

Physiological and biochemical analysis of heterosis in sorghum

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Received : 22 July 1982

ABSTRACT

The length and dry matter of the root and shoot, the relative rate of photosynthesis and the activity of ribulose 1,5-diphosphate (RuDP) and phosphoenol pyruvate (PEP) carboxylases were studied in the seedlings of 2 hybrids of sorghum [*Sorghum bicolor* (Linn.) Moench]. Heterosis for these attributes which was fairly high during the initial stages, declined with time, resulting in almost no advantage. The growth behaviour and photosynthesis, when split up at the components level, appeared to show Mendelian dominance in the F₁ hybrids. The complementation of all these processes in the F₁ and the interaction among them eventually appeared to make the F₁s outstandingly superior to their parents.

Several attempts have been made to reveal the mechanism of heterosis, but there is yet no co-ordinated explanation based on genetic, physiological, biochemical and environmental basis.

Some scientists do not believe in explaining heterosis based simply on the physiological and biochemical principles (Hageman *et al.*, 1967). Nevertheless, commercial hybrids are superior to their inbred parents both in dry-matter production and grain yield (Voldeng and Blackman, 1973). The higher yield potential of the hybrids is apparently the result of greater conservation of energy by them, because the harvest index of hybrids has not been found to be better than that of their parents (Sinha and Khanna, 1975). Therefore, the mechanisms related to energy conservation might be a very important determinant of heterotic yields. Heterosis in growth parti-

cularly in the initial stages after germination, might indicate the stage when the hybrid gets a potential superior to that of its parents (Sinha and Khanna, 1975).

Hence a study was undertaken on the post-germination growth behaviour and the photosynthetic-potential of the hybrids of sorghum [*Sorghum bicolor* (Linn.) Moench].

MATERIALS AND METHODS

'CSH 5' ('2077 A' × 'CS 3541') and 'CSH 6' ('2219 A' × 'CS 3541') were compared with their parents for different parameters.

Sterilized seeds were put in an incubator at 30°C for germination and humid atmosphere was provided. After 48, 72 and 96 hr of soaking, 3 replicates of a sample of 15 seeds each showing uniform growth were chosen from each genotype and the length of root and shoot measured. These roots and shoots from each sample were then oven-dried separately at 80°C for 48 hr to know the dry weight.

The relative rate of photosynthesis was studied in the hybrids and their parents when they were 2 and 4 weeks old, according to the method suggested by Jones and Osmond (1973).

Part of the M.Sc. (Agric.) thesis submitted in 1980 by the first author to the Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.

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The procedure outlined by Bjorkman (1960) was used to determine the RuDP carboxylase activity of the hybrids and their parents at 2-week-old and 4-week-old stages.

The assays for PEP carboxylase activity in the hybrids and their parents were done at 2-week-old and 4-week-old stages, according to the method given by Maruyama and Daniel Lane (1962).

RESULTS AND DISCUSSION

Post-germination growth behaviour

'CSH 5' showed a high degree of heterosis for root length over mid-parental

value 48 hr after soaking. The advantage gradually decreased with time, and 96 hr after soaking it was only marginally better than the mid-parental value (Fig. 1A). At 96-hr stage the hybrid was intermediate between the parents, suggesting partial dominance (Table 1). The root dry matter in hybrid ('CSH 5') was superior to that of either of its parents at the 48-hr stage, but later decreased. Ultimately, the hybrid, though intermediate, appeared to follow its poor parent, showing dominance for this character (Table 2; Fig. 2A).

In 'CSH 5' heterosis over the mid-parent for shoot length also showed a

Table 1. Length of root and shoot (mm) during the post-germination stages

	Hours after soaking					
	48		72		96	
	Root	Shoot	Root	Shoot	Root	Shoot
'2077 b'	9.56	1.56	18.50	3.56	38.30	8.92
'CSH 5'	32.90	7.40	45.70	18.50	68.00	18.70
'CS 3541'	23.30	1.10	49.20	7.80	93.90	7.90
'CSH 6'	22.70	0.90	40.40	27.10	65.70	31.90
'2219 B'	22.00	1.20	47.10	12.10	83.00	52.30
Mid-parental value for 'CSH 5'	16.43	1.33	33.85	5.68	66.10	8.41
Relative heterosis for 'CSH 5'	100.20	456.30	35.00	225.70	2.80	122.30
Mid-parental value for 'CSH 6'	22.65	1.15	48.15	9.95	88.45	30.10
Relative heterosis for 'CSH 6'	0.20	-21.70	-16.00	172.30	-25.70	5.90

Table 2. Dry weight of root and shoot (mg) during the post-germination stages

	Hours after soaking					
	48		72		96	
	Root	Shoot	Root	Shoot	Root	Shoot
'2077 B'	0.43	0.08	0.97	1.02	1.05	1.54
'CSH 5'	1.03	0.68	1.10	1.78	1.15	1.98
'CS 3541'	0.83	0.10	1.56	1.12	2.20	1.43
'CSH 6'	0.66	0.09	1.51	1.40	2.35	3.38
'2219 B'	0.43	0.04	0.55	1.90	0.99	2.75
Mid-parental value for 'CSH 5'	0.63	0.09	1.27	1.07	1.63	1.49
Relative heterosis for 'CSH 5'	63.40	655.50	-13.30	66.30	-29.40	32.80
Mid-parental value for 'CSH 6'	0.63	0.07	1.06	1.51	1.60	2.90
Relative heterosis for 'CSH 6'	4.70	125.00	42.40	-7.20	46.80	61.70

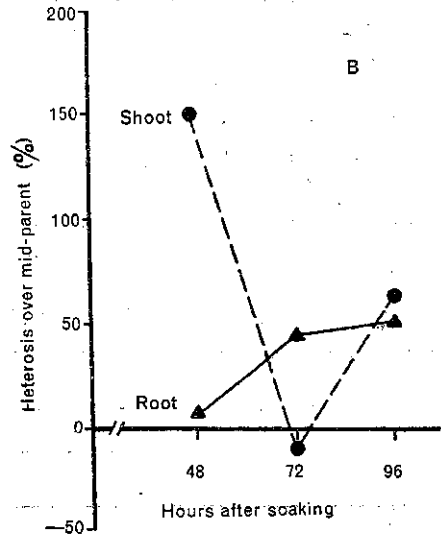
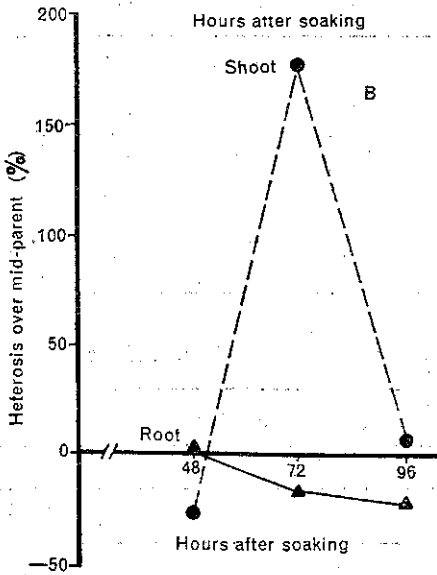
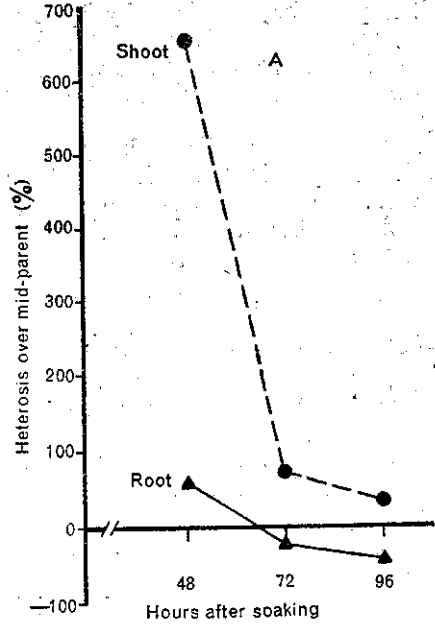
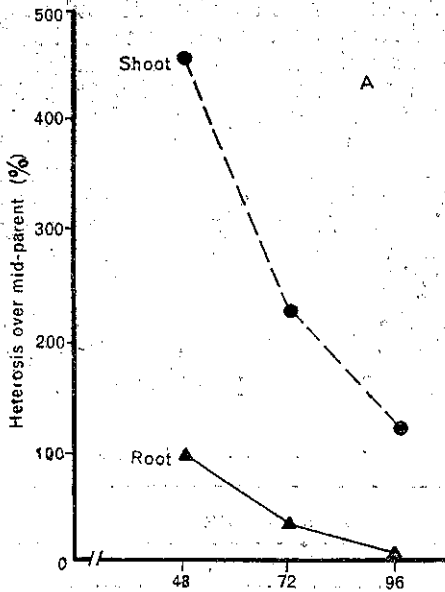


Fig. 1. Relative heterosis for root and shoot lengths during post-germination stages in (A) 'CSH 5' and (B) 'CSH 6'.

Fig. 2. Relative heterosis for dry-matter content during post-germination stages in (A) 'CSH 5' and (B) 'CSH 6'.

gradual decrease with time (Fig. 1A), although the hybrid was better than either parent at all stages showing over-dominance. Similar results were seen when

the dry matter in the shoot of 'CSH 5' was compared with the dry matter of its parents (Fig. 2A).

The root length in 'CSH 6' was inter-

mediate at the 48-hr stage but showed a negative heterosis at later stages, becoming poorer than the poor parent (Fig. 1B). The dry matter of the root in 'CSH 6', however, showed a positive heterosis over the mid-parental value and gradually increased with time (Fig. 2B). The dry matter of the hybrid was of the same amount as in the better parent, suggesting dominance for the trait.

The length of the shoot initially showed negative heterosis in 'CSH 6' compared with the mean of its parents but suddenly became far better than the shoot length of the better parent at the 72-hr stage. Nevertheless it became intermediate between the parental limits at the 96-hr stage. Just the reverse trend was observed for dry matter accumulation. The hybrid which appeared to follow one of the parents in the beginning became poorer than the poor parent at the 72-hr stage but subsequently shot up to show overdominance for the trait, becoming better than either of its parents.

Studies on heterosis in seedling growth have been very limited, and confined to the first 72-120 hr after soaking (Sarkissian and Srivastava, 1967; Hageman *et al.*, 1967; Ghose *et al.*, 1974). Nevertheless, seed germination and emergence of leaves were reported to take place earlier in heterotic hybrids than in their inbred parents (Ashby, 1937; Hageman *et al.*, 1967; Sarkissian, 1972). In our study this contention was true for 'CSH 5' only. In 'CSH 6' just the reverse trend was observed and the hybrid was later than either of the parents. The rate of growth initially was, however, higher in 'CSH 6' and it soon compensated for this initial disadvantage. However, in course of time one of the parents either equalled the hybrid in seedling vigour or ultimately exceeded it. Therefore the genetic potential for growth was the same in the hybrid and one of its parents, but the hybrid achieved the potential a bit earlier because of faster initial growth. This lag between parents and hybrids can be satisfactorily attributed to 2 causes; differential rate of water absorption and the quicker mobiliza-

tion of food reserves due to greater activity of digestive enzymes (Sinha and Khanna, 1975).

A clear evidence of Mendelian inheritance was obtained for the components of growth at least at the post-germination stages. However, the apparent advantage in seedling growth in the hybrid appeared only to be the result of complementation of better traits of both the parents.

Nevertheless, a detailed study of seedling growth as related to heterotic yields in the hybrids (considering the influence of growth processes intervening between seedling growth and yield at harvest) would give a better picture of the association between the two (Sinha and Khanna, 1975).

Relative rate of photosynthesis

For the relative rate of photosynthesis 'CSH 5' was similar to the better parent at both 2-week-old and 4-week-old stages, while 'CSH 6', which was initially intermediate between the parents, appeared to follow the poor parent at the 4-week-old stage (Table 3). Both, therefore, suggested dominance for this trait. When the rate of photosynthesis was expressed on unit chlorophyll basis, 'CSH 6' followed the poor parent at both the stages (Table 3).

Similar results were obtained by Sinha *et al.* (1976) in maize seedlings, in which the hybrids were found to be similar to one of the parents after an initial heterotic rate. However, Sinha *et al.* (1976) considered that the photosynthetic potential of the hybrid was higher than the potential of the parents on the basis of its larger leaf area. Nevertheless, the hybrids which inherit the length and width of better parents, and in which the leaves emerge earlier, have an initial advantage in photosynthetic potential over the inbred parents.

Both heterosis and no heterosis have been reported for the rate of photosynthesis (Moss, 1960; Fousova and Avratovsockova, 1969). The need to take into account the influence of developmental effects and sampling techniques was emphasized by Sinha and Khanna (1975).

Table 3. Relative rate of photosynthesis in sorghum hybrids 'CSH 5' and 'CSH 6' and their parents

	cpm/g fresh weight ($\times 10^4$)		cpm/mg chl ($\times 10^4$)		cpm/dm ² ($\times 10^4$)	
	2-week- old stage	4-week- old stage	2-week- old stage	4-week- old stage	2-week- old stage	4-week- old stage
'2077 B'	188.27	295.55	81.15	105.55	73.43	118.22
'CSH 5'	209.79	317.90	82.59	114.35	81.82	127.16
'CS 3541'	121.79	144.64	50.33	51.66	47.50	57.86
'CSH 6'	113.15	39.96	43.19	14.69	44.13	15.99
'2219 B'	97.46	21.00	41.30	8.02	38.01	8.40
Mid-parental value for 'CSH 5'	155.01	220.10	64.74	78.61	60.47	88.04
Relative heterosis for 'CSH 5'	35.30	44.40	25.60	45.40	35.30	44.40
Mid-parental value for 'CSH 6'	109.63	82.82	45.82	29.84	42.76	33.13
Relative heterosis for 'CSH 6'	3.20	-51.70	-5.70	-50.70	3.20	-51.70

Table 4a. Activity of ribulose 1,5-diphosphate (RuDP) carboxylase in sorghum hybrids 'CSH 5' and 'CSH 6', and their parents

	μ moles CO ₂ /g fresh weight/min		μ moles CO ₂ /mg chl/min		μ moles CO ₂ /dm ² /min		μ moles CO ₂ /mg protein/min	
	2-week- old stage	4-week- old stage	2-week- old stage	4-week- old stage	2-week- old stage	4-week- old stage	2-week- old stage	4-week- old stage
'2077 B'	1.290	1.533	0.556	0.548	1.985	2.433	0.078	0.088
'CSH 5'	1.618	1.765	0.637	0.635	2.489	2.802	0.112	0.114
'CS 3541'	1.200	1.805	0.496	0.645	1.846	2.865	0.083	0.117
'CSH 6'	1.628	1.788	0.621	0.657	2.505	2.838	0.121	0.123
'2219 B'	1.440	1.725	0.610	0.658	2.215	2.738	0.082	0.093
Mid-parental value for 'CSH 5'	1.245	1.669	0.526	0.596	1.915	2.649	0.080	0.102
Relative heterosis for 'CSH 5'	29.90	5.70	21.10	6.50	29.90	5.70	40.00	11.70
Mid-parental value for 'CSH 6'	1.320	1.765	0.553	0.651	2.030	2.801	0.083	0.105
Relative heterosis for 'CSH 6'	23.30	1.30	12.20	0.90	23.30	1.30	45.70	17.10

The averaging of rates over time which eliminated these effects resulted in no heterosis for the rate of photosynthesis (Sinha *et al.*, 1976). However, in our study even averaging did not affect the trend of the results.

Activity of photosynthetic carboxylases

Of the 4 components of photosynthesis (Sinha and Khanna, 1975) only carboxylation covering RuDP carboxylase activity

and PEP carboxylase activity was examined by us.

In 'CSH 5' the activities of both RuDP carboxylase and PEP carboxylase were found to be heterotic at the 2-week-old stage (Tables 4a, b). At the 4-week-old stage, RuDP carboxylase activity appeared to follow the better parent, but PEP carboxylase activity was poorer than that of the poor parent. In 'CSH 6' also both

Table 4b. Activity of phosphoenol pyruvate (PEP) carboxylase in sorghum hybrids 'CSH 5' and 'CSH 6', and their parents

	μ moles CO ₂ /g fresh weight/min		μ moles CO ₂ /mg chl/min		μ moles CO ₂ /dm ² /min		μ moles CO ₂ /mg protein/min ²	
	2-week- old stage	4-week- old stage	2-week- old stage	4-week- old stage	2-week- old stage	4-week- old stage	2-week- old stage	4-week- old stage
2077 B'	4.202	7.120	1.811	2.543	6.465	11.302	0.290	0.459
'CSH 5'	5.773	5.814	2.273	2.091	8.882	9.229	0.444	0.415
'CS 3541'	4.019	9.282	1.661	5.262	6.183	14.733	0.277	0.599
'CSH 6'	7.936	8.486	3.029	4.952	12.209	13.470	0.547	0.548
'2219 B'	6.569	5.916	2.784	2.258	10.106	9.390	0.526	0.438
Mid-parental value for 'CSH 5'	4.110	8.201	1.736	3.902	6.324	13.017	0.293	0.529
Relative heterosis for 'CSH 5'	40.40	-29.10	30.90	-46.40	40.40	-29.10	51.50	-21.50
Mid-parental value for 'CSH 6'	5.294	7.599	2.222	3.760	8.144	12.061	0.401	0.518
Relative heterosis for 'CSH 6'	49.90	11.60	36.30	31.70	49.90	11.60	36.40	5.70

the enzymes were heterotic over both the parents at the 2-week-old stage. At the 4-week-old stage, however, both of them followed the better parent. The extent of heterosis in both the cases for both the enzymes declined with time, and the PEP carboxylase activity in 'CSH 5' even showed negative heterosis at the 4-week-old stage.

Of the 2 enzymes, PEP carboxylase was more active and predominant in all the genotypes at both the stages.

Similar results were obtained by Sinha *et al.* (1976) for both the enzymes. In one of the hybrids, however, they observed that PEP carboxylase activity was higher than in either of the parents. They compared this behaviour to that of nitrate reductase, wherein 2 independent genes were reported to control the synthesis and decay of the enzyme (Warner *et al.*, 1969). But in our study the superiority of the hybrid over its parents in respect of both these carboxylase enzymes was found at the 2-week-old stage only. Afterwards, the PEP carboxylase activity became poorer than that of the poor parent. Hence this heterosis appears to be attributable more to developmental effects (Sinha and Khanna, 1975).

Heterosis in the activity of RuDP carboxylase and PEP carboxylase has been

reported at seedling stages (Nagy *et al.*, 1972; Khanna and Sinha, 1974; Sinha and Khanna, 1975; Sinha *et al.*, 1976). But this aspect requires further study since the activity of these enzymes is differentially influenced at various stages of physiological growth. A more comprehensive study that takes into account developmental effects would give a better picture of heterotic manifestations in the activity of RuDP carboxylase and PEP carboxylase.

Our results show that heterosis in growth components exhibit simple Mendelian inheritance. Nonetheless, the components of the process of photosynthesis are interdependent; consequently hybrids show high photosynthetic potential because of complementation rather than of multiplicative effects.

ACKNOWLEDGEMENTS

We are grateful to the Dean, College of Agriculture, and Director of Research, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, for providing facilities.

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