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SHORT COMMUNICATION

Cytological investigation on interspecific progenies of red fleshed *Saccharum robustum*

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Abstract
Saccharum robustum is one of the wild species and its chromosome number ranges from 2n =60 to 200. This group of *S.robustum* clones exhibit red flesh colour, robust growth and tolerance to abiotic stresses. In this study, 27 hybrid progenies developed from two polycrosses 'involving' red fleshed *S.robustum* clones were used as experimental material. The progenies were grouped into six categories based on the flesh colour and the cytological information was correlated. Six cytological groups were observed in the progenies. It was observed that all six progenies of NG 77-76 were of 2n=70 cytotype but among the progenies of NG 77-84, 57.1% were with 2n=70, 23.8% were with 2n=72 and the remaining four types were available in 0.83%. From this it is concluded that more cytotypes can be developed using NG 77-84 as female parent compared to NG 77-76. When the flesh colour was correlated with the diploid chromosome number, a significant negative correlation was observed. It is also inferred that the only one progeny having red flesh colour and 2n=60, might have developed as a result of un-intentional selfing.

Keywords: *Saccharum robustum*; Progenies; Cytology; Flesh colour

The genus *Saccharum*, derived its name from the Sanskrit word 'sarkara' meaning gravelly or gritty particle. Sugarcane is indigenous to tropical south and Southeast Asia. Different species of this genus are reported to be originated in different locations across the tropical region. Modern sugar cane varieties are derived from interspecific crosses involving many species and even related genera like *Sorghum*, *Erianthus* etc. (Sreenivasan et al. 1987). The complexity of modern varieties, the complex aneuploids with chromosome numbers ranging between 100 and 130 could be illustrated only by their cytology. Despite the cytological complexity, the chromosome behavior of some modern varieties is similar to that of allopolyploid (Price 1963). Among the six species of *Saccharum* five of them (*S.officinarum*, *S.spontaneum*, *S. barberi, S. sinense, S.robustum)* were widely utilized in the crop improvement programme.

S. robustum is believed to be the direct or cognate ancestor of *S. officinarum* (Artschwagar and Brandes 1958). Molecular studies also showed the close relation of *S.officnarum* with *S.robustum* (Vijayan Nair et al. 1999). The center of diversity of this species is South Pacific Island, Papua New Guinea. This is a wild species distributed from Borneo to the New Hebrides. Grassl (1946) reported that green form of *S. robustum* (normal flesh colour) was mainly distributed at the bank of Laloki River and the red flesh forms on the bank of Sepik River in New Guinea. These red fleshed forms closely resembles the noble cane in morphology, but its flesh is not soft, sweet or juicy, large pith in the interior, and some time hollow in the center. *S.robustum* is neither morphologically nor cytologically homogenous. The chromosome numbers of *S. robustum* clones range from 2n=60 to 2n= 200 (Sreenivasan et al. 1987). Grassl (1964) classified *Saccharum robustum* with

2n = 60 as S*accharum robustum* var *Sanguineum*.

One hundred and twenty eight accessions of *S.robustum* are available in the world collection of sugarcane germplasm maintained at Kannur. Out of these only nine clones which belongs to 'Forma' *sanguineum* are having red flesh with 2n=60 with the type form 28 NG 219. They are wild tall forms which were used by the aborigins of pacific island for fencing the homestead garden. They are characterized by very hard canes, with swollen nodes, raised growth ring, with less juice and they yield reddish purple juice during extraction. *S. robustum* are well adapted to adverse environmental conditions including drought and salinity (Vasantha et al. 2017). Though *S. robustum* is vulnerable to biotic stresses a few clones are reported to be resistant to red rot (Viswanathan et al. 2017) and many clones are free from foliar disease like leaf spot (un published). The red fleshed *S.robustum* genotypes are reported to have high amounts of antioxidant substances compared to *S.officinarum* and commercial hybrids (Rakkiyappan et al. 2012). 14 major antioxidant compounds from stem extracts of *S.robustum* clones have been identified and characterized using HPLC and TLC. Among these, five were flavanoids and flavanoids linked glucosides, three were anthocyanidin glucosides and six were unknown compounds. High amounts of catechins, quercetin, syringic acid and flavones were observed in nine red fleshed *S.robustum* (Rakkiyappan et al. 2012). As this pigmented *Saccharum* are the potential source of dietary antioxidants, the recent studies have been focused on utilization of these genotypes in crop improvement programme. In 2014 flowering season poly cross was effected on two red fleshed *S.robustum* (NG 77-84, and NG 77-76) and obtained 459 seedlings and were evaluated and reported wide variation for agro morphological traits (Chandran et al. 2019). The progenies were grouped into six categories based on flesh colour graded on 0-5 scale. The present study was done to find the variability for diploid chromosome number (2n) in a set of 27 selected progenies belonging to six grades of flesh colour (Table 1).

Pollen from 16 male parents including five *S.officinarum* (28 NG 51, 28 NG 220, 28 NG 287, 28 NG 288, 57 NG 24*),* six exotic hybrids (CP 63261, CP 62251, H44 3098, H 44-2818, LF 74-3152, LF 74-4010) and five Co canes (Co 210, Co 304, Co 775, Co 62020, Co 62024) were used for hybridization. The *S.officinarum* clones used as pollen parents were typical types with 2n = 80 except for 28 NG 287 which was atypical type with 120 chromosomes. All the other hybrid clones were having > 100 chromosomes.

For inducing roots single node cuttings of cane is treated with 0.75% Bavistin (organic mercurial fungicide) before planting in 3:1 Soil:FYM mixture. The potting mixture is supplemented with half kg potash and SSP 2kg/100kg. Single budded cane setts were planted and kept under ambient temperature (28-34°C) in sunlight with proper irrigation. Roots were attained proper growth within 5-7 days. The root tips were collected between 11.30 to 1PM. After removing, the dirt was cleaned thoroughly in running water and pre-treated with water saturated solution of α-Bromonaphthalene for two hours at refrigerated condition. After pre-treatment again washed in running water followed by rinsing in distilled water and fixed in 3:1 ethanol acetic acid mixture for 12 hrs. The roots are hydrolysed in 1N HCl at 60 °C for 12 minutes. After hydrolysis roots were washed thoroughly in distilled water for 2 or 3 times and transferred to 5% pectinase (in sodium citrate buffer) and incubated for 30 minutes at 37°C. The root tip was then washed with distilled water and transferred to Leuco basic fuchsin in dark for half an hour. The deeply stained portion was cut on a slide and squashed in 1% acetocarmine. The slides are observed under compound microscope

Table 1. The flesh colour, diploid chromosome number and cross details of the 27 progenies and two female parents.

S.no.	Progenies	Cross	Flesh colour (0-5 scale)	Chromosome number (2n)
1	GUK 14-559	NG 77-76 x Poly cross	0	70
2	GUK 14-578	"	0	70
3	GUK 14-798	"	0	70
4	GUK 14-117	NG 77 84 x Poly cross	0	72
5	GUK 14-100		0	70
6	GUK 14-698	"	0	70
7	GUK 14-24	"	1	70
8	GUK 14-673	"	1	70
9	GUK 14-664	"	1	80
10	GUK 14-16	"	1	70
11	GUK 14-14	"	1	70
12	GUK 14-19	"	1	72
13	GUK 14-605	NG 77-76 x Poly cross	2	70
14	GUK 14-833	"	2	70
15	GUK 14-33	NG 77 84 x Poly cross	2	74
16	GUK 14-47	"	2	72
17	GUK 14-41	"	2	70
18	GUK 14-37	"	2	72
19	GUK 14-824	NG 77-76 x Poly cross	3	70
20	GUK 14-7	NG 77 84 x Poly cross	3	86
21	GUK 14-722	"	3	70
22	GUK 14-129	"	3	72
23	GUK 14-13	"	3	70
24	GUK 14-30	"	3	70
25	GUK 14-745	"	4	70
26	GUK 14-111	"	4	70
27	GUK 14-48	"	5	60
28	NG 77-76	Female parent	5	60
29	NG 77-84	Female parent	5	60

(100x oil immersion) and photographed with canon EOS 1100D SLR Camera. The chromosome were counted directly from microscope and verified using AlphaView SA image analyzing software by manual counting option. The correlation was worked out using SPSS software package.

Twenty seven hybrids and 2 parental progenies were observed for diploid chromosome number. Based on the flesh colour the progenies were categorized into 6 groups and coded as 0-white flesh, 1-white flesh with red tinge, 2-light reddish white flesh, 3-reddish white flesh, 4- medium red flesh, 5- red flesh. The chromosome numbers of both female parents were confirmed as 2n = 60 (Fig. 1 and 2). The chromosome numbers of the progenies are given in table 1.

Six progenies were coded as '0' for flesh colour ie., with normal white flesh and the chromosome numbers of four of these hybrids are 2n = 70 (Fig.3-6) and two of them had 2n =72 (Fig.7 and 8).

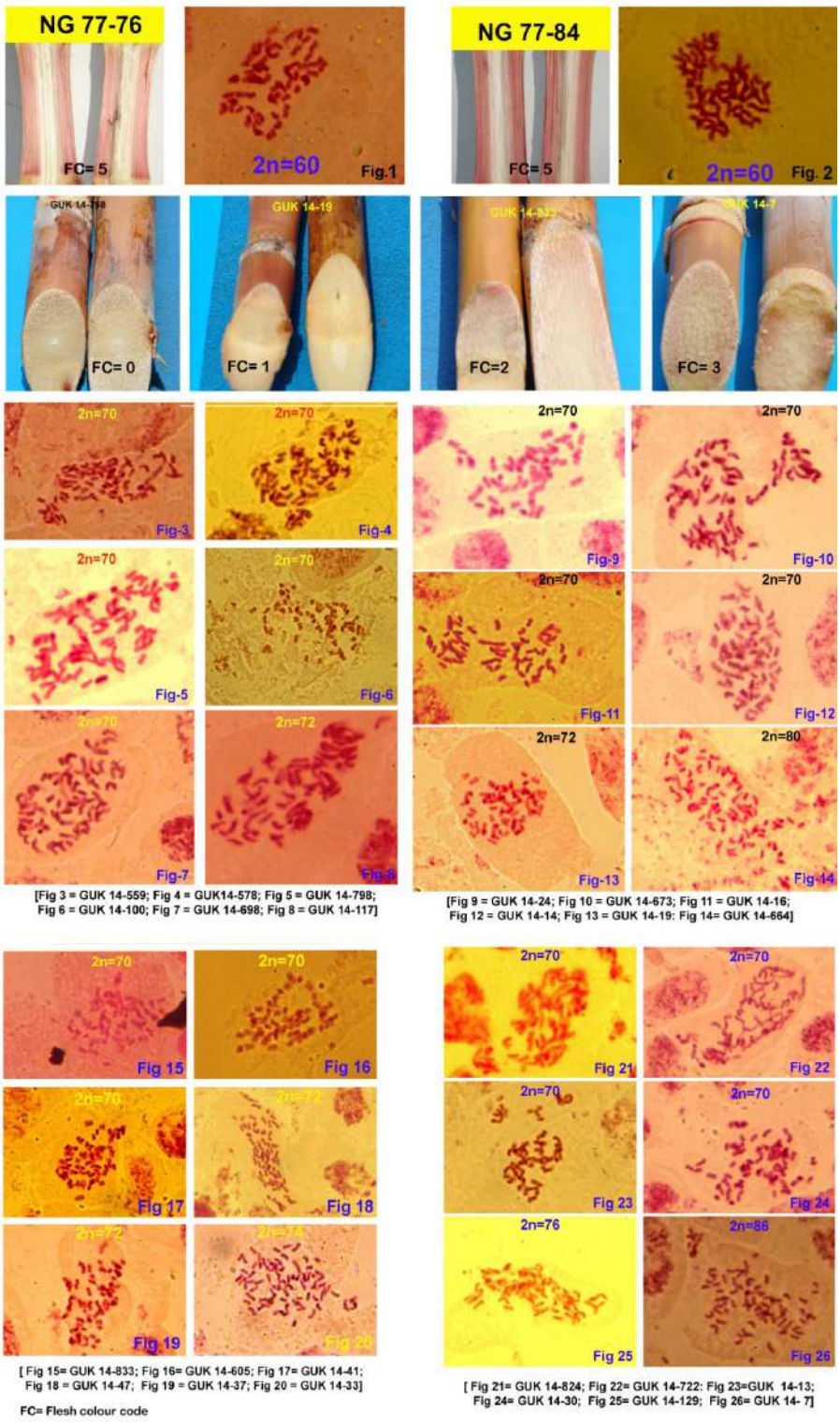

Figure 1-26. Diploid Chromosome number in female parents and progenies with flesh colour code 0-3

Among the six progenies that belong to the second category coded as "1" (white flesh with red tinge especially below the axilllary bud region,), three chromosome numbers were observed ie., 2n=70 in four of the progenies (Fig. 9-12); 2n=72 in GUK 14 -19 (Fig.13) and 80 in GUK 14 -664 (Fig. 14).

Another six genotypes belong to the third category coded as "2" (light reddish white flesh), the chromosome numbers were 2n =70 in three of the progenies (Fig.15 -17); 2n = 72 in two (Fig .18 and 19) and 2n = 74 in GUK 14 -33 (Fig. 20).

Next set of six genotypes were grouped into fourth category coded as "3" (medium reddish white flesh), their chromosome numbers were 2n =70 in four of the progenies (Fig 21-24); 2n = 72 in GUK 14- 129 (Fig. 25) and 2n =86 in GUK 14 -7 (Fig. 26).

There were only two progenies that belong to the fifth category, coded as "4" (medium red flesh) and had 2n =70 in both progenies, GUK 14 -745 and GUK 14 -111(Fig.27-28). In red flesh category coded as "5" only one progeny was available (GUK 14-48). In this progeny the flesh colour was similar to that of female parents and the chromosome number in this progeny was 2n=60 (Fig 29).

In total six cytological groups observed were 2n= 60, 70, 72, 74, 80 and 86. By comparing the progenies between the female parents, it was observed that all six of the progenies of NG 77-76 were 2n=70 cytotypes but in the progenies of NG 77-84, 57.1% were belongs to 2n=70 and 23.8% were having 2n=72. The remaining four cytotypes (60, 74, 80, 86) were available in one progeny each (0.83 %). From this it may be inferred that more cytotypes could be developed while using of NG 77 -84 as compared to NG 77-76.

All the *S.officinarum* clones used (28 NG 51, 28 NG 220, 28 NG 288, 57 NG 24) as pollen parents were typical types with 2n = 80 except for 28 NG 287 which was atypical type with 120 chromosomes. So the progenies with 2n= 70 introgression from typical *S.officinarum* may be the pollen source with normal n+n transmission and those progenies with higher than the 70 chromosomes might have resulted from the pollen source from the commercial hybrids, may be from the Hawaiian series of clones which had the *S.robustum* in their pedigree.

In natural population of *S.robustum,* Price (1957a, 1965) has conducted elaborate cytological studies where he recorded chromosome numbers of 2n=60, 63-70, 70, 80, 86, 92, 100-112, ca. 157, 164 and ca.

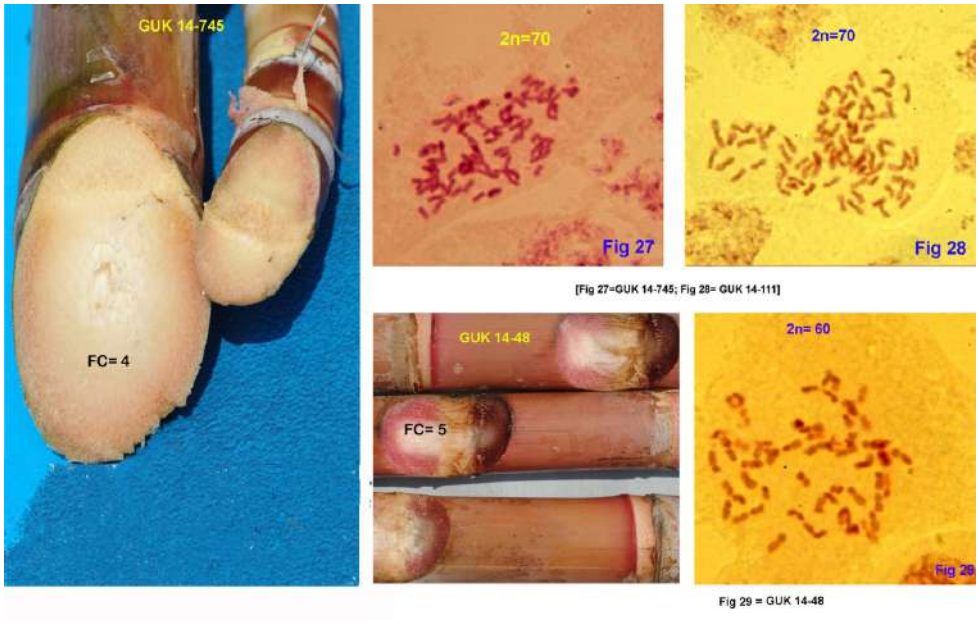

Figure 27-29. Diploid chromosome number in female parents and progenies with flesh code 4 and 5.

194 in *S. robustum* and natural hybrids of related species. He suggested that *S. robustum* has only two cytotypes 2n=60 and 2n=80. Genotypes with chromosome number other than 2n=60 and 80 need to be considered as natural hybrid between the species, *S. robustum* X *S. robustum*, *S. robustum* X *S.officinarum*, *S.robustum* X *S.spontaneum*, and intergeneric hybridization of *Saccharum* X *Miscanthus*. The diploid chromosome number observed in our studies also support this view that the chromosome numbers other than 60 and 80 are hybrids. Sreenivasan and Sreenivasan (1984) studied the meiosis of *Saccharum* species and reported that *S.robustum* clones NG 77-14, NG 77-76 and NG 77-170 with *2n* = 60 chromosomes showed a regular meiosis with 30 bivalents at diakinesis. This leads to our conclusion that one of the progenies GUK 14-48 in our experiment having the flesh colour exactly the same as that of female parent and 2n=60, might have resulted by normal diakinesis followed by unintentional selfing. When the flesh colour was correlated with the diploid chromosome number, a significant but negative correlation of 0.4*was observed. The reduction in the intensity of flesh colour in progenies may be as a result of the genetic introgression and some kind of epistatic control of the alien genes for this trait. In the first report of *S.officinarum x S. robustum* hybrid by Nishiyama (1956), where both parents were having 2n= 80, but the progeny showed 2n=93. The increase of 13 chromosomes was attributed to aneuploid transmission. The progenies with varying numbers of chromosomes in the present study corroborate with earlier studies. Price (1957b) reported that a crosses between S.*officinarum* (2n=80) × *S.robustum (2n=60)* produced hybrids with 2n=70 chromosomes confirming n+n transmission where red fleshed *S.robustum* was used as male parent. Our result also shows similar n+n transmission, despite being a reciprocal cross.

The red fleshed "*sanguineum* " group of S.*robustum* is having wider adaptation to abiotic stress factors and very robust in growth. But its utilization in the breeding programme was not popular due to its rare flowering characteristics. More over with respect to *S.robustum* the susceptibility to mosaic and smut also reported to be the discouraging factors for utilization. Only nine accessions represent the s*anguineum* group of *S.robustum* with red flesh colour in the world collection of sugarcane germplasm. These groups exhibit uniform number of chromosomes (2n=60) in the available collection and have very limited distribution in the natural population. Probably because of limited distribution and infrequent flowering, the genetic introgression in natural population might have been less, resulting in poor variability for economic traits. Hence, the studies on chromosome numbers in the progenies revealed that considerable variation could be induced for chromosome numbers and flesh colour. Some of these clones are regularly flowering types which also opens up further enhancement of these genotypes with the cytoplasmic background of the robust growing *sanguineum* group of *S.robustum*.

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