Responses of tepary bean plant under change in growth condition

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Abstract

Plants adjust physiochemical attributes to maintain sustainable growth under different agricultural practices such as the management of sowing date and planting pattern. The present experiment aimed to investigate the important role of sowing date (SD) and planting pattern (row arrangement) on physiochemical attributes of tepary bean (*Phaseolus acutifolius* L.) as a split-split plot design with three replications during 2017 and 2018. The main plots were two planting patterns (PP1: one-row and PP2: two-row plantation) and sub plots were three sowing dates (early, medium, and late planting, respectively in June 15:SD1, June 30:SD2, and July 15:SD3). Based on the results, increased chlorophyll (Chl) and carotenoid contents were observed under two-row plantation and medium sowing date. Although Chl content improved at PP2, carotenoid had no response to planting pattern The greater relative water content (RWC) was observed in plants under SD2, but the increased proline accumulation was reported at SD3. The least catalase and superoxide dismutase aggregations with 19 and 9.3 (Umg^{-1}protein) were obtained in plants under two-row plantation and medium sowing date, respectively. Photosynthesis pigments had a significant and positive correlation with RWC, but they negatively correlated with proline, CAT, and SOD. Sowing date and planting pattern influenced the microclimate of tepary bean, which affected the physiochemical properties. Therefore, physiochemical attributes are recommended to observe the optimal situation of tepary bean under the management of sowing date and planting pattern in arid and semiarid areas.

Keywords: Phaseolus acutifolius; photosynthetic pigments; proline; catalase; superoxide dismutase

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Introduction

Physiological and biochemical characteristics include the main changes in plants in response to environmental factors and human activities like climate change and pollution. These changes trigger reactive oxygen species (ROS) production in plants (Khosropour et al., 2018). In this regard, plants use different strategies to unrealized ROS through an elaborate antioxidant defense system consisting of ROS-scavenging enzymes and antioxidants (Saki et al., 2019). The physiochemical responses of plants to external factors are addressed in terms of changes in proline, antioxidant enzyme activity, photosynthesis pigments, water content etc. (Ghahremani et al., 2020). Plants produce a wide variety of free radical scavenging molecules, such as phenolic compounds, vitamins, terpenoids etc. against abnormal conditions (Rezaei and Ghasemi Pirbalouti, 2019; Ghahremani et al., 2020). Legume crops are widely used as the main component of most agricultural systems through

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providing multiple ecosystem services to meet the sustainability in agriculture. Legumes are used in various purposes such as grain for humans, forage for livestock, and soil amendments by improving nitrogen uptake and carbon sequestration (Baath et al*.,* 2020). Among the legumes, bean is the primary source of protein in many developing countries. Common bean (*Phaseolus vulgaris* L.) and tepary bean (*Phaseolus acutifolius* L.) are the main species of bean in the world (Leal-Delgado et al*.,* 2019). Although common bean is a main consuming legume in the world, it is fragile under drought stress conditions (Leal-Delgado et al*.,* 2019). On the other hand, satisfactory reports of tepary bean are well documented in the conditions with restricted soil moisture (Gujaria-Verma et al*.,* 2016). Tepary bean (*Phaseolus acutifolius* L.) is an annual plant and can be climbing, trailing, or erect with stems up to 4 m long. It consists of different local landraces and is appeared in various colors and sizes (Porch et al., 2017). Tepary bean (*Phaseolus acutifolius*) belongs to the genus Phaseolus as diploid and predominantly self-fertilizing crop (Gujaria-Verma et al., 2016). It is an edible bean and is resistant to adverse crop conditions such as drought, salinity, heat stress, pests, and microorganisms that affect ordinary beans (Heredia-Rodriguez et al., 2019). The nutritional quality of tepary beans is promising for human consumption due to its high grain protein (Mhlaba et al., 2018). Recently, the higher production of main crops is required to meet the population needs in the world (Saki et al., 2019). Therefore, the use of appropriate strategies to cope with theses limiting factors are helpful for farmers (Altieri et al., 2015; Wang et al., 2016). Sowing date and planting pattern can significantly affect plant yield (Matsuo et al., 2018). Planting pattern alters the interception of photosynthetically active radiation, the light rate and amount within the canopy, nutrients concentration, and moisture content, which finally affects the growth and yield of crops (Heitholt and Sassenrath-Cole, 2010). How to manage the sowing date and planting pattern, is a critical cropping practice to obtain high-quality products. Both sowing date and planting pattern represent different behavior on physiological properties to reach the optimum bean production in the world. For example, Molosiwa and Kgokong (2018) suggested that the best date for tepary bean in the southern part of Botswana is December and January. In the wet season of Rwanda, east Africa, the planting date should be delayed two weeks to reach the optimum bean production (Byiringiro et al., 2017). Changes in physiological and biochemical attributes are the main signal to understand plant tolerance range under different agricultural systems (Sadras et al., 2016). Until now, there has been little information on physiological and biochemical changes of the tepary been under sowing date and planting pattern in arid and semiarid conditions. Therefore, the present work was carried out to find the effect of sowing date and planting pattern on photosynthesis pigments, water content, antioxident enzymes activity, proline concentration, and their correlations in tepary been grown in semiarid conditions.

Material and Methods

Plant material and growth condition

Seeds of tepary bean (*Phaseolus acutifolius* L.) were obtained from Jiroft Agricultural Research Center, Iran. They were sown in the research farm of Agriculture and Natural Resource Faculty, Islamic Azad University, (1710 m above sea level, 34°01´45” N, 49°08´30”E) Arak Branch, Iran during June 2017 and 2018. The minimum and maximum temperatures were respectively 0 ℃ in January–February and 36 ℃ in July–August with mean annual value of 13.7 ℃ (Fig. I). Mean annual rainfall was 340 mm. pH and EC of the soil (0-20 cm) were 8.3 and 0.8 ds m^{-1}, respectively (Table 1).

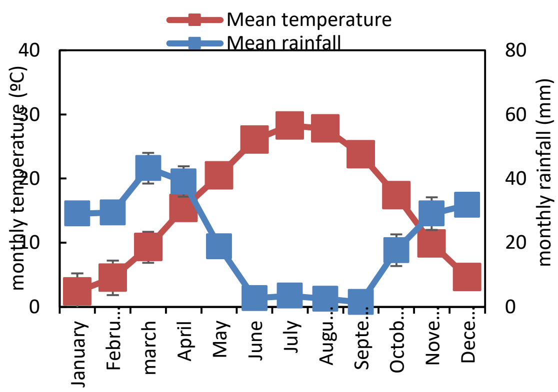

Fig. I. Mean temperature and rainfall in the case study

Table 1
The soil characteristics for cultivating the tepary bean grains

Year	Depth (cm)	PH	EC (dsm^{-1})	OC (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Sand (%)	Silt (%)	Clay (%)
2017	0-20	8.1	0.7	1.6	0.99	19	276	22	45	33
	20-40	8.4	0.6	1.5	0.98	17	255	21	42	37
2018	0-20	7.9	0.9	1.3	0.96	29	277	20	47	33
	20-40	8.2	0.5	1.2	0.95	19	251	21	43	36

Treatment details

The experiment was conducted as a split-split plot design with three replications during 2017 and 2018. Each plot was considered 4×3 m. The main plots were two planting patterns (PP1: one-row and PP2: two-row plantation) and sub plots were three sowing dates (early, medium, and late planting in June 15: SD1, June 30: SD2, and July15: SD3, respectively). During the experiment, no pesticide and chemical fertilizers were used and the weeds were controlled manually. In both years, plants were harvested at the end-pod filling stage in October and also the occurrence of flower was on September 4. Pod filling stage was on September 21 and physiological maturation stage was on October 4. The plant densities were set as 40 plants per square meter in all plots. Irrigation was performed using the drip irrigation system.

Chlorophyll (Chl) determination

The contents of chl *a,* chl *b* and carotenoids were extracted according to Arnon (1949) and Davies (1976). Briefly, 200 mg fresh samples were homogenized in 8 ml 80% acetone. The mixture was then centrifuged at 4 ℃ for 15 min (3000 rpm). Supernatants were used for analyzing chlorophyll content and the absorbance was determined at 480, 645, and 663 nm by the spectrophotometer.

Relative water content (RWC) measurement

At flowering stage, a number of fully developed leaves were taken from each experimental plot at 8 a.m. and were immediately transferred to the laboratory and weighed. The RWC of leaves was calculated as a percentage according to the method of Cohen et al. (2000) as follows:

$$RWC = \frac{WF - WD}{WS - WD} \times 100$$

where, *Wf* is fresh weight, *Ws* is leaf weight after soaking for 24 hours at room temperature, and *Wd* is leaf dry weight after drying for 24 hours at 75 ℃.

Proline concentration

To measure proline content, 0.5 g fresh leaf sample was mixed with 10 ml sulfosalicylic acid (3% w/v). The mixture containing the sample were centrifuged at 4000 × g for 20 min, and then 2 ml ninhydrin acid and 2 ml glacial acetic acid were added and mixed. Simultaneously, 2 ml of standard 0, 4, 8, 12, 16, and 20 mg/l proline and 2 ml ninhydrin acid and 2 ml acetic acid were mixed in a vortex mixer. All samples were heated in a hot water bath for 60 min and then placed on ice to cool completely. Then, 4 ml toluene was added to the solution and stirred with vertex mixer for 20 sec. Using 0, 4, 8, 12, 16 and 20 mgl^{-1} proline standards, the standard curve regression equation was determined spectrophotometrically at 520 nm. The toluene soluble proline was measured at 520 nm expressed as μmol proline g^{-1} FW (Bates et al., 1973).

Antioxidant enzyme assays

Frozen leaf samples (0.5 g) were ground with liquid nitrogen. The powder was homogenized in 5 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinyl pyrrolidone (PVP). The solution was centrifuged at 20000 g for 20 min at 4 ℃ and the supernatant was used for the following enzyme assay. BSA was used as standard to measure protein content based on the method of Bradford (1976).

Table 2
Analysis of variance for some physiochemical traits of tepary beans under sowing date and planting pattern

S.O.V.		MS							
	Df	chla	chl.b	total chl	carotenoid	RWC	Proline	CAT	SOD
rep	2	0.018 ns	0.0008 ns	0.027 ns	0.00035 ns	3.44 ns	18.6ns	0.0003 ns	2.19 ns
year	1	0.017 ns	0.00ns	0.017 ns	0.0004 ns	0.11 ns	12.2 ns	0.0003 ns	0.00ns
Year error	2	0.003 ns	0.0006ns	0.0037 ns	0.0045 ns	0.11 ns	0.083 ns	0.0011*	0.583 ns
Planting pattern	1	0.004 ns	0.0049**	0.0188ns	0.0001 ns	58.7 **	812.2 **	0.0038**	32.11 **
year* Planting pattern	1	0.001ns	0.00ns	0.0009 ns	0.0053 ns	0.11 ns	1.36 ns	0.0056**	0.11 ns
main error	4	0.011 ns	0.0028ns	0.011ns	0.0032 ns	20.2*	23.72 ns	0.00022 ns	1.44ns
sowing date	2	0.270**	0.0988 **	0.686**	0.0079 **	184.5 **	253.02 **	0.0096**	35.02 **
year*sowing date	2	0.021ns	0.00002ns	0.022 ns	0.00032 ns	0.52 ns	6.58 ns	0.0009*	1.75ns
Planting pattern * sowing date	2	0.001 ns	0.0018ns	0.0001 ns	0.00052 ns	0.52ns	19.08 ns	0.0003 ns	0.027ns
year* Planting pattern * sowing date	2	0.003 ns	0.0006 ns	0.0017 ns	0.0019 ns	0.19 ns	7.19 ns	0.0002ns	0.194ns
Sub-error	16	0.0098	0.00066	0.012	0.0014	4.6	20.3	0.00029	1.45
C.V	_	6.7	4.6	5.4	7.9	3.09	1.8	7.05	9.7

ns: non-significant, ** and * significant at 1% and 5%, respectively

Catalase (CAT, EC 1.11.1.6) activity

To measure CAT activity, the consumption of H_2O_2 (ε =39.4 / (mM cm)) was determined at 240 nm for 3 minutes. The reaction mixture 1 ml in volume contained 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2, and 10 µl enzyme extract (Bergmeyer et al., 1983).

Superoxide dismutase (SOD, EC 1.15.1.1) activity

SOD was determined according to the method of Elavarthi and Martin (2010). In the spectrophotometric assay, 1 ml of the reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 µM nitro blue tetrazolium, 2 µM riboflavin, and 50 µl of the extract. Riboflavin was added last, and the reaction was initiated by placing the tubes under white fluorescent light. The reaction was terminated after 10 min by removal from the light source. Reaction product was measured at 560 nm. The volume of the supernatant resulting in 50% inhibition of the reaction was taken as enzyme activity unit.

Statistical Analysis

The data (*n* = 3) were subjected to one-way analysis of variance (ANOVA) and 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 ($p<0.05$).

Results

Chlorophyll and carotenoid contents

Analysis of variance showed that the effect of sowing date was significant on chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid at 1% probability level; also, planting pattern had a significant effect on chlorophyll b at 1% probability level (Table 2). The result of mean comparison of physiochemical traits of tepary beans under planting pattern and sowing date

Table 3
Chlorophyll (Chl) and carotenoid content of tepary beans under planting pattern and sowing date

Year (Y)	Planting `pattern (PP)	Sowing date (SD)	Chl a (mg g^{-1} fw)	Chl b (mg g^{-1} Fw)	Total Chl (mg g^{-1} fw)	Carotenoid (mgg^{-1} fw)
2017	one-row	June 15	1.50±0.08^{a-c}	0.51±0.02^{c-e}	2.03±0.1^{c-e}	0.48±ab
		June 30	1.67±-0.04^{a}	0.65±0.01^{a}	2.34±0.04^{a}	0.53±a
		July 15	1.27±0.12^{d}	0.46±0.03^{g}	1.75±0.14^{g}	0.47±ab
	two-row	June 15	1.50±0.08^{a-c}	0.55±0.01^{b-d}	2.08±0.09^{b-d}	0.45±b
		June 30	1.67±0.05^{a}	0.66±0.01^{a}	2.34±0.05^{a}	0.47±ab
		July 15	1.30±0.08^{d}	0.48±0.01^{fg}	1.81±0.11^{fg}	0.47±ab
2018	one-row	June 15	1.43±0.09^{b-d}	0.51±0.02^{d-f}	1.97±0.08^{d-f}	0.45±b
		June 30	1.50±0.08^{a-c}	0.66±0.01^{a-c}	2.18±0.07^{ac}	0.51±ab
		July 15	1.33±0.05^{cd}	0.45±0.01^{fg}	1.81±0.06^{fg}	0.47±ab
	two-row	June 15	1.43±0.04^{b-d}	0.55±0.01^{c-f}	2.00±0.05^{c-f}	0.49±ab
		June 30	1.60±0.08^{ab}	0.64±0.05^{ab}	2.27±0.12^{ab}	0.53±a
		July 15	1.33±0.12^{cd}	0.50±0.03^{e-g}	1.86±0.09^{e-g}	0.47±ab

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

Table 4
The correlation among physiochemical traits of tepary beans

	Chl a	Chl b	Total Chl	Carotenoid	RWC	proline	CAT	SOD
Chl a	1							
Chl b	0.742**	1						
Total Chl	0.970**	0.884**	1					
Carotenoid	0.284	0.415**	0.351*	1				
RWC	0.712**	0.762**	0.774**	0.319	1			
proline	-0.618**	-0.634**	-0.665**	-0.177	-0.695**	1		
CAT	-0.524**	-.0663**	-0.610**	-0.266	-.0564**	0.525**	1	
SOD	-0.559**	-0.651**	-0.651**	-0.289	-0.649**	0.645**	0.740**	1

showed chlorophyll *a* as a main chlorophyll component in C4 plants varied from one-row planting and late sowing date (1.27 mgg^{-1}fw) to one-row plantation and medium sowing date in the first-year plants (1.67 mgg^{-1}fw). The highest chlorophyll *b* content was obtained in first-year plants under PP1/PP2 and SD2 increased by 27% and 40% in comparison to SD1 and SD2, respectively (Table 3). The amount of total chlorophyll in the first year under one-row plantation and medium sowing date increased by 15% and 33% in comparison to early and late planting dates, respectively. Increased carotenoid content was observed under medium sowing date by 10% and 12% in comparison to early and late sowing dates, respectively. However, no significant change was observed in carotenoid content under planting pattern (Table 3).

RWC and proline concentration

RWC and proline concentration significantly changed under planting pattern and sowing date (p≤0.01). There was no significant difference in relative water content between the first and second year but decline in relative water content in one-row plantation and late sowing date was significant. The highest RWC concentration (74%) was reported in two-row plantation and SD2 while the lowest RWC concentration (63%) was reported in one-row plantation and SD3 (Fig. II). Increased proline accumulation was reported at late sowing date. However, plants cultivated at SD2 represented lower proline concentration compared to SD1 and SD3. Under one-row plantation and SD3 proline concentration reached the highest values (250 and 251 µmol g^{-1}FW) in the first and second year, respectively (Fig. II).

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RWC	0.712**	0.762**	0.774**	0.319	1			
proline	-0.618**	-0.634**	-0.665**	-0.177	-0.695**	1		
CAT	-0.524**	-.0663**	-0.610**	-0.266	-.0564**	0.525**	1	
SOD	-0.559**	-0.651**	-0.651**	-0.289	-0.649**	0.645**	0.740**	1

Enzyme activity

Analysis of variance revealed the significant effect of planting pattern and sowing date on CAT and SOD activities ($p \leq 0.01$). CAT significantly changed under the influence of sowing date. The maximum catalase concentration (31Umg^{-1}protein) was recorded in first-year plants cultivated at SD3 under one-row plantation and the minimum catalase (19 Umg^{-1}protein) was recorded in two-row plantation and SD2 in the first year (Fig. III). In addition, superoxide dismutase activity was significantly higher at SD3 and one-row plantation in comparison with the other experimental treatments (15.66 Umg^{-1} protein) (Fig. III).

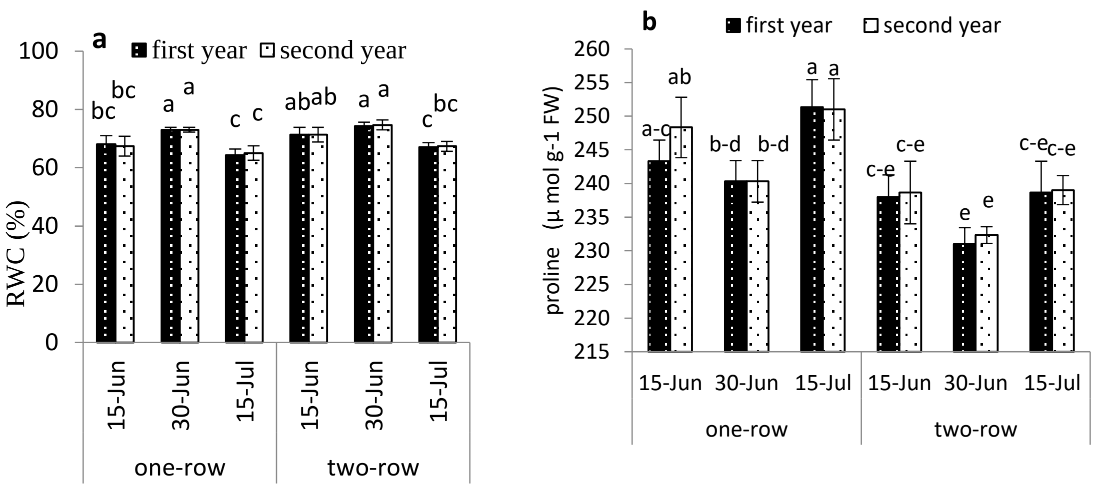

Fig. II. Relative water content (RWC) and proline concentration of tepary beans under planting pattern and sowing date

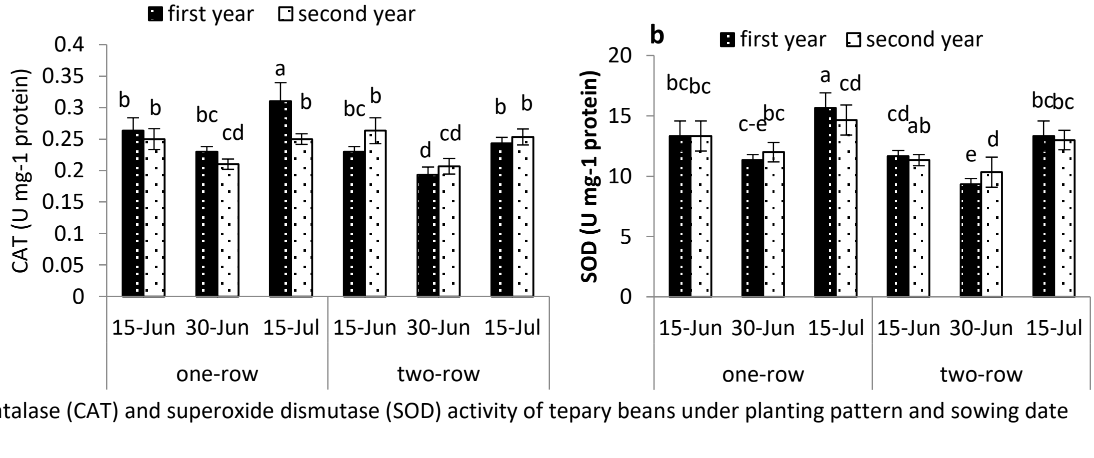

Fig. III. Catalase (CAT) and superoxide dismutase (SOD) activity of tepary beans under planting pattern and sowing date

However, plants cultivated at medium sowing date represented lower catalase and superoxide dismutase concentrations compared to early and late sowing dates in both years.

Correlations

Correlation results on the studied traits of tepary beans showed that Chl a significantly correlated with all traits except carotenoid (Table 4). However, Chl b and total Chl had a significant and positive correlation with carotenoid and RWC, but had a negative correlation with proline, CAT, and SOD. RWC negatively correlated with proline (-0.695), CAT (r=0.564), and SOD (r=-649). Proline had a significant and positive correlation with CAT (r=0.525) and SOD (r=0.645).

Discussion

Sowing date had a significant effect on tepary bean growth. The medium sowing date (SD2) due to efficient use of sunlight and soil moisture led to an increased growth in tepary bean. SD2 crops experienced a short reproductive period due to increased air temperatures and reduced canopy photosynthesis as a result of less radiation interception compared to early and late sowing dates (Liu et al., 2015; Gormus and Yucel, 2002). Planting pattern affected growth and yield in tepary beans by modulating leaf area and therefore, light interception and canopy photosynthesis (Ferreira et al., 2016). The narrow row tepary bean gives higher yield than the wider row tepary bean because of greater light interception. The significant response of tepary beans to planting pattern is in agreement with the results of Khalil et al. (2010), Bakry et al. (2011), and Ferreira et al. (2016), who reported remarkable changes in plant growth and development with altering row spacing. Regarding the significant effect of planting pattern, Osman et al. (2011) showed that this cropping system makes a competition among plants to obtain nutrients and water needed. The medium sowing date and two-row plantation led to an increased chlorophyll and carotenoid content of tepary beans. The changes in photosynthesis content under sowing date are related to the variation of water content in plant tissues and also the sunlight rate for photosynthesis. Energy input (sunlight) and water are the main photosynthesis components, which are strongly influenced by sowing date and planting pattern. During this stage of photosynthesis, as the sunlight excites the chlorophyll, water is split into oxygen and hydrogen and the oxygen is released into the atmosphere. The narrow row may cause more lodging, less light penetration in the crop canopy, and reduced photosynthetic efficiency (Soratto et al., 2017). Medium sowing date and two-row plantation could be an effective cropping strategy due to highlight interception and utilization and better spatial dissemination of leaf N to the carbon assimilation apparatus in leaves. Furthermore, higher photosynthesis pigment (Table 3) under medium and two-row plantation is due to the improvement in leaf gas exchange, total leaf area, and leaf area index, which finally lead to improvement in plant growth (Wang et al., 2016). RWC increased at medium sowing date, but decreased at early and late sowing date. At early sowing date in Arak with a semiarid condition, tepary bean seedlings are faced with lack of rainfall. In addition, at late sowing date, we can observe a high temperature, which adversely influences water content of plants particularly at their early growing stage. At late sowing date, RWC decreased due to the decreased soil moisture induced by high light rate. In delayed planting, the relative moisture content of the leaves decreases, which is due to the reduction of leaf water potential and reduced water uptake from the roots in dry conditions (Moosavifar et al., 2011). Application of wide–narrow row allowed quicker canopy coverage in the early growing stage, possibly a benefit of the integrated advantages of both one-row and two-row plantation. Although narrower row spacing could also cover soil in the early growing period due to much narrower spacing between inter and intra-row, there might be more mutual shade and competition for nutrients and moisture after the critical 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 while 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., 2016). We observed an increased proline content under one-row plantation and late sowing date compared to two-row plantation and medium sowing date. Proline is an essential component of plant cells. Cumulative proline in plant cells is perceived as a response to osmotic stress in a variety of plant species (Sharma and Verslues, 2010). In our study, proline increased at late sowing date due to the evaporation of soil water under direct sunlight. The most common pathway for proline synthesis in plants is the glutamate pathway and more glutamate is converted to proline during the water stress. Decreased proline degradation and disruption of the protein synthesis process also play a role in increasing proline in low water potential (Sharma and Verslues, 2010). When water solubility potential of the soil decreases, proline production increases, raising the osmotic pressure of the cell sap concentration (Kuznetsov and Shevyakova, 2006). 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). The main change in enzyme activity was related to sowing date, in which the highest value was found at late sowing date. As previously discussed, at late sowing date the sunlight rate makes a stress in plants. Under stress conditions, a collection of antioxidants act to suppress or prevent the formation of free radicals or reactive species in cells. They are efficient in neutralizing any molecule with the potential of developing into a free radical or any free radical with the ability to induce the production of other radicals. The SOD and CAT activity breakdown hydrogen peroxides and hydro peroxides to harmless molecules (H_2O_2alcoho^{-1} and O_2) (Ighodaro and Akinloye, 2018). Based on the correlations among treatments, CAT and SOD represented a similar behavior under planting pattern and sowing date. This can be due to the similar function of these antioxidant enzymes in scavenging the ROS produced under abnormal conditions in plants (Table 4).

Conclusions

Cropping systems like planting pattern and sowing date are factors that influence plant growth and development. According to the plant species and environmental factors, we can choose appropriate strategy to obtain high quality products. This two-year study showed the significant effect of sowing date and planting pattern on physiological properties of tepary beens (*Phaseolus acutifolius* L.) in semiarid area. Among the sowing dates (15 June, 30 June, and 15 July), 30 June was significantly better than early and late cultivation. In addition, two-row planting pattern was better than the one-row planting pattern. The results obtained from physicochemical traits as well as climatic conditions under study (semi-arid) suggested sowing the beans on 30 June with two-row planting pattern.

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