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EFFECT OF DIFFERENT HYDROLYTIC ENZYMES ON GERMINATION OF INTER AND INTRA SPECIFIC COTTON HYBRIDS AND PARENTS

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ABSTRACT

Cotton (*Gossypium* spp.; *Malvaceae* family) is the world's leading fiber crop and among the most important oilseed crops. It is the most essential textile fiber worldwide as it currently accounts for 90% of the commercially grown cotton. All the three hydrolytic enzymes such as amylase, protease and lipase are affecting the germination of cotton seeds. During germination of seeds, á-amylase and protease degrade starch granules and reserve proteins, respectively; thereby reducing the dietary bulk and improving the digestibility of starch and protein. Lipases are hydrolyzing triacylglycerols into fatty acids and glycerol. The present investigation revealed that hydrolytic enzyme (amylase, protease and lipase) activity of intraspecific hybrid (G.cot.Hy-12), interspecific hybrid (G.cot.Hy-102) and desi hybrid (G.cot.DH-9) are significantly increasing at 24, 48, and 72 hr in comparison of their parents. Among the different hybrids and parents intraspecific hybrid (G.cot.Hy-12) showed higher amylase (63.9 mg maltose/g tissue/min), protease (3.86 U/ml) and lipase (17.2 meq/min/g) activity respectively at 72 hr. Hydrolytic enzyme activity is decreases at 96 hr in all hybrids and their respective parents. Therefore, it can conclude that higher hydrolytic enzymes activity of all the hybrids shows higher germination percentage in comparison of their respective parents.

INTRODUCTION

Cotton is one of the important commercial crops cultivated in India for fiber and oil. Cotton, the "White Gold" is cultivated in different agro-climatic conditions in India constituting about 62 per cent of the raw material requirement of Indian textile industry. The Indian cotton crop is the most diverse in the world, both in terms of botanical status and fiber quality range. The world today has suddenly turned its attention towards the natural fibers that are environment friendly and biodegradable. Shukla, *et al*. (2013) noted that the information on suitable crop geometry and fertilization of new cotton hybrids is lacking at present and will be very useful for exploiting its potentiality to boost up the yield level. Cotton germination begins as the seed absorbs water and oxygen through its seed coat after planting. The water swells the dormant tissues, and cell growth and division begin to take place. The radical emerges through the micropyle, turns downward, and grows deeper into the soil, providing a taproot that will supply water and nutrients throughout the life of the plant. The hydrolytic enzymes *viz.,* protease, amylase and lipase are responsible for solubilizing reserve food material in form of starch, protein and lipid respectively in the seed and provide energy and other essentials nutrient to germinating embryo. During seed germination seed proteins are hydrolyzed by proteases (EC 3.4.21) into peptides and amino acids, which are translocated to growing embryo. Amino acids are used in the synthesis of enzymes, proteins, hormones, purines and pyrimidines bases. Amylases (EC 3.2.1.1) are enzymes which hydrolyze starch and are of great importance in germinating seedlings. The plant amylases are specially abundant in grains and in all germinating seeds. Kneen *et al*. (1942) showed that alpha and beta amylases are found in germinating seeds of wheat. The enzyme most frequently credited with the initial attack on starch granules is á-amylase (Trethewey and Smith, 2000). This enzyme is responsible for initiating the mobilization of starch in germinating seeds (Fincher, 1989). Lipases (EC 3.1.1.3) are enzymes that hydrolyze triacylglycerols into fatty acids and glycerol. Lipases are probably rate controlling during germination and the activity of the lipase is high during germination (Brockerhoff and Jensen, 1974; Ejedegba *et al*., 2007). During germination of the seed, the reserved triacylglycerols are disappeared, since the fatty acids can't be oxidized to provide energy until they are released from the triacylglycerol. Varietal differences in enzyme activity were related with density of seeds in triticale (Klassen *et al*. 1971) and varietal differences were also recorded in diastatic activity in wheat. It is quite probable that in cotton also the enzyme activity may be variety specific. The objective of present investigation is to find out the role of hydrolytic enzyme activity on germination of three inter and intra specific hybrids and their respective parents at different intervals.

MATERIALS AND METHODS

Plant materials and experimental design

Three inter and intra specific hybrids (G.Cot.Hy-12, G.Cot.Hy 102 and G.Cot.DH-9) and their respective parents of cotton were collected from Main Cotton Research Station, Surat and tested for hydrolytic enzyme activity in laboratory in completely randomized design (CRD) in four replications. The seeds of hybrids and parents are surface sterilized with 0.1% $HgCl_2$ and soaked in water in the petri plates for germination and the

enzyme activity observed at 24, 48, 72 and 96 hr after soaking (*i*.e. at 24 hr interval).

Enzyme Assay

Amylase

Extraction of enzyme was done according to the method of Bernfield (1955). One gram of sample material was crushed in 5-10 volume of ice-cold 10mM calcium chloride solution and keep it for 3hr at room temperature. Then centrifuged the extract at 54,000 g at 40ºC for 20 min. The supernatant is used as enzyme sources. Pipette out 1mL of starch solution and 1mL of diluted enzyme in test tube and incubate it at 27ºC for 15 min, stop the reaction by addition of 2mL of dinitrosalicylic acid reagent. Then heat the solution in a boiling water bath for 5 min, while the tubes are warm add 1mL of potassium sodium tartrate solution (Rochelle salt). Then cool it in running tap water after this make up volume to 10mL by addition of 5mL water and read the absorbance at 560 nm. Terminate reaction at zero time in control tube then prepared standard graph with 0-100 μg maltose.

Protease

Extraction of enzyme was done according to the method of Anson (1938). Homogenized 1 g of sample with cold distilled water and centrifuged then collect the supernatant. Take the 1mL of enzyme extract and add 3 ml of phosphate buffer and 2mL of 0.5 % casein solution in test tube. In control test tube add 1mL of distilled water, 3 ml of phosphate buffer and 2mL of 0.5 % casein solution. Incubate the test tube for 1hr at 30ºC in water bath. Pipette out 2 mL of reaction mixture and add 2 mL of 15 % of trichloroacetic acid then centrifuged the mixture after 20 min of incubation. Pipette out 1mL of reaction mixture from control and sample test tube. Add 4mL of 0.5 N NaOH and 1.2mL folin regent in both the test tube mix thoroughly. Incubate in dark for 20 min at room temperature then read the absorbance at 660 nm. Draw slandered graph using tyrosine (0-100 μg) and work out for the sample.

Lipase

Extraction of enzyme was done according to the method of Maliks *et al.* (2000). Grind 1 g quantity of sample with mortar and pestle. Homogenize the tissue with twice the volume of ice-cold acetone. Filter and wash the powder successively with acetone, acetone:ether (1:1) and ether. Air-dry the powder. This acetone powder can be stored in refrigerator. Extract 1g of the powder in 20mL ice-cold water or a suitable buffer. Centrifuge at 15,000 rpm for 10min and use the supernatant as enzyme source. Take 20mL of substrate in 500 m beaker. Add 5mL of phosphate buffer (pH 7.0). Set the beaker on the top of a magnetic stirrer cum hot plate and stir the content slowly. Maintain the temperature at 35ºC. Dip the electrodes of pH meter in the reaction mixture. Note the pH and adjust it to 7.0. Add enzyme extract (0.5mL), immediately recorded the pH and set the timer on. At frequent intervals as the pH drops by about 0.2 units add 0.1 N NaOH to bring pH to the initial value. Continue the titration for 30-60 min period. Note the volume of alkali consumed.

$$\text{Activity meq/min/g sample} = \frac{\text{Volume of alkali consumed x Strength of alkali}}{\text{Weight of sample in g x Time in min}}$$

Germination percentage (%)

Germination percentage was recorded as per ISTA rules at 12 days after sowing. This was done by counting the number of germinated seed in each paper pit.

$$\text{Germination percentage} = \frac{\text{No. of seeds sown}}{\text{No. of germinated seed}} \times 100$$

RESULTS

Amylase activity (mg maltose/g tissue/min)

The data indicated that the activity of enzyme increased upto 72 hours and declined thereafter. The data (Table-1) also revealed that all the hybrids recorded significantly higher amylase activity compared to their respective parents. Amongst the three hybrids, *hirsutum* hybrid G.Cot.Hy-12 recorded significantly higher amylase activity at 24h but later at 48, 72 and 96h, desi hybrid G.Cot.DH-9 became at par with it. As far as heterosis is concerned, positive heterosis over mid parent was recorded in all the three hybrids (*viz.,* G.Cot.Hy-12, G.Cot.Hy-102 and G.Cot.DH-9) for this character. The data also exhibited that, as the time increased the degree of heterosis declined.

Protease activity (U/mL)

The mean data presented in Table 2, showed significant differences in protease activity amongst various cotton hybrids and parents. The data revealed that at 24, 48 and 72 hr, all the hybrids exhibited significantly higher protease activity than their respective parents except for G.Cot.Hy-102 at 48 hr wherein its female parent GSHV-112 was at par to it. At 96 hr, *hirsutum* hybrid G.Cot.Hy-12 and desi hybrid G.Cot.DH-9 recorded significantly higher protease activity than their male parents but remained at par with their respective female parents, while in G.Cot.Hy-102, the protease activity was at par to both the parents. Positive heterosis over its mid parent was exhibited by all the three hybrids. Out of the three hybrids, *hirsutum* hybrid G.Cot.Hy-12 showed maximum positive heterosis than other hybrids except at 24 hr wherein desi hybrid G.Cot.DH-9 depicted maximum positive heterosis.

Lipase activity (meq/min/g)

Perusal of the data on lipase activity presented in Table-2 revealed significant differences amongst the hybrids and parents. *Hirsutum* hybrid G.Cot.Hy-12 recorded significantly higher lipase activity compared to its both parents at 24, 48 and 72 hr, whereas at 96 hr, its female parent was at par to it. ELS hybrid G.Cot.Hy-102 exhibited significantly higher lipase activity compared to its both parents at 24, 72 and 96 hr, whereas at 48 hr, it showed significantly higher activity only than the male parent, its female parent was at par. Desi hybrid G.Cot.DH-9 depicted significantly higher lipase activity compared to its both parents only at 24 hr, while later at 48, 72 and 96 hr, the hybrid recorded significantly higher activity than its male *arboreum* parent but female *herbaceum* parent remained at par to it. The data also indicated that the activity of enzyme increased with the increase in germination period and was maximum in 72 hr and declined thereafter. Amongst the three hybrids, *hirsutum* hybrid G.Cot.Hy-12 recorded significantly higher lipase activity. However G.Cot.Hy-102 was

Table 1: Hydrolytic enzyme activity (Amylase) in inter and intra specific cotton hybrids and parents

Hybrids/Parents	Amylase activity (mg maltose/g tissue/min)			
	At 24 hr	At 48 hr	At 72 hr	At 96 hr
Intraspecific hybrid (H x H)				
G.Cot. Hy-12 (F_1)	22.7	50.4	63.9	38.1
G.Cot. -16 (P_1) (*G. hirsutum*)	16.3	32.2	49.4	32.1
76 IH.-20 (P_2) (*G. hirsutum*)	15.7	43.2	53.2	30.3
Mid Parent heterosis (MPH)	41.9	33.7	24.5	22.1
Interspecific hybrid (H x B)				
G.Cot. Hy-102 (F_1)	20.5	33.6	48.3	22.9
GSHV-112 (P_1) (*G. hirsutum*)	17.1	29.2	44.2	21.8
GSB-39 (P_2) (*G. barbedence*)	15.1	25.3	35.1	21.4
Mid Parent heterosis (MPH)	27.3	23.3	21.8	6.03
Interspecific desi hybrid (h x A)				
G.Cot. DH-9 (F_1)	20.6	49.7	61.2	37.8
4011 (P_1) (*G. harbaceum*)	14.5	36.9	47.8	35.2
824 (P_2) (*G. arboreum*)	12.3	34.7	52.8	30.7
Mid Parent heterosis (MPH)	53.7	38.8	21.7	14.7
S.Em ±	0.59	1.10	1.21	0.91
C.D. at 5 %	1.73	3.20	3.51	2.64
C.V. %	6.92	5.92	4.68	6.06

Table 2: Hydrolytic enzyme activity (Protease & Lipase) and germination percentage in inter and intra specific cotton hybrids and parents

Hybrids/Parents	Protease activity (U/ml)				Lipase activity (meq/min/g)				Germinatio (%)
	24 hr	48 hr	72 hr	96 hr	24 hr	48 hr	72 hr	96 hr	
Intraspecific hybrid (H x H)									
G.Cot. Hy-12 (F_1)	1.84	2.82	3.86	1.24	1.19	8.20	19.6	17.2	95.0
G.Cot. -16 (P_1)	1.23	2.36	3.29	1.21	0.80	6.90	16.6	16.3	81.3
76 IH.-20 (P_2)	1.51	1.85	2.57	0.96	0.41	5.11	15.0	14.0	83.8
Heterosis (MP)	34.3	34.0	31.7	14.3	96.7	36.6	24.1	13.5	15.1
Interspecific hybrid (H x B)									
G.Cot. Hy-102 (F_1)	1.26	2.15	2.92	1.03	0.70	6.20	18.2	16.5	90.0
GSHV-112 (P_1)	0.93	2.01	2.46	0.97	0.50	5.80	15.6	15.2	85.0
GSB-39 (P_2)	0.58	1.35	2.39	0.98	0.35	4.10	13.5	12.8	83.8
Heterosis (MP)	66.9	28.0	20.4	5.6	64.7	25.3	25.1	17.9	6.6
Interspecific desi hybrid (h x A)									
G.Cot. DH-9 (F_1)	1.59	2.75	3.58	1.16	0.59	6.01	17.2	13.3	90.0
4011 (P_1)	0.80	2.28	3.17	1.07	0.48	5.42	15.9	12.6	78.6
824 (P_2)	0.58	1.93	2.95	0.99	0.20	4.30	13.6	11.6	82.5
Heterosis (MP)	130.4	30.6	17.0	12.6	73.5	23.7	16.6	9.9	11.7
S.Em ±	0.04	0.08	0.13	0.04	0.02	0.22	0.59	0.31	3.4
C.D. at 5 %	0.11	0.24	0.38	0.12	0.07	0.63	1.70	0.90	NS
C.V. %	6.78	7.52	8.68	7.52	8.48	7.50	7.27	4.31	7.9

at par with it at 72 and 96 hr.

As far as heterosis is concerned, positive heterosis over mid parent was recorded in all the three hybrids. The data also exhibited that, as the time increased the degree of heterosis declined as the differences in enzyme activity in the hybrid and the parents narrowed down. G.Cot.Hy-12 showed maximum positive heterosis at 24 and 48 hr, whereas G.Cot.Hy-102 depicted maximum positive heterosis at 72 and 96 hr.

Germination percentage

It can be seen from the data presented in Table-2 that the differences amongst the hybrids and parents were not significant. However all the three hybrids registered higher germination percentage than their respective parents? Amongst the three hybrids, intraspecific hybrid G.Cot.Hy-12 had highest germination percentage (95.0 %) followed by interspecific hybrid G.Cot.Hy-102 (90.0 %). Positive heterosis over its mid parent was exhibited by all the three hybrids. Out of the three hybrids, *hirsutum* hybrid G.Cot.Hy-12 showed maximum positive heterosis (15.1 %) over its mid parent than other hybrids.

DISCUSSION

Enzyme activity

The results (Table 1 and 2) on hydrolytic enzymes activity revealed that all the hybrids registered higher enzyme activity and a positive heterosis over mid parent. The hydrolytic enzymes *viz.,* amylase, protease and lipase are responsible for solubilizing reserve food material in form of starch, protein and lipid respectively in the seed and provide energy and other essentials to germinating embryo. Hydrolyzing enzymes activity increased upto seventy two hours due to continued utilization of reserve food content, after which the activity of enzyme decreased. Hybrids showed higher enzyme activity

in comparison to parents which results into greater and faster utilization of reserve food and ultimately higher germination in *hirsutum* hybrid which also showed a greater heterosis. Chitra Shukla and Saxena (2005) also observed heterosis for protease activity in H-6 cotton hybrid. The results also exhibited that as the time increased the degree of heterosis declined, infact at 96 hr in H x B hybrid G.Cot.Hy-102, the heterosis in amylase and protease activity declined to minimum, whereas in desi hybrid G.Cot.DH-9, the heterosis in lipase activity declined to minimum. Amongst the three hybrids, intraspecific hybrid G.Cot.Hy-12 recorded higher enzyme activity. Gupta (1991) also reported amylase activity was higher in *hirsutum* cultivars than that in the *arboreum*.

Germination percentage

The results (Table 2) showed no significant differences in germination percentage amongst the hybrids and parents. All the three hybrids recorded higher germination percentage and a positive heterosis over mid parent. Ashby (1930) and Whaley (1952) also observed a positive heterosis for germination percentage in cotton hybrids. Higher activity of hydrolyzing enzymes and subsequently soluble food in the hybrids in comparison to parents might possibly be the reason for it. Amylase is an important enzyme which has an active role in the hydrolysis of starch just before germination. By breakdown of starch, sugars are produced which provide readily available energy to the growing embryo and also play in important role in osmotic adjustment during early germination and seedling stages. Protease plays important role in mobilization of stored protein in seed as free amino acid are utilized in building necessary protein and enzyme (Ashraf *et al*., 2002 and Huang and Moreau, 1978). Similarly during germination of oily seeds, lipase hydrolyzes stored oils so that the required energy for growth and carbon skeleton for synthesis of new compounds are produced (Sadasivam and Manickam, 2008). All the three hybrids did show higher enzymatic activity and higher germination percentage in G.Cot.Hy-12 might be due to higher activity of hydrolyzing enzymes than parents which led to early and grater food availability for germinating seeds.

Thus, the enzyme activity and germination percentage indicated that the hybrids had higher enzymes activity (lipase, protease and amylase) than their parents which helped in faster and greater hydrolysis and mobilization of fats, proteins and carbohydrates. Availability of soluble food promoted higher seed germination. Such series of events seem to have occurred in hybrid G.Cot.Hy-12 and G.Cot.DH-9 as is evident from the results. However in the H x B hybrid G.Cot.Hy-102 despite greater hydrolyze enzyme activity. The heterosis in seedling growth was not manifested which is quite probable, since heterotic effect does not necessarily express at all stages and/or in all traits.

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