Full Length Research Paper

Effect of gibberellic acid on α-amylase activity in heat stressed mung bean (Vigna radiata L.) seedlings

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High temperature is a serious threat that can alter the number of biochemical processes in plants, which may lead to reduce crop yield. Gibberellic acid (GA$_3$) is a plant growth hormone, responsible for growth, stress tolerance and regulation of many enzymes like amylase. Amylase is responsible for growth by hydrolyzing starch into maltose. This experiment was carried out to study the effect of GA$_3$ on α-amylase activity under heat stress conditions of four genotypes of mung bean (NM 19-19, NM 20-21, NM 121-123 and NCM 89). Seeds were sown in Petri dishes and incubated at different temperatures with and without 100 µM GA$_3$. It was found that the lethal temperature was 50°C when exposed for 2 h and reduced α-amylase activity in all genotypes which increased when a mild temperature (40°C, 1 h) prior to lethal temperature was given. The application of GA$_3$ can further alleviate the effect of heat stress by increasing α-amylase activity. Genotypic variations was also observed and activity of amylase and heat stress tolerance index was highest in NM 19-19 and lowest in NM 20-21 for all treatments.

Key words: Mung bean, α-amylase, gibberellic acid, heat stress.

INTRODUCTION

Plant development is under the control of genes, profoundly affected by environment and number of stress conditions, where the most important is temperature (Scandalios, 1974). High temperature stress conditions can create a water deficit in plant tissues which in turn lead to injury of cell membranes and reduction in rate of transpiration, protein synthesis, enzymes and ion uptake and transport (Khalli et al., 2009). High temperature causes reduction in shoot dry mass, growth and net assimilation rates in number of plants (Wahid et al., 2007). Plants have evolved strategies like modulation of different enzymes (Thind et al., 1997) for preventing damage caused by rapid changes in temperature (Senthil-Kumar et al., 2007). Plants are able to survive, respond to, and acclimatize to mild temperatures which allow them to survive lethal temperature (Senthil-Kumar et al., 2007). α-Amylase is an enzyme which is involved in the hydrolysis of starch into maltose and is under nuclear gene control (Scandalios, 1974). It is responsible for active growth, and its activity increased in all plant organs with the progress of mung bean seedling growth (Kaur et al., 2001). Lethal temperature extremely retards seedling growth as well as amylase activity in winter wheat (Sultana et al., 2000). The exogenous application of GA$_3$ during heat stress is able to increase growth and amylase activity (Mitsui and Akazawa, 1986; Cavusoglu and Kudret, 2007). High temperature reduces the endogenous levels of GA$_3$ and its exogenous application counteracts the reduction in the endogenous level that was inhibiting the growth and related enzymes (Yakushikina and Tarasov, 1982; Kabar and Baltepe, 1990). Gibberellic acid was considered to promote seed germination; acting after abscisic acid (ABA)-mediated inhibition of germination has been overcome (Jacobsen et al., 2002).

The purpose of this study was to observe the effect of high temperature on α-amylase activity in the growth mung bean seedlings and to analyze the impact of GA$_3$ over α-amylase activity.

MATERIALS AND METHODS

Seeds of four mung bean genotypes (NM 19-19, NM 20-21, NM 121-123 and NCM 89) were obtained from National Agricultural...
Table 1. Mean sum of squares of amylase activity (µg maltose/mg protein/5 min) in mung bean (Vigna radiate. L) seedlings harvested after temperature and GA3 treatments.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>df</th>
<th>MS</th>
<th>0 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (T)</td>
<td>5</td>
<td>790.71**</td>
<td>518.5**</td>
<td>930.1**</td>
<td>2945.1**</td>
<td></td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>3</td>
<td>339.04**</td>
<td>341.17**</td>
<td>600.5**</td>
<td>1583.8**</td>
<td></td>
</tr>
<tr>
<td>T × G</td>
<td>15</td>
<td>10.81**</td>
<td>5.94**</td>
<td>23.6ns</td>
<td>24.77**</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>9.38</td>
<td>9.46</td>
<td>9.97</td>
<td>20.08</td>
<td></td>
</tr>
</tbody>
</table>

MS, Mean sum of squares; df, degree of freedom; ** highly significant; ns, non significant.

DISCUSSION

The present results reveal that amylase activity increased as the growth of seedlings was proceeded for all genotypes and treatments. Kaur et al. (2001) reported that with the progress of seedlings growth of mung bean, amylolytic activity increased in all organs like roots, shoots and cotyledons to hydrolyze starch. Current observed great reduction in mean a-amylase activity when seedlings were exposed to lethal temperature of 50°C for 2 h, but this decrease in a-amylase activity was somewhat improved when a pretreatment of 40°C (1 h) before lethal temperature was given. Sultana et al. (2000), reported the effect of various temperatures on winter wheat seeds and observed that a-amylase expression during germination was maximum at 29°C, although a high temperature of 38°C prevented the synthesis of a-amylase. Our results are also supported by the findings of Johnston et al. (2002) who reported that heat shock of 40°C induced the synthesis of heat shock proteins (HSPs) and suppresses the synthesis of a-amylase in barley aleurone, however if samples were treated with an increase of 2.5°C every 30 min from the normal temperature of 25°C to the heat shock temperature.
Figure 1a. Amylase activity (µg maltose/mg protein/5 min) in mung bean (Vigna radiata) seedlings of genotype NM 19-19, harvested after temperature and GA₃ treatments. Error bars represent ± SE of three replications. Similar alphabets indicate non significant difference (p≤0.05) among treatments of each harvest.

Figure 1b. Amylase activity (µg maltose/mg protein/5 min) in mung bean (Vigna radiata) seedlings of genotype NM 20-21, harvested after temperature and GA₃ treatments. Error bars represent ±SE of three replications. Similar alphabets indicate non significant difference (p≤0.05) among treatments of each harvest.
Figure 1c. Amylase activity (μg maltose/mg protein/5 min) in mung bean (*Vigna radiata*) seedlings of genotype NM 121-123, harvested after temperature and GA3 treatments. Error bars represent ±SE of three replications. Similar alphabets indicate non-significant difference (p≤0.05) among treatments of each harvest.

Figure 1d. Amylase activity (μg maltose/mg protein/5 min) in mung bean (*Vigna radiata*) seedlings NCM 89, harvested after temperature and GA3 treatments. Error bars represent ±SE of three replications. Similar alphabets indicate non-significant difference (p≤0.05) among treatments of each harvest.
of 40°C, synthesis of α-amylase would be over five folds greater than the heat shocked sample. Therefore the rapidity of the onset of the heat stress appears to be important for plant to acclimatize to high temperature stress in the field.

Present research showed that the activity of amylase was reduced under lethal temperature stress, however the mild temperature treatment prior to lethal temperature treatment induce amylase activity. Exogenous application of GA₃ was able to further induce α-amylase activity during temperature stressed as well as unstressed conditions therefore it is suggested that by the deficiency of amylase there could be the unavailability of maltose which is prerequisite of growth. It is supported by Zeid, (2011), who reported that amylase play an important role in germination by providing starch hydrolyzate, which was decreased due to stress and the deficiency in amylase and maltose was overcome by GA₃ treatment. Gibberellic acid can reduce the adverse effect of high temperature stress during seed germination (Cavusoglu and Kudret, 2007). It is reported that high temperature reduces the endogenous levels of cytokinins, gibberellins and auxins (Kabar and Baltepe, 1990). It is possible that exogenous GA₃ counteracts the reduction in the endogenous levels of plant hormones that promote growth and increase endogenous level of plant hormones. Toyomasu et al. (1994) reported that exogenous GA₃ promotes germination with a reduction in endogenous ABA content.

Heat stress indices have been developed for the evaluation of high temperature stress in plants, stress tolerance index (HST) has been used for comparing genotypic performance across years to environment and used to identify genotypes that perform well under both stress and non stress conditions (Fernandez 1993; Porch 2006). It is concluded that by the pretreatment of GA₃, thermotolerance can further be raised in all four mung bean genotypes, however the HSTI and amylase activity in NM 19-19 was highest and lowest in NM 20-21 (Figures 1a and b). Therefore by looking at the data, it may be suggested that NM 19-19 was considered to be heat tolerant and NM 20-21, as heat sensitive genotype.

REFERENCES
Jacobsen JV, Pearce DW, Poole AY, Pharris RP, Mander LN. (2002).
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