in some regions. The average proportion 50-55% is tropical while areas. approximately 40-45% in subtropical areas (Gomathi et al, 2013).

The cost of sugarcane production is much higher in Pakistan than in other countries including Brazil. Except for the low cost of arable land, better ecological and soil conditions, and the complete mechanical operations in sugarcane production, sugarcane variety with a strong strike is one of the most important reasons for the low cost in Brazil. In the Brazil agricultural cvcle practice. а consecutive rationing for 4-5 vears after the harvest of plant cane is generally adopted due to the standard cycle of plant-cane followed by three years of ratooning not being profitable. In India, it has been reported that the cost of ratoon crop is 25–30% lower than that of plant cane. However, due to low yields (40-50 t/ha)ratoon crop accounts only 40% for (Chumphu et al, 2019) or 40-45% of the total cane area and sugarcane is ratooned for one to two years in India (Singh et al, 2015), in relatively resulting the higher cost in sugarcane production. This is supported

another report, which bν suggests that ratoon cane contributes only 30% of the total cane production, though it accounts for over 50% of the acreage (Kumar et al, 2017). Another report also pointed out the problem of low yields of ratoon crop, indicating only 30-35 t/ha as compared to 65-75 t/ha of cane in India. Pakistan, ratoon crop can save 25-30% in past (Bashir et al, 2013) and 30-40% in production current costs (Aslam et al., 2020), indicating a widening gap in costs.

From all the above, ratoon production crop reduces costs and benefits growth through energy saving by the reduction of inputs utilization of residual manure and moisture. With the rising labor costs, the gap in cost ratooning between and replanting further will be widened. Additionally, ratooning is undoubtedly a simple and easy way to improve the efficiency production. sugarcane However, the yields of ratoon cane decline with age. In this review paper. we the achievements of sugarcane ratooning research, highlight shortcomings and propose research ideas. We hope that this review enhances the understanding of the research progress of ratooning ability and is beneficial to develop sugarcane variety with strong ratooning ability.

It is widely accepted that variations in ratooning ability exist in different genotypes and hybrid offspring. The definitions of ratooning ability that have been provided by

different authors are as follows: the yield of second ratoon as a percentage of the vield of newly planted sugarcane; ratoon crop performance as a percentage of a reference yield, usually that of the plant cane, first ratoon or the mean of these ability to crops; the with maintain yield the years increased ratooning (Abu-Ellail et al, 2019); the yield of the ratooning year as a percentage of the yield for the reference variety of that year; a joint evaluation based on the quantity, growth speed, strength, final stem formation rate, number of effective stems, and yield of ratoon cane in the last season (Ding et al, 2020). Although there are differences in description of ratooning ability, the core is similar, that is, a good performance in ration cane yield or ratoon can produce several profitable crops. In other words, the longer the ratooning cycle and the smaller yield decline in ratoon crops, the stronger ratooning ability.

Phenotypes of Ratooning Ability in Sugarcane

Ratooning increases the income of sugarcane growers due to the saving cost in cultivation and increases the income of industry because it mature earlier, better juice quality and thus improves sugar recovery at times of the crushing season compared with plant cane. For example, in plant cane and the second ratoon, the average sucrose content was 14.84% and 16.54%, respectively. Most studies sugarcane on

ratooning ability have focused on analysing the variation in ratooning ability based on phenotypic traits. Generally, the most effective way for the improvement of sugarcane ratooning ability is to select lines directly based on the yield performance of ratoon crops. However, it is not conducive to shortening the selection cycle, and the huge segregated population sugarcane hybrid F_1 limits this measure due to considerable and For time resources. example, to identify one commercial quality variety from the original F_1 population requires 11 years sequentially planted selection from approximately 75,000 genotypes (Milligan et al. 1996). An alternative approach is to select lines based on the yields of plant cane because varieties with high plant cane vields normally produce high ratoon crop yields. Indirectly selecting genotypes with strong resistance to diseases and insect pests may also increase the ratooning ability of the selected sugarcane breeding materials. In some cases, the ratooning ability has been indirectly evaluated by assessing the biomass or light utilization efficiency of sugarcane, and assessing drought tolerance in those arid or semi-arid cultivated regions is also suggested. Ratooning ability is a trait that a commercial quality variety must have indicative traits of

strong

ratooning

include both morphological

indicators of sugarcane root

residue/stubble and traits that

directly contribute to cane

vield and sugar output, such as a high number of stalks, high viability of buds, large number of viable buds, large number of viable roots, high cane yield, high sugar output, and high tillering rate in plant cane. (Milligan et al, 1996) found that stalk number was the only trait in the plant cane markedly correlated to the yield of the ratoon crop. (Qin et al. 2015) demonstrated in their research that sugarcane lines with strong ratooning displayed ability rapid germination, higher germination rate and tillering rate, and higher stalk number in plant cane. Additionally, a higher stubble germination rate and the larger shoot number were observed in the ratoon crops, which result in high stalk number and higher cane yields than those in plant-cane. Α similar observation was obtained by reports. also other lt is believed that the ratooning ability of sugarcane is mainly identified by four important factors, namely, root traits, the total number of strikes or shoot population. stalk number. and cane vield. Good performance on the four aspects above in its plant cane and the ratoon crops is necessary for the selection of varieties with a strong ratooning ability. In addition, a sugarcane variety considered to have a poor ratooning ability if the cane vield in ratoon crop is lower than that of its plant cane. The morphological characteristics of sugarcane stubble are closely related to the ratooning ability of the In addition, sugarcane.

sugarcane varieties with strong ratooning ability have a low stubble mortality rate and a short internodal length of underground stems, together with the obviously larger total number underground buds and the effective tillers. Generally, if there is an increased number of effective tillers formed by the lower buds of the main and there is stems. an increased total number of effective tillers on the main stems, then the variety likely has strong ratooning ability.

There was a significant interaction effect between growing varieties and seasons for all yield and qualitative traits except for the purity of sugarcane juice. In addition, in the second ratoon crop, both cane yield and total recoverable sugars (TRS) were significantly higher in the varieties with strong ratooning ability than in those with weak ratooning ability. Based on an investigation of later crop, it is also reported that single stalk weight, cane yield, total solids (Brix), soluble and sucrose percentage, were highly heritable traits that displayed the potential to obtain high genetic gain. Additionally, a study on the genetic relationships among sugarcane traits in a large population indicates that stalk number was the primary determinant of cane yield and thus became more important trait in determining cane yield in the ration crops (r = 0.77), much higher than those of stalk diameter (r = 0.52) and stalk length (r = 0.33).Research also indicated that,

for varieties with poor ratooning ability, the ratoon crops had a much lower cane vield than the plant-cane or a sharp decline in cane yield in the first ratoon compared with plant cane. Meanwhile, the vield decrease was observed in varieties with strong ratooning ability in the second ratoon crop; however, it is desirable to perform ratooning in sugarcane production for as many years as possible. In addition, a significant correlation found between the number of shoots and the genotype/ cane yield/harvesting time in sugarcane. In addition, the relationship between the ability ratooning and the changes in endogenous hormone contents durina germination of underground buds in stubbles have also been studied. Recent studies have also indicated that the experimental location is particularly important for evaluation of the ratooning ability.

In brief, for the selection of ratooning ability. direct indicators are the stubble morphology. stalk number. and the germination and tillering rates in the plant cane and the ratoon crops, while indirect indicators included disease resistance especially smut. pest resistance. biomass, light use efficiency, and hormone content during stubble bud germination. The number of indicators used in selection mav vary, researchers have the same or a similar opinion on those indicators. In addition, more attention should be paid to the selection of the experimental location, mostly due to the reason that the effect of the location on ratooning ability is visible.

Main Factors Influencing Longevity and Productivity of Ratoon Sugarcane

The ratooning ability or good ratooning potential is essential pre-requisite or the most critical factor for good ratoon. Α series investigations have been conducted on the factors that affect the longevity of ratoon cane. The genotype, cultivation management, and environment contribute to the ratoon crop in descending order. The ratoon crop yields decline typically with age. Studies have also shown that, subtropical regions. bottleneck major for improving ration productivity is the poor germination rate of buds in the stubble remaining after winter harvesting. There is a report that the poor germination rate of the ratoon crop not only affects the number of seedlings per unit area, but also leads to a large number of ineffective tillers throughout the growing season, resulting in the lower number at harvest (Hogarth and Berding, 2016). The trait of stalk number has the greatest impact sugarcane yield. Guangxi, the largest sugarcane producing area accounting for more than 60% of the total sugar in China, is located in the subtropical region, where germination is a severe challenge during ratooning due to the serious smut infection during growing frequent process and the

rainfall during harvest. The breeding and promotion of sugarcane varieties with strong ratooning ability is a technical approach to effectively solve this problem. This is because the ratooning ability directly affects the germination rate of the ratoon crop, thereby directly influencing the establishment of the high-yielding seedling population. and ultimately cane yield. Therefore, the ratooning ability is one of the most important target traits in sugarcane breeding and has always been valued bγ breeders.

From both the perspective of reducing production and improving the productivity of the ratoon crops, breeding and growing varieties with a strong ratooning ability is the most important prerequisite for extending the number of ratooning years and increasing the yield of the ratoon crops. Furthermore, in sugarcane-producing areas with low temperatures, frost, drought, pests, diseases (especially smut), stem borers. or extensive management, the ratooning ability of sugarcane varieties is particularly important for extending the number of ratooning years and increasing the yield of ratoon crops.

Genetic Research on the Variation in Ratooning Ability between Different Sugarcane Genotypes

Sugarcane genotypes with higher proportions of the genetic background of Saccharum spontaneum display stronger ratooning

ability (Oin et al, 2015) because the characteristics of a species can be affected by kinship, i.e., hereditary basis. Sugarcane 'nobilization' breeding aimed at bringing the genes controlling vigor, vitality, stress resistance, and strong ratooning ability from species into original species. cultivated 'noble' S. officinarum. A wild species S. spontaneum, the mostly used and studied, was the first species to naturally hybridize with S. officinarum. Other wild species that have desirable traits and have used in sugarcane breeding are S. rubustum and species in closely related including *Erianthus* genera arundinaceum and Narenga porphyrocoma. Of these, N. porphyrocoma was firstly utilized in China in recent to improve the vears ratooning ability and stress resistance, especially smut resistance of sugarcane varieties. The combining ability and heritability of traits including ratooning ability have been investigated in the aenetics and breeding sugarcane. The combining ability refers to the ability of parental traits to be combined in hybrid crosses, which is an important basis for selecting hybrid parents and making cross combinations. Studies have shown that the ability ratooning is jointly affected by the general and specific combining abilities of both the male and female parents. The inheritance of sugarcane rationing ability and the relationship of traits between plant cane ratoon crops has been well examined by (Milligan et al. 1996), which suggests that genetic coefficient of variation of ratooning ability is largest for cane yield and sucrose yield, and the selection for stalk number in vounger crops will increase the yields of older crops.

Mechanism Underlying the Variation in Sugarcane Ratooning Ability

Morphological basis. Previous studies on the morphology of sugarcane stubble reached the unanimous conclusion that strong ratooning abilityrelated stubble has deep roots (long roots), a large number of buds and live buds, and a large number of permanent roots, along with a rational leaf size and the reasonable tillering ability. However, no comprehensive quantitative morphological analysis has been reported for sugarcane root growth and development using modern technologies and equipment.

Molecular basis. There has been little research on the molecular basis of ratooning ability variation. ABA was found to regulate the withering of ineffective tillers through hormonal interactions in the form of molecular signals. Overexpression of the TB1 gene in a sugarcane line resulted in a reduced tiller number. So far, no report has published been on the molecular mechanism underlying the ratooning ability of sugarcane. However, related research has been conducted in rice, which is also monocotyledonous and

has tillering traits. This research in rice has provided a reference for the study of molecular mechanism responsible for the variation in sugarcane rationing ability. Differences in rhizosphere microorganisms. Plant-soil organisms. especially microorganism interactions, play important roles in crop health, crop yield, and soil However, quality. the relationship between the rhizosphere diversity of microorganisms and the ratooning ability is still unclear. In this field, most studies have focused on the investigation οf the differences of soil physicochemical properties, soil nutrient, and change of rhizosphere microorganism between cane plant ratoon crop, or among the different practices of cultivation management, including residue mulching, plant growth regulators bioagents and rotation. Lin et al. found 38 differentially expressed proteins rhizospheric soil of ratoon crop compared with plant significant cane. Recently, differences in the diversity of rhizosphere bacteria have been found between sugarcane varieties.

Shortcomings of Existing Research

Modern sugarcane varieties

(Saccharum spp. hybrids) are interspecific hybrids and the allopolyploids derived from the hybrids of homopolyploids, and may 120 have to up chromosomes. In fact, the number of chromosomes can different between genotypes, for example, from 106 to 111 among 10 main sugarcane cultivars in China. Of these, 70-80% of the chromosomes are from a tropical species (S. officinarum) that 80 has chromosomes (octaploid, 2n = 8×) and 10-20% are from species S. spontaneum that has 40–128 chromosomes $(2n = 4 \times to)$ 12×). Recombinants between the two species account for 10% of the genome in modern varieties. is lt precisely because of this highly heterozygous genetic background that the offspring sugarcane hybrids are widely segregated and the probability of aggregation of excellent traits is extremely (1/100,000-1/300,000).Therefore, for a long time, sugarcane cross breeding had to rely on large segregating populations. In China alone, in spite of more than one million F1 seedlings being planted in the field each year, a commercial cultivar yield, high with disease resistance, especially primary diseases including smut resistance, and strong ratooning ability has not been yet developed. Approximately 95-97% of planted F_1 seedlings are discarded after observation in the first year, without ratooning.

Research Prospects

ratooning ability sugarcane is not affected by a single factor, but by multiple factors including genotype. environment and temperature, humidity, water supply in the growing area), and cultivation technologies (Figure 1). Optimizing the sugar production per unit area is the ultimate goal of breeding sugarcane varieties with strong ratooning ability. Therefore. research sugarcane rationing ability should be integrated into the breeding of varieties with ratooning strong ability. Regarding the commercial cultivation, breeders need to consider at least three traits, namely, ratooning ability, main disease resistance in production (such as smut resistance in China), sugar production per unit area. To select these traits breeding. during it is necessary to identify and develop markers associated with the traits. Highthroughput technologies should then be used to analyse the populations.

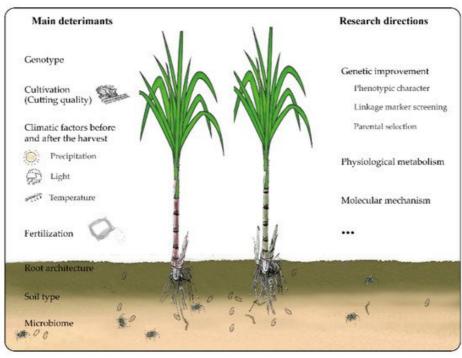


Figure-1 The main determinants and research directions for sugarcane rationing

Markers associated with target traits are valuable for breeding. sugarcane, ln markers associated with traits, including sugarcane sucrose content and sugar content, have previously been studied. In addition, markers associated with sugarcane yellow spot resistance have also been studied. However, only markers associated with the major gene, Bru1, which is responsible for sugarcane brown rust resistance, have been used in breeding. The first marker to be identified was 10 cM from the Bru1 gene. Furthermore, markers that were 1.9 cM and 2.2 cM from the gene were observed, but only the markers that were 0.28 cM and 0.14 cM from the Bru1 gene were identified and used to assist the selection in breeding. Nevertheless, these markers still have the problem of false positive, that is, the markers can be detected in a line without the actual disease resistance. It is difficult to identify markers closely associated with target traits because complete a reference genome is still unavailable for sugarcane; however, some progress has been made. Therefore, adding the full-length transcriptome unigene data of the hybrid parents supplement to the reference genome sequence, and using these data in biparental F₁ population mapping, may be beneficial for identifying target trait-associated genes. What should also be stressed here is that mining key regulatory genes associated with excellent traits, clarifying the allelic variation of these key genes, and analyzing the distribution and genetic effect haplotypes. are important for elucidating sugarcane ratooning ability and for gene editing breeding methods.

There are several technical approaches that are used to

identify associated markers and genes. First, polymorphic data of the whole genome obtained by Pool-seq can be compared to the corrected transcriptome unigene database to identify the key genes associated with sugarcane rationing ability. is а cost-effective strategy and needs to be considered. Second, biparental F₁ population and sugarcane single nucleotide polymorphism (SNP) chip can be used to perform genotyping marker and analysis. Successful cases using this approach have previously been reported. However, the actual application value of the obtained quantitative trait loci needs further (QTL) verification. A third approach involves the utilization of inbred populations, such as R570 inbred populations. This approach helps to tackle the fact that modern sugarcane varieties are highly

heterogeneous. and even selfing offspring are still highly heterozygous, and thus the target traits are segregating. A fourth approach, which has been successfully applied in sugarcane, involves the utilization of natural populations and the simplification whole of genome sequencing technology.

CONCLUSIONS

Ratooning can largely reduce production costs compared with replanting sugarcane. Labor costs increase yearly, and the cost difference between ratooning and replanting sugarcane widens. In this paper, previous studies sugarcane ratooning on ability were reviewed in terms of the definition, phenotypic major influencing traits. factors, genetic basis, and the formation mechanisms. In addition, the shortcomings of research existing on ratooning ability were highlighted and the focuses of studies future were suggested. We do hope that this review can provide a reference for understanding the mechanisms underlying sugarcane ratooning ability, and for breeding sugarcane varieties with strong ratooning ability.

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