Chickpea response to ascorbic acid foliar application at vegetative and reproductive stages

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Key words: Ascorbic acid, Chickpea, Foliar application, Reproductive stage, Vegetative stage.

Abstract

The growth and yield response of chickpea to foliar application of ascorbic acid (AA) at vegetative and reproductive stages of the plant under field conditions was studied in a factorial experiment with three factors based on randomized complete block design. The first factor was the stage of AA application with two levels of vegetative and reproductive stage application. The second factor was AA concentrations of 0, 100, 200 and 300 mg/l and two Desi type cultivars of chickpea including Pirooz and Kaka were studied as the third factor levels. Spraying of chickpea plants with AA during the vegetative stage of plant significantly enhanced the growth and crop productivity compared with reproductive stage application. By treating the plant with the higher concentrations of AA (i.e 200 and 300 mg/l) significant increases in seed yield were recorded at the rates of 30 and 27 percent in comparison with control treatment and through regression analysis the optimum concentration of AA to obtain maximum seed yield was estimated about 226 mg/l in this experiment.

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Introduction
Terminal drought stress and high temperature during reproductive stage of crop plants especially under rainfed conditions are considered as critical constraints to agricultural production in Iran. Chickpea (*Cicer arietinum* L.) as the most important grain legume in Iran is predominantly cultivated under rainfed conditions and subjected to terminal drought stress (Soltani *et al.*, 2005) which can be resulted in significant crop losses.

Among the environmental stresses, drought stress is one of the most critical factors to plant growth and productivity (Shao *et al.*, 2008). Water deficit stress, induces numerous biochemical and physiological responses in plants (Pattanagul and Madore, 1999). It has been shown that deleterious effect of various environmental stresses is at least partially due to the oxidative damage (Smirnoff, 1998; Lu *et al.*, 2008) resulting from reactive oxygen species such as superoxide anion radical, hydrogen peroxide and hydroxyl radical (Apel and Hirt, 2004). Plants have evolved some mechanisms to protect themselves from the effects of reactive oxygen species by using enzymes such as superoxide dismutase, catalase, peroxidase, glutathione reductase, polyphenol oxidase and non-enzymic ascorbate and glutathione (Agarwal and Pandey, 2004). Therefore one of the proper ways for promoting the stress resistance in plants may be increasing the level of antioxidant compounds. Ascorbic acid (AA) is regarded as one of the most effective antioxidants against various stresses in plants (Conklin, 2001; Khan *et al.*, 2011). It has a great effect on physiological processes such as cell division, plant growth and the biosynthesis of cell wall, metabolites and phytohormones. Moreover ascorbic acid plays a vital role in renovation of chloroplast and mitochondrion membranes (Barth *et al.*, 2004; Pavet *et al.*, 2005; Barth *et al.*, 2006). High endogenous ascorbic acid in plants is necessary to counteract oxidative stress in addition to regulating other metabolic processes of plant (Athar *et al.*, 2008). Ascorbic acid level in plant organs can be elevated by its exogenous application methods such as foliar spray, seed priming or rooting medium (Chen and Gallie, 2004; Athar *et al.*, 2008). In an experiment Farjam *et al.* (2014) demonstrated that the exogenous application of ascorbic acid and salicylic acid on chickpea plants increased the plant biomass significantly compared with control plants and cell membrane damage in chickpea leaves was decreased through foliar application of ascorbic acid and salicylic acid. Shalata and Neumann (2001) reported that the addition of 0.5 mM ascorbic acid could increased the survival percentage of tomato seedlings from 0% to 50% under severe salt-stress conditions of 300 mM NaCl around root system.

The problem of water deficit conditions and inappropriate dispersion of precipitations in Iranian farm lands have been resulted in an uncertain condition for crop production. In this condition protecting the crops from drought stress damages especially during the critical periods of plant growth can be of great value. Therefore this experiment was aimed to study the influence of ascorbic acid concentration and the time of its foliar application on chickpea under field conditions.

Materials and methods
Field conditions and treatments
The site of this experiment was the agricultural research farm of Islamic Azad University, Sanandaj Branch (35° 10′ N, 46° 59′ E; 1393 m above sea level) during the spring and summer 2013 under rainfed conditions. The long-term values of mean temperature and annual precipitation in the region are 13.35 °C and 471 mm respectively. Soil texture of the farm was clay loam and the electrical conductivity and pH were 0.40 dS/m and 7.9 respectively. The experiment was arranged in a factorial layout with three factors based on a randomized complete block design in three replications. The first factor was the stage of foliar application of ascorbic acid (AA) with two levels of vegetative stage application and reproductive stage application. The second factor was AA concentration in four levels of control (spraying with distilled water or 0 mg/l AA), 100, 200 and 300 mg/l AA and two Desi type cultivars of chickpea including Pirooz and Kaka were studied as the third
factor levels. Each experimental plot contained 4 sowing rows 2 m in length with 25 cm space between rows and 5 cm between plants in each row.

**Agronomic management and measurements**

Hand sowing operation was accomplished on 17 March 2013. Foliar spraying of the plants with distilled water (control) and ascorbic acid levels was done 3 times at each relevant stages (vegetative or reproductive) with 4 days interval. Different plant traits such as plant height, seed yield, biological yield and harvest index were recorded.

**Data analysis**

All collected data were subjected to the operations of ANOVA (analysis of variance) and treatment means were compared with Duncan’s multiple range test at $P \leq 0.05$. The statistical operations were accomplished using SAS software version 9.1 (SAS Institute, Cary, NC).

**Results and discussion**

The effects of application time and concentration of ascorbic acid on plant height were not significant but the cultivar effect on this trait was significant otherwise Kaka cultivar recorded a higher plant height than Pirooz (Tables 1 and 2).

### Table 1. Analysis of variance of chickpea characteristics affected by AA application stage, AA concentration and cultivar.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Plant height</th>
<th>Seed yield</th>
<th>Biological yield</th>
<th>Harvest index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>3.206&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>68027.324&lt;sup&gt;†&lt;/sup&gt;</td>
<td>735620.842&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>0.0002&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Application stage (A)</td>
<td>1</td>
<td>3.255&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>88665.43&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>420.199&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Concentration (B)</td>
<td>3</td>
<td>5.079&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>64225.456&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>73184.889&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.0179&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>501.167&lt;sup&gt;**&lt;/sup&gt;</td>
<td>12903.846&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>11257.753&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>A×B</td>
<td>3</td>
<td>14.482&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>5261.775&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>40729.815&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.0041&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>A×C</td>
<td>1</td>
<td>1.960&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1282.056&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>206684.561&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.0008&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>B×C</td>
<td>3</td>
<td>5.602&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>17968.769&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>60057.348&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.0012&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>A×B×C</td>
<td>3</td>
<td>1.220&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>17076.605&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>21075.344&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.0015&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>8.546</td>
<td>19157.106</td>
<td>166793.928</td>
<td>0.0012</td>
</tr>
<tr>
<td>CV (%)</td>
<td>9.76</td>
<td>21.28</td>
<td>16.71</td>
<td>12.76</td>
<td></td>
</tr>
</tbody>
</table>

<sup>ns, † and ‡**: Non significant and significant at 5 and 1% levels of probability, respectively.</sup>

### Table 2. Main effects of AA application stage, AA concentration and cultivar on growth and yield parameters of chickpea.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Seed yield (kg/ha)</th>
<th>Biological yield (kg/ha)</th>
<th>Harvest index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA application stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td>29.7 a</td>
<td>693.4 a</td>
<td>2441.6 a</td>
<td>28.3 a</td>
</tr>
<tr>
<td>Reproductive</td>
<td>30.2 a</td>
<td>607.4 b</td>
<td>2447.5 a</td>
<td>24.9 b</td>
</tr>
<tr>
<td>AA concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mg/l</td>
<td>30.5 a</td>
<td>545.8 b</td>
<td>2519.5 a</td>
<td>21.3 c</td>
</tr>
<tr>
<td>100 mg/l</td>
<td>30.4 a</td>
<td>655.8 ab</td>
<td>2466.1 a</td>
<td>26.8 b</td>
</tr>
<tr>
<td>200 mg/l</td>
<td>29.4 a</td>
<td>709.3 a</td>
<td>2334.8 a</td>
<td>30.4 a</td>
</tr>
<tr>
<td>300 mg/l</td>
<td>29.8 a</td>
<td>690.6 a</td>
<td>2457.8 a</td>
<td>28.0 ab</td>
</tr>
<tr>
<td>Cultivar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pirooz</td>
<td>26.7 b</td>
<td>634.0 a</td>
<td>2459.9 a</td>
<td>25.7 a</td>
</tr>
<tr>
<td>Kaka</td>
<td>33.2 a</td>
<td>666.8 a</td>
<td>2429.2 a</td>
<td>27.6 a</td>
</tr>
</tbody>
</table>

Values in each group of a column with the same letters are not significantly different at $P \leq 0.05$ according to Duncan’s multiple range test.

Seed yield was statistically influenced by the time of application and ascorbic acid concentration whereas the effect of cultivar on seed yield was not significant (Table 1). Foliar application of AA at vegetative stage significantly increased seed yield compared with reproductive stage application (Table 2). Application
of 200 mg/l AA resulted in the highest rate of seed yield (709.3 kg/ha) which was about 30 percent higher than the recorded seed yield of control (0 mg/l) treatment, however there was no significant difference between 200 and 300 mg/l treatments regarding seed yield (Table 2). The improvement of seed yield by the vegetative time application indicated that the plant could benefit from growth promotion of AA during its vegetative stage in order to establish a certain base to attain more productivity. Similarly in an experiment conducted by Dolatabadian et al. (2010) the effect of ascorbic acid foliar application in four concentrations of 0, 50, 100 and 150 mg/l on various traits of corn was studied and a significant increase in corn grain weight was recorded by treating the plants with 150 mg/l of AA.

The best-fitting regression equation between AA concentration and seed yield showed that the optimum concentration of AA to obtain maximum seed yield in this experiment was about 226 mg/l (Fig. 1).

![Fig. 1. Seed yield response to ascorbic acid concentration.](image)

Analysis of data showed that the experimental factors of application time, AA concentration and cultivar had no significant effect on biological yield of chickpea. On the other hand harvest index of the plant was statistically affected (P≤0.01) by the application stage and AA concentration (Table 1). Foliar application of AA at vegetative stage of chickpea plants significantly increased harvest index as compared with reproductive stage application and among the studied concentrations of AA, the treatment of 200 mg/l AA recorded the highest rate of harvest index (Table 2). The changing trend of harvest index was very similar to seed yield alterations. Harvest index is an indication of the rate of assimilates transport from source to sink and it is clear that with increasing the rate of photo-assimilates transport from green leaves to the seeds, the proportion of seed weight to the total biomass will increased.

**Conclusion**

The obtained results of this experiment indicated that spraying of chickpea plants with AA at the vegetative stage of plant compared with reproductive stage resulted in more improvement in crop productivity. Application of higher doses of AA (i.e 200 and 300 mg/l) led to significant increase in seed yield at the rates of 30 and 27 percent in comparison with control treatment and regression analysis revealed that the concentration of AA to optimize seed yield was 226 mg/l under conditions of this study.

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