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S.I. : HISTORY OF SUGARCANE BREEDING AND MOLECULAR GENETICS

Sugarcane Breeding, Germplasm Development and Supporting Genetic Research in Argentina

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Abstract Sugar production from sugarcane is one of the oldest agro-industries in Argentina and plays an important role on economic and social activities in Tucumán, Jujuy and Salta Provinces, located at the Northwest region of the country. Sugarcane production features are different between Tucumán and, Salta and Jujuy (Northern provinces), for that reason different breeding programs are developed in each region. The present review summarizes the main characteristics of sugarcane breeding programs of *Estacion Experimental Agroindustrial Obispo Colombres* (EEAOC) and *Chacra Experimental Agrícola Santa Rosa* (Chacra) that develop varieties for Tucumán and northern provinces, respectively. Breeding goals, history, germplasm collection, breeding methods, varieties currently grown in both productive areas, and molecular genetics and biotechnology supporting research are described. Efforts performed to develop sugarcane varieties adapted to diverse agro-ecological and management conditions have contributed to the profitability of industry as well as overcoming sanitary outbreaks. Improved varieties are key tools for the sugarcane production system, thus generating significant contributions for the socio-economic development of the region.

Keywords Sugarcane industry · Breeding programs · Variety selection · Biotechnology

Sugarcane Industry in Argentina

Sugar production from sugarcane is one of the oldest agro-industries in Argentina, starting in the late nineteenth century and constituting one of the most important economic and social activities in the Northwest (NW) region of the country (FET-Federación Económica de Tucumán 2020). Sugarcane has been concentrated in the Provinces of Tucumán, Jujuy and Salta. About 370,000 hectares are currently cultivated with sugarcane in Argentina where the 73% of the field crops are located in Tucumán, 16.8% in Jujuy, 9.2% in Salta, and 1% in the Northeast region, in the Provinces of Misiones and Santa Fe (Benedetti 2018; Fandos et al. 2020). The main features of sugarcane production are quite different between the NW Provinces with two well differentiated regions, Tucumán (between 26°21′24″ S and 27°56′25″ S) and the Northern provinces, Jujuy and Salta (between 22°33′53″ S and 25°2′14″ S) (Table 1).

Jujuy and Salta provinces have more favorable climatic conditions for the sugarcane crop than those of Tucumán, with a longer growth period and lower risk of frosts (Costa and Medina 2005). The sugarcane crop area in Tucumán is frequently exposed to severe frost, negatively affecting the maturation process and consequently also the commercial sucrose content (Romero et al. 2009). Tucumán presents a better rainfall pattern for sugarcane growing than Jujuy and Salta, which require irrigation (Volante et al. 2004).

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Table 1 Sugarcane and sugar production information of the two major regions of sugarcane industry in Argentina

	Tucumán	Salta and Jujuy
Current total sugarcane production area (ha)[1]	276,880	98,092
Total sugar production in 2019 (*t*)[2]	1,377,425	740,642
Average commercial sugar content (%). 2015–2019 period[3]	9.9	11.0
Number of sugar mills	15	5
Average number of growers[4]	7018	175
Average number of ratoon crops	5	4.5
% of area mechanically harvested[5]	98	99.5
% of area under irrigation[5]	10.2	98
Major abiotic stresses	Frosts in most of the sugarcane area with greater frequency and intensity in the eastern region	Water stress due to insufficient rainfall and poor water retention of soils
	Frequent water stress with greater severity and duration in the eastern region during Spring	Occasional frosts, more frequent in the southern region
	Edaphic limitations in the eastern region	
Major diseases	Brown rust (*Puccinia melanocephala*); orange rust (*Puccinia kuehnii*)[†]; smut (*Sporisorium scitamineum*); ratoon stunting disease (*Leifsonia xyli* subsp. *xyli*); leaf scald (*Xanthomonas albilineans*), red stripe (*Acidovorax avenae* subsp. *avenae*), sugarcane mosaic (SMV and SrMV) and yellow leaf (SYLV)	
Major pests	*Diatraea saccharalis, Elasmopalpus lignosellus, Mocis latipes, Acrotomopus atropunctellus, Ancistrosoma argentinum, Spodoptera frugiperda, Proarna bergi*	

[†]Only detected in the Misiones province (Funes et al. 2016)

[1]Fandos et al. (2020), Benedetti (2018)

[2]CARNA (2001)

[3]FET (2020)

[4]Ministerio de Desarrollo Productivo del Gobierno de Tucumán (2016) and Begenisic (2018)

[5]AybarGuchea et al. (2020)

The sugarcane industry structure also presents marked differences between both regions. Tucumán has a large number of small-scale sugarcane growers, many (88%) of them with less than 50 hectares (Ministerio de Desarrollo Productivo del Gobierno de Tucumán 2016). Technology adoption and agronomic management are less efficient in small farms compared to medium and large farms. The environmental and economic issues together result in three cane yield levels in Tucumán sugarcane growing area: low (< 56 t/ha; 36% of the growing area), medium (between 57 and 75 t/ha; 51% of the growing area) and high (> 76 t/ha, 13% of growing area) (Fandos et al. 2020). In the Northern provinces, there are fewer independent producers, the average farm size is larger, and the mills own nearly 85% of the production area, favoring more efficient agronomic management and rapid implementation of advanced agricultural technology.

Sugarcane in Argentina is affected by diverse diseases and pests (Table 1). The use of resistant varieties is the main strategy and the most efficient method for disease control in both regions with the exception of ratoon stunting disease (RSD). Most varieties currently grown in Argentina are susceptible to this bacterial disease. Sugarcane implantation with clean seed obtained from tissue culture or hydro-thermo therapy are common practices used by growers mainly to RSD control, as well as to manage other systemic diseases. On the other hand, the main strategies used for pest management in Argentina are cultural management and chemical and biological control (Salvatore et al. 2015; Isas et al. 2016; Iovane et al. 2018).

For many years, the Argentinean sugarcane industry has focused almost exclusively on sugar production. However, since the establishment of national laws promoting the use of biofuels in 2006, the ethanol production from sugarcane has become more important, reaching a production of about 520,000 m^3 in 2019 (http://datos.minem.gob.ar/dataset). Of the 20 sugar mills located in the NW, 13 have installed attached distilleries for bioethanol production. It is produced from molasses, high-grade molasses or a mixture of molasses and clarified juice, according to the production scheme of the sugar factory and the market price of sugar and bioethanol. In addition, some of these factories have further diversified its production. The five sugar mills in Jujuy and Salta produce sugar and bioethanol. Larger mills also produce other products such as paper from bagasse or co-generated electricity that is delivered to the electricity grid, with up to 32 MW of exported power in one of its mills. Three mills in Tucumán contribute with a total surplus of 21 MW of energy cogenerated from bagasse to be exported to the public energy grid (https://www.argentina.gob.ar/economia/energia/energia-electrica/renovables/). In addition, this province is also a pioneer in the country in the concept of biorefineries, with pilot production of other products such as dry yeast for animal feed and 2–3 butanodiol, compost for agricultural use and concentrated vinasse for use as fertilizer (Ruiz 2018).

Argentina is one of the leading countries worldwide in the use of genetically modified (GM) crops in agriculture. Currently, it grows around 24 million hectares of GM soybeans, corn, and cotton with different combinations of herbicide and insect resistance traits (ISAAA 2018). Transgenic sugarcane projects are in progress at research centers in different stages of the regulatory process in Argentina, although GM events have not been commercially released.

Overview of Breeding Programs

Three sugarcane breeding programs are currently operating in Argentina. *Estación Experimental Agroindustrial Obispo Colombres* (EEAOC) and *Instituto Nacional de Tecnología Agropecuaria* (INTA); both are located in Tucumán and *Chacra Experimental Agrícola Santa Rosa* (*Chacra*), in Salta.

EEAOC is the oldest scientific-technological institution in Argentina focused on agro-industrial production. It is an autarchic organization of Government of Tucumán and is governed by an *ad honorem* Board of Directors, representing different sectors of Tucumán agro-industry. Its financial support comes from the private and public sectors. Its main activities are focused on breeding, agronomy, phytopathology, entomology, biotechnology, chemistry and industry of sugarcane, citrus, grains and other crops of economical importance for Tucumán. The Sugarcane Breeding Program is the largest within the EEAOC and develops TUC varieties.

INTA is a state agency dependent on the national Government that aims to contribute to the sustainable development of the agricultural, agri-food and agro-industrial sector through research and extension. Since the creation of the *Estación Experimental Agropecuaria Famaillá* in Tucumán in 1958, this institution has carried out genetic improvement in sugarcane (INTA 2016). Its breeding program obtains the INTA sugarcane varieties, selected mainly from seeds produced by other breeding research centers (https://www.argentina.gob.ar/inta/variedades).

Chacra Experimental Agrícola Santa Rosa located in *Colonia Santa Rosa* is the only private institution dedicated to sugarcane breeding in Argentina and develops varieties specifically for the northern provinces. Initially established by CARNA (Northern Argentina Regional Sugar Center), Chacra is financially supported by Ledesma, Seaboard and Río Grande sugar mills to develop and promote new varieties, technology and innovation in sugarcane. Chacra activities focus on developing NA (*Norte Argentino*) varieties, research and development of new traits based on biotechnology research, and commercial propagation of clean seed cane.

The present review summarizes the main characteristics of the Sugarcane Breeding Programs of EEAOC and Chacra, which develop improved varieties for different sugarcane growing regions in Argentina. Supplementary materials contain contact information of both breeding programs.

The Sugarcane Breeding Program at EEAOC

Breeding Goals

The main objective of the EEAOC breeding program is to develop improved varieties with: (i) high cane yield; (ii) high sucrose content and early maturity; (iii) tolerance or resistance to major diseases prevalent in the region such as brown rust, red stripe, leaf scald, smut, and mosaic; (iv) acceptable fiber content (compatible with a good sugar recovery and bagasse); (v) suitability for mechanical green-cane harvesting (erectness, resistance to mechanical cutting) and good ratooning ability; and (vi) other agronomic traits including pith-less stem, rapid sprouting and good early growth.

History

The EEAOC breeding program was fully established in the 1960s with its own seed production and selection system (Mariotti et al. 1977). However, since its creation in 1909, the EEAOC has been responsible for the import, development and distribution of most of the varieties grown in Tucumán (Cuenya 2010; Ostengo et al. 2015b; Aybar Guchea et al. 2020). In its 111 years of history, different cultivars contributed significantly to the profitability of Tucumán sugar industry. In the 1920s, POJ 36 and POJ 213 overcame the mosaic crisis (Cross 1929). In the 1940s, TUC 2645 successfully solved a severe smut epidemic and also considerably increased sugar production per hectare, dominating the sugarcane growing area in Tucumán in the 1950s and part of the 1960s (Cross 1934, 1941; Guerineau 1961). Another Argentine variety, NA 56-79, selected by

Chacra, with high cane yield and wide environmental adaptability, became a dominant variety in Tucumán in the 1970s and 1980s (Ahmed et al. 2007). CP 65-357 (Breaux et al. 1974), imported from Florida, was adopted among growers in the 1990s and early 2000s due to its high sucrose content and early maturity. In the same period, TUCCP 77-42 (Mariotti et al. 1987, 1991) had a great impact due to its outstanding cane yield and wide adaptability to different types of soil. This variety was widely adopted in sugarcane areas of Tucumán and northern Argentina. TUCCP 77-42 was also the main variety cultivated in Uruguay for several years (Taulé 2011). LCP 85-384 (Milligan et al. 1994), imported by the EEAOC in 1990 and known for its high cane yield, early maturity, very high sugar accumulation throughout the harvest period and cold tolerance, is another key variety in the Tucumán region (Chavanne et al. 2000).

Between 1980 and 2019, the EEAOC breeding program bred and released 30 locally developed varieties (Anonymous 1980; Mariotti et al. 1981, 1987; Levi et al. 1985; Chavanne et al. 1993, 2000, 2002; Cuenya et al. 2009b, c, 2010,2011, 2013, 2015, 2019a, b, c) and three foreign cultivars (CP 65–357, LCP 85 384, and LCP 85-376) (De Faveri and Mariotti 1986; Chavanne et al. 2000). Among these, four varieties were released through UIMCA (a cooperative breeding project formerly integrated by EEAOC and INTA between 1995 and 2004).

The major focus of EEAOC breeding program is developing varieties with high sugar content, early maturity and accumulation of high sugar throughout the harvest period, fiber content suitable for the efficient recovery of sugar and adequate bagasse for co-generation of electricity. To achieve this outcome, considerable broadening of genetic base through ongoing introduction of foreign clones and its use in breeding is currently underway.

Germplasm Collection

The EEAOC germplasm collection mainly involves advanced clones and commercial varieties of national and foreign origin (Table 2). Currently, the collection has 789 accessions maintained in the experimental field of the EEAOC Central Research Station located in Las Talitas, Tucumán. All genotypes are characterized for sugar and fiber content, yield characteristics and disease resistance.

Materials from the USA breeding programs have historically shown good adaptation to Tucumán climatic and soil conditions (Mariotti et al. 1977; Díaz Romero and Cuenya 2001). For this reason, an active variety exchange with sugarcane breeding programs of Florida (USDA-ARS, Sugarcane Field Station, Canal Point) and Louisiana (Louisiana State University Agricultural Center and USDA-ARS, Sugarcane Research Unit, Houma) is in place.

Table 2 Clones in the EEAOC germplasm collection

Germplasm	Number of genotypes
TUC clones and varieties	503
Clones and varieties from other Argentine programs	18
Clones and varieties from foreign breeding programs	254
Saccharum spontaneum	4
F1, BC1	10
Total	789

Specially, from this last program the EEAOC has also introduced germplasm at different levels of backcrossing with species of the *Saccharum* genus in the recent past. Material exchange agreements were also established with breeding programs from other countries. In 2016, in order to increase the import of germplasm, EEAOC established a sugarcane quarantine facility (Funes et al. 2019).

Breeding Methods

Parental selection, hybridization and clonal selection are carried out by EEAOC. The whole process from crossing to variety release takes between 11 and 14 years and starts with parent selection. Parents are selected based on phenotypic characteristics (yield performance, sugar content, disease resistant and another agronomic trais) and progeny performance. Best Lineal Unbiased Predictor (BLUP) methodology is usually used to assess genetic merit and combining ability of parents based on mean family performance information obtained from progeny test. A high proportion of parents in the EEAOC breeding program includes clones selected from advanced stage trials and commercially grown cultivars (national and foreign). Materials from Louisiana programs constitute the main source of foreign parents. Sugarcane hybridization in Tucumán is difficult because it generally does not flower there naturally. Low night-time temperatures occur during late summer when the photoperiod is appropriate for induction. Artificial photoperiod treatments to induce and synchronize flowering of sugarcane are carried out in each crossing campaign between mid-November and the end of February. Photoperiod facilities include seven photoperiod chambers. Temperature control is carried out in heated greenhouses, once the photoperiodic treatments are finished. Two photoperiodic treatments (T1 and T2) are implemented to induce flowering. Both treatments start with 40 consecutive days of constant photoperiod (12.5 h), followed by 60 days of decreasing photoperiod. This decrease is 60 s/day for T1 and 30 s/day for T2 (Díaz

Romero and Cuenya 2002). Induction of adventitious roots in the stalk is facilitated by air-layering bagasse and perlite mixture in order to maintain flowering during the crossing. Biparental crossing is conducted in isolation cubicles within the greenhouse (under controlled humidity and temperature) from mid-March to late June (Fig. 1). The best crosses (parental combinations) are determined using information of progeny performance and complementary traits. The true seed production and seedling growing (greenhouse) are carried out at the EEAOC Central Research Station. Annual indicators of this area are presented in Table 3.

The clonal selection system in the EEAOC breeding program involves five stages. Each year, about 70,000 seedlings undergo selection (Stage I). In this stage, individual selection is made according to visual assessment (agronomic type and disease resistance) and subsequent brix evaluation on selected clones. All years, crossing appraisal is conducted where a sample of 64 genotypes per family is assessed for agronomic traits and disease resistance. Data from family performance (yield characteristic and sucrose content) obtained are used to support individual selection. The remaining stages comprise two early selection phases: first and second clonal multiplication

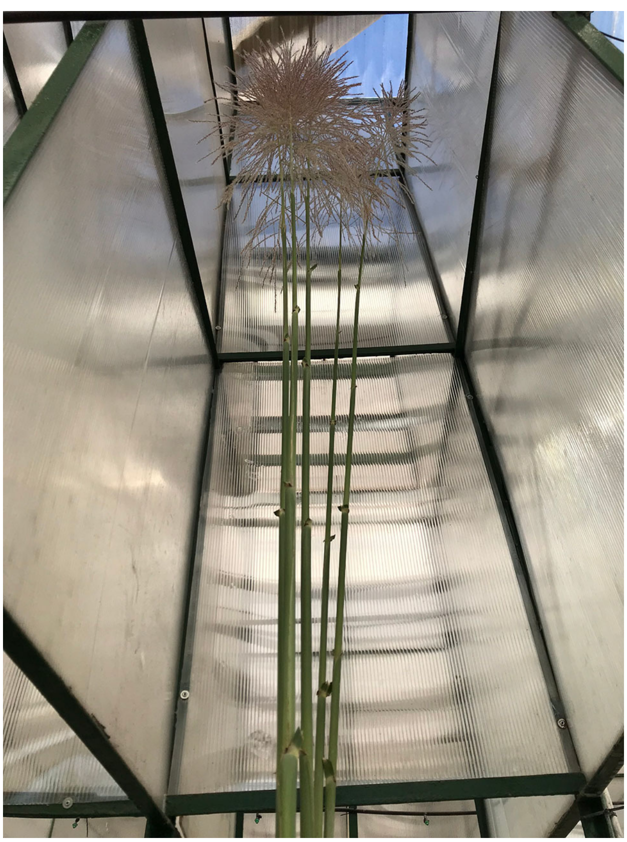

Fig. 1 Biparental crossing in isolated cubicles of the Sugarcane Breeding Program at EEAOC

Table 3 Annual estimates of true seed production activity in *Estación Experimental Agroindustrial Obispo Colombres* Sugarcane Breeding Program

	Average 1999–2019
Number of parents used for crossing	107
Number of stalks under flowering induction treatment	1620
Flowering stalk percentage	59.5
Biparental crosses	428
Mean germination/gm seed	117
Seed production (number of viable seeds)	308,751

(Stages II and III, respectively) and two stages of variety trials conducted in fields within breeding stations (Infield Variety Trials, Stage IV) and grower fields (Outfield Variety Trials, Stage V). Selection trials from Stage I to IV are located at the EEAOC Central Research Station and at the Santa Ana Station, 90 km far from EEAOC Central Research Station. Initial seedling population is divided between both sites, and the selection process at Stages II and III occurs independently within each station. Clones selected from Stage III are assigned as TUC varieties and planted in Infield Variety Trials replicated in the two experimental stations. At Stage V, advanced clones are evaluated in multi-environment trials planted in six sites representing contrasting environments of the sugarcane growing area in Tucumán. Different aspects of clonal selection stages are summarized in Table 4.

Special trials are carried out for pre-commercial clones in order to provide additional information for variety recommendation. This information mainly involves the cultivar response to frost (cold tolerance), ripener application and herbicide phytotoxicity (Ostengo et al. 2015c; EEAOC 2013, 2019).

TUC varieties are distributed to sugarcane growers by the EEAOC "Vitroplantas" Project, which provides high quality seed cane (free of systemic diseases and with genetic purity). New varieties are micropropagated by tissue culture (from pest and disease-free material) and the acclimatized plantlets are multiplied in the field, in nursery stages: Basic, Registered and Certified Nurseries (Díaz et al. 2019a, b; Noguera et al. 2019b; Díaz Romero et al. 2019a; Digonzelli et al. 2019). Currently, 73% of the sugarcane area in Tucumán is planted with high quality seed cane of the EEAOC "Vitroplantas" Project (Aybar Guchea et al. 2020).

In recent years, the EEAOC breeding program has conducted studies aimed at increasing the efficiency of the selection process and cultivar characterization. Most of this

Table 4 Clonal selection stages of the EEAOC Sugarcane Breeding Program

Stage (number of clones)	Plot size/trial design	Years	Sites	Selection criteria
Stage I: seedling (70,000)	Individual seedlings/mass selection	1	2	Visual assessment (agronomic type and resistance to diseases) and brix. Family performance information (cane yield parameters and sucrose content) from crossing appraisal is use as selection criteria
Stage II: first clonal stage (5000–7000)	1 row, 3 m long/ unreplicated	2	1	Visual assessment of agronomic type and resistance to diseases; number and weight of stalks; sucrose content in May (early maturity) and estimated sugar yield
Stage III: second clonal stage (500–700)	3 rows, 3 m long/ RCBD* (2 replicates)	2	1	Visual assessment of agronomic type and resistance to diseases number and weight of stalks; sucrose content in May (early maturity) and July and estimated sugar yield
Stage IV: infield variety trials (60–80)	3 rows, 8 m long/ RCBD (3 replicates)	4	2	Visual assessment of agronomic type and resistance to diseases; cane yield (whole plot weighing); number and weight of stalks; sucrose content in May (early maturity) and July and estimated sugar yield
Stage V: outfield variety trials (20)	3 rows, 10 m long/ RCBD (3 replicates)	4	6	Visual assessment of agronomic type and resistance to diseases; cane yield (whole plot weighing); number and weight of stalks; sucrose content in May (early maturity) and July; estimated sugar yield; fiber content; maturity curves and ratooning ability

Infield variety trials: trials conducted in fields within breeding stations. Outfield variety trials: trials conducted in grower fields

**RCBD* Randomized complete block design

research has focused on application of statistical genetics approaches. New strategies based on mixed models were developed to model data from multi-environment trials with multiple harvests (spatially and temporally correlated data) and to analyze triple interaction patterns (genotype-site-crop year) (Ostengo 2010; Ostengo et al. 2013a, 2013b, 2013c,2015a). In the same context, spatial analyses were implemented to increase efficiency in unreplicated early selection stages (Chavanne et al. 2019). In addition, different statistical techniques were used to propose an analysis methodology for genotype selection according to sucrose accumulation curves (Ostengo et al. 2020). This methodology allows to identify curves with maximum genetic variability in their accumulation parameters and ensure an efficient selection through: (i) the adjustment of non-linear models in the maturity curves, (ii) the classification of the accumulation curves according to the parameter maturity process, and (iii) estimation of the genetic contribution to the intragroup variability of each parameter. Another study was aimed to broaden the cultivar characterization in relation to processing quality. For this purpose, the genetic contribution of non-sugar components of juice (ashes, starch, phenols, phosphates) was studied in TUC varieties (Ostengo et al. 2019). Also, different traits associated with energy production were evaluated in commercial varieties (Díaz et al. 2019a, b).

Varieties Currently Grown in Tucumán

Since 1977, the EEAOC breeding program carries out periodic surveys to estimate the variety distribution in the Tucumán sugarcane growing area. Table 5 shows the five most cultivated varieties in Tucumán according to the 2019/2020 survey (Aybar Guchea et al. 2020). This table shows a predominance of LCP85-384 which is not a sustainable scenario for the sugar industry in Tucumán. Since its release in 2000, this cultivar has been distributed rapidly. In 2005, when its growing area reached 45%, LCP 85-384 became susceptible to *Puccinia melanocephala* (Cuenya et al. 2005). Since then, the inoculum pressure of brown rust, which was not considered a major disease before 2005, increased significantly over the whole sugarcane area of Tucumán (Cuenya et al. 2009a). Between 2009 and 2019, the EEAOC sugarcane breeding program released eight new cultivars that decreased the area occupied by LCP85-384 and made the agro-ecosystem more productive and sustainable.

Molecular Genetics and Biotechnology Supporting Research

The EEAOC biotechnology department has a special role to support the EEAOC breeding program activities. Different types of molecular markers, including Random Amplified Polymorphic DNA (RAPD) (Fontana et al. 2003), Amplified Fragment Length Polymorphisms (AFLP), Simple Sequence Repeats (SSR) (Perera et al. 2012a) and Target Region Amplified Polymorphism (TRAP) (Racedo 2014; Perera et al. 2016), were used to characterize and estimate the genetic diversity of sugarcane varieties and genotypes used as parents by EEAOC breeding program. In addition, SSR markers were also

Table 5 Major varieties grown in Tucumán, Argentina according to a 2019/2020 survey

Variety	Release year	% Area[†]	Parents (F × M)	Notes
LCP85-384	2000	67.7	CP77-310 × CP77-407	High cane yield; very high sucrose content and low fiber content. Early maturity. Resistant to red stripe and mosaic; intermediate resistance to leaf scald and smut, and susceptible to brown rust and RSD
TUC95-10	2011	18.1	CP72-370 × CP57-614	Very high cane yield; high sucrose content and moderate fiber content. Early to mid-maturity. Wide adaptability to different environments. Resistant to smut, red stripe and mosaic and intermediate resistance to brown rust and leaf scald, and susceptible to RSD
TUCCP77-42	1987	8.7	CP71-321 × US72-19	Very high cane yield; moderate to high sucrose content; moderate fiber content. Mid-maturity. Wide adaptability to different environments. Resistant to mosaic and smut; intermediate resistance to leaf scald and red stripe, and susceptible to brown rust and RSD
TUC97-8	2009	1.7	TUC87-21 × TUCCP77-42	High cane yield; high sucrose content and moderate fiber content. Early maturity. Resistant to mosaic and smut, intermediate resistance to brown rust, leaf scald and red stripe, and susceptible to RSD
TUC00-19	2015	1.4	HOCP92-675 × TUCCP77-42	High cane yield; very high sucrose content and moderate fiber content. Early maturity. Resistant to leaf scald, mosaic and smut; intermediate resistance to brown rust and susceptible to red stripe and RSD

[†]Survey based on 131,449 ha of the Tucumán sugarcane growing area (Aybar Guchea et al. 2020)

employed to confirm both the success of emasculation treatments and the hybrid character of the progeny obtained from crossing (Perera et al. 2012a). The use of TRAP markers allowed identifying male parents, which facilitates polycrosses (Perera et al. 2020a). Further, TRAP markers together with morphological traits proposed by UPOV (2005) were used for protecting intellectual property rights of newly released sugarcane varieties (Perera et al. 2020a).

Prevalence and effectiveness of the *Bru*1 gene for sugarcane brown rust resistance in the germplasm collection of EEAOC (Racedo et al. 2013) were analyzed through *Bru*1-specific molecular diagnostic markers. Additional resistance sources have been recently identified by biparental mapping, through intensive field and controlled conditions phenotyping, and Genotyping by Sequencing (GBS) with DArT-seq markers (Chaves et al. 2019).

Efforts are directed to develop tools to increase the selection efficiency of high yielding clones in the EEAOC breeding program. A previous Genome-Wide Association Study (GWAS) identified several DArT markers significantly associated with both high biomass and sugar yield in a population comprising clones at the last stage of the selection process (Racedo et al. 2016). Using the data from this study, a Genomic Selection strategy is currently being developed (Ostengo 2020).

In a multidisciplinary project, the EEAOC breeding program developed a transgenic sugarcane event of a local commercial variety RA 87-3, expressing *epsps* transgene conferring glyphosate tolerance. Several health and environmental regulatory studies were carried out to evaluate potential impact on agricultural environments and food safety for commercial deregulation of the transgenic event in Argentina (Noguera et al. 2015a; Perera et al. 2020b). This event has received three favorable recommendations from National Advisory Commission on Agricultural Biotechnology (*Comisión Nacional Asesora de Biotecnología Agropecuaria, CONABIA*), National Food Safety and Quality Service (SENASA) and Secretariat of Agricultural Markets (*Subsecretaría de Mercados Agropecuarios*) (Noguera et al. 2019a). Nevertheless, the approval of the Ministry of Agroindustry of the Nation for commercial cultivation remains pending. There are two other ongoing transgenic projects where local varieties were transformed to provide pest resistance and tolerance to abiotic stress (drought and salt tolerance), respectively.

The biotechnology department annually produces 85,000 sugarcane seedlings through in vitro meristem cultures and micropropagation techniques for EEAOC "Vitroplantas" Project. This program guarantees seed cane of high phytosanitary quality, and genetic purity by using TRAP markers (Noguera et al. 2015b; Perera et al. 2016).

Molecular detection of pathogens is another biotechnological application that helps the EEAOC breeding program with variety development. Molecular diagnosis of pathogens is used to evaluate meristem donor plants, micropropagated seedlings of EEAOC "Vitroplantas" Project (Perera et al. 2016) and sugarcane genotypes maintained in phytosanitary quarantine. Moreover, in order to develop future sustainable germplasm and disease management strategies, genetic diversity studies of several pathogens in Argentina such as SCMV and SrMV (Perera et al. 2009, 2012b), SCYLV (Bertani et al. 2014), *P.*

melanocephala (Bertani et al. 2019) and *A. avenae* subsp. *avenae* (Bertani et al. 2021) have been conducted.

The Sugarcane Breeding Program at Chacra Experimental Agrícola Santa Rosa

Breeding Goals

The main objective of Chacra's breeding program is to generate cultivars adapted to the agroecological conditions of sugarcane growing areas in Jujuy and Salta. Breeding target traits are (i) cane yield; (ii) sucrose content; (iii) resistance to local diseases such as smut, red stripe, mosaic, brown rust and leaf scald; (iv) early maturity; and (v) ratooning ability.

History

Chacra Experimental Agrícola Santa Rosa was established in 1951 as an experimental farm supported by CARNA (Northern Argentina Regional Sugar Center). In 1954, Chacra imported the cultivars CP48-103, widely adopted in commercial fields in Jujuy and Salta, and NCo310 (Cerrizuela 1988). The first series of NA cultivars (produced by Chacra) was selected among seedlings from crosses made by Fernández de Ullívarri in Puerto Rico in 1956 (CARNA 2001) and made a significant impact throughout the Americas. NA56-79, widely adopted in Brazil and Argentina, was one of the most extensively grown varieties worldwide in the 1980s (Tew 1987; Matsuoka 1990; Cursi et al. 2021). In Argentina, varieties NA56-79 and NA56-30 were dominant in Tucumán (Cerrizuela 1988; Dominguez 1989; Ahmed et al. 2007), whereas NA56-42, NA56-62 and NA56-83 were widely adopted in the northern part of Argentina (Cerrizuela 1988; Dominguez 1989). Another variety of this series, NA56-26, was the major variety in Bolivia for several years (ISSCT 2015, Carlos Costas Aguilera, Centro de Investigación y Transferencia de Tecnología de la Caña de Azúcar, personal communication). NA56-42 was the most adopted variety in Costa Rica in the 1990s and 2000s and is currently grown in a significant area (Subirós 1998; Chaves Solera 2018; ISSCT 2015). NA63-90, an early sugar variety, occupied nearly 25% of sugarcane hectares of Tucumán (Cerrizuela 1988; Dominguez 1989). Cultivars released after 1970 were registered in the National Registry of Cultivars (Registro Nacional de Cultivares (RNC) 2020). NA85-1602 was widely adopted in Jujuy and Salta (mill data) and is currently grown in Costa Rica (Chaves Solera 2018) and in a limited area in Mato Grosso, Brazil (Egydio Venturini, Itamaraty Sugar Mill, personal communication). More recently NA97-3152, occupied a large part of Jujuy and Salta (ISSCT 2015) but is now in decline with growers favoring more recently developed varieties such as NA03-3300 and the rapidly adopted NA05-860 in Jujuy and Salta.

Germplasm Collection

Chacra's germplasm collection has currently more than 1200 accessions currently maintained in Colonia Santa Rosa, Salta. The collection includes advanced clones and varieties from the Chacra breeding program, clones from national and foreign breeding centers, as well as some basic materials (species of the genus *Saccharum*, F1 and BC1) (Table 6). Germplasm and variety exchange are carried out through direct agreements with breeding programs worldwide or via the CIRAD-Visacane quarantine system. Chacra operates a SENASA (National Service for Agri-food Safety and Quality) approved quarantine facility since 2001.

An introgression effort was initiated by Chacra in 2014 from crossings between L79-1002 (F1 hybrid of CP 52-68 × Tainan, a *S. spontaneum* clone, Bischoff et al. 2008) and clones from the germplasm collection at Chacra. The progenies were evaluated and superior clones were selected for high biomass, intermediate sugar content and smut, leaf scald and red stripe resistance. They were subsequently crossed with the breeding program's elite parents. Progenies will be assessed for cane yield, sugar and fiber content, and resistance to the most relevant diseases in Jujuy and Salta.

Breeding Methods

Chacra's breeding program comprises (i) parental selection, (ii) hybridization and (iii) selection of superior genotypes. The process to obtain NA varieties currently takes approximately 15 years from the initial cross to the commercial release. Parents are selected based on the traits of sugarcane cultivars that have been successful in the region or in agroecologically similar areas. The predictor of crossing value (PCV) software (Uchino et al. 2015) is

Table 6 Number of genotypes of Chacra's germplasm collection

Germplasm	Number of genotypes
NA clones and varieties	542
Clones and varieties from other Argentine programs	209
Clones and varieties from foreign programs	413
Basic germplasm	15
F1, BC1	23
Total	1202

used to predict the best possible combinations (cross) and select high breeding value parents based on BLUP for male and female effects calculated from progeny historical records.

The photoperiod facility at Chacra has been operating since 1984. Flowering is induced in four photoperiod chambers (Fig. 2) and a supplementary greenhouse, allowing up to five photoperiod treatments, which are normally initiated with 12.5 daylight hours and decreasing 30, 45 and 60 s/day or combinations of these regimes. Photoperiodic treatments are initiated in February. Effective natural photoperiod occurs during February and March with a mean reduction of 78 s/day. This allows initiation of flowering in a limited number of clones that may be used for crossing only as female parents, as outside mean minimum temperatures are below the threshold required for pollen development. Stalks are marcotted with moss (*Tillandsia usneoides*) when the first signs of inflorescence are observed. Crosses are made from April to August, mainly of biparental type (with occasional poly-crosses) in a heated greenhouse. Photoperiodic treatments, hybridization and seedling growing in the greenhouse are conducted in Chacra's breeding facility in Colonia Santa Rosa (Province of Salta). Annual production indicators of this area are presented in Table 7.

Clonal selection is carried out in five successive stages (I–V). Stage I involves screening of approximately 250,000 seedlings each year. Individual (mass) selection is applied in this stage based on visual assessment and subsequent brix evaluation on selected clones. Stages II and III comprise un-replicated trials (first and second clonal selection, respectively). Stage II is conducted in experimental fields in Chacra, while clones in Stage III are planted in three different locations: Chacra, Seaboard Sugar Mill (Salta) and Ledesma Sugar Mill (Jujuy). Advanced clones are

Fig. 2 Photoperiod facility in Chacra Experimental Agrícola Santa Rosa

Table 7 Annual indicators of crossing and seed production of Chacra's sugarcane breeding program

	Average 1999–2019
Number of parents used for crossing	176
Number of stalks under flowering induction treatment	1613
Flowering stalk percentage	38
Biparental crosses	397
Mean germination/gm seed	132
Seed production (number of viable seeds)	323,604

evaluated in replicated multi-environment trials (Stage IV) planted in locations representing a range of soil and climate conditions in Seaboard Sugar Mill (4 locations), Ledesma Sugar Mill (4 locations), Río Grande Sugar Mill (1 location) and Chacra (1 location). This stage aims to identify stable and adaptable clones for different target environments. Finally, the promising clones selected from the multi-environment trials are tested in macroplots (Stage V; details in Table 8) in commercial fields to validate the clone response under standard productive crop management practices in locations selected according to the outcome of multi-environment trials. Different aspects of clonal selection stages of Chacra breeding program are summarized in Table 8.

NA varieties are distributed to Río Grande, Seaboard and Ledesma sugar mills as seed cane collected from the macroplot trials as well as clean seed generated by tissue culture in Chacra facility at Colonia Santa Rosa. Sugar mills also distribute seed cane to their growers.

Varieties Currently Grown in Jujuy and Salta

According to an extensive variety survey carried out by Chacra in the 2019/2020 campaign, nearly 80% of the sugarcane fields in Jujuy and Salta are planted with seven varieties (Table 9).

Molecular Genetics and Biotechnology Supporting Research

Chacra initiated sugarcane biotechnology research in 1999, focusing on developing technologies for practical applications. Initially, biotechnology efforts were directed towards obtaining transgenic sugarcane resistant to herbicide and mosaic disease. In partnership with Biosidus, a biotechnology company, Chacra generated the first transgenic sugarcane lines in Argentina (Fernández de Ullívarri and Serino 2008). In 2004, glyphosate-resistant transgenic lines were grown in the greenhouse, and they showed resistance

Table 8 Clonal selection stages of Chacra's Sugarcane Breeding Program

Stage (number of clones)	Plot size/trial design	Years	Sites	Selection criteria
Stage I: seedlings (250,000)	Individual seedlings/mass selection	3	1	Visual assessment (agronomic type and resistance to diseases), brix and ratooning ability
Stage II: first clonal stage (3000)	1 row, 6 m long/ unreplicated	2	1	Visual assessment (agronomic type and resistance to diseases); stalk number, stalk weight and brix (3 records between early and mid-harvest season)
Stage III: second clonal stage (250)	3 rows, 5 m long/ unreplicated	3	3	Visual assessment (agronomic type and resistance to diseases); stalk number, stalk weight and sucrose content (early, mid- and late harvest season)
Stage IV: multi-environment variety trials (20–25)	3 rows, 10 m long/ RCBD* (3 replicates)	3	10	Visual assessment (agronomic type and resistance to diseases); cane yield, sucrose content (early, mid- and late harvest season); maturity curves and ratooning ability
Stage V: macroplot (3–5)	6 rows, 70–100 m long/ RCBD (3 replicates)	3	10**	Visual assessment (resistance to diseases); cane yield; estimated sugar yield; herbicide phytotoxicity and maturity and tillering curves

**RCBD* Randomized complete block design

**Trials are not planted at every location every year

Table 9 Major varieties grown in Salta and Jujuy provinces (Argentina) according to a 2019/2020 survey

Variety	Release year	% Area†	Parents (F × M)	Notes
LCP 85-384	2000	17.8	CP77-310 × CP77-407	High cane yield; high sucrose and moderate fiber content. Moderate flowering. Resistant to smut, mosaic, red stripe and leaf scald, and susceptible to brown rust and RSD
NA 97-3152	2014	16.6	NA78-2186 × polycross	High cane yield; high sucrose and moderate fiber content. Low flowering. Resistant to smut and red stripe; intermediate to leaf scald and brown rust, and susceptible to mosaic and RSD
NA 96-2929	2014	9.8	SP70-1143 × polycross	Moderate to high cane yield; moderate to high sucrose and fiber content. Low flowering. Resistant to brown rust and mosaic; intermediate to smut, leaf scald and red stripe, and susceptible to RSD
CP 70-1133		8.9	CP56-63 × polycross	High cane yield; moderate sucrose and fiber content. Low flowering. Resistant to leaf scald and red stripe; intermediate to brown rust and susceptible to mosaic and RSD
TUCCP 77-42	1987	8.5	CP71-321 × US72-019	High cane yield; moderate to high sucrose content and moderate fiber content. Heavy flowering. Resistant to mosaic and red stripe; intermediate to smut and susceptible to leaf scald, brown rust and RSD
NA 90-1001	2009	8.4	SP70-1143 × NA76-128	High yield, moderate sucrose content, mid-late maturity, moderate fiber content, moderate flowering. Resistant to mosaic, smut and leaf scald. Susceptible to red stripe and RSD
NA 05-860	2014	8.3	NA91-2030 × NA90-1015	High yield, moderate sucrose content, late maturity, moderate fiber content. Low flowering. Resistant to smut, leaf scald, red stripe and brown rust. Intermediate to mosaic. Susceptible to RSD

†Survey based on 92,710 sugarcane hectares in Salta and Jujuy

to glyphosate (5L/ha) herbicide in the field in 2005 (Spedaletti et al. 2008). Currently, research efforts focuses on characterizing the gene inserts and expression as well as in generating regulatory data to support food, feed and environmental safety assessment of a glyphosate-resistant event, QAB016 (Romero et al. 2019).

To develop mosaic resistant sugarcane, a method for direct sequencing of RT-PCR products from crude extracts was developed (Gómez Maximiliano and Serino 2009) and used to obtain sequence data from more than 500 virus samples collected from Argentina and neighboring regions (Gómez 2012). RNAi gene constructs were designed based on sequence data and used to generate a large number of putative mosaic resistant transgenic lines (Gómez 2012). Successive field tests allowed selection of nine independent events that were not infected with mosaic in a three-year replicated trial alongside the corresponding non-transgenic donor lines. Virus immunity or high resistance was observed in the selected genetically modified (GM) lines, whereas control lines were highly infected. Cane yield was maintained in some of the GM lines, whereas it was significantly reduced in others (unpublished results). Mosaic resistant lines are being maintained in regulated plots for research purposes only. This project will not advance towards commercial release.

Recently, Chacra has initiated research on gene editing technology for sugarcane. Putative genome targets, gene editing constructs, tissue culture conditions and in vitro selection are developed using the high transformation efficiency commercial cultivar NA05-860 as a model.

Conclusion and Future Directions

Sugar production from sugarcane is one the most important economic and social activities in the Tucumán, Jujuy and Salta Provinces of the Northwest region of Argentina. As it is well known, progress in sugarcane breeding represents a challenge mainly due to its highly complex genome and long breeding/selection cycle. However, sugarcane breeding programs in Argentina have made big efforts to develop sugarcane varieties adapted to diverse agro-ecological and management conditions that contributed to the profitability of its industry as well as overcoming sanitary outbreaks. This review summarizes the main features of two of the three sugarcane breeding programs in the country: EEAOC and Chacra. Throughout the history, EEAOC and Chacra breeding programs have released key varieties that have been and currently are the most grown in Tucumán and the Northern provinces (Jujuy and Salta), respectively. Both programs aim to obtain sugarcane varieties mainly with high cane yield, high sucrose content, early maturity and tolerant to major diseases. Efforts to enhance both breeding programs include: exchange of material with breeding programs from other countries through their own quarantine facilities, research activities with the purpose to increase the selection efficiency and the implementation of projects for varieties distribution through clean seed obtained from micropropagation by tissue culture. Biotechnology area also plays an important role through the introduction by transgenesis of new traits into elite genotypes and the use of molecular markers for genetic studies and diagnostic of the main pathogens.

Future prospects are mainly focused in: (i) increasing pre-breeding activities by genetic broadening and introgression of traits associated with energy production and tolerance to biotic and abiotic stresses; (ii) developing selection models that allow optimizing the selection of parent for crosses and clones integrating a high precision phenotyping with molecular data derived from high throughput genotyping, and (iii) achieving the commercial release of a transgenic variety with traits of interest.

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Declarations

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

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