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PERFORMANCE EVALUATION OF BARBADOS FUZZ AT INITIAL STAGES

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ABSTRACT

In this research study, thirteen crosses of sugarcane fuzz received at Sugarcane Research Institute (SRI) Faisalabad from West Indies Central Sugarcane Breeding Station (WICSBS) Barbados were tested and evaluated for initial stages of varietal development program. This study was important as for the first time West Indian sugarcane germplasm was evaluated in Pakistan. The comparison was made with check (approved) varieties to promote into next selection stage. The sowing of fuzz at SRI started during 1st week of July 2019 as humidity level and temperature becomes more favorable for its germination. The selection of seedlings was made during the month of November 2019 and 234 clones were promoted to nursery-I. Similarly, 42 clones were advanced to nursery-II. The numbers were reduced to six in preliminary yield trial when red rot and other disease screening were applied during nursery-II. The clones were planted 1.2m x 1.2m at seedlings stage, 1.2m x 4m at N-I stage, 2.4m x 4m at N-II stage while three replications at preliminary yield trials. The clones showing better performance were carried out to next selection stage of varietal development program during selection process in the month of October each year. Further testing and evaluation of these clones is needed to check more adaptability and stability of general characteristics to proceed into net varietal development stages.

Key words: Sugarcane, Faisalabad. Fuzz, Barbados, Seedling, Selection

INTRODUCTION

Sugarcane is vital cash crop that plays vital role in overall GDP. In Pakistan it falls in top ranked valuable cash crop. The crop has been divide into three zones keeping in view the climatic, soil and other biotic and abiotic factors for successful production of crop. It is cultivated all over the country in irrigated areas. The production of sugarcane stood 81.009 million tones when cultivated on an area of 1.165 million hectare (Govt. of Pakistan, 2021). The climatic

conditions in country do not permit the profuse flowering with enough viability of the sugarcane true seed (fuzz). A specific set of conditions is necessary to induce the flowering in sugarcane plant that ultimately leads to flowering. A little bit natural conditions exist in Murree that favor flowering in some clones (Ahmed *et al.*, 2019). The changing climatic conditions are disturbing the sugar recovery, growth and overall tonnage of sugarcane crop and causing attack of insects pest, vulnerability to different diseases and lodging

of crop. These challenges urge the investigators to find out well suited clones having good adaptability in different ecological zones. Weather related events and climatic factors play important role to affect yield and production of sugarcane crop (Zhao and Li 2015). In *Saccharum officinarum* sucrose content range from 12–16% of fresh weight of sugarcane stalk (Bull and Glasziou 1963). Pakistan ranks 5th position by area and production among world sugar producing countries (FAO, 2017). Genetic variability is important

for developing new variety of any crop. Fuzz provide the opportunity to develop new variety in sugarcane as crossing over creates genetic variability at meiosis level during seed formation. The researchers try to fine better and improved sugarcane clones that have high sugar contents and good tonnage that are more profitable for growers and millers (Jackson, 2005). Bringing desirable parents in cross combinations during hybridization process is key to evolve new crop variety. The yield being quantitative is closely linked with environmental factors (Pandey *et al.* 2018).

Sugarcane Research Institute, Faisalabad Pakistan has been working on sugarcane varietal development program for many decades. Normally the sources of fuzz have been USA, Australia, South Africa, Brazil and Barbados for evolution of sugarcane varieties. However, a few varieties from local fuzz were also evolved but main source of varieties development have been exotic fuzz or introduction. Sugarcane Research and Development Board, Pakistan being a funding agency, have been providing fuzz to Sugarcane Research Institute, Faisalabad from last many years. During 2018-19, thirteen crosses of Barbados were provided to be included in varietal development program. The purpose of current study is to find out best suited clones having more adaptability in local climatic conditions and having

better or comparable performance with existing check varieties.

Plantation of Experiment

The experiment was planted at coordinate plane 31.4311° N and 73.0694° E during 2018, 2019 and 2020 at research farm of Sugarcane Research Institute, Faisalabad. The soil texture was loamy having electric conductivity (EC) 1.85, soil pH 7.9, organic matter 0.95%, available phosphorous 8.4 ppm, available potassium 189 ppm and saturation 30 %.

Seedlings Stage

The fuzz was sown during July, 2018 on raised beds. For proper germination of sugarcane fuzz requires relative humidity more than 60 % and temperature range from 30 to 38C. As monsoon season starts, the required temperature and humidity goes up to mark and provides favorable condition for good germination of fuzz. Thirteen crosses of Barbados were sown and all of them germinated providing 4077 seedlings. The seedlings were singled out in earthen pots and transplanted into field at the arrival of favorable environment when frost ended. During October 2019, selection of seedlings was made taking brix value in comparison to check varieties. During selection process 234 clones were selected and promoted to N-I stage for further evaluation at single line stage. The brix % of HSF 240 and CPF 249 stood 18 and 19 respectively. While the brix and other characters of each stool were

compared with check varieties and promoted to next stage for further evaluation (Table-1).

Nursery -1

The clones selected in seedlings stage were planted in single row of Nursery one stage along with three check varieties i.e HSF 240, CPF 249 and CPF 253 during October 2019. In this stage one row of 4 meter is planted by each promoted clone. Among 234 clones, 42 clones were found good and promoted to next stage called "Nursery-II" (Table-2). The selection index at this stage remained 18%. All other clones were not found good and eliminated from varietal development program. Liberal selection was made at this stage. The selection process of this stage was carried out during October, 2020. This stage is actually seed increase stage here only single replication is used.

Nursery-II

Nursery-II is double row single replication stage. The plot size of each entry kept 2.4m x 4m. At this stage 42 clones along with three check varieties were tested. Selection was made during October, 2021 to check the performance of these clones in comparison to check varieties. At this stage the reaction to red rot pathogen was also checked. The clones that showed susceptibility to red rot, showed lodging, high pithy, smut disease, poor growth and stand were rejected and only 6 clones were proved good according to selection criteria and

promoted to Nursery-III (Preliminary Yield Trial). These clones have been planted to Nursery-III stage using three replications deploying Randomized Complete Block Design (RCBD). Recommended seed

rate along with recommended cultural practices were applied to evaluate at this stage (Table-3). The plot size for single replication is kept 3.6m x 4 m. while promoting clones from Nursery-I to Nursery-II the selection index

remained 14%. These clones are under study current year and their performance will be evaluated at harvest time, January 2023. The clones showing good performance will be carried to Semifinal stage.

Table-1 Selected Clones in Seedlings to Promote in Nursery-1

Sr. No.	Code	Parentage	No. of Seedlings	Selected Clones	Brix (%)	Clone No. Allotment
1	9	D 98 398 x Poly CC	312	6	17-18	BDF 19-(1...6)
2	10	CR 10 2007 x Poly CC	342	35	16-22.5	BDF 19-(7...41)
3	2	BR 10 001 x Poly CC	216	4	15-22	BDF 19-(42...45)
4	4	CR 08 0014 x Poly CC	343	29	16-21	BDF 19-(46...74)
5	11	CR 10 2003 x Poly CC	229	20	17-22	BDF 19-(75...94)
6	5	B 09 2681 x Poly CC	290	17	16-22	BDF 19-(107...123)
7	1	CP 96 1252 x CR 11 0004	130	7	15-20	BDF 19-(124...130)
8	6	BJ 06 794 x Poly CC	230	27	17-22	BDF 19-(131...157)
9	7	BR 08 008 x Poly CC	420	12	16-22	BDF 19-(158...169)
10	8	D 93 224 x Poly CC	298	10	17-19	BDF 19-(170...179)
11	3	DB 04 01 x Poly CC	312	1	19	BDF 19-(180)
12	12	BJ 99 112 x Poly CC	940	66	15-25	BDF 19-(181...246)
13	13	DB 04 1009 x Poly CC	15	0	-	-
	Total		4077	234		

Table-2 Clones Promoted to Nursery-II from Nursery-I

Sr. No.	Clone No.	Brix (%)	Sr. No.	Clone No.	Brix (%)	Sr. No.	Clone No.	Brix (%)
1	BDF 19-1	15.3	16	BDF 19-41	16.3	31	BDF 19-112	18.3
2	BDF 19-7	16.7	17	BDF 19-45	20.7	32	BDF 19-139	21.3
3	BDF 19-10	18.7	18	BDF 19-48	20.3	33	BDF 19-146	19.3
4	BDF 19-11	18.7	19	BDF 19-50	19.0	34	BDF 19-177	18.3
5	BDF 19-12	20.7	20	BDF 19-52	17.0	35	BDF 19-181	21.0
6	BDF 19-15	19.3	21	BDF 19-54	18.0	36	BDF 19-186	19.0
7	BDF 19-16	20.0	22	BDF 19-56	17.3	37	BDF 19-205	20.7
8	BDF 19-22	18.3	23	BDF 19-67	20.3	38	BDF 19-223	17.7
9	BDF 19-23	19.3	24	BDF 19-70	19.0	39	BDF 19-227	15.0
10	BDF 19-24	18.3	25	BDF 19-77	18.0	40	BDF 19-230	19.7
11	BDF 19-25	17.3	26	BDF 19-79	15.7	41	BDF 19-245	19.3
12	BDF 19-28	19.0	27	BDF 19-92	19.3	42	BDF 19-246	18.3
13	BDF 19-29	19.3	28	BDF 19-102	19.3	43	HSF 240	19.3
14	BDF 19-31	17.3	29	BDF 19-105	19.7	44	CPF 249	19.0
15	BDF 19-35	18.0	30	BDF 19-110	21.0	45	CPF 253	19.7

Table-3 Clones promoted to Nursery-III (Preliminary Yield Trials)

Sr. No.	Clones	Brix (%)	Sr. No.	Clones	Brix (%)
1	BDF 19-25	17.7	6	BDF 19-230	16.0
2	BDF 19-45	15.7	7	HSF 240 (C1)	19.3
3	BDF 19-54	16.0	8	CPF 249 (C2)	18.0
4	BDF 19-92	16.3	9	CPF 253 (C3)	17.3
5	BDF 19-102	17.0			

CONCLUSION AND RECOMMENDATIONS

The varietal development program adapted at Sugarcane Research Institute, Faisalabad includes both fuzz and introduction for testing, evaluation, selection and promoting the clones to develop genetically improved

sugarcane varieties. The 1st way is extensively used for developing sugarcane varieties. Sowing of fuzz in July gives good germination results while its selection during the month of October is favorable when temperature lowers and brix percent starts to increase. Six clones BDF 19-25, BDF 19-

45, BDF 19-54, BDF 19-92, BDF 19-102 and BDF 19-230 from Barbados fuzz are under evaluation and will be promoted to semifinal for further study. The best performing clones may be released as variety while checking all required aspects in latter stage of varietal development program.

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A NEW APPROACH FOR INTEGRATED PEST MANAGEMENT OF SUGARCANE IN PAKISTAN

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ABSTRACT

Conventional Integrated Pest Management (IPM) is mostly described as “a decision-making process using multiple pest management tactics to prevent economically damaging out-breaks while reducing risks to human health and the environment”. Low-level IPM is the most often employed form, consisting of the most basic of IPM practices—scouting and insecticide applications according to economic thresholds. Some growers have progressed to medium-level IPM, the adoption of a few additional preventive measures, e.g. cultural controls and plant resistance, coupled with efforts to cut back on broad spectrum pesticide use in order to protect beneficial organisms. These IPM strategies are mainly targeted towards single pest species and do not consider all the pests in a specific agro-ecosystem. High-level or Bio-intensive IPM, is where multiple interventions are integrated in a bio-intensive approach targeting multiple pests. Bio-intensive IPM is based on holistic agro-ecosystem interactions, in which knowledge about insects, their symbionts, pathogens, natural enemies, plants, endophytes and interactions between all of these are combined to develop IPM in an area-wide, environmentally friendly manner. Reviewed here are advances in knowledge of, and of biotic interactions between direct, indirect and induced plant resistance, plant nutrition, habitat management, chemical ecology, natural enemies, soil-health, micro-organisms such as endophytic fungi and *Wolbachia* and phylogenetics and phylo-geography. All of these are potential building blocks of a bio-intensive IPM system under-construction at SRDB, SRI and MNSUAM. Also discussed are opportunities and challenges in these areas of research, considering bio-security threats to the Pakistan sugar industry and possible limitations in current sugarcane plant breeding material.

Keywords: *Chilo infuscatellus*, IPM, Induced Resistance, Sugarcane, Pakistan, Direct and Indirect Resistance.

INTRODUCTION

SRI Faisalabad has been working to improve control of the sugarcane stem borer *Chilo infuscatellus* (Snellen) since the early 2010s (Munir, 2014). A few cultural control measures and several less susceptible varieties have been developed against it (Munir, 2014). However, it still remains a pest throughout the sugar industry (Sikandar and Ahmad, 2021).

In order to build resilience

into the sugarcane agro-ecosystem, a refocusing of control efforts into a bio-intensive area-wide integrated pest management approach is necessary (Klassen, 2005). Such an approach marries conventional control options with ecologically based new technologies such as delineation of within species populations, chemical ecology, stimulo-deterrent diversion (push-pull) and enhancement of natural enemies through habitat

management and good soil health practices, to produce sustainable IPM strategies applicable across large areas involving multiple stakeholders (Conlong and Rutherford, 2009). There is also a need to refocus bio-security to again build resilience to invasion into agro-ecosystems, rather than building walls around them.

IPM—From the bottom up

Plant resistance to pests and diseases can be linked to optimal physical, chemical

and biological properties of soil (Zehnder *et al.*, 2007). 'Healthy' soil is described as having sufficient organic matter to support a high diversity of animal (arthropods, nematodes etc.) and microbial life. Soil can act as important reservoirs for a diversity of entomopathogenic fungi & nematodes, as well as predaceous arthropods, which can contribute significantly to the regulation of pest populations.

Ninety percent of insect pest species spend at least part of their life cycle in soil. In addition, pests that occasionally come into contact with soil can be attacked by predators or become infected by entomopathogens (Klingen *et al.*, 2002). After the harvest of heavily infested sugarcane, the residual *E. saccharina* population from which infestation of the following ratoon crop can be expected is found in the sugarcane stubble at soil level and in the stool below ground.

By minimizing compaction and tillage, and by mulching and increasing organic matter, soil can support increased populations of entomopathogenic fungi, entomopathogenic nematodes and predaceous arthropods such that these natural enemies of insects can be included in the conservation biological control strategy (Meyling and Eilenberg, 2007).

Direct and indirect host-

plant resistance

Insect resistance in grasses is the result of many defense mechanisms that act in parallel to limit the damage of herbivore attacks. Many of these defense mechanisms are based on plant secondary metabolites, or defensive proteins that directly affect the herbivore due to their toxic, deterring or anti-nutritional properties.

Structural resistance also occurs. Keeping and Meyer (2002) have shown that resistance to *E. saccharina* can be enhanced using soil-applied silicon, which becomes incorporated into the plant alongside lignin and fiber increasing resistance to penetration. These authors emphasize a relationship between nitrogen and silicon nutrition where by the ratio of these elements determined in leaf analyses can be used as an indicator of *E. saccharina* infestation risk. Keeping and Rutherford (2004) have reviewed mechanisms of direct resistance to *E. saccharina*. Two decades ago, a new type of defense mechanism, termed indirect defense, was first described in maize. Central to this type of defense is the release of a volatile plant SOS signal, a mixture of volatile secondary metabolites.

Plant volatiles are derived from complex biochemical processes and include fatty-acid-derived products [methyl-jasmonate, cis-jasmone, and green leaf volatiles (GLV) like hexenal and hexenyl-acetate], monoterpenes, sesquiter-

penes, and shikimic acid-derived products [e.g. methylsalicylate and indole] (Ferry *et al.*, 2004). These can serve as signals, not only to attract predators and parasites of attacking herbivores, but they can also repel the herbivore itself, and they can elicit responses in neighbouring undamaged plants (De Moraes *et al.*, 2001). The use of elicitors to directly activate or prime resistance shows much promise as an IPM tool (Zehnder *et al.*, 2007).

Habitat management

Therefore, it is very important to understand the role of plants in managing insect populations. An example comes from our experience in trying to control *E. saccharina* with indigenous and new association biological control agents.

Conlong *et al.* (2007) found that female *E. saccharina* moths will accept *Cyperus papyrus* and *Cyperus dives* as host plants in preference to the indigenous grass *Pennisetum purpureum*, with sugarcane being least preferred.

A preference was demonstrated by Keeping *et al.* (2007), that if given choice between older sugarcane & maize, *E. saccharina* would oviposit on maize even if it were Bt maize.

Keeping *et al.* (2007) further showed that larval survival on this Bt maize was zero. A hierarchical oviposition preference (Thompson and Pellmyr, 1991) is suggested in Southern African *E.*

saccharina females, with most ovi position found on or close to its indigenous sedge hosts, followed by indigenous grasses, and then sugarcane.

However, a large proportion of these eggs were not laid directly on the plants, but in crypticovi position sites in the vicinity of potential host plants (Kasl, 2004; Barker, 2008). Egg dumping is behavior of highly polyphagous species (*E. saccharina* attacks species of the Cyperaceae, Typhaceae, Juncaceae and Gram in aceae (Conlong, 2001; Mazodze and Conlong, 2003), orin species associated with super abundant host plants.

These are both possibilities with *E. saccharina* in sugarcane and its cyperaceous hosts, as both hosts occur in large essentially mono-specific stands. Adult females therefore may not be particularly attracted by host or 'pull' plants in an IPM system and conversely they may be more strongly repelled by non-host or 'push' plants, since the presence of these could indicate that the insect had reached the edge of the preferred mono-specific host plant stand.

Nevertheless, *E. saccharina* seems to have a hierarchical preference in choosing a host plant habitat to oviposit in, i.e. Cyperaceae and maize, both of which have *E. saccharina* population controls in place; natural enemies in the Cyperaceae

(Conlong, 1990, 1997, 2000) and genetically engineered Bt toxin maize (Keeping *et al.*, 2007).

Further evidence to promote habitat management as a control option, demonstrated the repellent 'push' properties of the indigenous grass *Melinis minutiflora* Beauv. to cereal stem borers, and also its attractant properties to their parasitoids. *M. minutiflora* produces volatiles similar to damaged maize, even in the absence of pest damage to itself (Gohole *et al.*, 2003).

In a glasshouse experiment at SASRI, *Xanthopim plastemmator* (Thunberg) (Hymenoptera: Ichneumonidae) parasitised more *E. saccharina* pupae in sugarcane in close proximity to this grass, than in sugarcane only (Figure 1) (Kasl, 2004). This suggests that the searching behaviour of the parasitoid was increased by *Melinis* volatiles.

The next phase in developing this habitat management approach for *E. saccharina* was to setup field trials using rows of *M. minutiflora* along either in irrigation or contour breaks as a repellent or 'push' plant. *E. saccharina* populations and damage were halved in field plots planted next to strips of *M. minutiflora* compared to control plots, suggesting that the pest was repelled by *Melinis* volatiles (Figure 2) (Barker *et al.*, 2006). Planting *Cyperus papyrus* as a trap, or 'pull' plant along drainage

lines of selected sugarcane fields resulted in significantly reduced damage in the cane associated with it (Figure 3) (Kasl, 2004).

Based on the success of these trials, a farm-based habitat management plan has been devised, incorporating indigenous host plants and Bt maize as 'pull' plants for *E. saccharina* and *M. minutiflora* as the 'push' component. This bio-intensive approach has been expanded into a Bio-intensive-PM plan, incorporating plant nutrition, soil health and the use of less susceptible sugarcane varieties.

An added aspect to the plan is to plant buck wheat at the time of sugarcane planting. This is to attract adult parasitoids and predators into the sugarcane environment by providing a pollen and nectar source for their survival during periods of low host availability, much the same as advocated by Wäckers *et al.* (2005) and Zehnder *et al.* (2007) in their conservation biological control approach to enhance the activity of indigenous natural enemies.

Does sugarcane emit SOS volatiles when attacked by *E. saccharina*?

In contrast to the situation in the natural hosts of *E. saccharina*, negligible parasitism has been recorded in sugarcane, even when this crop was planted adjacent to infested indigenous host plants with a

bundant parasitoids present (Conlong and Hastings, 1984). Many introduced parasitoid have also failed to colonise the sugarcane habitat (Conlong, 1997).

Using gas chromatography, Smith *et al.* (2006) showed different volatile emission patterns between *Cyperus papyrus* infested by *E. saccharina* and un-infested *C. papyrus*. Infested sugarcane was neither qualitatively or quantitatively different from un-infested sugarcane and both were different from *C. papyrus* (Figure 6). In addition, these authors showed that the parasitoid *Goniozus indicus* (Ashmead) (Hymenoptera: Bethyridae) was attracted to frass from *E. saccharina* that had fed on *C. papyrus*, and was not attracted to frass from *E. saccharina* that had fed on sugarcane. Adding this to the lack of parasitism recorded in sugarcane, even in the vicinity of natural host plants harbouring parasitoids, suggests that modern sugarcane genotypes may not attract natural enemies through the release of herbivore induced SOS volatiles, or that they may differ in the ability to do so.

Genotypic differences in plant volatile emission

The ability to mount indirect defence against *E. saccharina* may have been lost in sugarcane as a result of in advertently concentrating on direct resistance in amono culture oriented plant breeding selection program. Besides this possibility, the release of

plant volatiles is characterised by a large degree of genotypic variation within plant species, for example, maize genotypes and their closest wild relatives, *Zea mays* ssp. *Parviglumis* and *Mexicana* (collectively known as teosinte), show significant differences in emissions when attacked (Gouinguene *et al.*, 2001; Degen *et al.*, 2004).

An example of loss of indirect defence has been found below ground in maize. In response to feeding by the western corn rootworm, *Diabrotica virgifera virgifera* (LeConte) (Coleoptera: Chrysomelidae), maizeroots release (E)-b-caryophyllene that attracts the entomo pathogenic nematode *Heteror habditismegidis* (Rasmann *et al.*, 2005). Most North American maize lines do not release (E)-b-caryophyllene in response to rootworm attack, whereas many European lines and teosinte accessions do (Kollner *et al.*, 2008).

The existence of genotypic differences in the emission pattern of volatile compounds for Kenyan *M. minutiflora* cultivars has also been demonstrated (Goholeet *et al.*, 2003). The lack of response by *X. stemmator* in the presence of Australian *M. minutiflora* again points to variability within this species (Figure 1). Australian *M. minutiflorais* extensively used for cattle fodder.

The strong odour of the plant can be carried through to milk and, because of this,

there has been an extensive program to breed less volatile variety with similar nutritional quality. The volatile (s) that the parasitoid responded to in the African variety could have been bred out of the Australian variety.

Breeding for artificially primed and induced resistance

The loss of the ability to produce an SOS volatile and the observed genotypic variability in their production by maize points towards the exploitation of the phenomenon in sugarcane, by breeding varieties for enhanced attractiveness to natural enemies. This could be achieved through the application of an artificial elicitor followed by selection for enhanced direct and indirect resistance in a system that includes natural enemies.

Experimental application of elicitors is fairly simple and it is worth trying to make selections among plant breeding lines grown under the influence of plant defence elicitors, aiming for new cultivars optimized for artificially inducible resistance traits without significant yield penalty (Agrawal *et al.*, 2002; Ahman, 2006). Historically, induced resistance research has mostly concentrated on direct activation where resistance is expressed in advance of challenge by the pest. The possibility of priming as a mechanism of protection has often been overlooked because it only becomes apparent in challenged

plants. Priming equates to a 'heightened state of readiness', in that in the event of damage to a primed plant, resistance responses are faster and more intense (Conrath *et al.*, 2006).

Direct activation of resistance might best be employed where the target pest is widespread and has predictable outbreaks. An example is the sugarcane thrips, *Fulmekiolaserrata* (Kobus) (Thysanoptera: Thripidae) that affects more than two thirds of sugarcane plants in a particular field at the same time. Outbreaks occur in summer with numbers peaking every January since the pest was first discovered on the African continent in 2004 (Way *et al.*, 2006). Primed resistance would, however, be more suitable for *E. saccharina*, since a much lower proportion of plants is attacked and infestation tends to be patchy.

Ecology and phylogeography

The basic building block of IPM is still regarded as ecology (Gurr *et al.*, 2003). In a study of *E. saccharina*, Conlong (2001) found behavioural, host plant and natural enemy differences in population occurring between South, Central and West Africa, with them seemingly coming together in Uganda.

The second fusing factors between different populations of what is otherwise morphologically similar

species made it an ideal candidate for molecular systematic analyses. Assefa *et al.* (2006), using the cytochrome oxidase subunit 1 (CO1) region of the mitochondrial genome, separated *E. saccharina* into three distinct groups (west, south and Ethiopian). Two of these groups (west and south) were found in Uganda. The CO1 genetic diversity between these groups was larger than that between recognised species within the genus *Ostrinia* (Lepidoptera: Crambidae) (Coates *et al.*, 2005). In other insects, unexpected mt DNA CO1 patterns have led to the discovery of cryptic species (Hebert *et al.*, 2004; *et al.*, 2007). Such diversity should encourage us to confirm covarying genetic, behavioural and ecological characteristics which would lend support to the notion that cryptic species exist within the *E. saccharina* complex.

In IPM programs which use classical bio-control as one of their management options, or translocation of natural enemies, these aspects can be enhanced by using such techniques to identify cryptic species, or populations of species most closely related to each other, so that more informed decisions can be made regarding natural enemy selection for use against pests. This applies not only to pest species, but also to parasitoids (Ngi-Song *et al.*, 1998).

Since 1992, surveys for indigenous parasitoids of *E.*

saccharina in a variety of African habitats have been completed. Thirty species of larval parasitoids have been found in eight countries (Conlong, 2000). Several of these have failed to parasitise *E. saccharina* from South Africa due to incompatibility. For example, from West Africa, *Descampsinasesamia* (Diptera: Tachinidae) larva eareen capsulated by *E. saccharina* (Conlong, 1997). *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) from South Africa is also unsuccessful as egg eareen capsulated. Further collections of parasitoids from Central Africa, where southern and western populations of *E. saccharina* co-exist, could reveal biotypes of parasitoids that could be effective against this pest (Ngi-Song *et al.*, 1998).

The interaction between *E. saccharina* and *Fusarium*

When *E. saccharina* bores in sugarcane, the tissue surrounding the boring becomes reddish discoloured often affecting a whole internode. Following on from this, and work of Schulthess *et al.* (2002), McFarlane *et al.* (2009) cultured numerous *Fusarium* spp. isolates, from the red tissue surrounding *E. saccharina* borings, as well as from undamaged stalks as endophytes. Most of the isolates from borings were beneficial to *E. saccharina* in artificial diet in terms of larval survival and growth rate, and were attractive to neonates in olfactory choice assays.

A few of the endophytic isolates were antagonistic, with *E. saccharina* neonates repelled and growth retarded. Moths may also be attracted or repelled depending upon isolate. Ako *et al.* (2003) showed that West African *E. saccharina* females laid on average 32 eggs on maize stalks with *F. verticillioides* present as an endophyte, versus nine on stalks grown from fungicide or hot water treated seeds.

In an integrated control approach against *E. saccharina*, seed cane hot water treatment and/ or treatment with fungicides could reduce endophytic colonisation by *Fusarium* isolates beneficial to *E. saccharina*, thereby reducing the chance of infestation. Alternatively, the facilitation of endophytic colonization of sugarcane by *Fusarium* isolates antagonistic to *E. saccharina* could afford more sustainable and environmentally friendly protection from this stalk borer. Another approach could be to exploit the differences in volatiles between repellent and attractive isolates in the development of repellents and lures of use in the field.

CONCLUDING REMARKS

Khan *et al.* (1997a) described a 'push-pull' system effective against stem borers in sugarcane. This system includes the indigenous parasitoid *Cotesia sesamiae* as well as *C. flavipes* which were introduced against the exotic *Chiloptellus*

(Overholt *et al.*, 1997). *C. sacchari phagus*, now present in Mozambique, is being parasitised by both *C. flavipes* and *X. stemmatoron* larvae and pupae respectively in sugarcane. Ngumbi *et al.* (2005) showed that *C. flavipes* females respond to terpenoids and the green leaf volatiles which are released by maize plants damaged by *C. partellus*. This suggests that sugarcane may produce SOS volatiles in response to *C. sacchariphagus*.

There are alternative hypotheses to that of loss of ability to produce SOS volatiles. By boring the tops of stalks and leaf spindles in young cane, volatile emission could be elicited by *C. sacchariphagus*, whereas *E. saccharina* may avoid eliciting volatile emission by boring the bottoms of stalks in older cane. Another hypothesis is that the *Fusarium* associated with *E. saccharina* could interfere with the elicitation of volatile emission or change the composition of emitted volatiles. These possibilities are worthy of further investigation.

Nevertheless, a bio-intensive IPM strategy could be made more effective if the crop itself is capable of releasing appropriate SOS volatiles when attacked. Breeding varieties for enhanced attractiveness to natural enemies has potential if natural enemies are already present in the habitat management system. *Goniozus indicus* parasitises

E. saccharina in *C. papyrus*. It is also known to parasitise *C. partellus* (Keiji and Overholt, 1996) and has been found doing so in *Sorghum arundinaceum* growing in proximity to *C. papyrus* (Conlong, 1994; 1997). It is therefore possible that *G. indicus* could parasitise both *C. sacchariphagus* and *E. saccharina* in sugarcane. The same applies to the pupal parasite *X. stemmator*.

Conlong *et al.* (2004) showed that some of the varieties with the highest direct resistance to *E. saccharina* were the most susceptible to *C. sacchariphagus* and vice-versa. This has implications for variety choice in an IPM system designed to target both pests simultaneously, should *C. sacchariphagus* invade the South African sugar industry. Given increasing adoption of the 'push-pull' habitat management concept even in the absence of parasitoid activity against *E. saccharina* in sugarcane, and its success against *C. partellus* in maize, we are confident that 'push-pull' habitat management will also be effective against *C. sacchariphagus*.

The development of IPM strategies depends on a sound understanding of the chemical ecology of pest interactions with sugarcane, natural enemies and the habitat. Modern IPM is not only about insect/plant interactions, it is about holistic agro-ecosystem interactions, in which increased knowledge about the environment, soils,

plants, pathogens, endophytes, symbionts and insects are all combined to provide effective crop protection in an environmentally friendly manner.

As knowledge about, and interactions between, induced plant resistance, chemical ecology, micro-organisms such as endophytic fungi and *Wolbachia*, and phylogenetics and phylo

geography of arthropods becomes easily available; it is hypothesized that these will become important components of bio-intensive AW-IPM, thereby minimizing the impacts of synthetic pesticides even more.

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INTEGRATED WEEDS CONTROL IN SUGARCANE RATOON MANAGEMENT WITH BIOTECHNOLOGICAL AND MOLECULAR APPROACHES

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ABSTRACT

Sugarcane crop is a cash and industrial crop contributing 0.7% in Pakistan's GDP. It is providing raw material for sugar mills operating in the country. The average cane yield in Punjab is 742 mounds per acre. The progressive cane farmers are achieving more than 1500 mound per acre yield by growing latest varieties like CPF-249, HSF-240, CPF-234, CPF-250, CPF-251, CPF-252 and CPF-253 released by Sugarcane Research Institute, AARI, Faisalabad. Each variety has different features and needs different inputs and management requirements for plant and ratoon crop. Weeds management especially of narrow leaves is a difficult agronomic approach being faced in sugarcane. In most agriculture farmlands of sugarcane, weed management is predominantly reliant on herbicide application. Other agronomic methods and agro-technological manipulations were also being practiced for improving the productivity of sugarcane ratoons. It includes dismantling of ridges, stubble shaving, sub-soiling within rows, inter-culturing within rows and earthing up end of May. But these manipulations were adopted at small scale in farm area of sugar mills and few progressive farmers in Punjab. The weeds control is mainly done with use of weedicides of pre-emergence and post-emergence groups. However, the overuse and misuse of herbicides has resulted in the uptrend of herbicide-resistant weeds. Many biotechnological and molecular strategies can be focused on alterations of plant architecture, increased drought adaptation capabilities, increased salt tolerance, and increased pest and disease resistance and to reduce herbicide-resistant weeds. It is concluded that modern molecular approaches like Gene discovery, "omics," and genome editing technologies as a tool for current and future weeds management strategies in sugarcane plant and ratoon crop.

Keywords: Sugarcane, Weeds, Molecular, Biotechnological, Pakistan, Ratoon

INTRODUCTION

The world population is projected to increase from the current average of 7.6 billion people in 2020 to 8.6 billion people in 2030. The food security for increasing population is a great challenge for agriculture research and meet the demand of sugar of world population. One of the most significant challenges facing crop improvement programs globally is the capacity to adequately match crop

production with demand, thereby ensuring food security. Global crop production is affected by various abiotic and biotic stresses which are further worsened by climate change. Ratooning is ways of growing full cane crop from new growth of underground stubbles left in the field after reap of the plant crop (Singh *et al.*, 2013). Ratoon crop is cost-effective for the farming communities of Pakistan because making cost is 30% less than plant crop with

saving of seed material as an extra benefit. It saves the cost of seedbed preparation, seed material, irrigation and planting labour due reduced crop period. In Punjab, half of total sugarcane area is engaged as ratoon (Naeem *et al.*, 2019) but it contributes 30% to total cane production (Srivastava *et al.*, 2012) due to improper attention of the farmers towards ratoons. Low yield of ratoon crop is primarily because of peculiarity ratooning potential of cultivated varieties (Rafiq

et al., 2006) and pitiable ratoon management Techniques (Junejo *et al.*, 2010). Good ratoon management practices and inherent ratoon potential of a variety is of prime importance for sustaining high cane and sugar productivity (Cheong and Teeluck, 2015). Vast acceptance of a variety depends very much on its ratooning potential (Verma, 2002). The sugarcane varieties will show good performance in ratoon crop only if accompanied with best management techniques (Hemwong *et al.*, 2009). Otherwise, the variety will flop to perform in field (Singh and Singh, 2004). In world, sugarcane growing countries are taking two to five ratoons (Sundara *et al.*, 2006). Good improvement of ratoon crop be determined by high sprouting of underground buds after harvesting of plant crop (Bashir *et al.*, 2013). In multi-ratooning system, yield declined in successive ratoons can be enhanced by following good ratoon-management practices viz. loosening of inter-rows soil through chiseling, sub-soiling and earthing up to diminish soil compaction for root growth and preservation of trash to augment soil organic matter for resourceful utilization of water and nutrients (Hobbs *et al.*, 2008). Furthermore, in ratoon sugarcane, the mortality of facultative tillers usually happens, especially in case those sprout from the above-ground uneven portions of canes left after harvest. Therefore, stubble shaving are recommended within a week of harvest of sugarcane (Ahmed and Giridharan 2000).

Challenges in weed management

Despite the usefulness of integrated weeds

management (IWM), such strategies need to be heavily researched to determine the appropriate cultural, physical, and chemical methods that would be the most beneficial for the agro-ecological zone. Additionally, the change in the global climate has rendered some tried and true practices ineffective, leaving the door open to innovation in IWM. Climate change has raised complications in a number of different agricultural systems, and many of the challenges with weed management. Firstly, with the expected reduction in rainfall in already dry regions, the resilience of crops will be suffered. In this scenario, weeds have mechanisms to allow them to combat such stressors and out-compete the struggling crops, while also having extended periods of growth beyond their usual growing season (Ramesh *et al.*, 2017). Weeds have ability to quickly accumulate mutations to be better adapted to rapidly changing climate scenarios, in contrast to many crops which rely on breeding programs to introgression desired traits in a relatively slow manner. Focusing more on the management side, climate change is expected to result in the need for new weed management strategies that will need to be rapidly implemented to be an effective combatant to the rapid climate variance. The change in climate will also result in the increased instability of current herbicides.

Herbicide resistance in weeds

Continuous and non-judicious use of herbicides with the same mode of action creates herbicide resistance in weeds. From 1957 to 2020, the global reported number of unique cases of herbicide-resistant weeds has increased from 2 to 507 (Heap, 2022). In general, herbicide resistance mechanisms can be categorized into two broad types: (1) target-site resistance, and (2) non-target site resistance. Target-site resistance typically involves specific site mutations in the target enzyme, which prevents herbicide from binding to the target enzyme. Mutations could occur in the binding sites within the enzyme. Other forms of target-site resistance include target gene amplification (the increase in target gene copies) and the increase in target gene expression. These resistance mechanisms aim to increase the production capacity and abundance of the target enzyme, in which higher doses of a herbicide would be required to fully inhibit the target enzyme. Non-target site resistance stems from the physiological characteristics of the plant and how it absorbs, metabolizes, and/or sequesters the herbicide (Jugulam and Shyam, 2019). Another example of non-target site resistance is through reducing translocation of the herbicide, so once the herbicide enters the source leaves they are prevented from reaching the growing and meristematic

tissues *via* the phloem and/or xylem. Reduced translocation can be due to sequestration, which traps the herbicide molecules within the source tissues, or altered activity of transporter proteins, which either prevent or limit the entrance of the herbicide molecules into the phloem and/or xylem.

Weed seed bank persistence

Most weed species are known to be hardy and persistent in nature, producing thousands of seeds that can withstand various adverse environmental conditions, while staying dormant in the soil for long periods (Chauhan and Manalil, 2022). When optimal germination conditions are met, the seeds will germinate and compete with the crops sown on the same area of land. This makes weed management challenging. Seed dormancy is the main contributor to a persistent weed seed bank globally. It is a heritable genetic trait. Recent genetic and molecular studies on seed dormancy have provided important genomic information to aid the

understanding of seed dormancy in weeds.

Biotechnological and molecular approaches in weed management

Weeds are a detrimental threat to global crop production in both developing and developed countries (Chauhan, 2020). Overall, among the biotic factors causing crop losses, weeds contribute to the highest potential yield loss to crops. Some molecular approaches have been implemented in conjunction with herbicide application to reduce the proliferation of weeds in agricultural lands. Many molecular strategies for crop improvements have been largely focused on alterations of plant architecture, increased drought adaptation capabilities, increased salt tolerance, and increased pest and disease resistance. The development of glyphosate-resistant crops enables the application of glyphosate, a non-selective herbicide, to eliminate unwanted weeds in the field at various application timings, thus enhancing the level of weed control (Masselet *et al.*, 2021).

Gene discovery, “omics,” and genome editing technologies currently applied in crop research can be potentially applied to weeds as tools for weed management. Aside from GM methods, transient technologies relying on the non-transformative applications of RNA interference (RNAi) mechanism are also potential molecular approaches to control weeds instead of heavy reliance on herbicides.

These approaches could potentially manipulate expression of key genes in weeds to reduce its fitness and competitiveness, or, by altering the crop to improve its competitiveness or herbicide tolerance, by the molecular technologies in weed management. Genome editing may be used to improve crop resilience and adaptability to various environments, improve yields in suboptimal conditions. One such approach is the development of herbicide-resistant crops, such as the well-known Roundup Ready resistant crops (Barry *et al.*, 1997).

CONCLUSION

It is concluded from above discussion that Biotechnological and Molecular techniques, like, genome editing, CRISPR/Cas9, gene drives, OMICS and RNAi technology, may be used for future molecular research on weed management as a tool for integrated weeds management in ratoon and plant sugarcane crop along with agronomical manipulation approaches. It will improve level of weeds control, higher cane and sugar yield.

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REVIEW OF RECENT ADVANCEMENTS IN SUGARCANE GENOMICS

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ABSTRACT

Sugarcane is Pakistan's most important cash crop, primarily due to its industrial applications. In recent years, there has been a significant increase in sugarcane production, which has compensated for the decline in cotton production. Advances in sugarcane breeding have contributed to improving crop yield and agronomic traits, but further improvements are necessary to meet the rising demand for sugar and cope with changing climate conditions. However, conventional breeding methods have proven challenging due to sugarcane's complex and highly heterozygous genome. Despite these limitations, mapping techniques, genome-wide association studies, and genome editing techniques have identified key genes that enhance sugarcane's yield and resistance to disease and pests. Genome editing technologies, such as Zinc Finger Nucleotide (ZNF), Transcription activator-like effector nuclease (TALEN), and Cluster regularly interspaced short palindromic repeats (CRISPR/Cas associated protein 9), are efficient and precise tools that allow for rapid genome engineering. These technologies have been successfully applied in sugarcane to obtain new germplasm resources through gene-directed mutation. With whole genome sequencing data and knowledge of gene function, CRISPR-Cas 9 editing can rapidly generate new resources for key agronomic traits by precisely mutating important genes. This review focuses on the major differences and applications of these genome editing technologies in sugarcane plant engineering, emphasizing how they can facilitate molecular breeding and accelerate progress in basic research.

Keywords: Plant genome editing, crop improvements, Sugarcane breeding

INTRODUCTION

Sugarcane (*Saccharum* spp.) is a vital cash crop that is widely cultivated in countries with subtropical and humid climates such as the United States, India, Pakistan, China, Brazil, Australia, Cuba, and the Philippines. It is the primary source of global sugar production, accounting for 80% of the world's sugar supply. Sugarcane also yields various industrial high-end products like biofuel, waxes, and a diverse array of bio-fibers. There are two wild species (*S. robustum* and *S. spontaneum*) and four primarily cultivated species (*S. edule*, *S. barberi*, *S.*

sinense, and *S. officinarum*) within the *Saccharum* genus. Modern sugarcane varieties are genetically complex, highly polyploid, and aneuploid, with 70%–80% of the genome composition from *Saccharum officinarum* and 10%–20% from *S. spontaneum*. Recently, *Saccharum spontaneum* has emerged as a significant genetic resource for utilization in various sugarcane breeding programs, resulting in new polyploid and aneuploid varieties with chromosome counts ranging from 80 to 120. However, conventional breeding methods are challenging due

to the narrow genetic pool and complicated genome of sugarcane.

Recent research on sugarcane has focused on molecular biology, including genome editing techniques, to achieve higher yields, increased sucrose content, and biotic and abiotic stress tolerance. These approaches benefit from the understanding of the complex interactions among genes, proteins, metabolites, and the genome but rely heavily on analytical methods such as bioinformatics and computational analysis. Significant progress has been made in understanding the

molecular mechanisms of sugarcane resistance and tolerance to herbicides, cold, drought, and salinity stress, as well as plant development. Molecular marker approaches have elucidated the genome structure of modern sugarcane genotypes and derived phylogenetic relationships among the *Saccharum* complex. Additionally, sugarcane genome mapping experiments have helped detect marker-trait associations and validate the position of different essential genes. Top of Form

Gene-editing (GE) Technology:

Gene-editing Tools:

Gene editing tools have revolutionized the field of biology by allowing for precise targeting of specific genes, which has opened up new opportunities for optimizing food production in

plants. The ability to generate permanent mutations or insertions using gene editing has made it possible to create knock-out phenotypes, which are desirable in most applications. Previous gene editing systems such as zinc finger nucleases and transcription activator-like effector nucleases were complex and time-consuming to use, but the Clustered Regularly Interspaced Palindromic Repeat (CRISPR)-associated protein 9 system (CRISPR-Cas9) offers a low-cost, simple, and efficient method for eukaryotic genome manipulation. CRISPR-Cas9 induces site-specific double-strand breaks in genes of interest, which can be repaired through either non-homologous end joining or homology-directed repair pathway. Indels generated by NHEJ repair mechanisms disrupt the gene and create mutations, making

NHEJ more efficient than HDR for creating mutants.

The most widely used CRISPR-Cas system for genome editing is the CRISPR-Cas9 system, which consists of a single guide RNA and a Cas9 nuclease. The sgRNA binds to the target sequence and guides Cas9 to cleave the target sequence. The PAM sequence for the most commonly used CRISPR-Cas9 system is 5'-NGG, and the sgRNA is usually driven by U6 promoters while the Cas9 gene expression is driven by CaMV or ubiquitin promoters. The CRISPR-Cas9 system has been successfully used for genome editing in various plant species, and a platform called Plant Genome Editing Database has been created to consolidate information about CRISPR/Cas-generated mutants of plant species.

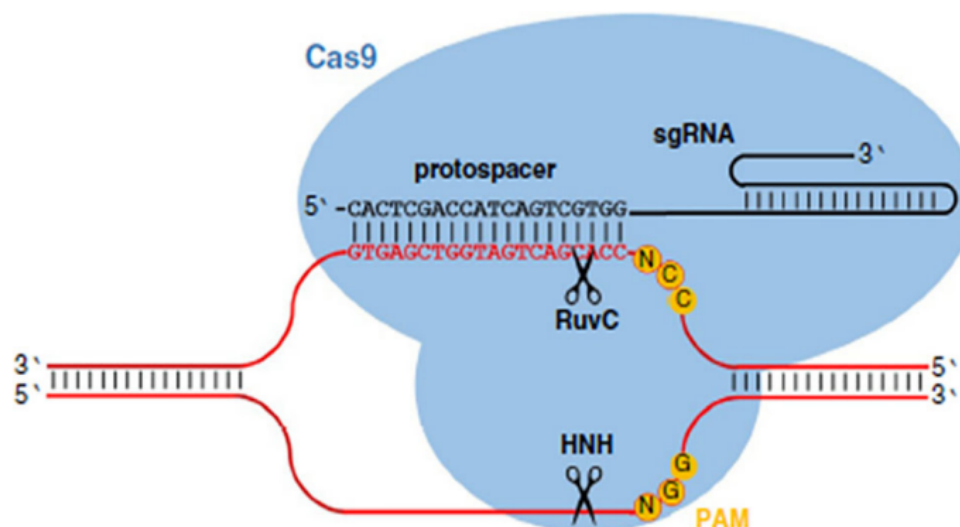


Figure-1: The CRISPR/Cas9 system of *Streptococcus pyogenes* (Figure adapted from Puchta 2017, an Open Access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license, <https://creativecommons.org/licenses/by/4.0/>)

Delivery of CRISPR Components:

CRISPR components can be introduced into plant genomes via different formats, including DNA, mRNA (in vitro transcripts or IVT), or protein. DNA format delivery allows for the use of various plant transformation techniques such as agro-infiltration, *Agrobacterium* infection, biolistics, electroporation, virus-based, and PEG-mediated transformation. The mRNA format is also efficient, leading to stable transgenic events with reduced off-target effects. Pre-assembled Cas9-gRNA ribo-nucleoproteins (RNPs) can be directly delivered, eliminating the introduction of foreign DNA into the host genome. This approach has been successfully used in various plants, including tobacco, *Arabidopsis*, lettuce, rice, petunia, grapevine, apple, potato, maize, and wheat, as well as in rice zygote cells. Lipofection, a mammalian DNA transfer technique, has also been used to deliver pre-assembled Cas9-gRNA RNPs into tobacco plant protoplasts for genome editing, with Lipofectamine 3000 and RNAiMAX achieving optimal delivery efficiencies of 66% and 48%, respectively (Liu et al. 2020).

Editing Efficiency:

The efficiency of CRISPR-Cas9-mediated knock-out can be influenced by several factors, including the gene's location on the chromatin, with editing being more effective in euchromatin than in heterochromatin (Jensen et

al. 2017), the selection of sgRNA sites from the gene to be edited (Zhao et al. 2017), the number and length of sgRNAs used for a single gene knock-out (Zhang et al. 2016), the form of Cas9 used for delivery (i.e., DNA, mRNA, or protein) (Kouranova et al. 2016), and the expression level threshold of Cas9/sgRNA (Yuen et al. 2017). As a result, researchers typically select multiple sgRNAs for in vitro testing and then choose the one with the best editing performance and the least off-targeting effect for further in vivo work (Liang et al. 2016; Zhao et al. 2017).

Applications and Limitations of CRISPR Systems:

The CRISPR-Cas9 system has various applications beyond gene knockouts, including the elimination of an entire chromosome (Zuo et al. 2017) or gene at a specific locus (Srivastava et al. 2017), removal of unwanted plant selectable genes, replacement and repair of a dysfunctional allele (Li et al. 2018a, b), and generation of an opening for gene integration at a specific locus (Zhao et al. 2016; Begemann et al. 2017). However, CRISPR-Cas9-mediated site-directed insertion is less efficient than CRISPR/Cas9-mediated site-directed deletions. Additionally, CRISPR technology can target multiple loci simultaneously (Rozov et al. 2019), regulate gene expression through specific gene activation or suppression, and create

genetic diversity for breeding (Miao et al. 2018). The use of GE techniques can lead to the achievement of homozygotes with bi-allelic and mono-allelic mutations in the T₀ generation, reducing breeding time significantly. Advanced imaging systems based on CRISPR/dCas9 have also been invented, such as fluorescence proteins fused to dCas9, which have been used to visualize telomere repeats in tobacco leaf cells. Plant breeders rely on genetic diversity to improve elite cultivars, and the CRISPR-Cas system offers promising new opportunities to create genetic diversity in an unprecedented way. For example, researchers have used CRISPR-Cas to edit regulatory regions and elements, generate novel alleles with varying expression levels, and optimize inflorescence architecture in tomato. Additionally, advanced imaging systems based on CRISPR/dCas9 have been developed, and homozygotes with bi-allelic and mono-allelic mutations can be achieved in the T₀ generation. This reduces breeding time and accelerates the rate of crop improvement, particularly for polyploidy crops with multiple alleles. Furthermore, scientists have developed nucleic acid diagnostic kits for plant and human diseases using the unique properties of Cas12 and Cas13 nucleases, such as the SHERLOCK™ CRISPR kit, which is easy to use, fast, accurate, and field-ready. However, limitations of using the CRISPR-Cas9

system remain, including specific PAM sequence requirements that may limit target sites for Cas9 at certain loci and the large size of Cas9.

Genome Editing and Sugarcane:

Jung and Altpeter (2016) reported the first successful genome editing of sugarcane using TALENs to knockout the COMT gene and reduce lignin content. Target mutations were achieved in 74% of transgenic lines, with up to 99% of wild-type COMT alleles mutated in various lines. However, transgenic events with 99% mutation frequency only resulted in a 29-32% reduction in lignin content, possibly due to biological plasticity. Nevertheless, Kannan et al. (2018) demonstrated that mutant lines in field conditions exhibited up to a 19.7% reduction in lignin content and increased saccharification efficiency without affecting biomass production. Recently, CRISPR-Cas9 technology has also been used for efficient and reproducible gene targeting in sugarcane (Eid et al. 2021; Oz et al. 2021), offering a simpler and more precise approach to editing complex polyploid genomes. However, high specificity and low off-target effects are still essential for effective genome engineering in sugarcane.

Prospects and Challenges of Genome Editing in Sugarcane:

Conventional breeding for developing sugarcane varieties is time-consuming

and laborious. It can take up to 12-15 years for a breeding cycle, and manipulating multiple genes or complex metabolic pathways is almost impossible. In contrast, the application of GE techniques, especially the CRISPR-Cas9 system, has many advantages. This system can accurately generate precise mutations on both single and multiple genes in a shorter time. However, despite being applied to various plant species, CRISPR-Cas9-mediated gene editing in sugarcane is limited due to the lack of genome sequencing and functional genomics studies on this crop. The recent availability of the early draft of sugarcane hybrid cultivar SP80-3280 and the monoploid reference genome of sugarcane, along with allele-defined genome sequencing, presents more opportunities for researchers to utilize CRISPR-Cas systems in sugarcane. However, the reference genome may not be enough as even a 1-bp difference between the real and the reference genome can prevent genome editing. Developing an efficient sugarcane transformation system for delivering GE components is also essential as sugarcane transformation efficiency is lower than other crops. Overexpressing maize developmental genes *Bbm* and *Wus2* has shown to increase transformation efficiency in sorghum, sugarcane, indica rice callus, and non-transformable maize inbred lines. The use of a tissue-specific promoter *ZmPLTP* has been employed

to generate fertile transgenic lines and avoid phenotypic abnormalities and sterility.

CONCLUSION

As the human population grows and available agricultural land remains limited, continuous crop improvement is necessary for food security. While conventional plant breeding techniques have been successful, they are slow and can take years to produce new varieties. Biotechnological methods, such as genome editing tools like the CRISPR-Cas9 system, offer faster and more precise ways to improve crop yields. This technology targets specific genes for modification, significantly reducing the time required to produce new varieties. Although transgenic techniques have been successful in enhancing crops, they remain controversial and have led to negative public perception. As a result, the emergence of CRISPR-Cas9-based genome editing is becoming a more accepted option. New Breeding Techniques (NBT), including CRISPR-Cas9, considering them indistinguishable from traditionally bred plants. This is good news for biotech companies, breeders, and researchers using CRISPR to modify plants. Researchers have already used CRISPR-Cas9 to improve the quantity and quality of biofuel feedstocks such as algae and plant cell walls, as well as to engineer fermentation yeasts

for more efficient bioethanol production. Sugarcane is a significant biofuel feedstock, and there has been substantial investment in improving its quality and quantity through transgenesis, with limited commercial success. We believe that genome editing will be used to identify important genes in sugarcane and their potential applications, particularly in bioenergy production. However, genetic improvement faces two

biological barriers: sexual reproduction in blooming plants and cellular equilibrium that ensures proper signaling between plant cells, tissues, and organs, ultimately affecting the plant's interaction with the environment. To overcome the former barrier, transgenic techniques can serve as a short-term solution, but it requires identifying the target gene for modification or introduction. Among the various breeding strategies, gaining a better

understanding of the biological network can effectively improve crop management, enhance the potential of existing crops, and facilitate the development of new ones through "assisted genomic selection." Utilizing biological systems and genomics tools can integrate knowledge from various fields and, with statistical analysis, lead to better management of new sugarcane varieties.

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ASSESSMENT OF SUGARCANE INTERCROPPING WITH DIFFERENT CROPS

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ABSTRACT

The rapidly growing population of Pakistan needs to fulfill its food and nutrition requirements, and to achieve this, a collaborative strategy must be adopted to increase productivity by intensifying land use. Intercropping, which involves cultivating multiple crops in the same space simultaneously, is an advanced management practice that improves soil fertility and increases yield on a given piece of land by utilizing a mixture of crops with different abilities in rooting, canopy structures, height, and nutrient requirements. Intercropping is particularly beneficial for smallholder farmers in the sub-tropics, where intercropping sugarcane and legumes is widespread due to the legume's ability to address declining soil fertility. This review paper focuses on the role of intercropping systems in improving the growth, yield, and nutrient status of sugarcane in smallholder farms in semi-arid areas of Pakistan and other countries. The study discusses the different intercropping systems used in sugarcane and their effectiveness in increasing productivity, profitability, water use efficiency, and controlling weeds, pests, and diseases. The findings of this study will be useful for researchers involved in this field.

INTRODUCTION

Sugarcane, which belongs to the *Saccharum* spp. hybrid complex, is a significant cash crop in Pakistan and is widely grown in tropical and subtropical regions of the world. The sugar industry is the second-largest agro-industry in Pakistan, providing a source of food, fuel, fodder, and fiber, and plays a crucial role in the national economy. Globally, sugarcane is the main sugar-producing crop and contributes nearly 75% to the total sugar pool. In Pakistan, sugarcane covers an area of 1.260 million hectares, with a production of 88.65 million tonnes and a yield of 70.34 kg/ha. In Punjab, it covers 7.76 lakh hectares, with a production of 577 lakhtonnes and a

productivity of 73.36 tonnes/ha (PSMA, 2021).

Intercropping was initially practiced as insurance against crop failure under rain-fed conditions. Nowadays, intercropping is mainly used to increase productivity per unit area and provide stability in production. The intercropping system efficiently utilizes resources and increases productivity. The primary advantage of intercropping is achieving greater yield on a given piece of land by making more efficient use of growth resources through the use of a mixture of crops with different rooting abilities, canopy structures, height, and nutrient requirements based on the complementary utilization of growth resources by the component crops.

Legumes, when used as an intercrop, increase soil conservation through greater ground cover than sole cropping and improve soil fertility through biological nitrogen fixation compared to monoculture. Sugarcane has a slow growth rate at the initial stage with low leaf canopy, providing sufficient uncovered area for some crops to be grown. As a long-duration and widely spaced crop, sugarcane offers good possibilities for growing early-maturing intercrops to harness the potentiality of the environment and use natural resources to increase production and net profit per unit area per unit of time. These features offer a potential scope to intercrop relatively short-duration and quick-growing crops to exploit

land resources more efficiently.

Literature Review:

Literature reviews on various aspects of intercropping in sugarcane, including growth, yield, economics, quality, soil nutrient status, as well as physical, chemical, and biological properties of soil, were collected in January, 2021 through internet searches using Google's search engines worldwide. The review encompassed published and unpublished sources such as reports, research papers, and theses from the past 25 years.

RESULT AND DISCUSSION

Growth, Yield and Economics of intercropping

The most profitable intercropping combination for sugarcane is cane and garlic. Garlic does not compete much with sugarcane for light and shade, and a companion crop of these two plants resulted in a cane yield of 111.47 tha^{-1} and 4.18 tonnes of garlic, with only a slight decline of 5.3% in cane yield compared to the sole crop of sugarcane in Pakistan (Bukhtiar and Muhammad in 1988). Ali et al. in 1987 and Ahmed et al. in 1991 made similar observations.

Garlic should be sown in between the rows of sugarcane at a spacing of 90 cm. To maintain proper spacing, three rows of garlic should be planted at a distance of 15 cm, with a plant-to-plant distance of 10 cm. If the cane row spaces are 120 cm wide, garlic can be planted in four rows (Ali et al. in 1987 and Ahmed et al.

in 1991). According to Patel et al., (1984), intercropping sugarcane with garlic resulted in significantly higher yields of cane, single cane weight, and commercial cane sugar. Meanwhile, sugarcane intercropped with onion had higher intercrop yield and net return. In order to cultivate a profitable combination of cane and maize, it is essential to plant both crops early in the season, preferably during the first two weeks of February. This allows the maize to grow and mature rapidly before the tillering phase of the cane. For cane, inter-row spaces of 90 to 120 cm should be used for planting, and the inter-row spaces of the cane should be cultivated as a seed bed for maize when irrigated. The maize seeds should be drilled in a single row if the cane row space is 90 cm and in two rows if the space is 120 cm, with a plant-to-plant distance of 15 cm. If trench planting is used, maize seeds should be dibbled on both sides of the trenches. To avoid the exhaustive effect of this intercrop combination, the cane field should be enriched with a significant amount of farm yard manure. Dual row planting of cane can accommodate exhaustive crops and produce profitable yields of both cane and maize (Balde, 2011) Rana et al. (2006) observed that sugarcane + maize intercropping produced significantly higher millable cane and cane yield, with cane equivalent yield being the highest under this treatment, along with maximum net return and B:C ratio. However, sugarcane +

maize gave equally high yield. Varghese et al. (2006) revealed that sugarcane intercropped with vegetable peas produced significantly higher cane yield, land equivalent ratio (LER), and B:C ratio, with higher cane weight. Peas are a legume vegetable that provides a profitable return and can be used for good biomass incorporation into soil. Additionally, all leafy vegetables have high biomass that is beneficial for soil incorporation. To achieve successful intercropping of vegetables, wider rows are recommended, but paired row planting is preferable for managing profitable intercropping. It is important to note that intercropping should aim to supplement cash returns without compromising cane yield.

Singh et al. (2010) found that single-bud vertical planted sugarcane + garlic had significantly higher cane yield and cane equivalent yield, followed by sugarcane + radish vegetable, with net return and B: C ratio being higher in the former treatment. Numerous studies have investigated the impact of cane and wheat intercropping systems on crop yield, with results showing that wheat has a negative effect on cane yield. For example, one study found that while a sole cane crop produced 133.97 tons per hectare, a combination of cane and wheat yielded 118.04 tons of cane and 3.385 tons of wheat per hectare, resulting in a 11.9% reduction in cane yield (Bukhtiar and Muhammad,

1988).Suryawanshi et al. (2010) reported that sugarcane + wheat intercropping gave higher net monetary return (NMR) and B: C ratio.

According to Islam and Islam (2016), the cane + potato combination is the most profitable intercropping system, provided that planting time, fertilizer needs, weed control, and earthing up operations are carefully managed. In particular, September-planted potato on ridges followed by cane in furrows produced higher yields than potato alone in September followed by cane in March. Furthermore, the highest cane yield was achieved with a September cane planting at 90 cm (101.16 t ha⁻¹) with potato (10.45 t ha⁻¹), followed closely by cane at 120 cm with potato (Malik and Kamoka, 1992). Kumar et al. (2011) noticed that sole sugarcane and sugarcane + potato intercropping had similar cane yield, while sugarcane + onion intercropping produced higher cane equivalent yield and net returns. Studies by Nayyar et al. (1987) and Ahmed et al. (1988) have shown that intercropping okra and sugarcane is a highly profitable combination, with an EMV of more than one. Although there was a reported reduction in cane yield of 6 to 17%, the monetary return from the okra crop compensated for this yield decline (Table-13). To minimize the shading effect of the intercrop, cane was planted in dual row strips with row spacing of 45-135-45 cm.

Paired row planting with a spacing of 30-150-30 cm was found to be more financially beneficial than the 45-135-45 cm row spacing. Therefore, wider inter row spaces are recommended to reduce light and shade competition, and two adjacent cane rows at 30/45 cm can make up the required plant population for cane. Keshavaiah et al. (2014) reported that sugarcane + french bean gave similar yields to pure sugarcane crop, while sugarcane + bhendi had significantly higher cane equivalent yield, with sugarcane + vegetable soybean having higher total income and B: C ratio.

Khippal et al. (2016) showed that sugarcane + pea intercropping had similar cane yield to sole sugarcane crop, with net return being higher in the former treatment. Rana et al. (2006) found that sugarcane + mash resulted in significantly higher juice sucrose levels, which were comparable to those of sole sugarcane, sugarcane + mustard, and sugarcane + maize. They also observed that this treatment produced the highest CCS.

Inter-cropping with lentil crops can yield a reasonably good profit margin without affecting the productivity of cane. Lentil has minimal competition for light and nutrients, and does not shade cane, and can even improve cane yield through symbiotic nitrogen fixation (Akhter et al., 2001). In fact, an EMV of 1.33 and 1.20 was obtained from intercropping cane with lentil (Table-4). Furthermore, it has been observed that lentil

intercropped in ratoon can improve cane yield compared to a sole crop (Singh et al., 2008).Singh et al. (2008) revealed that the highest amounts of available nitrogen and soil infiltration rates were obtained from the sugarcane + lentil intercropping system in both plant and ratoon sugarcane, with the lowest bulk density also observed in this treatment. Singh et al. (2011) found significantly higher commercial cane sugar levels in sole sugarcane, which were comparable to sugarcane + LP (1:3), with significantly higher purity percentages observed in sugarcane + LP (1:4), but comparable to all other treatments except sugarcane + LG (B). Brix readings were also higher in the same treatment.

Patel and Patel (2012) observed significantly higher values of available nitrogen and available phosphorus in the soil after sugarcane harvest with the application of a 100% recommended dose of phosphorus with green gram intercrop treatment, while available K₂O and S were found to be non-significant. Keshavaiah et al. (2014) reported significantly higher reducing sugar and ash levels in the sugar cane + French bean intercropping system, while sucrose levels were not significantly affected by various treatments.

CONCLUSION

Growing a variety of crops such as sunnhemp, maize, radish, linseed, pea, cucumber, wheat, soybean,

onion, amaranth, green gram, and french bean alongside sugarcane can increase sugarcane yield while also providing better economic returns. In addition, this intercropping system can enhance soil quality by improving nutrient status and physical and chemical properties of the soil, resulting in better quality crops.

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SUGAR INDUSTRY ABSTRACTS

Quantifying the effects of longevity of nitrification inhibitors on nitrogen losses in simulated sugarcane production

MP Vilas, K Verburg, JS Biggs and PJ Thorburn
Proceedings of the International Society of Sugar Cane Technologists, volume 30, 1179–1185, 2019

Nitrification inhibitors (NIs) are one form of enhanced efficiency fertiliser (EEF). They have the potential to reduce nitrogen (N) losses from sugarcane production systems. Their effectiveness in reducing N losses depends on the longevity of the inhibitory effect, which is, in turn, influenced by their persistence in an intact form in soils. Studies examining NIs have shown that their persistence can range between a few days to more than one year. In this study, we explore NIs of different persistence to assess their ability to reduce N losses for a case study in northern Queensland (Australia) by means of a modelling approach. We found that an increase in the persistence of NIs, is correlated with a reduction in N losses. However, we also found that this reduction in N losses does not always translate into yield benefits. We examine some specific cases to illustrate the situations that result in yield benefits in the presence of NIs.

Screening of sugarcane for high nitrogen-use efficiency at the seedling stage

Yang Liu, Liao Fen, Muhammad Anas, Li Qiang, Peng Lishun, Huang Dongliang and Li Yangrui
Proceedings of the International Society of Sugar Cane Technologists, volume 30, 1696–1702, 2019

This experiment screened high nitrogen-use efficiency (NUE) genotypes under low nitrogen pressure selection system. The important indexes affecting NUE of sugarcane were analysed, and the results provide a theoretical basis for the selection of high NUE in breeding and cultivation of sugarcane. In this study, seedlings of 58 sugarcane genotypes were evaluated in a hydroponic experiment with low-N (0.2 mmol/L N) and normal-N (2 mmol/L N) treatment. Growth, dry biomass and N accumulation and distribution characteristics in various sections of the plant were evaluated using descriptive statistic, principal component analysis and cluster analysis. The results indicated that morphology, biomass, nitrogen efficiency traits showed high genotypic variation for N treatments. Under low-N treatment, the dry weight of 58 genotypes varied from 0.64 to 14.75 g/plant, nitrogen accumulation varied from 5.53 to 63.00 mg/plant and

NUE varied from 115.4 to 279.3 g/g. Four factors contributed to 92.85% of the variance, according to 26 parameters such as dry weight and N uptake with N deficiency. Another five factors were contributed 82.21%, according to 19 parameters under both normal and low N treatment such as nitrogen transfer coefficient and genetic potential. The data revealed that dry weight (whole plant, leaf, root), N uptake (whole plant, leaf, shoot), NUE (whole plant, leaf), leaf relative NUE, shoot relative dry weight, shoot relative N uptake and shoot genetic potential were the key factors involved in sugarcane high NUE. Fifty-eight sugarcane genotypes were clustered into four groups: high NUE group, partial high NUE group, partial low NUE group and low group.

Value of the conversion of sugarcane-biomass xylans to alkyl glycosides

Narendra Mohan, Vishnu Prabhakar Srivastava and Anushka Agarwal
Proceedings of the International Society of Sugar Cane Technologists, volume 30, 1833–1841, 2019

Alkyl glycosides are a class of biodegradable non-ionic surfactants with wide applications in cosmetics, detergent, food and pharmaceuticals. Commercial production of these surfactants is carried out in

multiple steps through Fischer glycosylation reactions under extreme conditions. Issues of economic sustainability and environmental concerns have attracted the attention of the Indian sugar industry to explore the feasibility valuing by-products and biomass chain development in a bio-refinery concept. The Indian Sugar Industry crushes about 225-250 Mt of sugarcane each year, generating nearly 40-44% of lignocellulosic biomass residue, i.e. 90-100 Mt sugarcane bagasse and trash. Xylans are abundant polysaccharides in sugarcane biomass and remain underutilized to a great extent. Finding new applications for xylan-based molecules and value-added sustainable chemicals is of interest and challenging in developing economically viable industries based on sugarcane biomass. Our research was undertaken for selective transformation of sugarcane biomass-based xylans into surfactants. A one-step production of alkyl glycoside from sugarcane biomass has been developed by direct, selective alcoholysis in fatty alcohol media under acid catalysis at moderate temperature in a short time. The fatty alcohol serves as both solvent and reagent. The reactions conditions were broadly investigated and good conversion of xylan was achieved. The alkyl glycoside produced was characterized by NMR, FT-IR and mass spectrometry. Results indicate that the synthesized compound exhibits interesting

surfactant properties for applications as ingredients for detergents. This provides a new paradigm for the use of sugarcane biomass as a raw material for renewable chemical industries and will likely contribute towards economic sustainability of the sugar industry worldwide.

Optimization of a phenol typing system in sugarcane to evaluate different strategies against *Diatraea saccharalis* (Fabricius)

F Budeguer, MF Perera, G Michavila, J Racedo, G Gastaminza, MI Cuenya and AP Castagnaro
Proceedings of the International Society of Sugar Cane Technologists, volume 30, 193–199, 2019

Sugarcane borer *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) is the most important sugarcane pest in Tucumán, Argentina. Older larvae (L3, L4) bore into the stalks, disrupting the physiological integrity of the plant, facilitating fungi and bacteria colonization which indirectly reduce yield and quality of sugar. The aim of the present work was to optimize a sugarcane plant-infestation method with *D. saccharalis* under controlled conditions to help to evaluate different strategies to manage the pest. Different numbers of neonate larvae were placed in the leaf whorl of sugarcane seedling (2-months-old) of cultivars TUC 95-10, TUC 03-12 and LCP 85-384. 10 larvae of several instars were added in the ligule of second and third fully expanded leaves of single plants, 6-months old, of

TUC 95-10. Trials were conducted under controlled conditions (28-30°C; 50-70% RH). Each assay was repeated twice with 5-10 replicates per treatment. Several parameters were evaluated on 6-months-old plants: damaged sheath number, number of perforations in the internodes, total tunnel length, and dead-heart symptom; whereas on sugarcane seedlings only the last parameter was measured. Dead-heart symptoms were observed on sugarcane seedlings in all treatments with neonate larvae. Sugarcane plants (6-months-old) showed damage in the sheath and stem when infested with L2, L3 and L4 instars, whereas neonate larvae only produced sheath damage since they were unable to pierce and bore the sugarcane stem. Results suggested that different methods could be used to screen different strategies for sugarcane borer management.

Key considerations for high-performance continuous vacuum pans

BStC Moor, S Rosettenstein and N du Plessis
Proceedings of the International Society of Sugar Cane Technologists, volume 30, 25–35, 2019

The two most important objectives for a high-performance continuous vacuum pan (CVP) are good crystal quality and high exhaustions. To achieve these, the pan design needs to incorporate features that promote plug flow (a narrow

crystal residence-time distribution), a high heat-transfer coefficient (HTC) and vigorous circulation. Focussing on these will also achieve an energy efficient pan that can operate on a low steam-masseccuite temperature differential. Good plug flow is an essential for good crystal quality (low CV), which enables good purging with minimal washing and good exhaustions. This in turn minimises reboiling and its associated energy and sucrose losses. Low CVs also aid affination and for this reason are frequently included in raw-sugar specifications. Measures to achieve good plug flow include a good circulation profile and good inter-compartment masseccuite-transfer arrangements. Achieving plug flow has presented the greatest challenge to CVP designers, but the problem is shown to have been mastered in some horizontally-configured vertical tube pans. Key requirements for high exhaustions are appropriate seed supply, good feed (supersaturation) control, vigorous circulation and a high final Brix. Various ways in which circulation can be promoted are described. Recent information is that, contrary to previous belief, longer tubes perform as well as or better than shorter tubes in CVPs. Types of pans which do or do not have the desired attributes are mentioned. It is concluded that the type of pans best suited to meeting most of these requirements are horizontally-configured

vertical tube CVPs, at least one type of which is shown to come close to achieving true continuous spiral masseccuite flow.

Design and implementation of a web-based knowledge management system in the Sugarcane and By-products Development Company in Iran

Babak Radmehr and Elena Baninemeh

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 831–839, 2019

The Sugarcane and By-products Development Company includes seven agri-industry companies, with similar organizational structure and 18,000 skilled and experienced employees that assist in producing half of the sugar that is consumed in Iran. Because of its size and spread, it is necessary to have a knowledge management (KM) system to broadcast and share personal experiences and proficiency in order to improve organizational performance. The advantages of a KM system are in reducing redundancies, avoiding duplication of mistakes, preserving employee knowledge before leaving the organization, promoting successful experiences at all levels, and documenting explicit and implicit knowledge of personnel anywhere and anytime. The KM system in this company is based on the integration of two models – the Milton and Heisig models. A specific web application was designed and

implemented based on the proposed model that was integrated with a set of technologies, server-side and client-side programming languages. Frameworks and databases support the four main KM stages (discovery, capture, sharing and application). In the process of discovery, an efficient scoring system was implemented to motivate all personnel to participate, the performance of which was based on the algorithm defined according to importance of the user's activities. During the process of data capturing and sharing, it was established that the existing content management systems (CMS) did not meet the requirements of the company, resulting in implementation of a specific CMS from the outset. The CMS is an interface that allowed users to publish content directly to the Web-based KM system. This paper describes the implementation of a KM system in the Sugarcane Development Company.

Development of a further transgenic sugarcane cultivar resistant to glyphosate herbicide

J Racedo, F Budeguer, MF Perera, MJ Soria Femenías, SN Ovejero, MI Cuenya, AP Castagnaro and AS Noguera
Proceedings of the International Society of Sugar Cane Technologists, volume 30, 515–521, 2019

A previous development of transgenic glyphosate-resistant sugarcane suitable for commercial release was carried out through genetic

transformation of cultivar RA 87-3. During the time elapsed to develop this technology, new commercial cultivars produced by the local breeding program have been adopted by farmers. The complex genetics of modern sugarcane cultivars, which are interspecific hybrids, highly polyploid and frequently aneuploid, make the introgression by backcrossing of the transgene into other varieties extremely difficult. Direct transformation of new commercial cultivars or promising clones at final stages of a breeding scheme could greatly improve the development and adoption of transgenic sugarcane by both farmers and millers. The aim of this study was to obtain glyphosate-resistant transgenic events of the two recently released cultivars, TUC 95-10 and TUC 03-12, through the introduction via microprojectile bombardment of plasmids harbouring the epsps and nptII genes. A total of 23 and 8 independent bombardments experiments were carried out on TUC 95-10 and TUC 03-12, respectively. The stable transformation and integration of both epsps and nptII genes were determined by using PCR with specific primers. Transgenic events were evaluated for glyphosate tolerance under *ex vitro* conditions and for genetic similarity with donor plant by using target region amplification polymorphism (TRAP) molecular markers. Although plantlets of both varieties regenerated from calli, TUC 95-10 showed a

low tissue culture-response since only two events were obtained, whilst a total of 22 plantlets regenerated for TUC 03-12. One line of TUC 95-10 and three lines of TUC 03-12 were PCR-positive for both genes and different levels of herbicide-tolerance were observed. Genetic analyses of 213 TRAP loci from transgenic lines derived from TUC 03-12 indicate that two events show 99.30% of similarity with the non-transformed TUC 03-12 control and the third had 98.7% similarity. These are encouraging results as, based on our previous experience, the molecular-marker data suggest that the events are practically identical to their parental cultivar, and are suitable for future comparative field testing.

Multilocus Sequence Analysis highlights genetic diversity of *Acidovorax avenae* strains associated with sugarcane red stripe

PD Fontana, N Tomasini, CA Fontana, V Di Pauli, PS Cocconcelli, GM Vignolo and SM Salazar

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 1728–1735, 2019

Pathogenic species of *Acidovorax* cause economically important diseases in monocotyledonous and dicotyledonous crops, including sugarcane, corn, rice, oats, millet, foxtail, watermelon and orchids. Sugarcane red stripe, caused by *Acidovorax avenae*, is present in the main

production areas around the world. In Argentina, red stripe affects about 30% of stalks milled with important economic losses when severe infections occur. MLST was used to explore the genetic diversity of this bacterium associated with red stripe in Argentina, as well as their phylogenetic relationships. The MLST analysis included sequences from a total of 118 *Acidovorax*, 15 *A. avenae* strains isolated from Argentina sugarcane production areas, *A. citrulli* (93) from melon and watermelon, *A. avenae* (9) from rice, millet, corn, vasey grass and sorghum, and *A. oryzae* (1) from rice. MLST analysis revealed five novel sequence types (STs) for the sugarcane *A. avenae* strains, constituting a clonal complex with a common and close origin. When genetic relationships with other *Acidovorax* were explored, sugarcane strains were related to *A. avenae* from other hosts and more distantly to *A. citrulli*. Signals of frequent recombination in several lineages of *A. avenae* were detected and we observed that *A. oryzae* is closely related to *A. avenae* strains. This study provides valuable data in the field of epiphytological and evolutionary investigations of *A. avenae* strains causing sugarcane red stripe. Knowledge of the genetic diversity and host-strain specificity are important to select the genotypes with the best response to red stripe disease.

INTERNATIONAL EVENTS CALENDAR

2022 CONFERENCES & MEETINGS

February 16-19, 2022	7th IAPSIT International Sugar Conference & Sugarcon-2022 “Sustainability of the Sugar and Integrated Industries: Issues & Initiatives” Indian Institute of Sugarcane Research
April 17-19, 2022	S.I.T. Orlando Conference Sugar Industry Technologists Orlando, Florida, USA
April 19-22, 2022	Australian Society of Sugar Cane Technologists Conference Australian Society of Sugar Cane Technologists Mackay MECC, Queensland, Australia
June 14-16, 2022	ASSCT Annual Florida & Louisiana Joint meeting American Society of Sugar Cane Technologists Hyatt Regency Coconut Point Bonita Springs FL USA
June 20-24, 2022	XVI International Congress on Sugar and Cane Derivatives: Diversification 2022, AZCUBA Sugar Group, the Cuban Association of Sugar Technicians and the Cuban Institute of Research on Sugarcane Derivatives Cuba
June 30-July 1, 2022	Conferencia Bonsucro México 2022 Bonsucro Mexico City, Mexico
July 29- August 3, 2022	American Sugar Alliance Symposium American Sugar Alliance Seattle, WA, USA Location TBC
August 16-18, 2022	94th SASTA Congress 2022 SASTA – South African Sugar Technologists’ Association ICC, Durban, 45 Bram Fischer Rd, Durban, 4001 South Africa
August 16-19, 2022	28ª Feira Internacional da Bioenergia Fenasucro & Agrocana Centro de Eventos Zanini, Sertãozinho, Brazil
September 12-16, 2022	XII Congreso Tecnicaña (XII Tecnicaña Congress) Tecnicaña
November 22-23, 2022	31st ISO International Seminar, International Sugar Organization

GUIDELINES FOR AUTHORS

Dear Fellow Author(s),

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