RESEARCH ARTICLE

DEVELOPMENT AND EVALUATION OF HYBRID CLONES WITH DIVERSE CYTOPLASM IN SUGARCANE

V.A. Amalraj, Bakshi Ram and C.M. Radhakrishnan

Abstract

Modern sugarcane cultivars are genetically vulnerable having a narrow genetic base derived from a limited number of interspecific hybrids of S. officinarum (as female parent) and S. spontaneum. Although they possess nuclear genetic variability, they lack in cytoplasmic diversity since they are all derived from the cytoplasm of S. officinarum. A programme was initiated during 1988 to introduce the cytoplasmic diversity in sugarcane. Hybrid clones were developed for the first time at Sugarcane Breeding Institute, Coimbatore, using cytoplasm of S. spontaneum, S. barberi and S. sinense, and S. officinarum and commercial clones as pollen parents. Two to three backcrossings produced hybrid clones that were selected and designated as CD (cytoplasm diverse). These CD clones were evaluated for yield, juice quality and disease resistance under both sub-tropical (Karnal, North India) and tropical (Coimbatore, South India) conditions. Results indicated moderate to high variability for sugar yield, stalk yield and SSW (single stalk weight). However, the variability was low for pol %, stalk length and stalk diameter. Hybrids which performed on par with commercial canes in yield and quality, and resistance to red rot were identified. Two clones (CD-65 and CD-366) showed resistance to all the four races of red rot pathogen. Male sterility was observed in many of the clones that flowered.

***Key words**: Sugarcane, Saccharum spp., diverse cytoplasm, genetic stock, evaluation*

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Introduction

The present day commercial varieties of sugarcane have derived their maternal cytoplasm from a limited number of clones of *Saccharum officinarum*. Invariably, the noble cane (*S. officinarum*) has been used as pistil parent and *S. spontaneum*, *S. barberi*, *S. sinense* or other hybrids or commercial clones have been used as pollen parents in crop improvement programmes. Obviously, none of these pollen parents have contributed cytoplasm to their progeny. Though the commercial varieties of sugarcane have achieved chromosomal genetic diversity, they lack cytoplasmic diversity. According to Mangelsdorf (1983), 'after nearly 100 years of sugarcane breeding, we should still be completely in the dark as to whether the cytoplasm from related wild species may be able to contribute towards higher yields or towards more stable disease resistance'. He expressed concern about the lack of cytoplasmic diversity in the cultivated varieties subsequent to nobilisation. Berding and Roach (1987) remarked that modern cultivated varieties originated from a few parental clones and a considerable portion of the available genetic diversity remains to be exploited. Jie Arro *et al* (2004) have also stated that modern sugarcane cultivars are genetically vulnerable because the gene pool from which they are derived originated from a few interspecific hybrids between *S. officinarum* and its wild relative *S. spontaneum*. D'Hont *et al* (1993), evaluating cytoplasmic diversity using heterologous chloroplast and mitochondrial probes to assess the level of restriction fragment length polymorphism (RFLP) among the members of *Saccharum* complex, could not detect variability among the mitochondrial genome of *S. officinarum* but observed large variability within *S. spontaneum*, *Erianthus* and *Miscanthus*. Raghavan (1951) used *S. spontaneum* as pistil parent for the first time at Sugarcane Breeding Institute, Coimbatore and demonstrated the existence of cytoplasmic

inheritance in *Saccharum*. Based on his studies involving crosses between *S. spontaneum* and *S. officinarum*, he observed that the hybrids always resembled the maternal parent. It was also found that whenever *S. spontaneum* was used as the pistil parent, there was expression of male sterility as a maternally inherited character.

The lack of variability in cytoplasm of commercial varieties under extensive cultivation might lead to major pest and disease attacks (Tatum, 1971) as experienced in maize leaf blight epidemic in 1970. Sreevastava (1985) cited many examples of the use of cytoplasmic genes in breeding varieties of cereal and horticultural crops. The cytoplasm from wild and related species of wheat and rice was exploited for inducing male sterility, which helped in developing hybrid varieties. Walker (1978) reported that although *S. spontaneum* parent used in hybridization was resistant, F_1 and backcrosses with noble canes had high frequency of severe smut infections. He suggested genetic base broadening program to avoid genetic vulnerability of varieties to smut infection. Pan *et al* (2004) reported development of *Saccharum* hybrids with cytoplasm of *S. spontaneum* through a combination of conventional and molecular breeding approaches. They had selected 10 F_1 hybrids from two crosses between *S. spontaneum* (clone 'Djatiroto') and two sugarcane clones, CP 85-384 and CP 62-258. The present study, initiated during 1988, was aimed at broadening the genetic base by developing inter-specific clones with diverse cytoplasm of *S. spontaneum*, *S. barberi*, *S. sinense* and identifying genetic stocks resistant to biotic and abiotic stresses.

Materials and methods

Selected clones of *S. spontaneum, S. barberi and S. sinense* were used as pistil parents and *S. officinarum*, Co clones and other elite clones as pollen parents. Four clones of *S. spontaneum* (SES 2, SES 131A, SES 148 and SES 515/7) were selected as pistil parents and crossed with *S. officinarum* (57NG 66 with high pollen fertility), CoC 671, Co 6806 and Co 7704 as pollen parents. A total of 17 crosses comprising five unemasculated and 12 emasculated crosses, were attempted. In the latter group, nearly 150-200 spikelets were hand emasculated, tagged and pollinated for two days. Similarly, five clones of *S. barberi* (Kewali, Nargori, Katha, Pathri and Manjuri) were crossed with HR83-65, HR83-87, HR83-126, HR83-144 and HR83-159 as pollen parents. In *S. sinense*, Khakai and Uba were used as pistil parents. In all the cases, hybridity of the seedlings obtained in F_1 generation was cytologically confirmed by chromosome counts. Many of the progenies were found to be male sterile especially those derived from *S. spontaneum*.

On the basis of gross morphology and hybridity, seedlings were selected, evaluated for red rot and smut resistance, and further back crossed to CoC 671 or *S. officinarum* (57NG 66). Seedlings were selected on the basis of Hand Refractometer (HR) and general vigour, and one or two more cycles of back crossing were done. Selections were made on the basis of stalk diameter, number of millable canes (NMC), SSW and HR in the back cross generations and the selected clones were prefixed with CD. Two sets of field experiments were conducted. In the first experiment, F_1's along with 87 CD clones were evaluated for stalk yield, juice quality (sucrose and commercial cane sugar) and red rot resistance (in RBD with three replications) at Karnal (Haryana State, North India) under sub-tropical conditions. The second experiment was conducted under tropical condition in Coimbatore, with 95 CD clones which were evaluated for pollen fertility, stalk characters, NMC, HR , sucrose content in juice and for red-rot reaction. The data were analyzed statistically for variance and correlation.

To further improve the economic worth of the selected clones, 95 flowering CD clones were crossed with commercial canes in 2004 and the selected progeny was tested for red rot reaction (CoC 671 isolate of the pathogen). Again in 2007, the flowering progenies of 2004 crosses and CD clones were crossed with Co canes and the progenies tested for red rot in the year 2009. Based on single cane weight, sucrose and red rot reaction, the best clones were carried forward.

Results and discussion

Evaluation of inter-specific clones at Coimbatore

A total of 102 F_1 hybrids obtained from *S. spontaneum* x Co varieties in 1990 were evaluated. Of these, 74 were male sterile and the

Table 1. Evaluation of 95 CD clones at 10th month of growth in Coimbatore

Trait	Minimum	Maximum	Mean	SD	CV (%)
Plant height (cm)	115 (CD 25)#	300 (CD 264/59)	196.92	37.92	19.26
Stalk diameter (mm)	14.1 (CD 10)	29.2 (CD 52)	2.25	3.19	14.21
No. of millable canes	12 (CD 400)	105 (CD 77)	47.61	15.62	32.81

Clones are given in parentheses

remaining 28 clones showed partial anther development. In the first backcross seedlings, an improvement of 12 to 22% was observed for HR while the cane thickness ranged from 1.5 to 2.5 cm. Out of the 281 back cross clones obtained, 258 were male sterile; nine red rot resistant and 24 smut resistant clones were also identified. At Coimbatore, only one third of the CD clones flowered. Out of 33 flowered clones, 18 had normal fertile flowers and 15 had partly sterile flowers with staminodes or pistillodes and were mostly sterile. The hybrids CD 66 and CD 67 had ill-developed anthers extending into stigmatic lobes. Screening for red rot reaction at Coimbatore in July 2004 showed 19 CD clones as moderately resistant, eight moderately susceptible, 71 susceptible and nine highly susceptible.

Evaluation for plant height, NMC, stalk diameter and HR Brix at 10th month (Table 1) and for sucrose, purity and CCS % at 12th month (Table 2) showed that CD 75 was the best quality clone with 23.1%, 20.08% sucrose content, 87.63% purity and 13.83% CCS, while CD 52 was the best for cane thickness (2.9 cm) and CD 77 recorded the highest NMC (105). High mean number of millable canes (47.61) may have been contributed by *S. spontaneum* as this species has the ability to produce more number of tillers.

Of the 102 progenies from the 2004 crosses of CD clones screened for red rot, 38 clones were resistant and 64 clones susceptible. From 2007 crosses, 163 progenies were tested for red rot in 2009 and 78 clones were found to be resistant and 85 were susceptible (Table 3). It could be observed that there was an increase in the number of resistant clones and reduction in the number of susceptible clones in the 2007 progeny. Improvement in horizontal resistance to red rot in sugarcane clones due to the increased number of *S. spontaneum* in the chromosomal complement was well demonstrated by Natarajan et al. (2001).

Based on yield contributing characters, juice quality, and other agronomically important traits, 18 best clones were selected (Table 4) for the final clonal trial (Pre-Zonal varietal trial) at Sugarcane Breeding Institute, Coimbatore in 2010. Evaluation of the clones in Augmented Randomized Block Design along with standards resulted in the identification of one clone with resistance and 11 with moderate resistance. The results clearly indicated opening of new opportunities for developing commercially superior canes with diverse cytoplasm.

Evaluation at Karnal

Eighty-seven CD and F_1 clones were evaluated under subtropical conditions at Karnal. Range, mean,

Table 2. Evaluation of 95 CD clones at 12th month of growth

Trait	Minimum	Maximum	Mean	SD	CV (%)
HR Brix	13.30	23.10	18.02	1.87	10.39
Sucrose (%)	9.40	20.08	15.21	1.96	12.89
Purity (%)	67.64	87.63	80.15	4.09	5.11
CCS (%)	5.68	13.83	10.05	1.53	15.20

Table 3. Red rot reaction of 2004 and 2007 progenies

Reaction	2004 progeny	% of total	2007 progeny	% of total
Resistant	3	2.94	39	23.93
Moderately Resistant	35	34.31	39	23.93
Susceptible	16	15.68	43	26.38
Moderately Susceptible	3	2.94	38	23.31
Highly Susceptible	45	44.12	4	2.45

standard deviation, coefficient of variation and clones which were superior to general mean are presented in Table 5. F_1 clones recorded the lowest values for stalk diameter, single stalk weight (SSW), stalk yield, pol % at 10 and 12 months, and sugar yield but the maximum values for NMC and stalk length. Though F_1 clones produced higher NMC with more stalk length, cane yield was less because of their lower SSW and stalk diameter. The results indicated moderate to high variability for sugar yield, stalk yield and single stalk weight (SSW). However, the variability was low for pol %, stalk length and stalk diameter. Contrary to the general trend, the coefficient of variation (CV) for pol % at 12th month was higher than that for pol % at 10th month because many F_1 clones showed deterioration in juice quality from 10 to 12 months.

Table 4. Performance of elite clones identified for final clonal trial (PZVT) in 2010

S. No.	Clone	Red rot reaction*	SCW (kg)	NMC	Brix	Pol (%)	Sucrose (%)	Purity (%)
1	CD07-08	R	0.7	15	21.75	80.8	19.31	88.81
2	CD07-10	MR	0.7	7	21.05	79.3	19.01	90.32
3	CD07-15	MR	0.9	8	21.35	77.0	18.43	86.36
4	CD07-17	MR	0.5	7	21.75	76.0	18.16	83.52
5	CD07-18	MR	0.8	8	20.35	75.0	18.03	88.61
6	CD07-37	MR	1.5	8	21.45	79.0	18.90	88.15
7	CD07-38	MR	2.4	9	19.85	72.0	17.34	87.39
8	CD07-56	MR	1.4	10	21.05	78.9	18.91	89.86
9	CD07-57	MR	1.4	17	20.55	75.1	18.04	87.79
10	CD07-65	MR	0.7	14	20.05	74.1	17.83	88.97
11	CD07-67	MR	1.1	18	20.75	77.5	18.60	89.65
12	CD07-80	MR	1.3	17	22.15	82.2	19.61	88.57
13	CD07-19	MS	1.5	21	19.95	72.2	17.38	87.16
14	CD07-64	MS	0.8	18	21.35	80.8	19.34	90.62
15	CD07-49	S	0.8	19	22.65	86.2	20.53	90.64
16	CD07-55	S	1.3	6	20.55	77.0	18.46	90.02
17	CD07-61	S	0.8	20	20.75	76.5	18.36	88.50
18	CD07-72	S	1.0	12	20.55	75.0	18.01	87.68

* R=Resistant; MR=Moderately resistant; MS=Moderately susceptible; S=Susceptible

Table 5. Evaluation of F_1 s and 87 CD clones at Sugarcane Breeding Institute, Regional Centre, Karnal

Trait	Range (Clone)		Mean	SD	CV%	Superior (CD) clones
	Minimum	Maximum				
Sugar yield / (plot* (kg))	0.34 (F_1-7)	13.78 (CD-98)	6.71	2.56	38.15	CD-12, -50, -52, -58, -62, -67, -71, -75, -77, -95, -98, -132, -151, -374; F_1-33
Stalk yield / (plot (kg))	18.0 (F_1-42)	152.0 (CD-67)	62.70	22.87	36.48	CD-12, -16, -33, -37, -40, -50, -52, -57, -58, -62, -67, -71, -75, -77, -95, -98, -132, -263, -371, -374, -376, -383, -388; F_1-33
Pol % (10 months)	8.89 (F_1-51)	18.59 (CD-151)				13.71 1.73 12.62 CD-13, -43, -64, -75, -77, -95, -98, -119, -132, -151, -170, -173, -199, -374, -376; F_1-4, -21, -42, -52
Pol % (12 months)	4.97 (F_1-7)	20.48 (CD-151)	15.80	2.60	16.46	CD-35, -43, -50, -60, -64, -71, -75, -95, -98, -119, -132, -151, -170, -199, -374
NMC (/ plot)	51.5 (CD-49)	241.0 (F_1-33)	131.02	31.44	23.99	CD-21, -25, -27, -58, -62, -64, -170, -277; F_1-4, -5, -7, -21, -31, -33, -34, -41, -42, -43, -51, -52, -75, -90
Stalk diameter (cm)	0.91 (F_1-7)	2.35 (CD-371)	1.75	0.21	12.00	CD-12, -33, -40, -50, -52, -71, -98, -132, -371, -374, -376
Stalk length (cm)	147.5 (CD-43)	330.0 (F_1-31)	230.10	34.71	15.08	CD-2, -11, 12, -16, -37, -51, -57, -61, -62, -63, -65, -66, -67, -69, -71, -72, -97, -151, -279, -361, -383, -388; F_1-4, -5, -21, -31, -33, -34, -41, -43, -75, -90
Single stalk weight (kg)	0.09 (F_1-7)	1.17 (CD-52)	0.54	0.17	31.48	CD-4, -12, -33, -37, -40, -41, -44, -50, -52, -57, -62, -67, -71, -98, -119, -132, -151, -371, -374, -383, -388, -404

When compared with the standard varieties for yield and quality traits, eight clones *viz*. CD-52, CD-58, CD-067, CD-77, CD-97, CD-98, CD-228 and F_1-33 were significantly superior for stalk yield. For sugar yield, CD-67 and CD-98 (mainly due to their highest stalk yield) were on par with the standard Co 1148. CD-288 and CD-132 were numerically on par with Co 1148. None of the clones was superior to the best standard CoJ 64 for juice quality traits. However, CD-043, CD-060, CD-071, CD-119, CD-132, CD-151, CD-374 and CD-383 were numerically on par with CoJ 64 for pol %.

The clones were also tested for red rot resistance by plug method of inoculation against prevalent races of red rot. CD-37, CD-41, CD-66, CD-71, CD-366, CD-151, F_1-21, F_1-34 and F_1-90 were resistant and another 22 clones showed moderately resistant reaction. Only CD-65 and CD-366 showed resistance to all the four races of red rot pathogen. Clones CD-49, -56, -64, -75, -98, -151, -199, -277, -284 were resistant to three races, i.e. Cf01, Cf02, Cf03 of red rot pathogen. Many of these clones showed pithiness in the stalks. Parentages of superior CD clones based on cane yield and juice quality are given in Table 6.

Table 6. Parentage of superior CD clones

Cytoplasm donor	Parents (pistil parent x pollen parent)	Superior CD clones
S. sinense	Khakai x HR83-65	CD-50
S. barberi	Kewali 14G x HR83-7	CD-35, -37
	Kewali 14G x HR83-87	CD-16, -21, -25, -27, -33
	Kewali 14G x HR83-126	CD-2,-4,-11,-12,-13
	Kewali 14G x HR83-159	CD-361,-376,-383,-371,-374,-388
S. spontaneum	SES 2 x Co 6806	CD-65,-66
	SES 148 x CoC 671	CD-170,-173
	SES 515/7 x CoC 671	CD-60,-61,-62,-63,64,-69,-71,-72,-199,-263
	SES 515/7 x Co 7704	CD-52,-57,-58,-67,-75,-77,95,-98,119,132,-151

Correlation analysis for plant height, stalk diameter, NMC, HR Brix and CCS% showed highly significant positive correlation between stalk diameter and juice quality traits (HR Brix and CCS %) (Table 7). Therefore, selection for stalk diameter might result in improvement in juice quality of these clones with diverse cytoplasm.

However, caution must be taken to prove this correlation in larger variable population as no significant correlation was also reported between these two traits by Sukhchain et al. (1997). Stalk diameter was negatively correlated whereas cane height was positively correlated to NMC. Negative correlation between NMC and cane diameter was reported by Junejo et al., (2002), Afghan et al., (1993) and Khan et al., (2003) while Panhwar et al., (2002), Javed et al., (2001) and Khan et al., (2003) also obtained positive correlation between cane height and NMC with different sets of sugarcane hybrids. Hence, a compromise has to be made for NMC, stalk diameter and cane height while selecting better juice quality CD clones.

Conclusion

Although the related wild taxa such as *S. spontaneum*, *Erianthus* spp, *Miscanthus* spp. are extensively being used in sugarcane breeding programmes worldwide, the cytoplasm of species other than *S. officinarum* is not represented in the present day sugarcane varieties. Thus the purpose of this study was to evolve new genetic stocks with diverse cytoplasm, utilizing cytoplasm other than that of *S. officinarum*. Inter-specific clones have been developed with cytoplasm of *S. spontaneum*, *S. barberi* or *S. sinense* and the selected elite CD clones performed on par with commercial sugarcane varieties with respect to quality and 18 clones have qualified for evaluation in Pre-Zonal Varietal Trial (PZVT). Although work has been done earlier on diverse cytoplasm, this is the first report of successful development of diverse cytoplasm clones eligible for PZVT. Thus, this study has demonstrated the contribution of cytoplasm other than *S. officinarum* in the development of new desirable sugarcane genetic stocks which showed resistance to red rot.

Table 7. Correlation analysis of CD clones

	Height	NMC	Stalk diameter	HR Brix	CCS (%)
Height	1.000	0.201*	0.129	-0.061	-0.110
NMC		1.000	-0.238*	-0.190	-0.203*
Stalk diameter			1.000	0.357**	0.362**
HR Brix				1.000	0.770**
CCS (%)					1.000

* Significant at 0.05 level; ** significant at 0.01 level

These can be successfully utilized in the crossing programme for the development of resistant varieties with diverse resistance genes.

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