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Biochemical responses in fibre development of parental inbreds and hybrids in cotton (*Gossypium hirsutum* L)

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ABSTRACT : The present study was undertaken to study the fibre development of two intra *hirsutum* hybrids of cotton and their parental inbreds. Heterosis for fibre elongation manifested soon after anthesis and as early as 5 days after anthesis (DAA) the elongating fibre of two hybrids had attained greater lengths than their respective parental inbreds. LHH 144 achieved a final 2.5 per cent span fibre length of 28.1 mm compared with 26.3 mm of Fateh. After an initial lag period, the pattern of dry mass accumulation in bolls showed two maximas, the first around 10 DAA and another between 20-25 DAA. Heterosis for fibre dry matter accumulation and bundle strength value was also higher for LHH 144 than Fateh. There was little difference in heterosis for fibre sugar contents in case of Fateh while in case of LHH 144 it was 4-8 per cent. Soluble and wall bound acid invertase and neutral invertase increased in activity from 10 to 20 DAA followed by a decline at 30 DAA. High levels of sucrose synthases were detected in developing fibres of two hybrids and their parental inbreds. Both hybrids showed positive heterosis for enzyme activity with maximum expression at 20 DAA.

Key words : Fibre quality, invertase, peroxidase, soluble sugars, sucrose synthase

Though India occupies the foremost position among the cotton growing countries of the world in respect of acreage, it ranks fourth in the world production. Cotton is the worlds most important natural textile fibre and an important source of feed, foodstuff and oil with approximate world consumption put at 27 million metric tons/ year (Chen *et al.,* 2007).

Increased emphasis has been laid in recent years on heterosis breeding in cotton to achieve improved fibre productivity and quality (Dutt *et al.,* 2004). India has the distinction of having commercially exploited the phenomenon of heterosis extensively in cotton (Pathak and Kumar , 2009). A number of promising hybrids have been produced in cotton including H4, Varalaxmi , DCH 32, NHH 44, Fatch, HHH 81 and LHH-144 (Kranthi *et al.,* 2010). Although cotton breeders have made use of this phenomenon, the underlying physiological and biochemical mechanisms of heterosis remain largely unknown. Understanding the basis of heterosis will enhance our ability to develop new genotypes which will directly be used for F_1 hybrid development and will facilitate future selection programmes.

In F_1 hybrids, expression of hybrid vigour can be observed for fibre quality, yield, physiological and biochemical traits, subcellular structures and even for molecular mechanisms. It is, therefore, imperative that an integrated study of *in vivo* responses of inbred parents and their hybrids in cotton be undertaken with emphasis on the underlying mechanisms. Keeping this in view, kinetics of fibre growth, fibre quality traits (fibre strength, fibre length, micronaire, uniformity) and activity patterns of some important enzymes *viz.* invertases, sucrose synthase, peroxidase and the total soluble sugar content in fibres were determined.

MATERIALS AND METHODS

Raising of crop : Two intra *hirsutum* hybrids of cotton, namely, Fateh and LHH 144, along with their parental inbreds were raised in Department of Plant Breeding, Punjab Agricultural University, Ludhiana. For Fateh, female parent was LH 660 and male parent was Suman. For LHH 144, female parent was PIL 43 and male parent was PIL 8. Emergence occurred after six days of sowing and flowering started within another 50 days.

Sample collection : Individual flowers were tagged and tied on the day of anthesis and bolls were harvested at different days after anthesis (DAA). The data were taken for each set of parameters from flowers that bloomed within a narrow range, to escape the possible variations

that can be induced due to changing environmental conditions.

Fibre quality testing : The parameters of fibre quality studied included 2.5 per cent span length (mm), uniformity ratio (%), micronaire value (10^{-6} g/in), bundle tenacity (g/t) at 3.2 mm gauge length and fibre quality index. These were analyzed at Central Institute for Research on Cotton Technology (ICAR), Mumbai using high volume instrument (HVI).

Estimation of enzyme activities in cotton fibres

(a) Invertases extraction : Cotton fibres (1-2 g) harvested in duplicate at different stages of anthesis, *viz.*, 10, 20, 30 DAA were homogenized in a chilled pestle and mortar in 50 mM Hepes buffer (pH 6.5) containing 5 mM $MgCl_2$, 1 mM sodium EDTA, 2.5 mM DTT, 0.5 mg/ml BSA and 0.05 per cent (v/v) Triton X 100. The obtained homogenates were centrifuged at 15,000 g for 20 min. The pellet was resuspended in a small volume of the above buffer and centrifuged as before. The pooled supernatants were passed through Sephadex G 25 column to make it free from soluble sugars. All the steps of extraction were carried out at 0-4°C. The extracts collected from the column were used to assay acid invertase, alkaline invertase and sucrose synthase. The pellet stored in extraction buffer was used to determine wall bound acid invertase activity.

Assay soluble invertase : Alkaline invertase activity was determined by the above described assay procedure except that the acetate buffer was replaced by sodium phospahte buffer (pH 7.5).

Wall bound acid invertase : The washed pellet from the column was suspended in 1 ml of 50 mM sucrose in 0.2M sodium acetate buffer (pH 4.8) and incubated at 37°C for 20 min. The reaction was stopped by adding 1 ml of Nelson's reagent C and kept in a boiling water bath for 20 min. The contents were then centrifuged at 3000 g for 5 min. The supernatant was taken and one ml of arsenomolybdate was added to it. Rest of the procedure was same as for soluble invertases.

Sucrose synthase : Sucrose synthase activity in cleavage direction was assayed by taking a reaction mixture of 1 ml containing 0.2 ml of 250 mM sucrose, 0.1 ml of 20 mM UDP, 0.6 ml of 0.1M sodium phosphate buffer (pH 7.5) and 0.1 ml of enzyme extract. Rest of the procedure followed was same as that of invertase.

Peroxidase : This was done by the standard method.

Soluble Sugars : This was done by the standard method.

RESULTS AND DISCUSSION

Studies on physiological and biochemical basis of heterosis for fibre development in two intra *hirsutum* hybrids, namely, Fateh and LHH 144, and their parental inbreds were conducted.

Kinetics of fibre growth : The data for growth analysis showed that fibre elongation started soon after anthesis and as early as 5 DAA.The elongating fibres of two hybrids, had

Table 1. Fibre length / seed (mm) and per cent heterosis in two intra *hirsutum* hybrids, Fateh and LHH 144, and their parental inbreds at different developmental stages

	Days after anthesis (DAA)					
	5	10	15	20	25	30
Fateh (female parent)	4.75 ±0.24	11.0 ±0.66	21.75 ±1.08	25.50 ±2.04	26.50 ±1.33	26.70 ±1.34
Fateh (male parent)	4.13 ±0.21	9.62 ±0.58	19.13 ±9.6	24.76 ±1.98	25.25 ±1.26	25.38 ±1.26
Fateh (hybrid)	5.37 ±0.26	11.75 ±0.71	25.50 ±1.28	26.37 ±2.11	26.75 ±1.34	26.80 ±1.35
Heterosis (%)	20.94	13.96	24.75	4.93	3.38	2.91
LHH 144 (female parent)	5.25 ±0.27	13.37 ±0.81	23.75 ±1.05	24.25 ±1.394	25.80 ±1.29	26.00 ±1.24
LHH 144 (male parent)	5.37 ±0.27	11.0 ±0.66	21.13 ±1.24	23.37 ±1.87	24.50 ±1.23	24.75 ±1.18
LHH 144 (hybrid)	6.50 ±0.31	13.63 ±0.82	24.50 ±1.23	25.00 ±1.25	26.81 ±1.34	27.30 ±1.30
Heterosis (%)	22.41	11.85	9.18	4.99	6.60	7.58

Table 2. Dry mass / boll (mg) and per cent heterosis in two intra *hirsutum* hybrids, Fateh and LHH 144, and their parental inbreds at different developmental stages

	Days after anthesis (DAA)					
	5	10	15	20	25	30
Fateh (female parent)	27.0 ±1.35	84.0 ±3.36	210.0 ±4.20	512.0 ±10.24	850.0 ±16.67	1167.0 ±29.18
Fateh (male parent)	28.0 ±1.69	82.0 ±2.46	253.0 ±7.59	423.0 ±12.70	756.0 ±22.68	1023.0 ±22.73
Fateh (hybrid)	30.0 ±0.90	90.0 ±3.60	248.0 ±5.17	491.0 ±10.23	842.0 ±17.54	1153.0 ±24.02
Heterosis (%)	9.09	8.43	7.12	5.02	4.85	5.29
LHH 144 (female parent)	25.0 ±0.75	73.0 ±2.19	198.0 ±7.92	513.0 ±10.91	804.0 ±17.11	1044.0 ±22.69
LHH 144 (male parent)	27.0 ±1.08	79.0 ±2.52	231.0 ±9.24	482.0 ±10.71	847.0 ±20.17	1029.0 ±24.50
LHH 144 (hybrid)	30.0 ±1.50	84.0 ±2.52	236.0 ±8.43	526.0 ±11.19	853.0 ±17.77	1134.0 ±23.62
Heterosis (%)	15.38	10.52	10.02	5.72	3.33	9.40

attained greater lengths in comparison with their respective parents (Table 1). This trend continued upto 15 DAA. In all the genotypes, fibre elongation was nearly completed by 25 DAA. Hence, the total period of fibre elongation was not affected. However, when measured at 30 DAA, the final fibre length of both the hybrids was only slightly better over its parents.

Apparently, the heterosis for fibre elongation is manifested in the hybrids mainly during the period of rapid elongation, *i.e.* upto 15 DAA, because the increase in fibre length of hybrids was detectable as early as 5 DAA, an early onset of elongation and/or an increased rate of elongation might lead to the observed increased in fibre length upto 15 DAA. This point needs to be resolved by further research.

Fibrograph measurements of 2.5 per cent span length for mature fibres were also similar (Table 3). Majority of the studies indicated that extreme parent heterosis for fibre length can occur in cotton but generally the magnitude of transgressive expression is small. The heterosis (H) for fibre length expressed as H= [(hybrid – mid parent)/mid parent] x 100 for Fateh was 2.73 per cent and for LHH 144 5.8 per cent.

The mature fibre quality traits of the two cotton hybrids and their parental inbreds are depicted in Table 3.

Dry matter accumulation by developing fibre : The data for fibre dry mass accumulation *versus* age showed a distinct lag phase in all the genotypes (Table 2), as opposed to fibre elongation (Table 1). Generally, there was a rapid increase in dry matter accumulation around 10 DAA and another between 20-25 DAA or about the time when elongation ceased. The increase at 10 DAA possibly corresponded with an increase in primary wall synthesis required for sustaining rapid elongation (Basra and Malik, 1984). The second peak between 20-25 DAA coincided with extensive secondary cell wall cellulose deposition and cessation of fibre elongation. A varying degree of overlap between the elongation and secondary wall thickening phases in various genotypes has been reported by several workers.

Per cent heterosis for fibre dry matter accumulation was observed in both the hybrids at the three developmental stages investigated (Table 3). Because the final fibre lengths of LHH 144 is greater than Fateh, which would require greater synthesis of new cell wall materials, it is worth noting that per cent heterosis values of

Table 3. Fibre quality traits and per cent heterosis in two intra *hirsutum* hybrids, Fateh and LHH 144 and their parental inbreds

Variety	2.5 per cent span length (mm)	Uniformity ratio	Micronaire value (10^6 g/in)	Bundle tenacity (g/t) at 3.2 mm gauge length
Fateh (female parent)	25.8	51	4.7	20.2
Fateh (male parent)	25.1	54	5.4	21.4
Fateh (hybrid)	26.3	52	4.8	20.5
Heterosis (%)	2.73	0.95	-4.9	1.44
LHH 144 (female parent)	26.4	48	4.1	20.6
LHH 144 (male parent)	26.7	54	4.4	21.8
LHH 144 (hybrid)	28.1	52	4.2	22.2
Heterosis (%)	5.8	1.96	1.17	4.7

Table 4. Content of total sugars (mg / g FW) and per cent heterosis in two intra-*hirsutum* hybrids, Fateh and LHH 144 and their parental inbreds at three developmental stages

	Days after anthesis (DAA)		
	10	20	30
Fateh (female parent)	8.0±0.35	14.0±0.70	14.5±0.73
Fateh (male parent)	5.2±0.26	9.6±0.58	10.1±0.71
Fateh (hybrid)	6.7±0.35	11.9±0.56	12.3±0.59
Heterosis (%)	1.5	0.84	0.0
LHH 144 (female parent)	7.1±0.33	11.2±0.56	12.0±0.60
LHH 144 (male parent)	4.5±0.23	14.8±0.89	15.0±0.75
LHH 144 (hybrid)	6.3±0.31	13.6±0.82	14.1±0.64
Heterosis (%)	8.6	4.6	4.4

Table 5. Changes in activity of soluble acid invertase (µg sucrose hydrolysed/ min/ g fresh mass) and per cent heterosis in two intra *hirsutum* hybrids, Fateh and LHH 144, and their parental inbreds at three developmental stages

	Days after anthesis (DAA)		
	10	20	30
Fateh (female parent)	48.71±2.44	50.35±3.03	41.07±2.89
Fateh (male parent)	65.28±3.26	68.84±3.44	58.36±2.91
Fateh (hybrid)	69.40±3.30	74.11±3.70	60.00±3.86
Heterosis (%)	21.76	24.35	20.69
LHH 144 (female parent)	44.37±2.67	49.78±2.99	35.24±2.48
LHH 144 (male parent)	45.30±2.06	56.19±2.81	39.76±0.36
LHH 144 (hybrid)	52.46±3.69	61.22±4.89	42.09±3.37
Heterosis (%)	17.00	15.54	12.24

LHH 144 were also higher over Fateh at 10 DAA and 25 DAA (Table 1) denoting the active periods of fibre elongation and secondary wall thickening, respectively. Increased fibre dry mass may translate directly into fibre strength or tenacity. Fibre strength in turn is directly related to yarn strength, which is a critical factor in efficient manufacturing of textile fabrics. Fibre strength is very much a genetic property and for this genotype x environmental interactions are smaller than genetic influences. Similar to the data of fibre length, there is also a small amount of heterosis for fibre strength.

Heterotic expression of enzymes and total soluble sugar content in developing fibres : There has long been interest in physiological or biochemical mechanisms which underlie genetic difference in growth, including differences associated with heterosis or hybrid vigour. Although heterosis for fibre properties is small, there have been no studies comparing biochemical changes in developing fibres of parental inbreds and hybrids in cotton. This information is essential for selecting the right parents in hybrid production.

The metabolism of imported photo assimilate (sucrose) from the leaf to the cotton fibre is of key importance for fibre development. Therefore, it was analyzed changes in total sugars and the enzymes of sucrose metabolism, *i.e.* invertase (soluble and wall bound) and sucrose synthase at three developmental stages: (1) 10 DAA, the stage of rapid fibre elongation. (2) 20 DAA, denoting the cessation of elongation and the beginning of secondary wall deposition, and (3) 30 DAA, the stage of active secondary wall synthesis.

The female parent of hybrid Fateh had relatively greater sugar levels at the three developmental stages in comparison to the male parent or the hybrid itself (Table 4). However, there was no difference in per cent heterosis values among the parental inbreds at either of the stages.

For LHH 144, the male parent was superior over the female parent at 20 and 30 DAA stages. Per cent heterosis varied between 4-8 per cent (Table 4). This may be related to increased fibre length and dry matter accumulation of the hybrid over its parents at the three developmental stages (Table 1, 2). Whether the developing fibres of the hybrid receive a greater proportion of translocated assimilate from the leaf or there is a greater proliferation of plasmodesmata at the fibre base is worthy of future investigation.

Invertase activity was found in both the soluble and wall bound fractions forms at the three developmental stages investigated (Table 5, 6). Generally, the activities increased from 10 to 20 DAA and decreased at 30 DAA. Hence, the total activity in fibres was maximum at the onset of massive secondary cell wall formation, coinciding with the maxima found for glucose and fructose. However, the inversion of sucrose is not a pre requisite for its uptake from the cotton fibre

Table 6. Changes in activity of acid wall-bound invertase (µg sucrose hydrolysed /min/ g fresh mass) and per cent heterosis in two intra *hirsutum* hybrids, Fateh and LHH 144, and their parental inbreds at three developmental stages

	Days after anthesis (DAA)		
	10	20	30
Fateh (female parent)	15.2±0.92	31.4±1.49	14.25±0.86
Fateh (male parent)	21.4±0.97	40.9±2.04	24.13±1.21
Fateh (hybrid)	20.2±1.42	42.5±1.93	20.50±1.44
Heterosis (%)	10.38	17.56	6.82
LHH 144 (female parent)	16.70±1.18	28.70±1.72	19.20±1.35
LHH 144 (male parent)	19.95±1.20	36.08±2.17	30.72±2.16
LHH 144 (hybrid)	18.60±0.93	38.0±2.29	23.40±1.87
Heterosis (%)	1.50	17.32	6.25

Table 7. Changes in activity of alkaline invertase (µg sucrose hydrolysed/ min / g fresh mass) and per cent heterosis in two intra *hirsutum* hybrids, Fateh and LHH 144, and their parental inbreds at three developmental stages

	Days after anthesis (DAA)		
	10	20	30
Fateh (female parent)	36.07±2.17	46.68±2.33	32.00±2.25
Fateh (male parent)	25.53±1.28	61.37±2.56	14.62±1.17
Fateh (hybrid)	34.80±2.45	62.51±3.77	24.59±1.98
Heterosis (%)	13.00	15.70	5.50
LHH 144 (female parent)	23.36±1.87	44.08±2.66	25.10±2.26
LHH 144 (male parent)	40.62±2.03	54.76±3.29	29.06±2.62
LHH 144 (hybrid)	34.00±2.72	51.04±2.22	30.79±2.46
Heterosis (%)	6.28	3.27	13.70

apoplast.

Soluble acid invertase activity was more than alkaline invertase, and the soluble activity was predominant over the wall-bound activity (Table 5, 6, 7). The hydrolysis of sucrose by invertase produces glucose and fructose which can then be activated by hexokinases and used for respiratory metabolism of developing fibres.

Soluble as well as wall bound acid invertase activity of male parent was more than the female parent in both the hybrids (Table 5, 6).However, in respect of alkaline invertase, the activity was again higher in the male parent of LHH 144 at 10 and 20 DAA stages but the converse was true for Fateh.

Positive heterosis for soluble acid invertase was observed in both the hybrids which ranged from 21 to 24 per cent in Fateh and between 12 to 17 per cent in LHH 144 (Table 5). However, the extent of heterosis for wall bound acid invertase activity was relatively small (Table 6). Stimulation of invertase activity in tissues undergoing cell elongation and leading to an increase in sink activity have been reported from other plant species. The sink activity increased in this way may thus trigger fibre growth.

High activities of sucrose synthase were detected in developing fibres of the two hybrids and their parental inbreds (Table 8). In particular, there was dramatic increase in enzyme activity from 10 to 20 DAA, which continued upto 30 DAA. Both the hybrids showed positive heterosis for enzyme activity at the three developmental stages, with the maximum expression at 20 DAA (Table 8; Ruan *et al.,* 2003)

A massive increase in sucrose synthase activity at 20 DAA corresponds to the transition phase from primary to secondary wall synthesis. This phase initiates in most varieties of *G. hirsutum* around 16 DAA and is characterized by an abrupt change in the rate of cellulose synthesis that rises over hundred fold at this stage of development. Another peculiarity of elongation period is that elongation of fibres continues unabated for atleast 5 days in *G. hirsutum* varieties.

UDP glucose is known to be the substrate for cellulose synthesis. It can potentially be synthesized by either of two reversible reactions that catalysed by sucrose synthase or UDP glucose pyrophosphorylase. Cotton fibres contain high levels of both the enzymes. Hence, it is likely that the rate of sucrose utilization and the growth of fibres may be controlled by an interplay of invertase and sucrose synthase. Invertase is probably of greater importance during the period of rapid fibre elongation, whereas a subsequent massive increase in sucrose synthase activity is linked to the onset and most active stage of secondary wall deposition. The combined action of invertase and sucrose synthase will result in an increase in sink activity, triggering thereby the fibre elongation and secondary cell wall cellulose synthesis.

In all the genotypes, soluble peroxidase activity remained low at 10 DAA and increased

Table 8. Changes in activity of sucrose synthase (µg sucrose hydrolysed /min / g fresh mass) and per cent heterosis in two intra *hirsutum* hybrids, Fateh and LHH 144, and their parental inbreds at three developmental stages

	Days after anthesis (DAA)		
	10	20	30
Fateh (female parent)	18.87±1.72	194.08±11.69	347.37±15.10
Fateh (male parent)	38.75±2.33	251.24±12.53	306.05±14.57
Fateh (hybrid)	30.72±3.01	277.0±19.50	364.65±21.96
Heterosis (%)	6.62	24.40	11.61
LHH 144 (female parent)	28.22±1.42	185.51±11.18	318.12±19.16
LHH 144 (male parent)	17.45±1.66	244.68±17.23	414.09±20.70
LHH 144 (hybrid)	26.68±2.13	264.73±13.23	398.27±20.96
Heterosis (%)	16.83	23.07	8.78

Table 9. Changes in activity of peroxidase (D / min / g fresh mass) and per cent heterosis in two intra *hirsutum* hybrids, Fateh and LHH 144, and their parental inbreds at three developmental stages

	Days after anthesis (DAA)		
	10	20	30
Fateh (female parent)	1.30±0.13	3.14±0.19	3.26±0.16
Fateh (male parent)	1.28±0.12	3.25±0.19	3.29±0.26
Fateh (hybrid)	1.22±0.09	2.97±0.21	3.13±0.19
Heterosis (%)	-5.42	-7.04	-4.43
LHH 144 (female parent)	1.52±0.11	2.63±0.14	2.74±0.13
LHH 144 (male parent)	1.61±0.11	2.51±0.13	2.53±0.18
LHH 144 (hybrid)	1.49±0.13	2.46±0.15	2.57±0.15
Heterosis (%)	-4.79	-4.28	-2.46

sharply at 20 DAA corresponding with the cessation of elongation (Table 9). The activity remained high upto 30 DAA. Apparently, there is an inverse correlation between fibre elongation and peroxidase activity. Therefore, a negative heterosis for all the genotypes was observed at all the stages of fibre development (Table 9). The role of peroxidse in cessation of elongation growth of cotton fibres has been postulated. Peroxidase is a ubiquitous enzyme that reduces H_2O_2 in the presence of electron donor. Peroxidase restricts growth by rigidifying the cell-wall, and auxin oxidation. Interestinly, H_2O_2 has been proposed to be a signal molecule which is initiated exactly coincident with the onset of secondary wall formation and termination of elongation. H_2O_2 levels remain high throughout the transition as well as into the phase of true secondary wall synthesis. Hence a decreased peroxidase activity during development may be associated with improved fibre length of cotton cultivars and hybrids.

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