**Physiological Response of Lupine Plant (Lupinus termis L.) To Heat Hardening**

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**Abstract:** Two pot experiments were conducted in two successive seasons (2007-2008) in the screen of National Research Centre, Dokki, Giza, Egypt to study the effect of heat hardening treatments on growth and biochemical constituents of lupine plant. The obtained results indicated that heat hardening treatments significantly affected vegetative growth criteria and biochemical constituents (Carbohydrate %, nitrogen %, crude protein % and protein pattern in the seeds). The most effective treatment in this respect was that of heat hardening at 50 % for 10 minutes which resulted in the highest values of the studied characteristics.

**Key words:** Lupine (Lupinus termis L.) • Heat hardening • Biochemical constituents

**INTRODUCTION**

Lupine plant (*Lupinus termis* L.) was used for green manuring on the light lands. Seeds after boiling and prolonged steeping in water to get rid of their bitter and poisonous alkaloids are used as stock feed and for human consumption as important source of protein. The germ meal of white lupine seeds contained about 44 % crude protein and the quality of protein is similar to soybean protein [1, 2]. In addition, most of lupine species contain appreciable amounts of oil compared to other legumes with exception of soybean and peanut [3]. The medicinal value and uses of lupine described by Grieve [4], who stated that bruised seeds after soaking in water, could be sometimes used as an external application to ulcers and internally as anthelmintic, diuretic and emmenagogue. Recently, lupine extract is used for the recovery of diabetes mellitus.

Hardening and protection of seeds of plants against heat stress could be induced by exposure of seeds or seedlings to relatively higher sublethal temperature above the maximum temperature of environment resulted in better vegetative growth, favourable accumulation of certain secondary metabolites values as for *Hyoscyamus muticus* L. [5] and for wheat growth, yield and protein pattern [6].

The present study aimed to investigate the effect of heat hardening treatment on vegetative growth, seed production and changes in important biochemical constituents of lupine plant.

**MATERIALS AND METHODS**

Two pot experiments were carried out during two successive seasons at the screen of National Research Centre, Dokki, Giza, Egypt. Seeds of lupine (*Lupinus termis*, L.) were secured from Agriculture Research centre, Ministry of Agriculture, Giza, Egypt. Seeds were sown at October 2007/08 and 2008/09, respectively in pots 30 cm in diameter filled with loamy and sand soil (2:1 in proportion). Each experiment was laid out in a completely randomized design with four replicates, after one month from sowing, plants were thinned leaving 3 plants per pot. Each pot received equal amounts of water and fertilizers. Phosphorus as calcium super phosphate (15.5 % P₂O₅) was added to the soil before sowing at the rate of 4 g/pot, while nitrogen as ammonium sulphate (20.5 % N) at the rate of 2 g/pot and potassium sulphate (48 % K₂O) at the rate of 1 g/pot were added to the soil, 30 and 60 days after sowing.

**Treatments:** Lupine seeds were soaked in water till they obtained moisture 55 % of their original dry weight to activate the embryo before exposure to heat hardening, which were carried out in an water-bath previously adjusted to the required heat hardening treatments at 45, 50 and 55°C for the periods of 10, 20 and 40 minutes. These pre-sowing treated seeds in addition to the untreated ones (attained the same moisture content and kept at room temperature) were sown at pots as above-mentioned.
Sampling and Growth Criteria: Three samples were drawn out during the growth season; the first sample was at vegetative stage, the second one was at flowering and the third stage was at harvest stage (full mature seeds). Three replicates were drawn from different pots of each treatment; each replicate consisted of four plants. Vegetative characteristics were plant height, number of leaves/plant, fresh and dry weights of different plant organs (g/plant), straw yield (g/plant) and 1000 seeds weight (g).

Determination of Biochemical Constituents:
- Total carbohydrate content % (on dry weight basis) was determined in the seeds according to the methods of Dubois et al. [7].
- Nitrogen and crude protein percentage in the seeds were determined according to method of A.O.A.C. [8].
- Protein patterns were estimated in fresh vegetative part (Kept at -20°C till analysis). Protein pattern was conducted according to SDS-PAGE method, Laemmli [9] using sodium dodecyle sulphate, polyacrylamide gel electrophoresis (SDS-PAGE). Marker protein of known molecular weights was used for identification of protein of the present samples.

Statistical Analysis: Data obtained was subjected to standard analysis of variance procedure. The values of L.S.D. were calculated whenever F-values of significant at 5 % level as reported by Snedecor and Cochran [10].

### RESULTS AND DISCUSSION

#### Effect of Heat Hardening on Vegetative Growth and Yield:
Data presented in Table 1 indicated that all heat hardening treatments significantly increased plant height (cm) at all used temperature degrees and times during both sampling dates (vegetative growth and full flowering stages). The most effective treatments in this respect was that of heat hardening at 50°C for 10 min. which recorded the highest values of plant height (44.3 and 56.5 cm at vegetative and full flowering stages) compared to 36.0 and 44.3 cm of untreated control at the same stages. Numbers of leaves/plant showed the same trend of plant height and markedly increased as a result of all pre-sowing heat hardening treatments. Heat hardening of the seeds at 50°C for 10 minutes resulted in the highest number of leaves produced by the plant, recorded 30 leaves/plant at full flowering compared to 18.7 for untreated plants. As for the effect of heat hardening on dry weight of leaves and stems of lupine plants, the obtained results given in Table 1 indicated that leaves and stems dry weights of lupine plants were significantly increased as a result of heat hardening at all used degrees of temperature at both vegetative and full flowering stages. Generally, the dry weights of leaves and stems were slightly higher during full flowering stage than that of vegetative one. The most effective treatment of heat hardening in this respect was 50°C for 10 minutes either in the vegetative or full flowering growth stage. This positive effect of heat hardening on plant growth was previously reported by Gamal El-Din [5] on *Hyoscyamus muticus* L. Gamal El-Din

<table>
<thead>
<tr>
<th>Characters</th>
<th>Full vegetative stage</th>
<th>Full flowering stage</th>
<th>Yield (Harvest stage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height (cm)</td>
<td>Leaves number / plant</td>
<td>Dry wt. leaves g/plant</td>
<td>Dry wt. branches g/plant</td>
</tr>
<tr>
<td>Control (Untreated)</td>
<td>34.3</td>
<td>16.7</td>
<td>7.17</td>
</tr>
<tr>
<td>45°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min.</td>
<td>36</td>
<td>19.3</td>
<td>7.28</td>
</tr>
<tr>
<td>20 min.</td>
<td>39.7</td>
<td>20</td>
<td>7.38</td>
</tr>
<tr>
<td>40 min</td>
<td>39.7</td>
<td>20</td>
<td>7.39</td>
</tr>
<tr>
<td>50°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min.</td>
<td>44.3</td>
<td>20.7</td>
<td>7.71</td>
</tr>
<tr>
<td>20 min.</td>
<td>43.5</td>
<td>20.5</td>
<td>7.66</td>
</tr>
<tr>
<td>40 min</td>
<td>43.5</td>
<td>19</td>
<td>7.64</td>
</tr>
<tr>
<td>55°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min.</td>
<td>36</td>
<td>18.7</td>
<td>7.63</td>
</tr>
<tr>
<td>20 min.</td>
<td>35</td>
<td>17.5</td>
<td>7.53</td>
</tr>
<tr>
<td>40 min</td>
<td>34.7</td>
<td>17.5</td>
<td>7.46</td>
</tr>
</tbody>
</table>

L.S.D. 0.05 | 0.08 | 0.08 | 0.61 | 2.7 | 2.7 | 0.02 | 0.11 | 0.09 | 0.42 | 0.4 | 0.47 | 0.87 | 8.04 |
Table 2: Effect of Heat Hardening on some biochemical constituents in lupine seeds (Means of two seasons 2007 and 2008).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carbohydrate (%)</th>
<th>Total nitrogen (%)</th>
<th>Crude protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Untreated)</td>
<td>12.04</td>
<td>6.50</td>
<td>40.63</td>
</tr>
<tr>
<td>45°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min.</td>
<td>16.97</td>
<td>7.12</td>
<td>48.88</td>
</tr>
<tr>
<td>20 min.</td>
<td>17.97</td>
<td>7.37</td>
<td>46.06</td>
</tr>
<tr>
<td>40 min</td>
<td>20.63</td>
<td>7.83</td>
<td>44.5</td>
</tr>
<tr>
<td>50°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min.</td>
<td>21.29</td>
<td>8.43</td>
<td>52.69</td>
</tr>
<tr>
<td>20 min.</td>
<td>19.97</td>
<td>8.21</td>
<td>51.31</td>
</tr>
<tr>
<td>40 min</td>
<td>16.30</td>
<td>7.86</td>
<td>49.13</td>
</tr>
<tr>
<td>55°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min.</td>
<td>14.97</td>
<td>7.03</td>
<td>43.94</td>
</tr>
<tr>
<td>20 min.</td>
<td>13.92</td>
<td>6.82</td>
<td>42.63</td>
</tr>
<tr>
<td>40 min</td>
<td>12.64</td>
<td>6.77</td>
<td>42.31</td>
</tr>
<tr>
<td>L.S.D. 0.05</td>
<td>0.44</td>
<td>0.26</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Table 3: Protein banding patterns as molecular weight (KDa) in fresh vegetative part of lupine as affected by heat hardening treatments.

<table>
<thead>
<tr>
<th>Temp.</th>
<th>45°C</th>
<th>50°C</th>
<th>55°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>10 min</td>
<td>20 min</td>
<td>40 min</td>
</tr>
<tr>
<td>Duration</td>
<td>10 min</td>
<td>20 min</td>
<td>40 min</td>
</tr>
<tr>
<td>97.32</td>
<td>81.33</td>
<td>95.55</td>
<td>90.58</td>
</tr>
<tr>
<td>74.48</td>
<td>60.96</td>
<td>82</td>
<td>51.36</td>
</tr>
<tr>
<td>37.36</td>
<td>41.2</td>
<td>59.59</td>
<td>37.22</td>
</tr>
<tr>
<td>32.12</td>
<td>34.99</td>
<td>40.22</td>
<td>29.09</td>
</tr>
<tr>
<td>--</td>
<td>--</td>
<td>--</td>
<td>17.61</td>
</tr>
<tr>
<td>--</td>
<td>--</td>
<td>--</td>
<td>14.06</td>
</tr>
</tbody>
</table>

and Reda [6] on wheat and Gamal El-Din [11] on fenugreek plants. The promoting effect of heat hardening might be attributed to more synthesis of IAA and root induction under heat shock treatment comparing with control plants as reported by Karas et al. [12]. Concerning, the effect of heat hardening on yield of lupine plant, the present results indicated that all heat hardening treatments resulted in significant increases in seed yield and straw yield of lupine plant. It could be observed that heat hardening at 50°C resulted in higher yield than 45°C or 55°C. The most effective treatment in this respect was that of 50°C for 10 minutes which recorded the highest value (4.75 g/plant) compared to 2.57 g/plant for the untreated plants. Heat treatment at 55°C for 40 min was ineffective in this concern. Weight of 1000 seeds was also positively affected by different treatments of heat hardening. Hardening of lupine seeds at 50°C resulted in higher increases in 1000 seeds weight than the two other hardening temperatures (45, 55°C). The most effective treatment in this respect was that of hardening at 50°C for 10 minutes. This positive effect of heat hardening on the yield of seeds and straw was previously reported by Gamal El-Din [11] on fenugreek plant.

Effect of Heat Hardening on Chemical Constituents of Lupine Plant: Data presented in Table 2 indicated that total carbohydrate and total nitrogen contents of lupine seeds were significantly increased as a result of heat hardening at all temperature degrees and time used. Heat hardening at 50°C was more effective in this respect than the other two temperature degrees (45, 55°C). The most effective treatment in this respect was that of heat hardening at 50°C for 10 minutes. Crude protein content followed the same trend of total carbohydrate and nitrogen content, reaching the maximum values by heat hardening at 50°C for 10 minutes which recorded 52.69% as compared by 40.63% in the untreated control. The positive effect of heat hardening on increasing carbohydrate content in the seeds of many plants was previously reported by Talaat and Shalaby [13] on Calendula officinales L. Tarraf [14] on fenugreek, Gamal El-Din and Reda [6] on wheat plant and Gamal El-Din [11] on fenugreek plants. This promoting effect might be attributed to increasing photosynthetic pigments activity and enzymatic activity (Chlorophyllase and peroxidase activity, Reda and Mandoura [15]. The positive effect of pre-sowing heat hardening on total nitrogen and crude
protein was indicated by Tarraf [14] on fenugreek, Gamal El-Din and Reda [6] on wheat and Gamal El-Din [11] on fenugreek. This promoting effect could be attributed to membrane changes during hardening or due to the accumulation of membrane stability compounds and increasing most of amino acids [14].

**Effect of Heat Hardening on Protein Pattern:** Data presented in Table 3 illustrated that the total number of protein bands separated by SDS-PAGE in vegetative parts of lupine plant varied according to heat hardening treatments. The maximum number of protein bands (8 bands) was separated at treatment of heat hardening at 50°C for 20 minutes. Protein of molecular weights ranged of 15-30 KDa appeared at most treatments as well as control and more increased in treatment of heat hardening at 50°C for 20 minutes. Detection of protein of mol.wt ranged from 15-30 KDa in most of heat hardening treatments partially explain the role of heat hardening in promotion of growth and enhancement of metabolism. Brag et al. [16] stated that the range of molecular chaperons (15-30 KDa) prevented aggregation of protein. It could be suggested that changes evoked by heat hardening in the pattern of protein in lupine plant might be due to induction of changes in protein complements. In this concern, the products of some genes were increased and other was decreased [17].

In conclusion, heat hardening of lupine seeds especially at 50°C for 10 minutes increased vegetative growth, seed yield, total carbohydrates, total nitrogen, crude protein content and protein pattern in the produced seeds.

**REFERENCES**