



Breeding methodology for improvement of grain and fodder yield and quality traits in dual purpose sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract

A total of 100 genotypes including 75 F_1 's with 20 parents and 5 checks were planted in simple lattice design with two replications during *Kharif* 2015 and *Kharif* 2016 in two intra row spacing viz., 12.5 cm and 20 cm to analyze the buffering ability of genotypes. Data were collected for seven dual purpose attributing traits i.e. grain yield, dry fodder yield, protein content in grain, protein content in fodder, TSS, juiciness and stay greenness. On the basis of *per se* performance, economic heterosis and SCA effects for both grain yield and dry fodder yield, two crosses viz., ICSA 202 x SU 1570 and ICSA 474 x SU 1561 were identified as promising for normal spacing environments (E_1 and E_3) and ICSA 202 x SU 1561 for wider spacing environments (E_2 and E_4). The days to 50 per cent flowering of ICSA 202 and SU 1570 were almost equal. Therefore, heterosis breeding is recommended for ICSA 202 x SU 1570, whereas to exploit ICSA 474 x SU 1561 through heterosis breeding staggered sowing is essential. The dual-purpose cross, ICSA 202 x SU 1561 in wider spacing was having significant SCA and involved at least one good general combiner parent and equal days to 50 per cent flowering in the parents. Therefore, this could also be exploited through heterosis breeding.

Keywords: Combining ability, Dry fodder yield, GCA, Heterosis, Protein content, SCA

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal crop in the world belonging to the grass family *Poaceae* (Poehlman and Sleper, 1995). It is an important crop providing food and fodder under moisture stress conditions. The performance of dairy animals depends on the continuous availability of quality forage in an adequate amount. Therefore, the critical limitation on profitable animal production in developing countries is the insufficient availability of quality forage (Sarwar *et al.*, 2002). Sorghum is becoming an increasingly important dual-purpose crop in many regions of the world (Zerbini

and Thomas, 2003). Its high tolerance to drought makes it a suitable crop for semi-arid areas, especially in light of its higher productivity under dry conditions compared to corn. Fodder yield in quantity alone cannot measure the feeding value of the crops. So the quality value of forages like the nutritional value of fodder must be determined for measuring the feeding value. Improving the nutritive value of sorghum for productive ruminants is an important goal. The palatability and quality of forage can be improved by increasing the sugar content of sorghum stalk. Sorghum green fodder is one of the cheapest sources of feed for milch, meat and draft animals. Among the cereals, sorghum plays an important role in India, as a major grain cum fodder crop. It is extensively grown as a fodder crop in north India and as a dual-purpose crop in south India. Sorghum is widely used as fodder crop on account of its quick growth, tillering ability, high dry matter content, leafiness, high palatability, hardiness and suitability for silage making. In comparison to pearl millet, it is having lower oxalate and fibre content (Prakash *et al.*, 2010).

Maintenance of plant population per unit area is very difficult. If genotypes possess buffering ability to cope with the available space, the productivity can be maintained. Therefore, breeding for buffering capacity is another important aspect in the genetic improvement of crop plants. Therefore, development of such hybrids/variety, which gives consistent and desirable performance over a wide range of spacing, is needed. For this, it is desirable to see the impact of different spacings on the sorghum genotypes to identify the stable genotype. Sorghum is an often-cross pollinated crop, and both varieties and hybrids can easily be developed. Combining ability is an efficient way for the selection of parents for hybridization and following breeding methodology for each cross— a key for a successful breeding programme. The maximum potential of any genotype is preserved in F_1 . Therefore, to judge the potentiality of any genotype, evaluation of F_1 is essential.

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Further, the breeding methodology is based on the genetic cause of superiority *i.e.* combining the ability of parents and F_1 's. Accordingly, the potential crosses were identified based on economic heterosis, and breeding methodology was suggested based on combining ability.

Materials and Methods

Plant material and experimentation: On the basis of days to flowering and suitability for dual purpose 15 lines *viz.*, ICSA 202, ICSA 208, ICSA 349, ICSA 356, ICSA 357, ICSA 363, ICSA 380, ICSA 399, ICSA 474, ICSA 481, ICSA 552, ICSA 29001, ICSA 29002, ICSA 29003 and ICSA 29004 were obtained from ICRISAT. Five restorer testers *viz.*, SU 1557, SU 1561, SU 1565, SU 1570 and SU 1571 were identified from station trials on the basis of days to flowering. To identify superior genotypes checks *viz.*, CSH 16 (grain type hybrid), CSV 23 and CSV 27 (dual purpose varieties) and CSV 21 F and PC 1080 (fodder type varieties) were included. These 15 MS lines were crossed with 5 restorers in line x tester fashion during *Kharif* 2014 at RCA Udaipur and during *Rabi*, 2014 -15 at off season sorghum breeding nursery of IIMR, Hyderabad situated in Warangal to obtain 75 hybrids. The 75 crosses along with the parents *i.e.* 15 lines and 5 testers and 5 checks were planted in a simple lattice design with two replications during *Kharif* 2015 and *Kharif* 2016 in two intra row spacing *viz.*, 12.5 cm and 20 cm. The recommended intra row spacing for sorghum in the zone is 12.5 cm. The genotypes were also tested in wider spacing to identify the genotypes having high buffering ability to cope up with the environment. The environments were denominated as E_1 (45 x 12.5 cm in *Kharif* 2015), E_2 (45 x 20 cm in *Kharif* 2015), E_3 (45 x 12.5 cm in *Kharif* 2016) and E_4 (45 x 20 cm in *Kharif* 2016). The inter row spacing was 45 cm. Each genotype was sown in single row plot of 2 m length. Data were collected for dual purpose attributing traits *viz.*, grain yield, dry fodder yield, protein content in grain, protein content in fodder, TSS, juiciness and stay greenness. The assessment of sorghum for various quality traits was taken up as per Singh *et al.* (2018).

Data recording: Grain yield and dry fodder yield were measured on plot basis in kilogram per plot and converted to quintal per hectare. Protein content in grain and fodder was estimated by using Micro Kjeldahl's method given by Lindner (1944). Juiciness was measured at days to 50 per cent flowering on five randomly tagged plants on the basis of juiciness of leaf midrib color on 1-5 scale, where 1= non-juicy (pithy) and 5= juicy and average was worked out. TSS was measured

on border plants with hand refractometer at days to 50 per cent flowering. Stay green was scored on 0 and 1 scale for each plot. Where, 0 indicate senescence and 1 indicate non-senescence on the visual basis for each plot at maturity. The analysis of variance for simple lattice design was performed according to Petersen (1994). Heterosis, heterobeltiosis and economic heterosis were calculated according to the method suggested by Shull (1914), Fonseca and Patterson (1968) and Meredith and Bridge (1972), respectively. Griffing's method of diallel (1956) analysis for individual environment and Singh's method (1979) for over the environments were extended for Line x Tester mating design to calculate the GCA and SCA effects.

Results and Discussion

Analysis of variance for lattice design revealed that the relative efficiency of lattice over randomized block design was upto 108.70 per cent only. Therefore, blocking was not much effective, and further analysis was performed as per the RBD. Analysis of variance for RBD revealed that mean square due to genotypes and its components *viz.*, parents, crosses and checks were significant for all the characters. This indicates sufficient variation in parents, in crosses and checks. Mean squares due to genotype x environment interactions were significant for all the characters. This showed that different genotypes were influenced by the environments differentially. The Bartlett test revealed that error variance was homogeneous for protein content in grain, juiciness and TSS. Therefore, the pooled analysis was carried out for these characters only and the rest of the characters were discussed on individual environment basis and further breeding methodology was suggested for only individual environments for these characters.

Frequency and magnitude of heterosis, heterobeltiosis and economic heterosis:

The heterosis and heterobeltiosis for grain yield were in a positive direction for the majority of crosses. Out of the crosses, 7, 2, 8 and 5 exhibited positive significant economic heterosis, the highest magnitude being 28.92% in E_1 , 18.42% in E_2 , 33.05% in E_3 and 33.67% in E_4 respectively. Similarly for dry fodder yield, the majority of the crosses expressed positive direction of heterosis and heterobeltiosis. Out of them, 7, 14, 4 and 12 crosses exhibited positive significant economic heterosis, the highest magnitude being 25.46% in E_1 , 125.67% in E_2 , 25.48% in E_3 and 152.82% in E_4 . Likewise, for protein content in grain, the majority of the crosses expressed heterosis and heterobeltiosis in a positive direction. Out of them, 14

crosses expressed positive significant economic heterosis, and the highest magnitude was being 13.01 per cent over the environments. For protein content in fodder, most of the crosses exhibited heterosis and heterobeltiosis in a positive direction. Out of them, 4, 13, 17 and 15 crosses showed positive significant economic heterosis, the highest magnitude being 12.27%, 13.39%, 17.46% and 14.24% in E_1 , E_2 , E_3 and E_4 respectively. Concerning to juiciness, majority of the crosses were having heterosis in a negative direction, and none of the crosses exhibited significant economic heterosis in any environment for juiciness and TSS (Table 1). Similar findings of heterosis, heterobeltiosis and economic heterosis for quality traits in sorghum was earlier reported by Jain and Patel (2013) and Rini *et al.* (2016).

Breeding methodology: Finally the appropriate breeding methodology was suggested only for consistent economic heterotic crosses based on their SCA and GCA effects. In sorghum both varieties and hybrids are popular. For both types of cultivars, consistent superiority of F_1 is essential. Then such crosses are to be classified based on SCA and GCA. The consistent heterotic crosses with non-significant SCA effects and good general combining parents, provide an opportunity for obtaining transgressive segregants. Whereas, in heterotic crosses with significant SCA effects and poor general combining parents heterosis breeding is the only option left. The heterotic crosses with significant SCA effects and both

good general combiner parents leave the opportunity of exploiting the cross through both breeding strategies *i.e.* heterosis and pure line breeding. Probability of good transgressive segregants will get reduced with the reducing numbers of good general combiner parents in the crosses *i.e.* $G \times G > G \times A > G \times P > A \times A > A \times P > P \times P$.

Grain yield: With respect to grain yield, four crosses *viz.*, ICSA 356 x SU 1570, ICSA 357 x SU 1571, ICSA 349 x SU 1561 and ICSA 29002 x SU 1561 were having consistent positive significant economic heterosis in normal spacing (E_1 and E_3). These crosses were also having higher *per se* performance and good SCA effects. These crosses involved at least one good general parent except ICSA 356 x SU 1570 and ICSA 29002 x SU 1561 in E_3 where GCA of parents was average and poor. These crosses also exhibited significant economic heterosis and good SCA effects for other traits in same environments *viz.*, ICSA 356 x SU 1570 for protein content in grain (Table 2).

Dry fodder yield: For dry fodder yield, two crosses ICSA 202 x SU 1571 and ICSA 481 x SU 1571 in normal spacing (E_1 and E_3) and six crosses *viz.*, ICSA 349 x SU 1570, ICSA 481 x SU 1561, ICSA 349 x SU 1565, ICSA 481 x SU 1565, ICSA 357 x SU 1565 and ICSA 29001 x SU 1570 in wider spacing (E_2 and E_4) were expressing consistent positive significant economic heterosis. Among them, ICSA 202 x SU 1571, ICSA 481 x SU 1571, ICSA 349 x SU

Table 1. Frequency and magnitude of heterosis, heterobeltiosis and economic heterosis

Character		Heterosis				Heterobeltiosis		Economic heterosis	
		Frequency		Magnitude (%)		Frequency	Maximum value (%)	Frequency	Maximum value (%)
		-	+	-	+				
Pool basis									
Protein content in grain		19	49	53.03	95.89	39	84.36	14	13.01
Juiciness		44	6	69.81	42.22	1	18.52	-	-
TSS		10	40	25.65	76.36	18	55.20	-	-
Individual environment basis									
Grain yield	E ₁	52	52	38.65	205.62	37	178.00	7	28.92
	E ₂	14	52	49.75	184.81	39	181.25	2	18.42
	E ₃	12	47	61.79	175.19	37	131.52	8	33.05
	E ₄	7	49	61.49	192.65	37	175.61	5	33.67
Dry fodder yield	E ₁	9	59	31.89	214.86	45	213.05	7	25.46
	E ₂	8	53	39.17	253.58	38	227.53	14	125.67
	E ₃	9	53	47.96	290.32	45	283.71	4	25.48
	E ₄	7	47	53.86	386.72	35	364.56	12	152.82
Protein content in fodder	E ₁	16	50	67.44	158.99	39	135.91	4	12.27
	E ₂	17	52	61.19	157.54	41	148.80	13	13.39
	E ₃	17	52	62.95	140.30	43	126.49	17	17.46
	E ₄	17	57	57.25	156.09	46	136.01	15	14.24

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Table 2. Economic heterotic crosses for grain yield and their status with economic heterosis, GCA and SCA effects for other traits

Hybrids	Env.	Grain yield		PCG		Breeding methodology
		<i>Per se</i> (q ha ⁻¹)	GCA	SCA	GCA SCA	
ICSA 356 x SU 1570	E ₁	78.00	PG	G		Heterosis breeding>pure line breeding
	E ₃	75.83	PA	G		Heterosis breeding
	PI				PG G	
ICSA 357 x SU 1571	E ₁	82.66	GG	G		Pure line and heterosis breeding
	E ₃	84.30	GG	G		Pure line and heterosis breeding
ICSA 349 x SU 1561	E ₁	80.00	GG	G		Pure line and heterosis breeding
	E ₃	76.56	GA	G		Heterosis breeding>pure line breeding
ICSA 29002 x SU 1561	E ₁	78.36	PG	G		Heterosis breeding>pure line breeding
	E ₃	73.50	PA	G		Heterosis breeding

G, A and P: Good, average and poor general and specific combining ability effects respectively and PI: Pool; PCG: Protein content in grain; PCF: Protein content in fodder

Table 3. Economic heterotic crosses for dry fodder yield and their status with economic heterosis, GCA and SCA effects for other traits

Hybrids	Env.	Dry fodder yield		PCG		PCF		Breeding methodology
		<i>Per se</i> (q ha ⁻¹)	GCA	SCA	GCA SCA	GCA SCA		
ICSA 202 x SU 1571	E ₁	327.50	GG	G				Pure line and heterosis breeding
	E ₃	300.00	GG	G				Pure line and heterosis breeding
ICSA 481 x SU 1571	E ₁	331.83	GG	G				Pure line and heterosis breeding
	E ₃	302.50	GG	G				Pure line and heterosis breeding
	PI				GG G			
ICSA 349 x SU 1570	E ₂	241.50	GG	G				Pure line and heterosis breeding
	E ₄	194.50	GA	G				Heterosis breeding>pure line breeding
ICSA 481 x SU 1561	E ₂	183.84	GG	G				Pure line and heterosis breeding
	E ₄	205.22	GA	G				Heterosis breeding>pure line breeding
ICSA 349 x SU 1565	E ₂	200.50	GG	G				Pure line and heterosis breeding
	E ₄	179.50	GG	A				Pure line and heterosis breeding
ICSA 481 x SU 1565	E ₂	165.94	GG	P		AP G		Pure line and heterosis breeding
	E ₄	168.84	GG	A		AP G		Pure line and heterosis breeding
	PI				GA G			
ICSA 357 x SU 1565	E ₂	338.50	GG	G		GP G		Pure line and heterosis breeding
	E ₄	367.00	GG	G		GP G		Pure line and heterosis breeding
ICSA 29001 x SU 1570	E ₂	226.00	GG	G				Pure line and heterosis breeding
	E ₄	261.00	GA	G				Heterosis breeding>pure line breeding
	PI				GG G			

G, A and P: Good, average and poor general and specific combining ability effects respectively and PI: Pool; PCG: Protein content in grain; PCF: Protein content in fodder

1570, ICSA 481 x SU 1561, ICSA 357 x SU 1565 and ICSA 29001 x SU 1570 were having higher *per se* performance and good SCA effects. Crosses ICSA 349 x SU 1565 in E₄ and ICSA 481 x SU 1565 were having non-significant SCA effects along with higher *per se* performance. These crosses involved at least one good general parent. These

crosses also exhibited significant economic heterosis and good SCA effects for other traits in same environments viz., ICSA 481 x SU 1571 and ICSA 29001 x SU 1570 for protein content in grain, ICSA 357 x SU 1565 for protein content in fodder and ICSA 481 x SU 1565 for both protein content in grain and fodder (Table 3).

Table 4. Economic heterotic crosses for protein content in grain

Crosses	Per se (%)	GCA	SCA	Breeding methodology
ICSA29004 x SU1561	15.76	GG	G	Pure line and heterosis breeding
ICSA208 x SU1561	15.40	GG	G	Pure line and heterosis breeding
ICSA481 x SU1571	15.16	GG	G	Pure line and heterosis breeding
ICSA29002 x SU1570	15.06	AG	G	Heterosis breeding>pure line breeding
ICSA363 x SU1557	15.03	AP	G	Heterosis breeding
ICSA481 x SU1570	14.91	GG	G	Pure line and heterosis breeding
ICSA356 x SU1565	14.90	PA	G	Heterosis breeding
ICSA474 x SU1557	14.76	PP	G	Heterosis breeding
ICSA481 x SU1565	14.71	GA	G	Heterosis breeding>pure line breeding
ICSA29002 x SU1571	14.59	AG	G	Heterosis breeding>pure line breeding
ICSA29001 x SU1570	14.56	GG	G	Pure line and heterosis breeding
ICSA202 x SU1565	14.54	GA	G	Heterosis breeding>pure line breeding
ICSA356 x SU1570	14.44	PG	G	Heterosis breeding>pure line breeding
ICSA357 x SU1570	14.43	GG	G	Pure line and heterosis breeding

G, A and P = Good, average and poor general and specific combining ability effects respectively

Table 5. Economic heterotic crosses for protein content in fodder

Crosses	Per se (%)				GCA				SCA				Breeding methodology			
	E ₁	E ₂	E ₃	E ₄	E ₁	E ₂	E ₃	E ₄	E ₁	E ₂	E ₃	E ₄				
For all four environments-																
ICSA363 x SU1557	9.54	9.61	9.93	9.81	PP	PP	PP	PP	G	G	G	G	H	H	H	H
ICSA208 x SU1561	9.53	9.37	9.79	9.12	GG	GG	GG	GA	G	G	G	G	H&P	H&P	H&P	H>P
ICSA202 x SU1565	9.93	9.52	9.53	9.75	GP	GP	AP	GP	G	G	G	G	H>P	H>P	H	H>P
ICSA29003 x SU1571	9.53	9.18	9.75	9.38	GG	GG	GG	GG	G	G	G	G	H&P	H&P	H&P	H&P
For wider spacing environments-																
ICSA349 x SU1557		9.27		9.40			AP		AP		G	G		H		H
ICSA474 x SU1557		9.04		9.49			PP		PP		G	G		H		H
ICSA202 x SU1561		9.52		9.82			GG		GA		G	G	H&P		H>P	
ICSA357 x SU1565		9.11		9.74			GP		GP		G	G	H>P		H>P	
ICSA481 x SU1565		9.38		9.39			AP		AP		G	G	H		H	
ICSA357 x SU1570		9.21		9.71			GG		GG		G	G	H&P		H&P	
ICSA399 x SU1570		9.48		9.56			AG		AG		G	G	H>P		H>P	
ICSA29002 x SU1570		9.12		9.60			PG		PG		G	G	H>P		H>P	
ICSA481 x SU1571		9.13		9.40			AG		AG		G	G	H>P		H>P	

G, A and P: Good, average and poor general and specific combining ability effects respectively. H: Heterosis and P: Pure line

Protein content in grain: For protein content in grain, crosses ICSA29004 x SU1561, ICSA208 x SU1561, ICSA481 x SU1571, ICSA29002 x SU1570, ICSA363 x SU1557, ICSA481 x SU1570, ICSA356 x SU1565, ICSA474 x SU1557, ICSA481 x SU1565, ICSA29002 x SU1571, ICSA29001 x SU1570, ICSA202 x SU1565, ICSA356 x SU1570 and ICSA357 x SU1570 were found promising having positive significant SCA effects. Crosses ICSA29004 x SU1561, ICSA208 x SU1561, ICSA481 x SU1571, ICSA481 x SU1570, ICSA29001 x SU1570 and ICSA357 x SU1570 were having both parents with good GCA effects. Similarly, the crosses ICSA29002 x SU1570, ICSA481 x SU1565, ICSA29002 x SU1571, ICSA202 x SU1565 and ICSA356 x SU1570 involved at-least one good

general parent. In crosses viz., ICSA363 x SU1557 and ICSA356 x SU1565, GCA of the parents was average and poor and in ICSA474 x SU1557, GCA of the parents was poor and poor (Table 4).

Protein content in fodder: Likewise for protein content in fodder, ICSA363 x SU1557, ICSA208 x SU1561, ICSA202 x SU1565 and ICSA29003 x SU1571 in all the four environments and ICSA349 x SU1557, ICSA474 x SU1557, ICSA202 x SU1561, ICSA357 x SU1565, ICSA481 x SU1565, ICSA357 x SU1570, ICSA399 x SU1570, ICSA29002 x SU1570 and ICSA481 x SU1571 in wider spacing of both the years (E₂ and E₄) exhibited consistent significant economic heterosis. All of them

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Table 6. Promising dual-purpose crosses and their status with economic heterosis, GCA and SCA effects for other traits

Spacing	Hybrids	Env.	GY	DFY	GY	DFY	GY	DFY	Protein content in fodder		Days to 50 % flowering	
			<i>Per se</i> (q ha ⁻¹)		GCA		SCA		GCA	SCA	L	T
Normal	ICSA 202x	E ₁	83.67	311.29	GXG	GXP	G	G			78.50	83.00
	SU 1570	E ₃	74.45	289.67	GXA	GXP	G	G			77.50	81.50
	ICSA 474x	E ₁	86.38	317.51	AXG	GXG	G	G			70.50	79.50
	SU 1561	E ₃	74.84	330.00	AXA	GXG	G	G			69.00	77.00
Wider	ICSA 202x	E ₂	69.50	272.60	GXG	GXP	G	G	GG	G	78.00	78.50
	SU 1561	E ₄	60.50	256.67	GXG	GXA	G	G	GA	G	78.00	76.50
		PI										

G, A and P = Good, average and poor general and specific combining ability effects respectively and PI = pool and GY = grain yield and DFY = dry fodder yield

exhibited positive significant SCA effects. Among these, crosses ICSA208 x SU1561, ICSA29003 x SU1571, ICSA202 x SU1561, ICSA357 x SU1565, ICSA357 x SU1570, ICSA399 x SU1570, ICSA29002 x SU1570 and ICSA481 x SU1571 were having both or at least one good general combiner parents. The crosses *i.e.* ICSA363 x SU1557 where GCA of both the parents was poor, ICSA202 x SU1565 in E₃ where GCA of the parents was average and poor, ICSA349 x SU1557 and ICSA481 x SU1565 where GCA of the parents was average and poor and ICSA474 x SU1557 where GCA of both the parents was poor, exploitation through heterosis breeding will be most rewarding. Consisted economic heterotic crosses were not found in normal spacing (E₁ and E₃) (Table 5).

Thus crosses like ICSA 202 x SU 1570 and ICSA 474 x SU 1561 were found promising for normal spacing environments (E₁ and E₃) and one cross ICSA 202 x SU 1561 for wide spacing environments (E₂ and E₄) for both grain and dry fodder yields. These crosses also exhibited significant economic heterosis and good SCA effects for other traits in same environments *viz.*, ICSA 202 x SU 1561 for protein content in fodder (Table 6). Similar findings of identifying promising dual purpose crosses were also reported earlier by Yadav and Pahuja (2007) and Mohammad and Talib (2008).

Conclusion

For dual-purpose sorghum breeding, both grain yield and dry fodder yield are considered. Therefore, based on *per se* performance of economic heterosis and SCA effects for both grain yield and dry fodder yield two crosses *viz.*, ICSA 202 x SU 1570 and ICSA 474 x SU 1561 were identified as promising for normal spacing environments (E₁ and E₃) and ICSA 202 x SU 1561 for wide spacing environments (E₂ and E₄). The crosses identified for normal spacing had significant SCA effects but no consi-

-stency in GCA effects. The days to 50 per cent flowering of ICSA 202 and SU 1570 were almost equal. Therefore, heterosis breeding is recommended for ICSA 202 x SU 1570, whereas to exploit ICSA 474 x SU 1561 through heterosis breeding staggered sowing is essential. The dual-purpose cross, ICSA 202 x SU 1561 in wide spacing had significant SCA and involved at least one good general combiner parent and equal days to 50 per cent flowering in the parents. Therefore, this could also be exploited through heterosis breeding. The cross ICSA 202 x SU 1561 could also be exploited after attempting crosses between B line of ICSA 202 and tester SU 1561 to identify transgressive segregants if any.

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