In the present study, the effect of temperature (20/10 °C and 35/20 °C) on the activities of esterase, peroxidase, protein profile and growth parameters in two different plants (Vicia faba cv. Assuit 95/2 and Zea maize cv. Hi Tech 20*30) was investigated. Vicia faba cv. Assuit 95/2 was grown in pots at optimum temperature 20/10 °C exposed to heat stress 35/20 °C. While Zea maize cv. Hi Tech 20*30 was grown in pots at optimum temperature 35/20 °C exposed to cooling stress 20/10°C for 15 days. Behavior of esterase, peroxidase, protein profile, growth parameters, and metabolic constituents of Vicia faba and Zea maize plants varied between increase and shortage under heat stress and also between two organs shoot and root. Under high temperature, the growth parameters (fresh weights, dry weights, length and leaf area) were greatly reduced. It can be observed that the two plants responded to the unfavorable conditions (heat stress) by the change in their activities of enzymes, in the number of isoenzymes which include esterase (EST) and peroxidase (PX) and also changes in their biochemical composition actor in the amount of protein, carbohydrates and proline.

Keywords: Heat stress, Protein profile, Isoenzymes, Proline, Vicia faba cv. Assuit 95/2, Zea maize cv. Hi Tech 20*30.

Introduction

Growth, physiology and metabolism of plants are known to be altered by heat stress (high temperature and chilling (Laudencia-Chingcuanco et al., 2011 and Han et al., 2014). The degree of these alterations depends mainly on the plant type and the plant growth stage. In most cases, there are quantitative and qualitative differences in the degree of plant responses to heat stress (Bita & Gerats, 2013).

Changing temperature beyond limits decreased elongation, shoot length, shoot dry weight and fruit yield (Audusseau et al., 2013). This depression of the relative growth rate varied in various types (Allen et al., 2013). Morphologically the most typical symptom of temperature stress injury to plant is the reduction of growth, and also the reduction of the leaf growth rate and shortening of the period of rapid leaf elongation by producing shorter leaves.

The chlorophyll and total carotenoids contents of leaves decrease in general under temperature stress. Photosynthesis, one of the most important metabolic pathways in plants, is a target of temperature stress. Abscisic acid produced in response to temperature stress decreases turgor in guard cells and thus limits the CO₂ available for photosynthesis (Vikender et al., 2016). During temperature stress, reduction of chloroplast stromal volume and generation of active oxygen species also are thought to play an important role in inhibiting photosynthesis (Vikender et al., 2016). Photosynthesis depends on leaf chlorophyll content and stomatal conductance (Borjigidai & Yu, 2013).

Photosynthesis involves a long chain of mechanisms, enzymes and intermediate products and is regulated by several external and internal factors. Photosynthetic efficiency depends on the sequence of metabolic events such as photochemical reactions on the enzymes involved in carbon assimilation, on the structure of the photosynthetic apparatus and on the transport of photosynthetic intermediates between the subcellular compartments (Vikender et al., 2016). Photosynthetic rate is lower in the temperature-treated plants, but the photosynthetic...
potential is not greatly affected when the rates are expressed with regard to chlorophyll or leaf area. Decreases in photosynthetic rate are due to enhanced photooxidation and changes of enzyme activity induced by temperature stress (Waraich et al., 2012).

Temperature influences most plant processes, including photosynthesis, transpiration, respiration, germination, and flowering (Vikender et al., 2016). As temperature increases (up to a point), photosynthesis, transpiration, and respiration increase. When combined with day-length, temperature also affects the change from vegetative (leafy) to reproductive (flowering) growth. Depending on the situation and the specific plant, the effect of temperature can either speed up or slow down this transition (Collins & Parent, 2017).

Heat stress causes oxidative stress which is marked by the generation of reactive oxygen species (ROS). These species include singlet oxygen, superoxide anion, peroxide hydrogen and hydroxyl radical; all of them are said to damage the cell membrane (Kumar et al., 2012). Plants have developed a series of enzymatic and non-enzymatic detoxification systems to counteract ROS, and protect cells from oxidative damage (Sairam et al., 2002 and Nagesh Babu & Devaraj, 2008). The Antioxidant enzymes such as SOD, CAT, PX, and GR function in detoxification of super oxide and \( \text{H}_2\text{O}_2 \) (Mittler, 2002 and Adak & Datta, 2005). Moreover, He (2010) found that differential responses to heat stress in activities and isozymes of four antioxidant enzymes for two cultivars of Kentucky bluegrass contrasting in heat tolerance.

Therefore, the present work was carried out to study behavior or mechanism tolerance of two plant \( Zea \) maize \( \text{cv. Hi Tech 20*30} \) and \( Vicia \) faba \( \text{cv. Assuit 95/2} \) under heat stress. This study can help in identification of heat tolerance of the plant and better understanding of physiological and biochemical defenses mechanisms associated with heat tolerance.

**Materials and Methods**

The present work was carried out to study the effect of different temperature degrees (20°C day/10°C night) and (35°C day /20°C night) on \( Zea \) maize \( \text{cv. Hi Tech 20*30} \) and \( Vicia \) faba \( \text{cv. Assuit 95/2} \). 

**Results**

**Fresh and dry weight**

\( Vicia \) faba \( \text{cv. Assuit 95/2} \)

The data presented in Fig.1 reveal that, in root and shoot of \( Vicia \) faba \( \text{cv. Assuit 95/2} \),
there is decrease in fresh and dry matter. This reduction was more pronounced in root than shoot. The percent of reduction in fresh weight of root and shoot were about 33.1% and 84.5%, respectively in comparison with the corresponding control plant at the (35°C /20°C). Moreover, the percent of reduction in dry matter yield was about 49% and 78.8% in root and shoot, respectively in comparison with the corresponding control plant at the (35°C /20°C).

**Zea maize cv. Hi Tech 20*30:**
Fresh, dry weights of shoot and dry weight of root were sharply decreased. On other hand, fresh weight of root was slightly increased under (20°C day/10°C night). The percentage of increasing in fresh weight was about 104.7% in root, while the percentage of reduction in fresh weight of shoot was 69.6%. And also, the percentage of reduction in dry matter was about 65.3% and 55.6% in root and shoot, respectively in comparison with the corresponding control plant (Fig 2).
Length of root and shoot:

\textit{Vicia faba} cv. Assuit 95/2

Shoot length was more or less unchanged at (35°C/20°C) degree. On other hand, the root length was sharply decreased with increasing temperature (35°C/20°C) (Fig.1).

\textit{Zea} maize cv. Hi Tech 20*30:

The lengths of root and shoot were sharply decreased with decreasing temperature (20°C/10°C). The percentage of reduction in length was about 86.1% and 57.1% in root and shoot, respectively as compared with the corresponding control plant (Fig. 2).

Leaf area:

\textit{Vicia faba} cv. Assuit 95/2

The results concerning the leaf area (cm²/plant) of the various treatments are presented in Fig. 1. It revealed that the leaf area of cv. Assuit 95/2 exhibited a marked and progressive decrease with increasing temperature and the percentage of this reduction was 34.9 % relative to the control.

\textit{Zea} maize cv. Hi Tech 20*30:

The leaf area of \textit{Zea} maize exhibited a marked and progressive decrease with decreasing temperature and the percentage of reduction was 51.1 % as compared with the control (Fig 2).

Photosynthetic pigments

\textit{Vicia faba} cv. Assuit 95/2

The concentration of Chl. \(a\), Chl. \(b\) and carotenoids were decreased at high temperature and the percentage of reduction was 46.7 %, 20.6% and 34.5%, respectively as compared with absolute control (Fig. 3).

\textit{Zea} maize cv. Hi Tech 20*30

The concentration of Chl.\(a\) decreased at low temperature the percent of reduction were 16.2 % as compared with absolute control. On other hand, the content of Chl. \(b\) and carotenoids increased at low temperature (Fig. 4).

Soluble carbohydrate

\textit{Vicia faba} cv. Assuit 95/2:

Soluble carbohydrate increase in root and shoot. This accumulation was more pronounced in shoot than in root. The percent of increasing 129.6% and 123.7% in shoot and root as compared with absolute control, respectively (Fig. 3).

\textit{Zea} maize cv. Hi Tech 20*30

Soluble carbohydrate highly reduced at low temperature. The percent of reduction 98% and 70% in shoot and root as compared with absolute control, respectively (Fig. 4).

Soluble protein

\textit{Vicia faba} cv. Assuit 95/2:

Soluble protein, in root and shoot marked and progressive increase. The accumulation of soluble protein more pronounced in shoot than root as compared with control (Fig. 3).

\textit{Zea} maize cv. Hi Tech 20*30:

In root and shoot a marked and progressive decrease in soluble protein. These reductions more pronounced in shoot than root as compared with control (Fig. 4).

Proline

*Vicia faba* cv. Assuit 95/2:

The accumulation of proline was observed in two organs but it was more pronounced in root than shoot under high temperature (Fig. 3).

*Zea maize* cv. Hi Tech 20*30:

In shoot a marked and progressive decrease in proline, while the accumulation of proline more or less unchanged in root at low temperature as compared with control (Fig. 4).

Protein profile

Heat stress caused an induction in the synthesis of some new polypeptides in two plant compared to control one (Tables 1,2 and Fig. 5). Generally, the electrophoretically separated protein under heat stress as compared with control revealed (i) Quantitative decline in certain proteins, (ii) Rise in levels of other proteins (density of protein bands), (iii) Some proteins remained unchanged, and (iv) *de novo* induction of specific proteins. In *Vicia faba* cv. Assuit 95/2, levels of proteins with molecular weights 19.766, 13.106 and 11.687 kDa polypeptides in shoot and 60.843, 15.369, 12.982 and 9.777 kDa in root, were common bands under different treatment. Application of low temperature (20/10°C) caused changes in the levels of proteins with molecular weights of 176.845, 89.45, 53.39 and 29.81 kDa in shoot while, 167.542, 101.928, 59.691 and 33.753 kDa in root. These alterations ranged in molecular weight from as low as 8 kDa to as high as 200 kDa. Moreover, the intensity of bands was changed in two organs of both plants (Fig. 5). From the general picture of stress proteins emerging from this work, one point is noteworthy, more protein alterations were scored in stressed plant than unstressed plant in both plant, moreover, theses alterations in protein highly observed in two oranges under study in both plant. It is possible that this differential response in both plants reflect their relative sensitivities to stress conditions.

Isozymes activity

The electrophoresis profiles of PX and EST isozymes showed that isozymes activity was affected by heat stress and a differential PX and EST isozymes profiles between two plants and also in two organs were observed (Fig. 6 and 7).

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Fig. 4. Photosynthetic pigments (mg plant⁻¹), soluble protein and soluble carbohydrate (mg plant⁻¹) of shoot and root of *Zea maize* cv. Hi Tech 20*30 under different degrees of temperature (35/20°C) and 20/10°C).
TABLE 1. SDS electrophoresis analysis of protein bands produced by Vicia faba cv. Assuit 95/2 from the shoot and root under different temperature 35/20°C and 20/10°C.

<table>
<thead>
<tr>
<th>Vicia faba</th>
<th>Shoot</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW/KDa</td>
<td>20/10°C</td>
<td>35/20°C</td>
</tr>
<tr>
<td>Band1</td>
<td>81.298</td>
<td>54.947</td>
</tr>
<tr>
<td>Band2</td>
<td>59.123</td>
<td>29.810</td>
</tr>
<tr>
<td>Band3</td>
<td>33.432</td>
<td>19.766</td>
</tr>
<tr>
<td>Band5</td>
<td>13.106</td>
<td>11.687</td>
</tr>
<tr>
<td>Band6</td>
<td>11.687</td>
<td>12.982</td>
</tr>
<tr>
<td>Band7</td>
<td>9.777</td>
<td>9.777</td>
</tr>
<tr>
<td>Number of bands</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

Electrophoresis patterns of esterase EST and peroxidase PX isozymes showed differences in density and number of bands among control and under heat stress in two plants and also in root and shoot. And also, this difference in density and number of bands are more pronounced under heat stress (Tables 3, 4 and Fig. 6, 7). Peroxidase PX electrophoresis patterns are illustrated in Table 1 and Fig. 6. In Vicia faba, isozyme of esterase under the heat stress show some increase in activity and in the number of bands in root under heat stress treatment at Rf 0.293.

TABLE 2. SDS electrophoresis analysis of protein bands produced by Zea maize cv. Hi Tech 20*30 from the shoot and root under different temperature 35/20°C and 20/10°C.

<table>
<thead>
<tr>
<th>Zea maize</th>
<th>Shoot</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW/KDa</td>
<td>35/20°C</td>
<td>20/10°C</td>
</tr>
<tr>
<td>Band1</td>
<td>140.158</td>
<td>176.845</td>
</tr>
<tr>
<td>Band2</td>
<td>56.365</td>
<td>89.450</td>
</tr>
<tr>
<td>Band3</td>
<td>31.569</td>
<td>53.394</td>
</tr>
<tr>
<td>Band4</td>
<td>25.020</td>
<td>29.810</td>
</tr>
<tr>
<td>Band6</td>
<td>12.258</td>
<td>12.258</td>
</tr>
<tr>
<td>Band7</td>
<td>9.871</td>
<td>9.871</td>
</tr>
<tr>
<td>Band8</td>
<td>8.663</td>
<td>8.663</td>
</tr>
<tr>
<td>Number of bands</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Esterase electrophoresis patterns are illustrated in Table 4 and Fig. 7. In Zea maize cv. Hi Tech 20*30, six bands (monomorphic bands) exhibited with different densities and intensities in root under control and heat stress. In shoot, six bands with different intensities and densities were observed among the profiles of all treatments (control and heat stress). One band was absent at Rf 0.529 in shoot under heat stress. In Vicia faba, isozyme of esterase under the heat stress show some increase in activity and in the number of bands in root and shoot. Also, one and two isozymes bands were induced in shoot and root under heat stress treatments at Rf 0.529 and (0.234 and 0.529), respectively.
Fig. 5. Electrophoretic banding profile of protein and Scanogram of protein profiles extracted from the leaves and root of *Vicia faba* cv. Assuit 95/2 and *Zea maize* cv. Hi Tech 20*30 under different temperature 35/20°C and 20/10°C. Lane 7: shoot under 20/10°C, lane 8: shoot under 35/20°C, lane 6: root under 20/10°C and lane 5: root under 35/20°C of *Vicia faba* cv. Assuit 95/2.
Fig. 7. Electrophoretic banding profile of esterase from the leaves and root of *Vicia faba* cv. Assuit 95/2 and *Zea maize* cv. Hi Tech 20*30* under different temperature 35/20°C and 20/10°C. Lane 7: shoot under 20/10°C, lane 8: shoot under 35/20°C, lane 6: roots under 20/10°C and lane 5: root under 35/20°C of *Vicia faba* cv. Assuit 95/2.


**Discussion**

The plants under study differ in their acclimation to heat stress which exerts adverse effects on plant growth and development. One of the major environmental factors limiting the productivity of crops is heat stress which negatively affects the metabolism of plants and causes important modification in different biochemical and molecular processes (Baurle, 2016). The two plants agree on the negative effects of heat stress on root/shoot length, fresh and dry mass production (Baghour et al., 2002 and Waheed et al., 2007). Reduced growth in stressful medium is a typical phenomenon that has been interpreted as a change in metabolism initiated to resist stress. Moreover, heat stress may induce osmotic stress, oxidative stress and protein denaturation in two plants, which lead to cellular adaptive responses and accumulation of compatible organic solutes such as soluble carbohydrates, amino acids and proline (Dufoo-Hurtado et al., 2013). In the present study, adaptive responses in *Vicia faba* cv. Assuit 95/2 were represented in accumulation of soluble protein in root and shoot, soluble carbohydrate in two organs and also proline. On the other side, in *Zea maize* cv. Hi Tech 20*30*, it was achieved in accumulation of soluble protein in shoot only and reduction of soluble protein in root. On the other hand, soluble carbohydrate and proline in two organs were reduced. In addition to their role in cell water relations, organic solute might helped towards the removal of free radicals, and stabilization of macromolecules, such as proteins, protein complexes and membranes (Bohnert & Shen, 1999; Bray et al., 2000 and Bita & Gerats, 2013). Metabolic imbalances caused by ionic toxicity under heat stress may also lead to oxidative stress and cause accumulation of reactive oxygen species (ROS). Plants employed antioxidants compounds and detoxifying enzymes e.g., (superoxide dismutase, catalase, and enzymes of ascorbate-glutathione cycle) to resist oxidative stress (He, 2010 and Mansoor & Naqv, 2013). Transgenic plants over expressing ROS scavenging enzymes, such as superoxide dismutase (Alschler et al., 2002), ascorbate peroxidase (Wang et al., 1999) and glutathione S-transferase/glutathione peroxidase (Roxas et al., 2000) showed increased tolerance to osmotic, temperature, and oxidative stresses.

The results showed that, three bands were exhibited with different densities and intensities in two plants grown under controlled temperature and under heat stress treatment. Two bands of peroxidase are common bands. Peroxidase activity in roots of two plants under study increased under heat stress treatment. These results are similar to those of Mohamed & Abdel–Hamid (2013) who found that three bands were exhibited with different densities and intensities in cotton genotypes grown under control temperature and heat stress treatment. Two bands are common.
bands which detected at Rf 0.30 and 0.55. Peroxidase activity in the tolerant genotypes of cotton (Giza 85 and Giza 92) increased under heat stress treatment (40°C). Peroxidases are heme-containing oxidoreductases that participate in a number of metabolic processes, such as regulation of cell elongation, lignifications, cross linking of cell wall structural proteins and phenolic oxidation (Kumar et al., 2012 and Silva et al. 2015). Seven bands of esterase electrophoretic patterns were observed among the profile of all treatments. Five bands were common present in some treatments with substantial differences in their intensities and densities. Also, two isozyme bands were induced in two organs of *Vicia fava cv. Assuit* 95/2 and *Zea maize cv*. Hi Tech 20*30* under heat stress treatments. These results are in accordance with those of Mohamed & Abdel –Hamid (2013) who found four bands in some treatments and absence of others (polymorphic) with substantial differences in their intensities and densities in cotton plants. One band which has Rf 0.02 was present in all treatments (monomorphic bands). In addition, two unique bands were detected in the tolerant genotype (Giza 92) under heat stress treatment (Silva et al., 2015).

Three types of modifications are observed in the protein patterns of two organs of *Vicia fava cv. Assuit* 95/2 and *Zea maize cv*. Hi Tech 20*30* some protein bands disappeared, other proteins selectively increased and synthesis of new set of proteins was induced. Some of these responses were observed under heat stress treatments. These results are in accordance with those of Mohamed & Abdel –Hamid (2013). These new protein bands may be the HSPs or the enzyme of the antioxidant systems which plays very important role in providing tolerance against oxidative burst which is in conformity with the observation made by Singh & Khurana (2016). Another approach to understand the molecular basis of heat stress tolerance is to identify stress induced changes in the protein expression (Bita & Gerats, 2013 and Silva et al. 2015).

**Final conclusion**

In conclusion, both non enzymatic and enzymatic antioxidant mechanisms responded distinctly to temperature stress. In two plant under study have divergent of response mechanisms to heat stress. Response two plant (*Zea maize cv. Hi Tech* 20*30* and *Vicia fava cv. Assuit* 95/2) to heat stress involves few common enzymatic and non enzymatic components. This is reflected in plant growth and plant resistance to stress.

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**References**


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Slack of ascorbic acid peroxidase, trifoliate, and total leaf area in plants under oxidative stress.

DOAA MOSTAFA
