Agro-physiological responses of Tepary bean to planting patterns and plant densities

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ABSTRACT- Planting pattern and plant density are efficient management tools for maximizing crop yield by reducing soil surface evaporation and optimizing resource utilization such as light, nutrients, and water. A two-year (2017-18) field trial was conducted to determine the effects of plant density and planting pattern on some agro-physiological and biochemical traits of Tepary bean (Phaseolus acutifolius L.) at the Research Station of Islamic Azad University, Arak Branch, Iran. The experiment was performed as a split-plot arranged in a randomized complete block design with three replications. The treatments were two planting patterns (one-row and two-row plantations) and three plant densities (30, 40 and 50 plants per m²). The results showed that one-row planting pattern and 40 plants per m² improved seed yield by 75% and 65% compared to 30 and 50 plants per m² in one-row planting pattern in the second year, respectively. The plant chlorophyll content increased by 20% with two-row plantation and medium plant density compared to high and low plant density in the first year. The highest accumulation of seed carbohydrates (38.26 mg. g⁻¹ FW) was obtained with two-row cultivation and medium plant density in the second year. The relative seed water content significantly decreased with one-row plantation and low plant density compared to a two-row plantation and medium plant density. The least malondialdehyde and proline aggregation (3.2 and 225 µ mol. g⁻¹ FW, respectively) was achieved with two-row plantation and medium plant density. Finally, a two-row planting pattern with medium-density cultivation is recommended to obtain optimal Tepary bean seed yield in the region.

INTRODUCTION

Tepary bean (Phaseolus acutifolius L.) belongs to the genus Phaseolus as a diploid and predominantly self-fertilizing crop (Gujaria-Verma et al., 2016). Its genome (647 Mbp) is slightly greater than that of common bean (P. vulgaris L.) (637 Mbp) and Lima bean (P. lunatus) (622 Mbp) (Assefa et al., 2019). This plant is an edible bean and it is resistant to adverse environmental conditions including drought, salinity, heat stress, pests, and microorganisms affecting ordinary beans (Pinto Americano and Black Jamapa) (P. vulgaris L.) (Heredia-Rodriguez et al., 2019). The nutritional quality of Tepary beans is promising for human consumption due to their high seed protein content (Mhlaba et al., 2018).

Plant density is a predominant management factor that affects plant growth, adjusting capacity to capture solar radiation, water, and nutrients (Venugopalan et al., 2014). Plant density is one of the main factors that have an essential role on the growth, yield, and quality of plants (Onat et al., 2016). At low plant densities, seed yield in faba bean (Vicia faba) is limited by the number of plants; however, it is decreased due to increasing number of aborted pods and barren stalks at higher plant densities (Gezahegn, 2019). Bennet and Adams (1977) reported that the intensity of light penetration into the canopy was insufficient at high densities of beans (P. vulgaris L.). Under high densities of beans, production of photosynthetic material in the plants decreased, and the number of hollow pods increased, resulting in yield reduction.

The spatial pattern of plants can significantly affect competition between plants and weeds (Liu et al., 2017). Low light intensity at high plant density causes less light penetration, reducing photosynthetic efficiency and bean (Phaseolus vulgaris L.) pod number and quality.
In a study conducted by Ikeda (1992), the effect of planting arrangement in soybean (Glycine max L.) was investigated. It was found that the seed yield decreased with increasing the distance between rows and decreasing the distance between seeds on rows (Ikeda, 1992). It was reported that the spatial distribution of maize (Zea mays L.) leaf area index significantly affected the sunlight rate and soil moisture, and therefore determining corresponding weeds and soil microorganisms with changes in leaf area index (Aquino Portes and Melo, 2014).

Iran has arid and semiarid climates which mostly characterized by low annual precipitation averages of 25 cm or less. There was a lack of information regarding the plant density and row spacing of Tepary bean. Therefore, the experiments of this study were conducted in a semiarid area of Iran to determine the effects of plant density and planting pattern on biochemical traits of Tepary bean, including seed yield and photosynthetic pigments.

MATERIALS AND METHODS

Experimental Location, Plant Material, and Growth Conditions

Black Tepary bean seeds (P. acutifolius L.) were received from Pakan Bazr Company and sown at the research station of Arak Islamic Azad University (34° 30’ N, 40° 41’ E and altitude of 1779 m above sea level) during 2017-2018. The minimum and maximum temperatures were 0°C in January–February and 36 °C in July–August, respectively, with a mean annual temperature of 13.7 °C (Fig. 1). The mean annual rainfall was 340 mm. Soil properties of the experimental field are presented in Table 1.

Treatments

The experiment was conducted as a split-plot based on randomized complete blocks design with three replications. The main plot included two planting patterns (one-row and two-row plantation), and the subplots included three plant densities (30, 40 and 50 plants per m²) with a 4×3 m plot size. The seeds were hand-sown at a depth of 5 cm in the soil on 22nd June 2017 and 16th June 2018. The plants were irrigated using a drip system. During the experiment, no pesticide and chemical fertilizers were used, and weeds were manually removed. In both years, plants were harvested at the end of the pod filling stage in October.

Seed Yield

Seed yield was estimated by measuring the total weight of seeds after threshing (Toker, 2004).

Harvest Index (HI)

HI is a correlation between seed yield and biological yield (plant total weight including stem, leaf, pod and seed weights) which determined using the following equation.

\[
HI (%) = \frac{\text{seed yield}}{\text{biological yield}} \times 100
\]

Measuring the Soluble Sugar (Carbohydrate)

The soluble sugar was measured using solutions of 95% and 70% ethanol as discussed below: 0.5 g. of grain was crushed with liquid nitrogen and ground with 5 ml of 95% ethanol to release sugar. Next, 70 % ethanol was added two times, each time 5 ml. The solution was centrifuged at 3500 rpm for 10 min, and kept in the refrigerator for one week. Subsequently, 0.1 ml of the stored stock was mixed with 3 ml Antron (150 mg Antron in 100 ml 72% sulfuric acid). The solution was placed in the boiling water bath at 90 °C for 10 min. Light absorption of samples were measured at 625 nm using a UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan) and the amount of sugar (mg.g⁻¹ FW) in the leaf solutions were determined using the light absorptions of samples at 625 nm and a standard glucose curve as reported by Irigoyen et al., (1992). For this, solutions with 0, 1, 2, 3, 4, 5, 7, 10 ppm concentrations were used to prepare a standardized diagram (Irigoyen et al., 1992).
Chlorophyll (Chl) Assay

The leaf chlorophyll a, chlorophyll b, and carotenoids contents were extracted according to Arnon (1949) and Davies (1976). 200 mg of fresh leaf samples were homogenized in 8 ml of 80% acetone. Subsequently, the mixture was centrifuged at 4°C for 15 min (3000 rpm). Supernatants were used to analyze chlorophyll content. Absorbance was determined at 480, 510, 645, and 663 nm utilizing a spectrophotometer. Because the maximum light absorption is done by chlorophyll a and b in purple, blue and red regions and by carotenoids in purple and blue regions, the plant pigments of chlorophyll a, chlorophyll b, and carotenoid were determined using the following equations.

Chlorophyll a (mg·g⁻¹ FW) = [12.7× (A663) - 2.69 × (A645)] V/1000W
Chlorophyll b (mg·g⁻¹ FW) = [(22.9 × A645) - 4.68 × (A663)] V/1000W
Carotenoids (mg·g⁻¹ FW) = [7.6 × (A480) - 1.49× (A510)] V/1000W

V: Volume of filtered solution (centrifugate solution)
A: Absorption of light at wavelengths of 480, 510, 645 and 663 nm
W: Wet weight of the sample in terms of grams

Relative Water Content (RWC) Measurement

Leaf relative water content was determined using the following equation (Barris and Weatherley, 1962)

RWC% = (FW - DW) / (TW - DW) × 100

where FW is fresh weight, TW is leaf weight after soaking at room temperature for 24 hours, and DW is leaf dry weight after drying at 75 °C for 24 hours.

Malondialdehyde (MAD) Content

The samples were extracted with phosphate buffer and centrifuged at 14,000 rpm for 30 min. To determine the MAD content, the thiobarbituric acid (0.5% w/v) containing 20% w/v trichloroacetic acid was added to the mixture. Subsequently, samples were placed in a hot water bath for 30 min and then were immediately cooled with ice and finally centrifuged at 10,000 rpm for 10 min. The absorbance of samples were read at 532 and 600 nm wavelengths and the malondialdehyde concentration was determined as discussed by Heath and Packer (1969).

To measure leaf proline content, first 0.5 g fresh leaf sample was mixed with 10 ml of sulfo salicylic acid (3% w/v). The mixture containing the sample was centrifuged at 4000 × g for 20 min. Then, two ml of ninhydrin acid and two ml of glacial acetic acid were added to the mixture. Simultaneously, two ml of standard 0, 4, 8, 12, 16, 20 mg l⁻¹ proline and two ml ninhydrin acid and two ml acetic acid were mixed and vortexed using a Vortex Mixer. All samples were heated in a hot-water bath for 60 min and then placed on ice to be cooled completely. Four ml of toluene was added to the solution and mixed for 20 sec. The standard curve regression equation was determined spectrophotometrically at 520 nm using 0, 4, 8, 12, 16, and 20 mg, l⁻¹ proline standards. The toluene soluble proline was sufficiently measured at 520 nm and expressed as mol proline g⁻¹ fresh weight (FW) (Bates et al., 1973).

Statistical Analysis

The data (n = 3) were subjected to one-way analysis of variance (ANOVA) using the SAS software package for Windows (SAS, version 9.3, SAS Institute, Cary, NC). The mean values were subjected to Duncan’s multiple range tests when statistical significance (p < 0.05) was detected.

RESULTS

The data of variance analysis of the effects of planting patterns and plant densities on agro-physiological traits of Tepary bean and the interaction between treatments are presented in Table 2.

Seed Yield

The seed yield of Tepary bean was the lowest (1.01 t ha⁻¹) with one-row plantation and 50 plants per m² and the highest (1.8 t ha⁻¹) seed yield was obtained with one-row plantation and 40 plants per m² in the both years of the experiment (Table 3). Seed yield in the treatment of plant density of 40 plants per m² improved by 75% and 65% compared to the treatments of plant densities of 50 plants per m² and 30 plants per m² in one-row plantation in the second year, respectively (Table 3). The plant density of 40 plants per m² in both planting patterns had a higher seed yield than those of other plant densities. There was no significant difference in seed yield of these treatments between the two years of the experiment.

Table 1. Some soil properties of the experimental location of this study

<table>
<thead>
<tr>
<th>Year</th>
<th>Soil Depth</th>
<th>pH</th>
<th>EC (dS.m⁻¹)</th>
<th>Organic carbon (%)</th>
<th>N (%)</th>
<th>P (mg.kg⁻¹)</th>
<th>K (mg.kg⁻¹)</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>0-20 cm</td>
<td>8.2</td>
<td>0.8</td>
<td>1.2</td>
<td>0.98</td>
<td>18</td>
<td>272</td>
<td>21</td>
<td>45</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>20-40 cm</td>
<td>8.3</td>
<td>0.5</td>
<td>1.1</td>
<td>0.98</td>
<td>17</td>
<td>255</td>
<td>21</td>
<td>42</td>
<td>37</td>
</tr>
<tr>
<td>2018</td>
<td>0-20 cm</td>
<td>8.1</td>
<td>0.9</td>
<td>1.3</td>
<td>0.96</td>
<td>21</td>
<td>277</td>
<td>20</td>
<td>47</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>20-40 cm</td>
<td>8.2</td>
<td>0.4</td>
<td>1.2</td>
<td>0.95</td>
<td>19</td>
<td>255</td>
<td>21</td>
<td>43</td>
<td>36</td>
</tr>
</tbody>
</table>
Table 2. The data of variance analysis of the effects of planting patterns and plant densities on agro-physiological traits of Tepary bean

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>df</th>
<th>Seed yield</th>
<th>Harvest index</th>
<th>Chl. a + b content</th>
<th>Chl. a/b content</th>
<th>Carotenoid content</th>
<th>Carbohydrate content</th>
<th>Relative water content</th>
<th>Malondialdehyde (MAD) content</th>
<th>Proline content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication (R)</td>
<td>2</td>
<td>1073.5 ns</td>
<td>3.12 ns</td>
<td>0.006 ns</td>
<td>0.002 ns</td>
<td>0.003 ns</td>
<td>0.41 ns</td>
<td>19.44 ns</td>
<td>0.041 ns</td>
<td>160.03*</td>
</tr>
<tr>
<td>Year (Y)</td>
<td>1</td>
<td>225.0 ns</td>
<td>0.04 ns</td>
<td>0.084 ns</td>
<td>0.026 ns</td>
<td>0.030 *</td>
<td>0.04 ns</td>
<td>0.11 ns</td>
<td>0.340 **</td>
<td>6.25 m</td>
</tr>
<tr>
<td>RxY</td>
<td>2</td>
<td>761.6 ns</td>
<td>6.31 ns</td>
<td>0.007 ns</td>
<td>0.151 ns</td>
<td>0.006 ns</td>
<td>1.07 ns</td>
<td>0.11 ns</td>
<td>0.081 ns</td>
<td>1.58 m</td>
</tr>
<tr>
<td>Planting pattern (PP)</td>
<td>1</td>
<td>51680.4 **</td>
<td>20.96 ns</td>
<td>0.084 ns</td>
<td>0.382 *</td>
<td>0.007 ns</td>
<td>0.74 ns</td>
<td>513.78 **</td>
<td>1.480 **</td>
<td>434.03 **</td>
</tr>
<tr>
<td>Y* PP</td>
<td>4</td>
<td>3211.1 ns</td>
<td>2.26 ns</td>
<td>0.008 ns</td>
<td>0.116 ns</td>
<td>0.000 ns</td>
<td>1 ns</td>
<td>0.44 ns</td>
<td>0.007 ns</td>
<td>0.03 *</td>
</tr>
<tr>
<td>main error (PD)</td>
<td>2</td>
<td>73668.8 **</td>
<td>13.20 ns</td>
<td>0.145 *</td>
<td>0.056 ns</td>
<td>0.054 **</td>
<td>6.1 **</td>
<td>127.69 **</td>
<td>1.694 **</td>
<td>752.69 **</td>
</tr>
<tr>
<td>Y* PD</td>
<td>2</td>
<td>1430.3 ns</td>
<td>6.35 ns</td>
<td>0.057 ns</td>
<td>0.002 ns</td>
<td>0.007 ns</td>
<td>0.64 ns</td>
<td>0.36 ns</td>
<td>0.134 *</td>
<td>5.58 m</td>
</tr>
<tr>
<td>PD × PP</td>
<td>2</td>
<td>4315.4 ns</td>
<td>9.06 ns</td>
<td>0.041 ns</td>
<td>0.144 ns</td>
<td>0.006 ns</td>
<td>1.46 ns</td>
<td>75.53 **</td>
<td>0.310 **</td>
<td>37.03 *</td>
</tr>
<tr>
<td>Y* PP × PD</td>
<td>2</td>
<td>1858.8 **</td>
<td>8.92 ns</td>
<td>0.017 ns</td>
<td>0.026 ns</td>
<td>0.006 ns</td>
<td>2.6 *</td>
<td>0.19 ns</td>
<td>0.177 *</td>
<td>2.03 *</td>
</tr>
<tr>
<td>Sub-error</td>
<td>16</td>
<td>2515.08</td>
<td>7.65</td>
<td>0.02</td>
<td>0.052</td>
<td>0.006</td>
<td>0.48</td>
<td>8.3</td>
<td>0.03</td>
<td>26.95</td>
</tr>
<tr>
<td>C.V</td>
<td></td>
<td>6.08</td>
<td>6.42</td>
<td>7.76</td>
<td>10.83</td>
<td>17.03</td>
<td>1.9</td>
<td>3.82</td>
<td>4.98</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* and ** mean significant at 5% and 1% probability, respectively. ns means non-significant.

Table 3. Seed yield and harvest index data of Tepary bean in different planting patterns and plant densities used in this study

<table>
<thead>
<tr>
<th>Year (Y)</th>
<th>Planting patterns</th>
<th>Plant densities (Plants m⁻²)</th>
<th>Seed yield (t. ha⁻¹)</th>
<th>Harvest index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>One-row</td>
<td>30</td>
<td>1.10±0.09b</td>
<td>35.7±2.3cd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>1.68±0.13a</td>
<td>46.5±2.5a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>1.01±0.06b</td>
<td>34.1±1.7cd</td>
</tr>
<tr>
<td></td>
<td>Two-row</td>
<td>30</td>
<td>1.10±0.08b</td>
<td>33.0±2.3d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>1.77±0.09a</td>
<td>45.7±2.6ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>1.07±0.05b</td>
<td>33.0±1.9d</td>
</tr>
<tr>
<td>2018</td>
<td>One-row</td>
<td>30</td>
<td>1.09±0.08b</td>
<td>36.2±2.2b-d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>1.80±0.07a</td>
<td>51.2±2.6a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>1.03±0.05b</td>
<td>35.7±2.4cd</td>
</tr>
<tr>
<td></td>
<td>Two-row</td>
<td>30</td>
<td>1.17±0.013b</td>
<td>36.1±2.2cd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>1.70±0.16a</td>
<td>43.7±2.1a-c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>1.07±0.10b</td>
<td>33.8±2.3cd</td>
</tr>
</tbody>
</table>

Means followed by the same letters in each column are not significantly different (Duncan multiple range test 5%)

Harvest Index (HI)

HI was not significantly different in most treatments with plant densities of 30 and 40 plants per m². The highest (51.2%) HI was obtained with one-row plantation and 40 plants per m² in the second year. However, the lowest (33.0%) HI was obtained with two-row plantation and 30 and 50 plants per m² in the first year. HI improved by 40% in one-row plantation and 40 plants per m² compared to 30 and 50 plants per m² in the second year (Table 3).

Chlorophyll and Carotenoid Contents

The lowest (1.30 mg. g⁻¹ FW) and the highest (1.60 mg. g⁻¹ FW) contents of leaf Chlorophyll a were...
obtained in two-row plantation with 30 (and also 50) and 40 plants per m² densities, respectively (Table 4). Chlorophyll b content in two-row plantation and 40 plants per m² with 0.81 mg. g⁻¹ FW was higher than those of other treatments. Chlorophyll a + b contents varied from 1.98 to 2.41 mg. g⁻¹ FW. Plant Chlorophyll a + b contents were significantly higher in two-row plantation and 40 plants per m² compared to other treatments. In the first year, Chlorophyll a + b contents in 40 plants per m² increased by 21% and 20% compared to 50 and 30 plants per m², respectively. Chlorophyll a to b ratio (1.86) was lower in two-row plantation and 30 plants per m² than those of other treatments in the first year.

Carotenoid content was significantly changed with various planting patterns and plant densities. 40 plants per m² had higher carotenoid contents, followed by 30 and 50 plants per m², respectively. Besides, two-row plantation with a plant density of 40 plants per m² was significantly better than one-row plantation. The highest carotenoid content (0.59 mg g⁻¹ FW) was obtained with two-row plantation with 40 plants per m² in the second year (Table 4).

**Carbohydrates and Relative Water Contents**

Seed carbohydrate content was significantly changed with some planting patterns and plant densities. There was a dramatic increase of seed carbohydrate content in 40 plants per m² plant density in both planting patterns in the first year and in two row planting pattern in the second year. The highest seed carbohydrate content (38.27 mg. g⁻¹ FW) was achieved with two-row plantation and 40 plants per m² in the second year and improved by 7% compared to one-row plantation and 40 plants per m² in the first year. Seed carbohydrate content did not differ significantly in two-row plantation with plant densities of 30 and 50 plants per m² (Fig. 2, a). There was no significant change of relative water content (RWC) between the first and second year in each plant density treatment; however, a significant decline of RWC occurred in one-row plantation and 30 plants per m² compared to all other treatments in both years. The highest (82%) RWC was obtained in two-row plantation and 40 plants per m² in both years, and the lowest (66%) RWC was reported in one-row plantation and 30 plants per m² in the first year (Fig. 2, b).

**Malondialdehyde (MDA) and Proline Content**

Significant increase of MDA (4.53 and 4.50 µmol. g⁻¹ FW in the first and second years, respectively) were obtained in one-row plantation with 30 plants per m², which were approximately 40% more compared to those of two-row plantation with 40 plants per m² in both years. There was no significant difference in MDA content between plants in one-row plantation with plant densities of 40 and 50 plants per m² and two-row plantation with plant densities of 30, 40, and 50 plants m² in the second year (Fig. 3, a). A similar trend was found for proline concentration.

There was no significant change for proline content between the first and the second year; however, a meaningful increase of proline occurred in one-row plantation with 30 plants per m² in the first-year. The highest proline content (247 and 250.3 µmol g⁻¹ FW) was achieved in one-row plantation with 30 plants per m² in the first and the second year. The lowest proline content (224.6 and 225.6 µmol g⁻¹ FW) was achieved in two-row plantation with 40 plants per m² in the first and the second year (Fig. 3, b).
DISCUSSION

The seed yield of Tepary bean was changed under different farming practices used in this study. It was reported that higher plant density might cause more lodging, lower light penetration in the crop canopy, and reduced photosynthetic efficiency, resulting in lower seed yield (Soratto et al., 2017). Similarly, Khalil et al. (2011) found that seed yield of faba bean (Vicia faba L.) decreased under higher plant density condition. Soratto et al. (2017) reported that the high plan density of common bean (Phaseolus vulgaris L.) resulted in decreasing pod yield without changing the seed yield.

In this study, it was shown that photosynthetic pigments improved with two-row plantation and 40 plants m⁻² plant density treatments. Similarly, Yao et al. (2015) showed that cotton (Gossypium hirsutum L.) plants improved photosynthetic capacity by increasing photosynthetic nitrogen-use efficiency and adjusting leaf expansion per area by changing plant density. In this research, two-row plantation with a plant density of 40 plants per m² was the optimum planting pattern for the high light interception and utilization and better spatial dissemination of leaf nitrogen to the carbon assimilation apparatus of leaves. Furthermore, higher photosynthesis pigment (Table 4) in two-row plantation with a plant density of 40 plants per m² was due to improved leaf gas exchange, total leaf area, and leaf area index, which finally improved the plant growth (Wang et al., 2015).

Leaf relative water content (RWC) and seed carbohydrate content of Tepary bean increased with 40 plants per m² and decreased with 30 and 50 plants per m² in this study. At 30 plants per m², RWC appears to be reduced due to decrease in soil moisture which induced by higher light penetration. Abu-Grab et al. (2019) realized that the RWC of maize (Zea mays L.) leaf changed with plant various densities and planting patterns and increased with increasing plant spacing between inter-row and intra-row plantations. They concluded that the wide–narrow row allowed quicker canopy coverage in the early growing stage of maize, possibly because of a benefit of the integrated advantages of both one-row and two-row plantations. Although narrower row spacing could also cover the soil in the early growing period due to much narrower spacing between inter and intra-row, there might be more mutual shade. Consequently, competition for nutrients and moisture after the critical leaf area index (LAI) is achieved, bringing about possible detrimental impacts on crop lower canopy leaves (Turgut et al., 2005). On the other hand, wide row-spacing may contribute to more solar radiation transmission down to lower canopy, better ventilation and less competition for nutrients among plants, but it is unlikely to make the best use of solar energy largely due to later canopy closure to obtain the critical LAI (Wang et al., 2015). In this study, MDA and proline content increased in one-row plantation with 30 or 50 plants per m² compared to the two-row plantation with 40 plants per m². Increasing MDA content under moisture deficiency conditions has also been reported in other studies (Mao et al., 2011; Eskandari Zanjani et al., 2010). Proline is an essential component of plant cells. Some researchers concluded that cumulative proline in plant cells could be a response to osmotic stress in various plant species (Sharma and Verslues, 2010).
In the present study, proline content increased in plant densities of 30 plants per m$^2$ (decrease in vegetation and evaporation of soil water due to direct sunlight) and 50 plants per m$^2$ (high competition between plants) due to low water content in the soil. The most common pathway for proline synthesis in plants is glutamate, and during the water stress more glutamate is converted to proline (Bagheri and Najafi Zarini, 2015). Decreased proline degradation and disruption of the protein synthesis process also increase proline with low water potential (Sharma and Verslues, 2010). It has been shown that proline stabilizes the membranes and macromolecules and helps maintain their natural shape and structure under water scarcity. In addition to its direct effect on stabilizing macromolecules, proline also has an indirect protective effect due to its antioxidant properties (Aleksza et al., 2017).

CONCLUSION

A plant density of 40 plants per m$^2$ was the most effective treatment to achieve a higher seed yield and an excellent Tepary beans yield. In addition, the two-row planting pattern was much better than the one-row planting pattern due to decreasing in soil moisture storage and increasing in chlorophyll and carbohydrate contents. Therefore, planting Tepary bean with a two-row planting pattern and 40 plants per m$^2$ can offer the highest seed yield and quality in the region.

REFERENCES


### Table 4. Chlorophyll and carotenoid contents of Tepary bean in different planting patterns and plant densities used in this study

<table>
<thead>
<tr>
<th>Year (Y)</th>
<th>Planting patterns</th>
<th>Plant densities (Plants per m$^2$)</th>
<th>Chl a content (mg g$^{-1}$ FW)</th>
<th>Chl b content (mg g$^{-1}$ FW)</th>
<th>Chl a + b content (mg g$^{-1}$ FW)</th>
<th>Chl a/b ratio</th>
<th>Carotenoid content (mg g$^{-1}$ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>One-row</td>
<td>30</td>
<td>1.40±0.11ab</td>
<td>0.60±0.02d</td>
<td>2.00±0.03bc</td>
<td>2.37±0.32a</td>
<td>0.41±0.04bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>1.50±0.17ab</td>
<td>0.68±0.08b-c</td>
<td>2.18±0.16a-c</td>
<td>2.23±0.14ab</td>
<td>0.45±0.07a-c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>1.37±0.12ab</td>
<td>0.65±0.09c-d</td>
<td>2.02±0.17bc</td>
<td>2.11±0.28ab</td>
<td>0.36±0.05c</td>
</tr>
<tr>
<td>2018</td>
<td>Two-row</td>
<td>30</td>
<td>1.30±0.14b</td>
<td>0.70±0.05b-d</td>
<td>2.00±0.13bc</td>
<td>1.86±0.11b</td>
<td>0.34±0.11c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>1.60±0.15a</td>
<td>0.81±0.07a</td>
<td>2.41±0.08a</td>
<td>1.97±0.14ab</td>
<td>0.53±0.02ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>1.30±0.09b</td>
<td>0.68±0.03b-d</td>
<td>1.98±0.10c</td>
<td>1.92±0.18ab</td>
<td>0.45±0.01a-c</td>
</tr>
<tr>
<td>2017</td>
<td>One-row</td>
<td>30</td>
<td>1.40±0.12ab</td>
<td>0.60±0.05d</td>
<td>2.00±0.08bc</td>
<td>2.34±0.27a</td>
<td>0.45±0.02a-c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>1.50±0.13ab</td>
<td>0.66±0.03b-d</td>
<td>2.16±0.09a-c</td>
<td>2.27±0.10ab</td>
<td>0.55±0.09ab</td>
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<td></td>
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<td>50</td>
<td>1.47±0.09ab</td>
<td>0.76±0.03a</td>
<td>2.23±0.06a-c</td>
<td>1.92±0.03a</td>
<td>0.41±0.02bc</td>
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<tr>
<td>2018</td>
<td>Two-row</td>
<td>30</td>
<td>1.50±0.11ab</td>
<td>0.73±0.03a-c</td>
<td>2.23±0.20a-c</td>
<td>2.04±0.10ab</td>
<td>0.47±0.05a-c</td>
</tr>
<tr>
<td></td>
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<td>40</td>
<td>1.57±0.15ab</td>
<td>0.76±0.04a</td>
<td>2.33±0.15ab</td>
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<tr>
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<td>2.21±0.14a-c</td>
<td>2.16±0.04ab</td>
<td>0.41±0.02bc</td>
</tr>
</tbody>
</table>

Means followed by the same letters in each column are not significantly different (Duncan multiple range test 5%).


Sharma, S., & Verslues, P. E. (2010). Mechanisms independent of abscisic acid (ABA) or proline feedback have a predominant role in transcriptional regulation of proline metabolism during low water potential and stress recovery. *Plant, Cell & Environment*, 33(11), 1838-1851. DOI: 10.1111/j.1365-3040.2010.02188.x


نتایج نشان داد که در سال دوم در کاشت یک ردیف، تراکم 30 بونه در متر مربع به ترتیب 25 و 45 درصد عمده‌دار را در مقایسه با تراکم 20 بونه در متر مربع در همین کلوه کاشت افزایش داد. مقدار کلروفیل در سال اول تحت کاشت 20 ردیف و کاشت با تراکم متوسط در مقایسه با کاشت با تراکم زیاد و رکورد 30 درصد افزایش یافت. بیشترین میزان تجمیع کربوهیدراتهای بدن با 37/96 میلی گرم در گرم در کشت و رکورد کاشت با تراکم متوسط در سال دوم به دست آمد. در مقایسه با کاشت 20 ردیف و کاشت با تراکم متوسط، محصولات نسبی این گروه به طور قابل توجهی تا 24 درصد در کاهش تحت کاشت یک ردیف و کاشت با تراکم کم کاهش یافت. کم‌ترین محصولات مالون دی آدنی و تجمیع پرولیف پر نیز با 27/3 و 34 میکرومول در گرم به ترتیب در کاشت 20 ردیف و کاشت با تراکم متوسط در سال دوم در نهایت، کلوه کاشت 20 ردیف و تراکم متوسط برای دست اورده عامل رکورد عامل برای توری تیاری نصبیه می‌گردد.

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