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Effect of Heat Stress on Amylase Activity in Chalky and Translucent Rice Genotypes

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Abstract*: Occurrence of chalkiness is a major problem as it is an important parameter in determining the quality of rice grains. High temperature during the grain filling period results in loose packing starch granules that could be due to the involvement of starch degrading enzymes i.e., amylases. Therefore, two rice genotypes viz., PAU 3699-13-2-1-1 and PR122 were sown at two different dates. Thirty-day-old seedlings of the two genotypes were transplanted in the field at two different times in order to expose the plants to different temperature regimes during grain filling. A Significant increase in total amylase activity was observed during early transplanting. The chalky genotypes i.e., PAU-3699-13-2-1-1 possessed higher α-amylase activity than translucent genotype (PR122) during heat stress. Whereas, PR122 possessed higher β amylase activity than PAU-3699-13-2-1-1. High temperature encountered during early transplanting resulted in induction of activities of amylases.

Keywords: Chalk, Heat Stress, Rice, Amylase.

1. INTRODUCTION

Grain chalkiness is a major concern in rice (*Oryza sativa* L.) crop as it is one of the major factors in determining quality and price. Percentage of grains with chalkiness is one of the main indices of rice determining appearance quality. Varietal differences in chalkiness have been reported in several studies [1]. Among environmental factors, the temperature has the largest influence on chalkiness [2]. Formation of chalky grains is greatly enhanced by high temperature during ripening phase [3]. High temperature during the grain filling period leads to a reduction in grain filling-related enzymatic activity, increase in respiratory consumption of assimilation products, water imbalance, decreased sink activity of luminous flowers, and increased loosely-packed starch granules [4]. The loos packing of starch granules suggests the involvement of starch degrading enzymes.

Amylases are starch degrading enzymes. They are widely distributed in microbial, plant and animal kingdoms. They degrade starch and related polymers to yield products characteristic of individual amylolytic enzymes. Initially, the term amylase was used originally to designate enzymes capable of hydrolyzing α -1, 4- glucosidic bonds of amylose, amylopectin, glycogen and their degradation products [5, 6]. They act by hydrolyzing bonds between adjacent glucose units, yielding products characteristic of the particular enzyme involved. They can specifically cleave the O-glycosidic bonds in starch. Plant hormones and other metabolites regulate starch degradation which is a complex biochemical process [7].
Grain filling of cereals is vulnerable to environmental stress including high temperatures. For japonica cultivars of rice, a temperature above 26ºC during the first half of the ripening period adversely affects yield through a decrease in grain size [8] and quality due to impaired deposition of starch. Cereal α- amylases play a very important role in the starch metabolism in developing as well as germinating cereals. The enzymatic activity and gene expression of α- amylase increase during the grain filling period [3]. It has been reported that the expression of α- amylase genes, *Amy1A*, *Amy3D*, and *Amy3E*, was increased more than twofold to an elevated temperature during the ripening period, while the expression of many genes for starch biosynthesis was regulated [9] preventing the starch accumulation. Furthermore, the expression of several α-amylase mRNA species was detected in ripening seeds of rice using a transcriptomic analysis. Moreover, the mRNA expression of *Amy1A*, *Amy1C*, *Amy3D*, and *Amy3E* genes, as well as α-amylase activity, was increased under HT stress [10]. The present study was conducted to observe the effect of heat stress encountered at different DAA in chalky and translucent genotypes.

2. MATERIALS AND METHODS

Rice genotypes viz., PAU 3699-13-2-1-1 and PR122 were sown at two different dates i.e. the first week of March and last week of May in the experimental field of Punjab Agricultural University, Ludhiana (Punjab, India).

Thirty-day-old seedlings of the two genotypes were transplanted in the field at two different times in order to expose the plants to different temperature regimes during grain filling, in a complete randomized block design with three replications. The recommended agronomic practices were followed to raise a healthy crop. The mean weekly temperature under two transplanting dates starting from the date of anthesis recorded by the field meteorological laboratory is represented in Fig. 1. The plants were tagged at the time of anthesis and the developing grains on 10, 15, 20 and 28 days after anthesis (DAA). The developing grains were used for the extraction and estimation of amylases.

2.1 Extraction and Estimation of amylases

The extraction of α-amylase was carried out following the method of Doehlert and Duke [11]. 50 mM sodium acetate buffer (pH 5.0) containing 1 mM $CaCl_2$, and for β-amylase with 100 mM sodium acetate buffer (pH 3.6) containing 0.1 mM EDTA was used for extraction. The homogenate was centrifuged at 10,000 x g for 30 minutes at 4°C. The supernatant obtained was used as an enzyme extract. For the α-amylase assay, the extract was incubated with 0.067% starch in the presence of 0.05 M glycine-NaOH buffer (pH 9.5) containing 1mM $CaCl_2$ for one hour at 30°C. The reaction was stopped by the addition of iodine solution [12]. The activity was determined by subtracting the concentration of starch left after hydrolysis from the starch present in the substrate blank.

In the enzyme extract used for assaying α-amylase activity, β-amylase was inactivated by heating the extract at 70 °C for 20 min. β-amylase was assayed according to Duffus and Rosie [12], using starch as substrate. Total protein was estimated by Bradford method [13] using BSA as standard.

3. RESULTS AND DISCUSSION

High temperature during grain development has a significant impact on starch degrading enzymes viz., amylases. PAU 3699-13-2-1-1 is a long slender grain rice that generally forms chalk under the climate of Ludhiana, Punjab (India). PR122 is another long slender grain which is moderately resistant to forming chalk. In rice, amylase activity is mainly induced during germination but it affects the quality of stored rice grains [14, 15]. A significant increase in total amylase activity was observed during ET when crop encountered heat stress. Overall, total amylase activity was observed to be higher in PR122. During ET, both PAU- 3699-13-2-1-1 and PR122 possessed the highest activity at 10 DAA whereas, during NT, highest activity was observed at 20 and 10 DAA (i.e., 1.98 nmoles of sucrose hydrolyzed min^{-1} mg^{-1} protein and 7.49 nmoles of sucrose hydrolyzed min^{-1} mg^{-1} protein) in PAU-3699-13-2-1-1 and PR122 respectively (Table 1). The induction in total amylase activity was 65% and 23% in PAU- 3699-13-2-1-1, and PR122 respectively. Higher amylase activity during heat stress resulted in the formation of chalk, which was observed to be 19.5% compared to 8.3% and 97% compared to 86% during NT in PR122 and PAU-3699-13-2-1-1 respectively.

Alpha Amylase is endoamylases, also known as 'liquefying' enzymes that cleave α -1,4 glycosidic bonds in amylose, amylopectin and related polysaccharides such as glycogen. The products of hydrolysis, which are oligosaccharides of varying chain lengths, have α configuration at the C1 of the reducing glucose unit, hence the name α-amylase. As the name suggests, endoamylases hydrolyze the bonds located in the inner regions of the substrate resulting in a rapid decrease of the viscosity of the starch solution as well as a decrease in iodine staining power [16]. In our study, α amylase activity observed to be significantly increased during heat stress (Table 2). Induction in α-amylase activity has been reported to cause chalkiness in rice grains [10]. In our study, chalky genotypes i.e., PAU-3699-13-2-1-1 (genetically chalked genotype) possessed higher α-amylase activity than translucent genotype (PR122) during heat stress encountered at ET (Table 2). The maximum activity was observed in PAU-3699-13-2-1-1 at 10 DAA (6.82 nmoles of sucrose hydrolyzed min^{-1} mg^{-1} protein) during ET, whereas PR122 possess minimum activity at 20 DAA (0.27 nmoles of sucrose hydrolyzed min^{-1} mg^{-1} protein) during NT. Earlier, SEM investigations evident α-amylase attack as micro-pores occurred on the surface of the compound granules, indicating the involvement of starch-degrading enzymes in grain chalkiness [17, 18] under heat stress, thereby suggesting induced α-amylase due to high temperature causes starch degradation hence resulting in the formation of grain chalkiness [3, 10].

β amylase is exoamylases, also known as 'saccharifying' enzymes which cleave a-1,4 glucosidic bonds in amylose, amylopectin, and glycogen from the non-reducing end by successive removal of maltose/glucose in a stepwise manner. The products of hydrolysis have the β - configuration at the C1 of the reducing glucose unit due to inversion of the product. In contrast to the action of endoamylases, this results in a slow decrease in the viscosity and iodine staining power of starch. Cereal and bacterial b-amylases and fungal glucoamylases come under this category [19]. β amylase activity followed the similar trend as that of α-amylase and total amylase activity. During ET, both genotypes possessed highest β amylase activity at 20 DAA whereas, during NT, it was observed to be highest at 10 (2.79 nmoles of sucrose hydrolyzed min^{-1} mg^{-1} protein) and 20 DAA (3.21 nmoles of sucrose hydrolyzed min^{-1} mg^{-1} protein) in PAU-3699-13-2-1-1 and PR 122, respectively. Overall, PR122 possessed higher β amylase activity than PAU-3699-13-2-1-1 (Table 3).

From above results, it can be concluded that heat stress encountered during early transplanting has a great impact on starch degrading enzymes which are amylases. The activity of amylases increases to a higher level during heat stress.

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Table 1: Effect of transplanting dates on total amylase activity

Variety	DOT		
	Stage	ET	NT
PAU 3699-13-2-1-1	10DAA	8.24 ± 0.19	1.44 ± 0.30
	15 DAA	4.19 ±0.53	1.92 ± 0.11
	20 DAA	3.19 ±0.65	1.98 ± 0.03
	28DAA	2.88 ±1.09	1.16 ± 0.28
PR122	10DAA	8.17 ±1.16	7.49 ± 0.78
	15 DAA	3.95 ± 1.57	3.53 ± 0.27
	20 DAA	5.93 ± 0.97	2.61 ± 0.22
	28DAA	2.14 ± 0.75	1.96 ± 0.03
LSD (5 %)	A =Genotypes= 0.65 B = Date of transplanting = 0.91 C= Stage of sampling = 0.65 A*B = 1.29 B*C = 1.29 A*C = 0.91 A*B*C = 1.82		

Values are mean ± S.D of three replicates, total amylase activity is expressed as nmoles of starch hydrolyzed $min^{-1}mg^{-1}$ protein

Table 2: Effect of transplanting dates on α - Amylase activity

Variety		DOT	
	Stage	ET	NT
PAU 3699-13-2-1-1	10DAA	6.82 ± 1.52	0.40 ± 0.05
	15 DAA	0.88 ± 0.04	0.34 ± 0.11
	20 DAA	1.50 ± 0.64	1.16 ± 0.71
	28DAA	0.24 ± 0.17	1.04 ± 0.05
PR122	10DAA	1.40 ± 0.95	0.88 ± 0.12
	15 DAA	1.36 ± 0.37	0.91 ± 0.10
	20 DAA	1.95 ± 0.67	0.27 ± 0.04
	28DAA	2.27 ± 0.30	1.60 ± 0.21
LSD (5 %)	A =Genotypes= NS; B=Date of transplanting = 0.47 C=Stage of sampling= 0.34 A*B = 0.67 B*C = 0.67 A*C = 0.47 A*B*C = 0.95		

Values are mean ± S.D of three replicates, α - amylase activity is expressed as nmoles of starch hydrolyzed $min^{-1}mg^{-1}$ protein

Table 3: Effect of transplanting dates on β - Amylase activity

Variety		DOT	
	Stage	ET	NT
PAU 3699-13-2-1-1	10DAA	0.65 ± 0.33	2.79 ± 0.80
	15 DAA	3.06 ± 1.51	1.86 ± 0.46
	20 DAA	4.84 ± 1.39	1.31 ± 0.42
	28DAA	1.65 ± 0.61	0.95 ± 0.56
PR122	10DAA	1.97 ± 1.09	1.91 ± 0.64
	15 DAA	6.21 ± 0.52	2.26 ± 0.71
	20 DAA	4.74 ± 0.93	3.21 ± 0.84
	28DAA	4.21 ± 1.15	1.49 ± 0.52
LSD (5 %)	A =Genotypes= 0.46 B = Date of transplanting = 0.65 C= Stage of sampling = NS A*B = 0.92 B*C = 0.92 A*C = 0.65 A*B*C = 1.29		

Values are mean ± S.D of three replicates, β - amylase activity is expressed as nmoles of starch hydrolyzed $min^{-1}mg^{-1}$ protein

