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Full Length Research Paper

Isoamylase profile of mung bean seedlings treated with high temperature and gibberellic acid

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Heat stress is one of the common abiotic stresses and is the most harmful factor affecting the growth and productivity of any crop. Heat stress can reduce number of morphological and physiological properties of any living organism. Gibberellic acid (GA_3), a plant growth hormone is also known for resistance of number of abiotic stresses like heat stress. Effect of high temperatures and GA_3 were evaluated in terms of amylase isozymes in four mung bean genotypes. Twenty four hours old seedlings of four mung bean genotypes (NM 19-19, NM 20-21, NM 121-123 and NCM 89) were exposed to lethal temperature (50°C), pretreated with 40°C prior to lethal temperature with and without 100 µM GA_3. Polyacryamide gel electrophoresis (7.5%) revealed five isoamylases all together in different samples; however, variations were seen among control and treated samples of different genotypes. It was observed that Amy 2 was present in all samples. A very light band of Amy 5 was seen only in treated samples of 0 h of all genotypes, similarly Amy 4 was specific to treated samples at 0 h harvest of NM 20-21 and NCM 89. Application of GA_3 during unstressed condition (A1), showed no prominent induction in amylase activity for all genotypes except for NCM 89 at 24 and 72 h. However, the induction in amylase was seen when the pretreatment of 100 µM GA_3 was given during mild (B1or B2) or lethal temperature (C) for all genotype at some harvests.

Key words: Gibberellic acid, heat stress, Isoamylase, mung bean.

INTRODUCTION

Mung bean is the most important and popular pulse crop, especially in Asian countries including Pakistan. In nature, plants are subjected to changes in temperature, both during changes in seasons and more rapidly over the course of individual days. The temperature can change much rapidly than any other factor that can cause stress. The effect of heat stress on crops is significant morphologically and physiologically, which ultimately can reduce crop yield. Plants may have inherent thermotolerance against temperature above optimum or may develop acquired thermotolerance by protecting plants exposed to preliminary mild heat stress and lethal high temperature treatment (Queitsch et al., 2000).

Temperature stress can reduce enzyme activity through changes in intra- and inter- cellular bonds of proteins, which can be broken at elevated temperature and the activities of several enzymes like amylase was negatively affected by heat treatment (Anderson and Sonali, 2004). Amylase is an enzyme which is involved in starch hydrolysis into mono and oligosaccharides in cotyledons of legume seeds (Koshiba and Minamikawa, 1981), and sugar availability plays an important role in plant growth and tolerance to abiotic stresses (Vartapetian and Jackson, 1997). Gibberellic acid (GA_3) is a plant hormone that takes part in various metabolic processes and important in stress tolerance. It has been well established that the expression of α-amylase is regulated by plant hormone GA_3 and ABA (Mitsui and Itoh, 1997). Temperature stress reduces amylase activity but the exogenous application of GA_3 during heat stress is able to increase amylase activity hence the growth (Mitsui and Akazawa, 1986; Cavusoglu and Kudret, 2007).

Amylase can be present in multiple molecular forms called isozymes with same substrate specificity. Isozymes

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may be used for studying genetic variability within and between populations of plants. Native gel electrophoresis revealed variable number of isoamylases in different species. Yemeni bean seeds showed the presence of two isoforms of amylase (Al-Maqtari et al., 2011). Wagenaar and Lugtenborg (1973) reported five isoamylases in germinating rye seeds. Germinating barley seeds contain two isoamlases (AMY 1 and AMY 2), both of which are involved in starch degradation to provide energy for the development of plant embryo (Leah and Mundy, 1989; Vallee et al., 1998).

We conducted this study to examine the effect of heat stress and GA_3 on α-amylase isozymes in seedlings of four mung bean genotypes harvested after various time intervals.

MATERIALS AND METHODS

Four mung bean genotypes (NM 19-19, NM 20-21, NM 121-123 and NCM 89) were obtained from National Agricultural Research Centre (NARC), Islamabad, Pakistan. Seeds were sterilized with 1% sodium hypochlorite solution for 2 min, rinsed several times with distilled water (d/w).

Temperature and GA_3 treatments were given according to the method of Chen et al. (1986) with minor modifications. Sterilized seeds were imbibed in d/w for 5 h, sown in Petri dishes of 6 inch diameter lined with two layers of filter papers soaked with 6 ml of d/w. Seeds of all four genotypes were incubated at 30°C for 24 h. These 24 h old seedlings were incubated at 30°C (GA_3) (treatment A1), mild temperature prior to lethal, that is, 40°C 1 h + 50°C 2 h (treatment B), 40°C 1 h (GA_3) + 50°C 2 h (treatment B1), 40°C 1 h + 50°C 2 h (GA_3) (treatment B2) and lethal temperature 50°C 2 h (treatment C), keeping 30°C (treatment A) as control. All treatments were given in the presence of 6 ml of incubating buffer (0.001 M sodium phosphate buffer pH 6.0, containing 1% sucrose with or without 100 µM GA_3). Seedlings were transferred in new Petri dishes, lined with filter paper moistened with 6 ml of d/w and incubated at 30°C, harvested 0, 24, 48 and 72 h after treatments and saved for 7.5% non denaturing gel electrophoresis (Davis, 1964).

Frozen tissues were crushed in a pre-chilled pestle and mortar by taking 4 mL of cold homogenizing buffer (0.25 M sucrose, 0.02 M 2-mercaptoethanol, 0.003 M EDTA in 0.02 M Tris HCl buffer of pH 7.5) per gram fresh weight of plant tissue. The homogenate was strained through two layers of muslin cloth and filtrate was centrifuged for 30 min at 10,000 rpm and 4°C. Bromophenol blue (0.0002%) and sucrose (10%) was added in the resulting supernatant to load on 7.5% non denaturing polyacrylamide gel. Scie plas (TV 100Y) vertical slab gel electrophoresis unit was used for the separation of Isoamylases. Pre-run without samples was performed for 15 min at 60 V. Equal amount of protein (300 µg) from prepared samples was loaded under electrode buffer (Tris-glycine). A constant voltage of 100 V was supplied. Electrophoresis was conducted until the tracking dye reached the end of glass plates. Gels were taken out from plates by inserting a needle of syringe into the sides of the plate and applying water with force.

Gels were stained according to the method described by Przybylska et al. (1982), incubated for 5 to 6 h at room temperature in 1% soluble starch, rinsed with distilled water and stained with iodine solution [1.5% of potassium iodide and 0.004% iodine in 0.2 M acetate buffer (pH 5.3)]. Colourless zones of α-amylase were detected against a blue background of unhydrolyzed starch and iodine complex. Bands were numbered in increasing order, downwards from the top of the gel. The upper bands had low Rf values which increased downwards (Rf = distance travelled by the isozyme /distance travelled by the tracking dye ×10).

RESULTS

When twenty four hours old etiolated mung bean seedlings of all four genotypes were exposed to different temperatures with or without 100 µM GA_3 and subjected to 7.5% native gel, they revealed five isoamylases all together (Figure 1). Isoamylases in zymogram were named according to their Rf values as Amy 1 (Rf, 1.07 cm), two variant bands of Amy2, that is, Amy 2a (Rf, 2.28 cm) and Amy 2b (Rf, 2.51 cm), Amy 3 (Rf, 2.85 cm), Amy 4 (Rf, 3.07 cm) and Amy 5 (Rf, 5.57 cm). It was detected that in NM 19-19, Amy 2 either as variant band of Amy 2a or Amy 2b were present in most of the samples at all harvests, which was present in other genotypes as well. A new band of Amy 3 was specific to 50°C (treatment C) at 0 h only (Figure 1; lane 6 of NM 19-19). It was also noticed that the intensity of Amy 2 was reduced in lethal temperature treatment (50°C) at 0 h of almost all genotypes (Figure 1, lane 6) and induction was seen at 48 and 72 h harvest (Lanes 18 and 24, respectively), except at treatment C of NM 19-19 at 72 h. A very light and thin Amy 4 band was detected in treated samples (B, B1, B2 and C) of NM 20-21 and NCM 89 at 0 h only. Another very weak band of Amy 5 was also observed in B, B1, B2, C of NM 19-19 and NCM 89, while it was found in treatment B1 and B2 of NM 20-21 and B, B1 and B2 of NM 121-123 at 0 h only. It was also noticed that Amy 1, 4 and 5 were specific to temperature treatments with or without GA_3 irrespective of the genotype and harvests. Furthermore, Amy 4 and 5 were specific to treated samples of early developmental stage only. It was also detected that exogenous application of 100 µM GA_3 during 30°C (A1) caused no prominent induction in isoamylase for all genotypes except for NCM 89 at 24 and 72 h; however caused induction when applied with temperature (B1 or B2) as compared to treatment B at different harvests.

Varietal variation was also detected. There was presence of Amy 3 in the treatment C at 0 h of NM 19-19, A1 at 72 h of NM 20-21, C at 72 h of NM 121-123 and A1, B1 and C at 24 h of NCM 89. Amy 4 was absent in NM 19-19 and NM 121-123 but present in NM 20-21 and NCM 89 of treated samples at 0 h harvest only.

DISCUSSION

High temperature is one of the major factors that reduce yield and number of physiological functions including the synthesis of enzymes. It is reported by Anderson and Sonali (2004) that heat stress reduced protein synthesis and many enzymes like α-amylase. Similarly, current results showed reduced intensity of amylase isozymes during heat stress (50°C) in early developmental stages. These results were also supported by Brodl et al. (1990) who reported that heat shock in barley (*Hordeum vulgare*

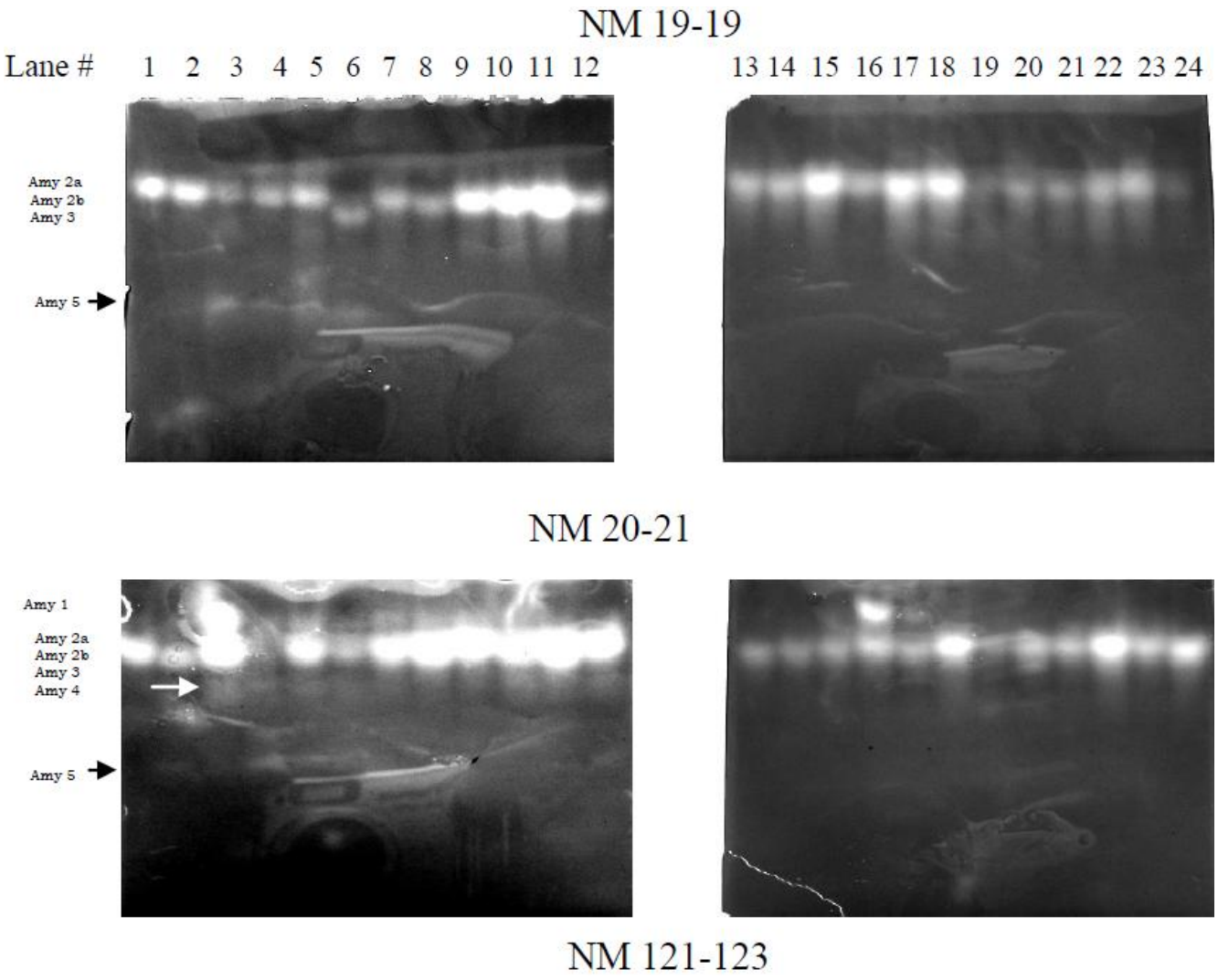

Figure 1. Isoamylase profile of seedlings of four genotypes of mung bean, harvested after temperature and GA_3 treatments at various time intervals. Amy 4 and 5 are very weak bands present only at 0 h, represented by white and black arrows, respectively.

L. cv. Himalaya) aleurone layers induces the synthesis of heat shock proteins (HSPs) and suppresses the synthesis and secretion of α-amylase. It was also observed that the activity of amylase was more at 50°C at later developmental stages. It was supported by the findings of Commuri and Duke (1997) who reported that heat stress causes four to six folds induction in apoplastic α-amylase in pea seedlings.

The assessment of amylase isozyme profile on 7.5% native polyacrylamide gel electrophoresis revealed five isozymes all together in different samples. Similarly, Wagenaar and Lugtenborg (1973) also reported five isoamylases in germinating rye seeds. However Chiba et al. (1990) observed three main groups of isoamylases that were separated from rice cell culture.

Variable response in amylase expression at temperature as well as control samples at different harvests was seen. As shown in our results, GA_3 was unable to cause induction in amylase at 30°C except for NCM 89, it could be suggested that abscisic acid may cause antagonistic effect on GA_3 at this temperature. It is reported by Koornneef et al. (2002) that the temperature controlled responses are mediated via the manipulation of endogenous plant hormone levels and/or signal transduction. For example, gibberellic and abscisic acids have been shown to be important factor in the regulation of seed dormancy and growth. The present study shows the induction in amylase when GA_3 was applied during temperature stress which could be responsible for improving the growth as compared to those exposed to temperature alone. Similarly, Nilufa et al. (2000) reported that the pretreatment of GA_3 was effective in promoting germination and increasing amylase expression in low and high temperature stress in wheat seedlings. We observed certain bands which were specific to control or treatment or genotypes; so one can use isozymes for studying genetic variability

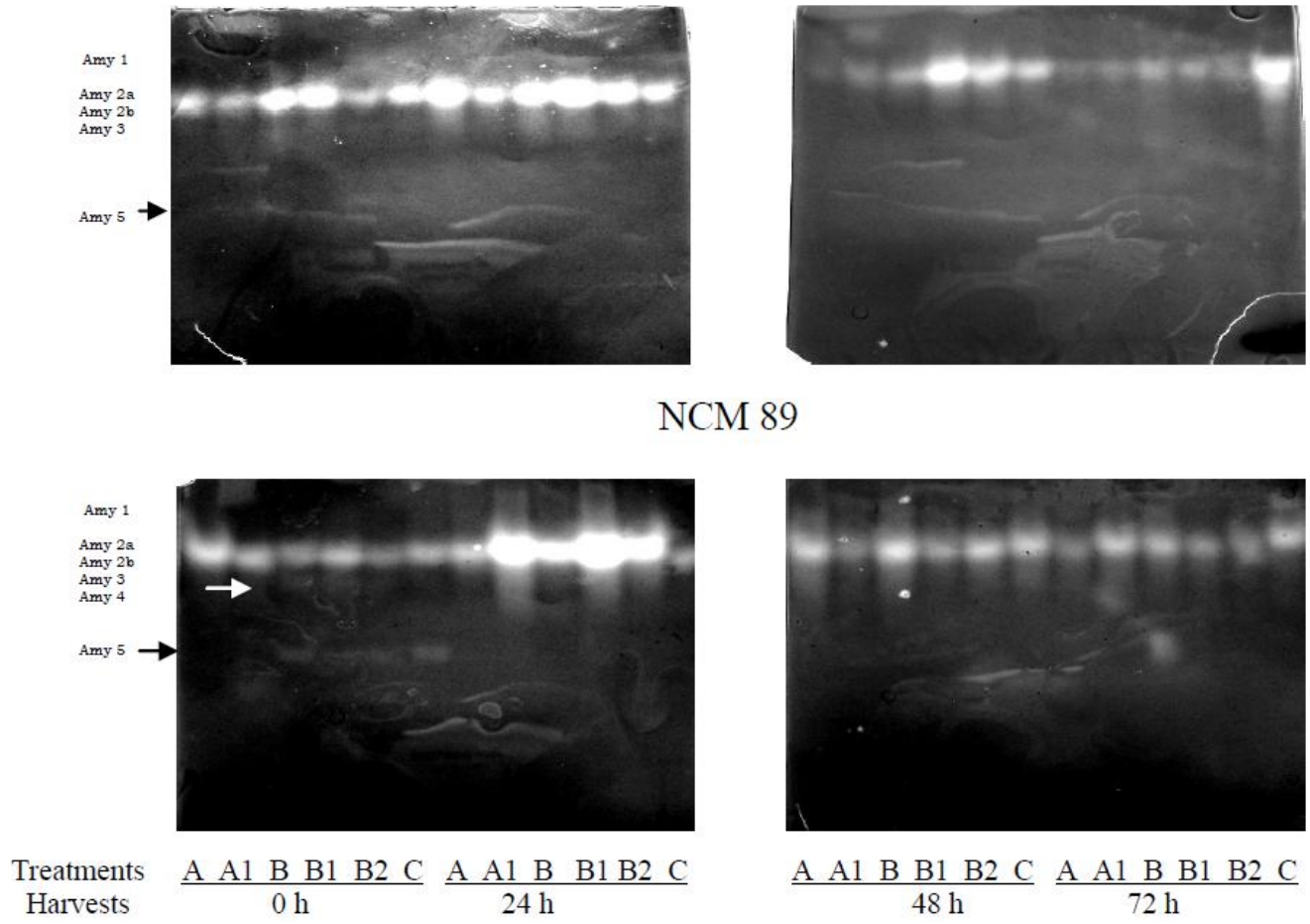

Figure 1. Contd.

between and within different genotypes.

Conclusion

Temperature is one of the most important factors that can affect plant performance in many aspects including the synthesis of amylase enzyme which in turn affect the growth. The current research is an attempt to study the zymogram pattern of amylase of different genotypes of mung bean under lethal as well as pretreatment of mild temperature prior to lethal stress. Furthermore the effect of GA_3 during stress and unstressed conditions on amylase enzyme was also studied and variability was found between treatments as well as genotypes.

REFERENCES

Al-Maqtari MA, Nagi KM, Al-Hazmi SM (2011). Identification and characterization of α-amylase from Yemeni bean (*Dilichos Lablab L*.) seeds. Jordan J. Chem. 6:219-229

Anderson AJ, Sonali PR (2004). Protein aggregation, Radical scavenging capacity, and stability of Hydrogen Peroxide Defense system in heat stressed *Vinca* and sweet pea leaves. J. Amer. Soc. Hort. Sci. 129:54-59.

Brodl MR, Faith CB, Ho DH (1990). Heat shock proteins are not required for the degradation of α-amylase m RNA and the delamellation of endoplasmic reticulum in heat stressed barley aleurone cells. Plant Physiol. 92:1133-41.

Cavusoglu K, Kudret K (2007). Comparative effects of some plant growth regulators on the germination of barley and radish seeds under high temperature stress. Eur. Asia. J. Bio. Sci. 1:1-10.

Chen YM, Seiichiro K, Masuda Y (1986). Enhancing effect of heat shock and gibberellic acid on the thermotolerance in etiolated *Vigna radiata*.I. Physiological aspects on thermotolerance. Physiol. Plant.66:595- 601

Chiba Y, Miki U, Yoji K, Tasuki N, Eiji I (1990). Two groups of intracellular α-amylase isozymes from cultured rice cells. Phytochem. 29:2075-2078

Commuri PD, Duke SH (1997). Apoplastic α -amylase in pea is enhanced by heat stress. Plant Cell Phyisol. 38:625-630.

Davis, B (1964). Disc electrophoresis.II. Method and application to human serum proteins .Ann. N.Y. Acad. Sci. 121:404-427.

Koornneef M, Bentsink L, Hilhorst H (2002). Seed dormancy and germination. Curr. Opin. Plant Biol. 5:33-36

Koshiba T, Minamikawa T (1981). Purification by affinity chromatography of α-amylase. A main amylase in cotyledons of germinating *Vigna radiata* seeds. Plant cell Physiol. 22:979-987.

Leah R, Mundy J (1989). The bifunctional α -amylase/subtilisin inhibitor of Barley: nucleaotide sequence and patterns of seed specific functions. Plant Mol. Biol.12:673-682.

Mitsui T, Akazawa T (1986). Preferential Secretion of R-Type α-Amylase Molecules in Rice Seed Scutellum at High Temperatures. Plant Physiol. 82:880-884.

Mitsui T, Itoh K (1997). The α-amylase multigene family. Trends Plant Sci. 2:255-261.
Nilufa S, Takeshi I, Toshiak M (2000). GA_3 and proline promote germination of wheat seeds by stimulating α-Amylase at unfavorable temperatures. Plant Prod. Sci. 3:232-237
Przybylska J, Blixt S, Parysibaz H, Przybylsk ZZ (1982). Isoenzyme variation in the genus *pisum*. I. Electrophoretic patterns of several enzyme systems. Polon.23:103-21.
Queitsch C, Hong S.W, Vierling E, Lindquist S (2000). Heat shock protein plays a crutial role in thermotolerance in Arabidopsis. Plant Cell, 12:479-792.
Vallee F, Anders K, Yves B, Michel J, Kees WR, Birte S, Richards H (1998). Barley α–amylase bound to endogenous protein inhibitor BASI: crystal structure of the complex at 1.9A^o resolution. Curr. Biol. 6:649-659.
Vartapetian BB, Jackson MB (1997). Plant adaptation to anaerobic stress. Annals Bot. 79:3-20.
Wagenaar S, Lugtenborg TF (1973). α-amylasees of rye seeds. Phytochemistry.12:1243-1247.