RESEARCH ARTICLE

GENETIC VARIABILITY FOR AGRO MORPHOLOGICAL TRAITS AMONG THE INTERSPECIFIC HYBRIDS OF *SACCHARUM*

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Abstract

The extent and pattern of variation for various agromorphological traits were studied in 1346 progenies of six crosses involving *S. officinarum*, *S. spontaneum*, *S. robustum*, Indian and foreign hybrids. The progenies along with the parents were evaluated for traits like tillering, plant height, number of millable canes (NMC), Hand Refractometer (HR) Brix %, internode length, pithiness and red rot resistance. We observed a considerable amount of variation among the progenies for all the traits. The initial tillering in all the progenies was very high compared to their parents and highest tiller count was in *S. officinarum* x *S. spontaneum* cross. Progenies were generally thin especially among the crosses with *S spontaneum* as male parent. The mean HR Brix % at 8th month of the progenies having *S spontaneum* in their parentage was low (8-10%) which needs further improvement through backcrossing with *S. officinarum*, the female parent. The hybrid progenies of some of the crosses showed heterotic vigour for traits like NMC, internode length and plant height. Many progenies of the crosses Azul de caza (*S. officinarum*) x IK 76-10 (*S. spontaneum*), IJ 76-315 (*S.officinarum*) x IJ 76 280 (*S. robustum*), IJ 76-315 x IMP 558 (*S. spontaneum*) were resistant to red rot. The wild species of *Saccharum* are known to impart disease resistance; the study resulted in introgression of red rot resistance genes to the progenies from *S. spontaneum* and *S robustum*. The red rot resistant progenies can be further utilized in the back breeding program to improve the yield and quality traits.

Key words: Sugarcane, genetic variability, interspecific hybrids

Introduction

Inter-specific hybridization has always remains as the backbone of sugarcane breeding to improve and sustain the productivity. The sugarcane varieties grown throughout the world are products of inter-specific hybridization involving *S. officinarum, S. barberi, S. sinense* and wild species of S. *spontaneum* and *S. robustum* (Price, 1963). In general, *S. officinarum* clones are known to impart ability to accumulate high level sucrose and clones of *S. spontaneum* confer vigour, high tillering, resistance to biotic and abiotic stresses and wider adaptability (Sreenivasan and Amalraj 2004). *S. robustum* clones are hard, woody, pithy with little juice and can grow up to 10m height (Stevenson 1965). *S. barberi* / *sinense*, being considered to be derivatives of natural hybridization between *S. officinarum* and *S. spontaneum* (Parthasarathy 1946) might have contributed gene complexes for adaptiveness and sucrose.

Though the genetic variability created through interspecific hybridization and back crossing involving these major species has sustained the sugarcane improvement activities over the years, the sugarcane varieties have a narrow genetic base with only 19 *S. officinarum* and 2 *S. spontaneum* clones as parents in their lineage (Arceneaux 1965, Roach 1968). Because of the small number of clones/ species used in primary crosses, genetic base of modern sugarcane varieties has been reported to be narrow and is thought to be the reason for the present slow progress in sugarcane breeding (Arceneaux 1965, D'Hont et al. 1995).

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Widening of the genetic base is need of time for almost all crops and can be attained by the use of wild species and primitive cultivars in breeding programme (Hawkes 1977). In the recent years the importance of unutilized noble and wild species clones of *Saccharum* in broadening the genetic base has been recognized. Considering the fact that the crop has to cope with many adverse environmental conditions in the changing climate scenario introgression of wide adaptive genes from the wild and related species by conventional method is of utmost importance. This necessitates the sugarcane breeders to explore new possibilities to increase genetic variability by identifying and effecting crosses between the diverse parents.

With the objective of utilizing diverse germplasm resources for developing genetic stocks, hybridization program is being regularly carried out at Sugarcane Breeding Institute Research Centre, Kannur which houses world's largest sugarcane germplasm collection. In the present study the extent and pattern of variation for various agromorphological traits were studied in 1346 progenies of six crosses involving *S. officinarum*, *S. spontaneum*, *S. robustum*, Indian and exotic hybrid. The progenies along with the parents were evaluated for traits like tillering, plant height, NMC, HR Brix %, Internode length, red rot resistance and pithiness

Materials and Methods

The materials consists of 1346 F_1 seedling obtained from six crosses. Of the six crosses three crosses were made between Azul de caza, *S officinarum* as female parent and IK 76-10 (*S. spontaneum*), CP 96-1252 (Foreign Hybrid) & WL 07-757 (waterlogging resistant hybrid) as pollen parents. Similarly other three crosses were made between IJ 76- 315, *S officinarum* as female parent and IMP- 558 (*S. spontaneum*), IJ-76-280 (*S robustum*) and IA 3306 (Indo American hybrid) as pollen parents. The fluffs were germinated and individual seedling was raised in polybags during the crop season 2012-13. The seedlings were transplanted to the field with a spacing of 90 cm between rows. All the cultural operations were given as per the normal package and practices followed in sugarcane cultivation. Pre-monsoon tillering and plant height recorded at 3rd month, number of millable cane (NMC), cane thickness and HR Brix at 8th month. Internode length, flowering behavior, internode colour, pithiness and reaction to red rot were recorded. Pithiness was recorded by spitting the cane in 0 (low) - 9 (high pith) scale (Evans 1966). The data were tabulated and statistically analysed to derive mean standard deviation and coefficient of variation for each cross combination for all traits studied. The relative heterosis (heterosis over mid parent) was calculated for all the traits under study.

The progenies were screened for red rot resistance (*Colletotrichum falcatum* pathotype CF06) under controlled conditions testing (CCT) at ICAR-SBI, Coimbatore (Viswanathan et al., 1998, 2018). Three canes per clone were inoculated along with a standard susceptible variety CoC 671. The inoculated canes were kept inside the red rot testing chamber and incubated under ideal conditions of for red rot development on the canes as reported earlier. After 10 days of incubation, the disease development and its severity were assessed based on red rot lesions on leaf scar and growth ring, infections on buds and root eyes, nature of lesions on the rind, internal spread of the lesions and fungal growth on the affected tissue and the disease reactions were scored as resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS).

Results and Discussion

The pre-monsoon tiller count ranged from 1 to 25 in the progenies of the three crosses where Azul de caza (*S. officinarum*) was the female parent. The highest number of tillers was observed in one of

Table 1. Mean performance of progenies involving Azul de caza as female parent

Cross	Tiller count	Plant Height(cm)	NMC	Thickness (cm)	HR Brix %	Internode length (cm)
Azul de caza x IK 76-10 N=408						
Azul de caza	3	146.7	3	2.5	18.9	19.2
IK 76-10	6	73.3	17	1.6	10.5	19.2
F1 Mean	6	110.2	5	1.7	8.9	18.8
F1 Range	1.0- 15	13-165	1.0- 14	0.9-2.7	5-17.2	9.5-27.3
Relative Heterosis	33	0.2	-50	-17	-39	-2.1
SD	2.5	21.7	2.3	0.3	3.1	3
CV	45	19.7	46	15.4	35.2	16
Azul de caza x CP 96-1252 N=302						
Azul de caza	3	146.7	3	2.5	18.9	19.2
CP 96-1252	4	112.7	5	2.2	19.2	15.2
F1Mean	4	70.3	3	2.2	17.7	16.8
F1 Range	1.0-25	9-140	1.0-16	1.0-3	7.0-22	9.0-26
Relative Heterosis	14	-45	-25	-6.3	-7.1	-2.3
SD	2	19.7	2	0.3	2.6	2.3
CV	56	28	55	14.4	14.5	13.8
Azul de caza x WL 07-757 N=211						
Azul de caza	3	146.7	3	2.5	18.9	19.2
WL 07-757	4	94.3	3	2.4	18.2	18
F1 Mean	5	62.4	4	2.3	17.8	18.7
F1 Range	1.0- 15	16-110	1.0-11	2.0-3	12.0-22	10.0-24
Relative Heterosis	43	-48	33	-6	-4	0.5
SD	2	15.2	2	0.3	2	2.4
CV	46	24.3	47	13.2	11.4	12.9

the progenies Azul de caza x CP 96-1252 (Table 1). Among the progenies of IJ 76-315 as female parent, the tiller count ranged from 1 to 24 with highest tiller count in the combination of IJ 76-315 x IMP 558 (*S. spontaneum*) (Table 2). In all the six crosses, the average tiller count of progenies was either higher or on par with the parents indicating initial vigour of the progenies. The SD value among the crosses were 2 to 5 with highest in the cross IJ 76-315 x IMP 558. The relative heterosis for tiller count was positive for all the six crosses and it ranged from 14 to 150%. The F_1 progenies from the IJ 76 315 x IA 3306 and IJ 76-315 x IMP 558 had tillers more than two times as of the mid parental value hence showed high relative heterosis. The tiller count was high among the progenies of the crosses where *S. officinarum* was crossed with *S. spontaneum, S robustum* and Indo American hybrid, as compared to crosses where *S. officinarum* was crossed with Indian or Exotic hybrids, indicating the suitability of utilizing the wild species for improved tiller count.

Table 2. Mean performance of progenies involving IJ 76-315 as female parent

Cross	Tiller count	Plant Height (cm)	NMC	Thickness (cm)	HR Brix %	Internode length (cm)	Pithiness
IJ 76 315x IMP 558 N=141							
IJ 76 315	3	126.7	2	2.4	18	16.2	0
IMP 558	5	61.3	26	1	5.2	16.5	9
F1Mean	10	60.6	10	1.4	10	16.4	4
F1 Range	1.0-24	22-92	1.0-32	0.9-2.5	4-17.8	9-25.5	0-7
Relative Heterosis	150	-36	-29	-17	-13.8	1.3	-11
SD	5	13.4	6	0.3	4.1	2.9	2
CV	50	22.1	59	18.1	41.3	17.8	48
IJ 76 315 x IA 3306 N=166							
IJ 76 315	3	126.7	2	2.4	18	16.2	0
IA 3306	5	100	3	1.3	17.6	16.1	5
F1 Mean	10	59.1	8	1.7	18.6	16.8	4
F1 Range	1.0-19	4.0-98	1.0-18	1-2.3	7.2-22.8	12.2-23.5	0-5
Relative Heterosis	150	-48	220	-8.1	4.5	0.4	60
SD	3	17.1	3	0.2	2.1	2.2	1
CV	31	28.9	39	13.2	11.2	13.3	38
IJ 76 315 x IJ 76-280 N=118							
IJ 76 315	3	126.7	2	2.4	18	16.2	0
IJ-76-280	5	147.7	4	1.7	15.8	17.1	5
F1 Mean	6	58.3	5	1.8	15.7	16.3	3
F1 Range	1.0-12	28-105	1.0-12	1.8-2.8	7.8-21.6	9-22.5	0-5
Relative Heterosis	50	-57	66	-12	-7.1	-2.1	20
SD	2.3	14.5	2.4	0.2	2.2	3	2
CV	39	24.9	49.5	13.4	14.2	18.2	66.5

The mean pre-monsoon plant height an indicator of the waterlogging resistance of the progenies which ranged from 58.3 cm (in the cross IJ 76-315 x IJ 76-280) to 110.2 cm (in the cross Azul de caza x IK 76-10) (Table 1 and 2). The height of the progenies from the cross Azul de caza x IK 76-10 ranged from 13 cm to 165 cm. The mean height of the progenies were on par with the mid parental value in the cross Azul de caza x IK 76-10, but in other crosses it was less compared to the parents. Thus the relative heterosis was on the negative for all the crosses except in the cross Azul de caza x IK 76-10. The variability among the progenies for pre-monsoon plant height was low. Pre-monsoon plant height was highest when *S. officinarum* was crossed with *S. spontaneum* as male parent.

The mean NMC of F_1 seedlings varied from 3.0 (Azul de caza x CP 96-1252) to 10 (IJ 76-315

Table 3. Percentage distribution of progenies for agromorphological traits

Cross	>10 No.s /clump	NMC 5 to 10 No.s / clump	< 5 No.s /clump
Azul de caza x IK 76-10	4	49	47
Azul de caza x CP 96-1252	1	25	74
Azul de caza x WL 07-757	1	32	67
IJ 76-315 x IMP 558	42	34	24
IJ 76-315 x IA 3306	35	29	36
IJ 76-315 x IJ 76-280	5	48	47
Cane thickness	**> 2.5 cm**	**2.5-2.0 cm**	**< 2.0 cm**
Azul de caza x IK 76-10	0.2	16.7	83.1
Azul de caza x CP 96-1252	20.5	45	34.4
Azul de caza x WL 07-757	35.5	55.5	9
IJ 76-315 x IMP 558	0.7	1.4	97.9
IJ 76-315 x IA 3306	0	12.7	87.3
IJ 76-315 x IJ 76-280	0.8	18.6	80.5
HR brix at 8th month	**> 20.0 %**	**15.0-20.0 %**	**< 15.0 %**
Azul de caza x IK 76-10	0	2.2	97.8
Azul de caza x CP 96-1252	12.6	72.8	14.6
Azul de caza x WL 07-757	16.1	73.9	10
IJ 76-315 x IMP 558	0	12.1	87.9
IJ 76-315 x IA 3306	28.3	65.1	6.6
IJ 76-315 x IJ 76-280	3.4	63.6	33.1

x IMP 558) with the highest NMC of 32 canes recorded in the progeny of IJ 76-315 x IMP 558 (Table 1 and 2). The NMC of progenies for all the crosses were either superior or on par with their female parent. The percentage of seedling having more than 10 millable canes /clump was 42 and 35% in the crosses IJ 76-315 x IMP 558 and IJ 76 315 x IA 3306, respectively (Table 3). In the progenies of both the crosses IJ 76 315 x IA 3306 and IJ 76-315 x IMP 558, the presence of *S spontaneum* genes is obvious as IA clones are hybrids between American commercial hybrids and Indian *S. spontaneum* clones. This clearly substantiates that the *S. spontaneum* contributes genes for higher tiller count to their progenies. It is in concordance with the increased NMC in the F_1's of commercial hybrids x *S. spontaneum* compared to the female parent reported by Babu and Ethirajan (1962). The NMC of the progenies were less (below 5) when *S. officinarum* was crossed with exotic and Indian hybrids.

The cane thickness ranged from 0.9 (IJ 76-315 x IMP 558 and Azul de caza x IK 76-10) to 3.0 cm (Azul de caza x CP 96-1252 and Azul de caza x WL 07-757) (Table 1 and 2). Thin canes were mainly observed in the progenies with *S. spontaneum* and *S. robustum* as the male parent where as the thickness was high when hybrids were used as male parent (Table 3). The mean cane thickness of the progenies was less compared to the mid parental value, whereas it was higher in comparison with the male parent. The variability was very less among progenies with CV of around 13-18% only. Similar observations were recorded in F_1 of *S. officinarum* x S. *spontaneum* and commercial hybrid *x S. spontaneum* (Roach, 1968; Babu and Ethirajan, 1962).

Length of internode was highly variable among the seedlings. It ranged from 9.0 (Azul de caza x CP 96-1252 and IJ 76-315 x IJ 76-280) to 27.3 cm (Azul de caza x IK 76-10), the highest internode length of 27.3cm was recorded in the progeny of the cross involving IK 76-10 (*S. spontaneum*) as male parent. The means of the progenies were on par with the mid parental values but were higher as compared to the male parent.

HR Brix % is the most reliable early stage selection criterion as it is one of the important attributes of juice quality and is also known to have high correlation between different stages of selection cycle. The HR brix ranged from 8.9 Azul de caza x IK 76-10 to 18.6 % IK 76-315 x IA 3306 (Table 1 & 2). In individual crosses, the Brix ranged from 7 to 22 %. The brix % was low in the progenies with *S. spontaneum* as male parent whereas the brix was above 20% in seedlings when hybrids were used in the cross (Table 3). Variability found to be low to moderate with CV ranging from 11.2 % in (IJ 76-315 x IA 3306) to 41.3 % in IJ 76-315 x IMP 558. In all the crosses except Azul de caza x IK 76-10, progeny mean was on par with the mid parental value. Similar results were also reported in F_1 progenies of *S. officinarum x S. spontaneum* (Roach, 1968, 1977). The male parents (*S. spontaneum* clones) had a marked effect on the F_1 hybrids, which all had lower brix compared to F_1 hybrids with higher brix when hybrids used as male parents. This is in accordance with those of Tai et al. (1990) for F_1 hybrids from crosses between three commercial sugarcane cultivars and three *S. spontaneum* clones.

Out of the 1346 seedlings randomly selected 155 seedlings of four crosses were tested for their reaction to red rot under CCT method. The four crosses for red rot testing was selected based on the criteria that the male parent used are wild species either *S. spontaneum* or *S robustum*. Among the parents used in the crosses IJ 76 315 and IMP 558 showed susceptible reaction whereas IA 3306, IJ 76 280 and IK 76-10 had resistance reaction to red rot pathogen. Of the 155 progenies tested 65.8% of clones showed resistance/moderately resistant reaction to red rot and 34.2 % showed susceptible/ moderately susceptible/ highly susceptible reaction to red rot pathogen (Table 4). More number of resistant/ moderately resistant progenies was observed in the cross IJ 76-315 x IMP 558. In the other crosses the proportion of resistant/ moderately resistant progenies were less

Table 4. Percentage distribution of progenies for red rot pathogen reaction

Progenies	No. of clones tested	R	MR	RRR MS	S	HS
Azul de caza x IK 76-10	15	26.7	20	6.7	13.3	33.3
IJ 76-315 x IMP 558	71	57.1	30	2.9	10.	0.0
IJ 76-315 x IA 3306	50	4	4	10	62	20
IJ 76-315 x IJ 76-280	19	52.6	10.5	0.0	10.5	26.3

R-Resistant, MR- Moderately Resistant, MS-Moderately Susceptible, S-Susceptible, HS-Highly Susceptible.

Table 5. Percentage distribution of progenies for flowering behavior

Progenies	Flowering Behaviour	
	Flowering	Non flowering
Azul de caza x IK 76-10	54.0	46.0
Azul de caza x CP 96-1252	33.0	67.0
Azul de caza x WL 07-757	20.0	80.0
IJ 76-315 x IMP 558	71.0	29.0
IJ 76-315 x IA 3306	80.0	20.0
IJ 76-315 x IJ 76-280	47.0	53.0

compared to susceptible/ moderately susceptible/ highly susceptible. It implies that red rot resistance could be introgressed to sugarcane from wild species *S. spontaneum* and *S robustum*. The red rot resistance has also been incorporated in these interspecific hybrids as the sugarcane clones IMP 558 used as parents were susceptible to red rot.

Selection against pith is one of the major criteria to eliminate inferior sugarcane cultivars in early stages of evaluation as pith is considered to be associated with reduced sugar yield (Gravois et al 1990). Pith is composed of white, cottony, dead parenchyma cells. Pith formation is due to the rapid growth of large cells in the centre of the stalk where the density of fibre-vascular strands is low and subsequent maturation of these cells result in dry cavities within the internodes, which can affect sucrose extraction during processing. Though it is markedly a varietal phenomenon environmental factors also influence the pith formation. As per earlier studies, continued irrigation until harvest of the crop and increased nitrogen fertilization cause the increased pith development (Evans 1966, Verma 1948). The pithiness was recorded for progenies of three crosses involving IJ 76-315 as female parent (Table 2). The pith was absent in IJ 76-315 whereas the pithiness was high (Scale 9) in IMP 558(S. *spontaneum*) and scale 5 for the other two female parents IA 3306 (IA hybrid) and IJ76-280 (*S robustum*). The pithiness was in the range of 0-7 scale in the progenies of the cross IJ 76-315 x IMP 558 and 0-5 scale among the progenies of the other two crosses (Table2). It is reported that the proportion of progenies having pith is high when both parents have pith and it considerably decreased even when one of the parents is non pithy (Dutt and Rao 1950).

Flowering in sugarcane is a physiological process which affects yield and quality thus in tropical breeding programmes importance is given to selection against flowering (Berding and Hurney, 2005). The flowering percentage of the progenies varied from 20% (Azul de caza x WL-07-757) to 80% (IJ 76 315 x IMP558). The percentage of flowering progenies was less when hybrids are used as male parents as in the case of the crosses Azul de caza x CP 96-1252 and Azul de caza x WL 07-757 and is high when *S. spontaneum* and *S. robustum* were used as male parent (Table 5). Flowering behaviour of clones in a particular environment is genetically controlled and are therefore heritable. (Stevenson 1965). Stevenson (1965) reported that heavy flowering is a dominant character and hy brids obtained from crosses between *S. officinarum* and *S. spontaneum* are characteristically heavy flowering. Though flowering is considered unfavourable for production point of view, it is essential for further utilization in the development of new improved varieties of sugarcane.

Summary

The study of agronomic and commercial traits of interest in the progenies of interspecific hybrids of sugarcane is of great importance to select better performing progenies for further utilization in the breeding programme. In the present study F_1 progenies of interspecific hybrids involving *S. officinarum, S. spontaneum, S. robustum* and hybrids were evaluated for traits like tillering, plant height, NMC, HR Brix %, internode length, pithiness and red rot reaction.

The progenies clearly showed the superiority for cane population over two *S. officinarum* clones used as female parent (IJ 76-315 and Azul de caza) as well as two hybrid clones (WL-07-757 and CP 96-1252) utilized in the study. In comparison with the *S. spontaneum* parents, only very few clones had a higher cane population. Variation was also very substantial for this important attribute which is necessary for effective selection.

Though there was a good increase in NMC, average cane thickness in all the crosses showed a considerable reduction especially when *S. spontaneum* is involved in the parentage. In general, canes were thin which is normally expected and certainly back crossing either to a different *S. officinarum* clone or a good commercial clone is required to improve the cane diameter. Variation for cane diameter was found to be low in the study. Stalk thickness and Brix are the most repeatable and important traits used as selection criteria in early selection stages of the sugarcane varietal improvement program (Miller and James 1974; Tai and Miller 1989). However, HR brix was low in the crosses involving *S. spontaneum* as a male parent. The Brix % of progenies reached above 20 when hybrids and *S. robustum* were used as male parents. This clearly substantiates the effect of male parents on the progenies performance. Wide variation exists among progenies for brix % indicating the scope for further improvement which is very vital for quality improvement. The progenies from *S. officinarum* x *S. spontaneum* cross was found to have more pith compared to female parent (*S.officinarum*) and the presence of pith may be contributing to low Brix % among the progenies . The present investigation has identified a large number of progenies with red rot resistance especially among the progenies of *S. spontaneum* and *S. robustum*. Since wild species of *Saccharum* is known to impart disease resistance, the study resulted in introgression and incorporation of red rot resistance genes to the progenies. These red rot resistant progenies can be further utilized in the back breeding program to improve the yield and quality traits.

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