

RESEARCH ARTICLE

Yield and quality evaluation of First-generation progenies of red-flesh *Saccharum robustum* Brandes and Jeswiet ex grassl

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Abstract

Saccharum robustum, a wild species of sugarcane which shares a common natural habitat with the *Saccharum officinarum* in Pacific Islands. The cytotype of *S.robustum* with $2n=60$ and having red flesh are placed in 'forma' *sanguineum*. These genotypes possess unique deep red flesh canes. In spite of the close similarity and kinship to the noble canes, the utilization of the red-flesh *S. robustum* clones in commercial breeding programmes is relatively less owing to its rare flowering habit. They are well adapted to wide environmental conditions including waterlogging situations, and can be a potential source for waterlogging resistance. The red-fleshed *S. robustum* has a high quantity of anti-oxidants, flavonoids and anthocyanin content in the cane. Anti-oxidants are free radical scavenging compounds which prevent or slow cell damage. Bringing these traits to the sugarcane background will be beneficial by way of fortifying the nutrient-rich sugarcane juice. The first successful hybridization of red-fleshed *S. robustum* resulted in 459 progenies from two polycrosses. Eight selected progenies based on the initial performance were further evaluated across four crop seasons for flesh colour, yield and quality traits to identify potential genetic stocks. The clone GUK 14-48 had red flesh similar to the female parent (NG 77-84) and had significantly higher cane yield and juice extraction percentage than the female parent. Based on the flesh colour, cane yield and CCS yield, the clones GUK 14-48, GUK 14-754 and GUK 14-130 are identified as potential inter-specific hybrids for further utilization in the breeding programme.

Keywords: *Saccharum robustum*; Red-flesh; Polycross; Progenies; Yield evaluation

Introduction

Saccharum robustum, a wild species originated from New Guinea and the neighbouring Islands and the type specimen was collected from the banks of Laloki River near Port Moresby in the expedition led by Jeswiet (Grassl, 1946). Its natural habitat is in the tropics, but it is adaptable to a wide range of environmental conditions and also played a principal role in the origin of the cultivated species *S.officinarum*. They are characterized by high vigour, low juice content, low sucrose, high fibre, tillering, fairly deep root system and medium thick stalks. Bremer (1930) and Price (1957) studied the cytology of *S.robustum* and reported several cytotypes, mainly $2n=60$ and $2n=80$ types.

Forms deviating from these numbers are considered as aneuploids or probable inter-specific hybrids (Grassl, 1946). The cytotype $2n=60$ consists of three groups, *forma sanguineum* (red-fleshed) distributed on the bank of Sepik and Digul rivers, 'Teboe Salah' which had a distribution in Indonesia, Teboe Salah and Tanangge, and Wau/Bulolo type distributed in New Guinea (Grassl,1946). The world collection of sugarcane germplasm maintained at Kannur, Kerala, India has nine red-fleshed *S.robustum* (*forma sanguineum*) accessions. Red fleshed *S.robustum* genotypes possess high amounts of antioxidants, flavonoids and anthocyanin content compared to *S.officinarum* and commercial hybrids. Fourteen

major antioxidant compounds were identified from stem extracts of red-fleshed *S. robustum* clones and characterised using High Performance Liquid Chromatography and Thin Layer Chromatography. Among these, five were flavonoids and flavanoid-linked glucosides, three were anthocyanidin glucosides and six were unknown compounds. High amounts of catechins, quercetin, syringic acid and flavones were also reported in red-fleshed *S. robustum* (Rakiappan et al. 2012, Suresha et al. 2017). The red-fleshed *S. robustum* clones were restricted to a limited geographic area and the variation within this group of clones is very limited. Moreover, these clones are not regular flowering types and hence natural genetic introgression and the chance of subsequent genetic diversification were very low. Conventional breeding programmes to transfer this specific trait to other species of *Saccharum/hybrids* were limited to the report by Chandran et al. (2020) and these progenies with higher antioxidant content and higher juice quality will be a potential clones in crop improvement of sugarcane (Chandran et al. 2023). In the present study, eight promising progenies out of 459 progenies developed from two polycrosses of red-fleshed *S. robustum* genotypes (Chandran et al. 2020) were evaluated further for four crop seasons to identify genetic stocks of improved red-fleshed *S. robustum* for further utilization in crop improvement programme of sugarcane.

Materials and methods

Eight selected progenies viz., GUK 14-30, GUK 14-41, GUK 14-48, GUK 14-69, GUK 14-129, GUK 14-130, GUK 14-732 and GUK 14-754 from NG 77-84 polycross were evaluated along with female parent and commercial variety (Co 86032) for four crop seasons 2014-15, 2016-17, 2017-18, 2021-22 in replicated trials with plot size of 10ft

two rows. The progenies were multiplied by clonal propagation in sufficient quantities and designated as clones with respective progeny number. Data on flesh colour, number of millable canes (NMC), cane thickness, single cane weight, cane length and cane yield and quality traits at 11th month viz., HR Brix 7th month at bottom middle and top, extraction percentage, spindle Brix and sucrose were recorded every year. The NMC, CCS yield and yield were estimated per hectare. The mean of observed traits and estimated data was used for ANOVA. Four year data of cane yield, CCS yield and flesh colour (based on RHS colour chart) of nine genotypes were also subjected to genotype and genotype-environment interaction (GGE) biplot analysis (Yan and Kang, 2003) using 'R' and using 'metan' package (Olivoto and Lúcio, 2020).

Results and discussion

The progeny GUK 14-48 had red flesh colour (RHS 57A), followed by light red (RHS 57C) in GUK 14-130 and GUK 14-754 and other clones showed less intensity in colour. During the evaluation in 2015-16 crop season, high NMC was observed for GUK 14-30, GUK 14-48, GUK 14-69, and GUK 14-754 (45000/ha), compared to the *S. robustum* parent (30000/ha) and GUK 14-754 had significantly high NMC (60000/ha) than the commercial variety Co 86032 (52500/ha). The red-fleshed *S. robustum* clones are very shy tillering types including the female parent used in this study. Since yield is positively correlated with NMC in sugarcane (Parihar 2020), the improvement in NMC of these shy tillering genotypes is very important in the pre-breeding programme of sugarcane. GUK 14-130, GUK 14-754 and GUK 14-732 were superior for Brix and sucrose content in comparison with the female parent. The clones GUK 14-69, GUK 14-48 and GUK 14-30 recorded high cane yield and for CCS yield of GUK 14-69

and GUK 14-30 were higher compared to the female parent.

Flesh colour recorded during the 2017-18 crop season was the same as in previous year. In this trial, clones GUK 14-754, GUK 14-732 and GUK 14-41 had significantly higher NMC than female parent and Co 86032. *S. robustum* clones have generally low sucrose content (5 to 13.8%) and in many clones sufficient juice extraction was not possible due to low juice content (Chandran et al. 2023). The female parent had an average of 33.8% juice. All progeny clones had significantly higher extraction percentage than female parent. For cane yield, the clones GUK 14-41, GUK 14-732, GUK 14-130 and GUK 14-754 were significantly superior to the female parent. For Brix and sucrose % GUK 14-754, GUK 14-732, and GUK 14-130 were superior and GUK 14-69, GUK 14-30, GUK 14-130 and GUK 14-732 showed significantly higher values than the female parent.

In the trial conducted during 2018-19, tillering and cane yield were better than the previous crop seasons. The clones *viz.*, GUK 14-754 and GUK 14-130 had significantly higher NMC than Co 86032. All clones had significantly higher juice extraction % than the female parent. In female parent the average Brix was 8.7% with a sucrose content of 3.9%. Brix percent of the clones ranged from 7.0 % to 13.3% and sucrose from 1.7 % to 6.4 %. GUK 14-754, GUK 14-732, GUK 14-69 and GUK 14-130 had higher Brix content than the female parent in this crop season. Cane yield of GUK 14-754, GUK 14-69 and GUK 14-41 were higher than Co 86032.

In the 2021-22 evaluation trial also, flesh colour was consistent as in the previous years in all the clones. Significant improvement was observed for all the traits in comparison with the female parent. For NMC, the clones GUK 14-754, GUK 14-732,

GUK 14-130 and GUK 14-69 were superior to the female parent. For Brix content, the clones GUK 14-754, GUK 14-732, GUK 14-130 and GUK 14-30 were significantly higher than female parent. Sucrose content of the clones GUK 14-754, GUK 14-130 was higher than the female parent and was found promising. The female parent was poor in single cane weight though the cane thickness was medium (2.5cm). All the evaluated clones showed higher cane yield compared to the female parent NG 77-84. The clones GUK 14-754, GUK 14-732, GUK 14-69, and GUK 14-130 recorded higher cane yield than the commercial variety Co 86032. CCS yield was correlated with Brix %, sucrose % and single cane weight. Hence, most of the progenies had higher CCS yield than the female parent. During 2021-22, GUK 14-754, GUK 14-732, GUK 14-30 and GUK 14-130 were promising for CCS yield.

Combined analysis of the clones over four years indicated significant differences between the clones for all the traits except for cane length (Table 1). Flesh colour was very consistent across the years and only one clone GUK 14-48 had red flesh colour similar to the female parent (Fig 1). The yearwise



Figure 1 LS of Cane (GUK 14-48)

Table 1. ANOVA table for the combined analysis of data over four crop seasons.

Particulars	df	Mean Square	F	Sig.
NMC/ha	9	547606336.806	15.441**	0.000
CTK(cm)	9	0.139	3.576**	0.005
HRB(%)	9	35.241	14.357**	0.000
CL(cm)	9	1669.950	1.568	0.175
EXT(%)	9	93.022	11.923**	0.000
SCWT(kg)	9	0.187	4.184**	0.002
BRIX(%)	9	36.119	31.410**	0.000
POL(%)	9	67.621	54.427**	0.000
CCS (%)	9	44.660	47.155**	0.000
YLD/ha (kg)	9	485923121.854	5.895**	0.000
CCS/ha(kg)	9	21173075.325	23.370**	0.000

**significant at 1% level

(NMC =Number of millable canes, CTK= Cane thickness, HRB= Hand refractometer brix, CL=Cane length, EXT= Juice extraction percent, SCWT=Single cane weight, BRIX= Spindle brix at 11th month, Pol =Pol percent, CCS= Commercial cane sugar, YLD= Cane Yield/ha, CCS/ha= Commercial cane sugar yield/hectare).

analysis of yield and quality data showed that NMC was the most variable yield trait across the years and this reflected in the cane yield/ha. The quality traits viz., Brix %, and sucrose % did not vary significantly across the years. Across the years GUK 14-48 had significantly higher cane yield (61.5t/ha), and it had higher juice extraction percentage (38.9%) than the female parent (Table 2). The clones GUK 130, GUK 14-30 and GUK 14-754 had light red flesh, GUK 14-129, GUK 14-732, GUK 14-41 had light reddish-white flesh and GUK 14-69 had only red tinge on flesh. For number of millable canes the clones GUK 14-754 and GUK 14-130 were significantly higher than the commercial variety Co 86032. In all the test clones, yield was significantly higher than the female parent. The clones GUK 14-41, GUK 14-130 and GUK 14-754 had cane yield was 62.48 t/ha, 69.6

t/ha and 58.6 t/ha respectively against 35.7 t/ha in the female parent. The clone GUK 14-754 recorded cane yield which was on par with the commercial variety Co 86032. For CCS yield, all the test clones were superior to the female parent. The clones GUK 14-130, GUK 14-41, GUK 14-732 and GUK 14-754 recorded CCS yield of 3.93 t/ha 2.6 t/ha, 2.9 t/ha and 4.1 t/ha respectively against 0.484 t/ha in the female parent. These clones recorded five to nine fold improvement over the female parent. All the test clones had significantly higher extraction percentage. Except for the clone GUK 14-48, all the other clones had significantly higher Brix % (11.91% to 13.6%), sucrose % (6.71% to 9.95%) and CCS % (3.38 to 6.15 %) than the female parent with 8.71% Brix, 3.87 %, sucrose and 1.41 CCS %.

Table 2: Average yield and quality data of four crop season

GENOTYPE	FC	NMC/ha	CTK (cm)	HRB (%)	EXT (%)	SCWT (kg)	BRIX (%)	POL (%)	CCS (%)	YLD t/ha	CCS t/ha
1 GUK 14-129	2	36250.0	2.3	12.7	39.5	1.24	12.0	7.5	4.2	43.95	1.77
2 GUK 14-130	3	58281.3	2.4	12.9	39.9	1.2	13.0	9.2	5.7	69.69	3.94
3 GUK 14-732	2	47578.1	2.3	13.1	39.6	1.1	13.4	8.8	5.1	52.67	2.93
4 GUK 14-754	3	63125.0	2.1	14.2	41.5	0.92	13.8	10.0	6.2	58.65	4.18
5 GUK 14-69	1	43750.0	2.6	12.0	44.4	1.41	11.9	6.7	3.4	61.42	2.60
6 GUK 14-30	3	27968.8	2.7	12.0	45.3	1.55	12.6	8.2	4.3	43.54	2.06
7 GUK 14-41	2	48125.0	2.3	13.0	48.3	1.29	12.3	7.6	4.2	62.49	2.64
8 GUK 14-48	5	37187.5	2.5	8.3	39.0	1.68	8.8	4.1	1.6	61.57	1.01
9 NG77-84	5	28671.9	2.4	9.2	33.8	1.23	8.7	3.9	1.4	35.74	0.48
10 Co 86032	0	49843.8	2.6	19.3	49.8	1.33	19.6	18.7	13.4	64.79	8.69

The genotype and genotype-environment interaction analysis was satisfactory as the first two principal components (PC1 and PC2) of the GGE explained 87.79%, 92.99% and 99.06% variation for cane and CCS yield and flesh colour. Mean performance, stability and genotype rankings analysed by GGE biplot for the selected variables are depicted in Figures 2-4. Accordingly, the clone GUK 14-130 was ranked as a high cane yielder followed by the clones GUK 14-48 and GUK 14-41 but their yields varied between the environmental years. Whereas, GUK 14-30 and GUK 14-129 were poor in cane yield. The clone

GUK 14-754 had a comparatively high yield and was stable across environments. Similarly, the clones GUK 14-130 and GUK 14-754 were found to be the best performers for CCS yield (kg/ha). The clone GUK 14-732 had comparatively high CCS yield and was also found to be stable across years. The clone GUK 14-48 was found to be comparable with the parental genotype NG 77-84 and found to be highly stable over four years and inherited red flesh colour.

Though wild species harbour desirable traits like resistance to biotic and abiotic stresses their utilization in crop improvement programme was

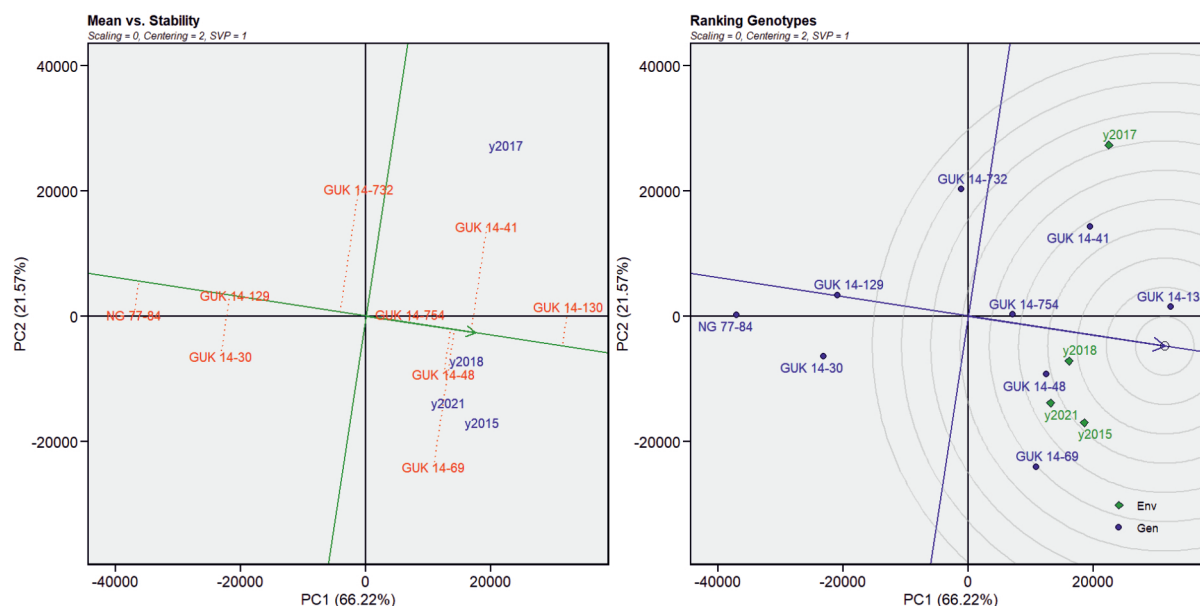


Figure 2. GGE biplot based on a set of yield per hectare of nine genotypes over four years. Mean vs. stability biplot (left), Ranking genotypes biplot (right)

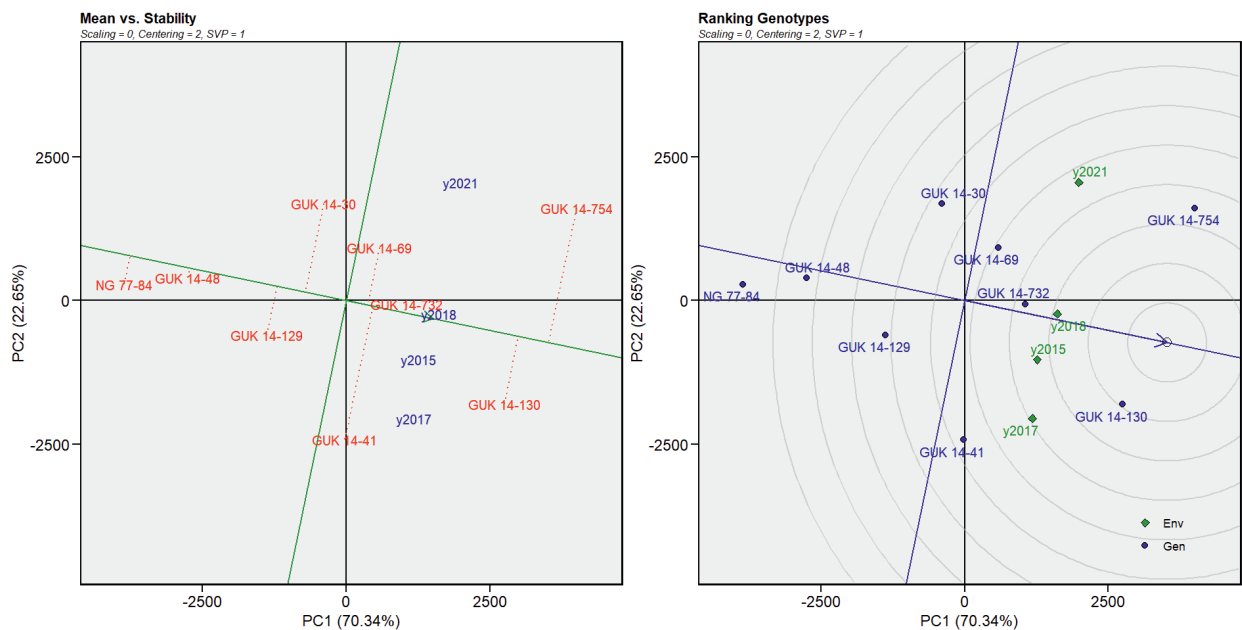


Figure 3. GGE biplot based on a set of CCS yield per hectare of nine genotypes over four years. Mean vs. stability biplot (left), Ranking genotypes biplot (right)

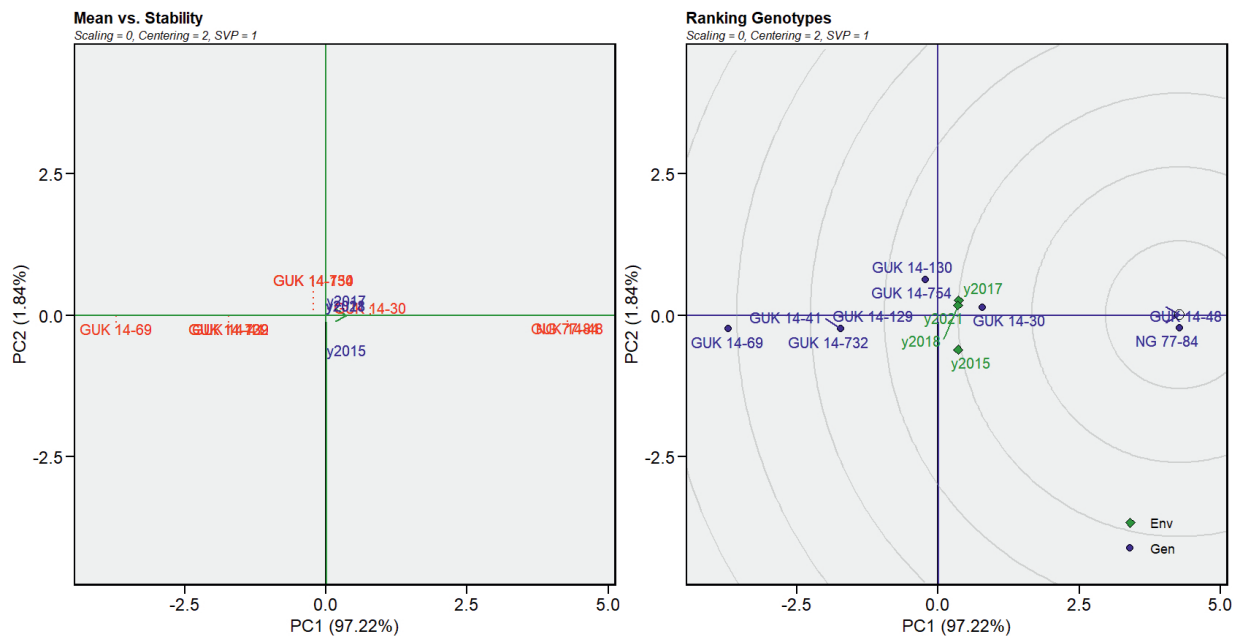


Figure 4. GGE biplot based on a set of red flesh rating of nine genotypes over four years. Mean vs. stability biplot (left), Ranking genotypes biplot (right)

hindered by the strong linkage with undesirable traits which are very difficult to break (Hawks 1977). In sugarcane many varieties for water logging tolerance have been developed in Hawaii

utilizing *S. robustum* clones. Similarly in India also many Co canes (Co 07020, Co 07032, Co 12012) were developed with the background of *S. robustum* clones and it indicate the potential of this

species in crop improvement programme of sugarcane. However, the utilization of red fleshed types of *S. robustum* was limited because of rare flowering habit of these genotypes (Chandran et al 2020). The flesh characteristic was unique to the germplasm under the forma *sanguineum* and this trait could be successfully introgressed in their progenies for the first time (Chandran et al 2020). Even after exhaustive exploration in the centre of diversity, only a few germplasm belonging to red-flesh types could be collected and maintained in the gene bank, indicating the low variability in the natural condition at the centre of origin and diversity (Grassl 1974, Daniel and Roach 1987). It may be because the natural population was restricted to the Laloki River basin in New Guinea and natural introgression might have been constrained by its rare flowering habit. The cytological uniqueness of these clones was established by karyotype analysis (Jagadeesan and Ratnambal 1969). *S. robustum* clones are in general widely adapted to various environmental stresses and more particularly to waterlogging stress. The progenies evaluated and identified with red flesh and light red flesh will be very much useful for developing varieties with red flesh and high juice quality. The juice based value added products can be manufactured with juice having health beneficiary compounds like antioxidants from these clones. The jaggery found in the market often adulterated with synthetic colour. Varieties developed with red flesh can be useful for manufacturing jaggery with red colour without addition of artificial colour. The sugar content of these developed progenies are higher than the red fleshed *S. robustum* clones, many health products can be directly prepared from the promising progenies for commercialization. Considering all these the variation created is very important in direct commercialization and for genetic enhancement

and the clones with better sugar content, extraction percentage and biomass yield. The clone GUK 14-48 is a potential genetic stock with a unique red flesh colour with better yield and quality characters than the female parent, NG 77-84. GUK 14-754 and GUK 14-130 were with light red flesh and had high NMC and cane yield across the crop seasons and has commercialization potential.

Conclusion

The red-fleshed genotypes possess high quantity of anti-oxidants that are known free radical scavenging compounds which prevent or slow down the cell damage. These clones also possess wider environmental adaptations and more particularly promising under waterlogged conditions. Hence, these three red-fleshed progenies (GUK 14-48, GUK 14-754 and GUK 14-130) have a high potential for direct commercialization and for further genetic improvement of sugarcane.

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