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Effect of Plant Pattern and Density on Yield and Amino Acid Profile of Tepary Bean (*Phaseolus acutifolius*) in A Semiarid Area

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ABSTRACT

Background: Tepary bean is recognized as a deal crop for cultivation in arid and semi-arid regions. The spatial distribution of plants is an important farming practice. A study was conducted in tepary bean (*Phaseolus acutifolius* A. Gray) to investigate the effect of planting pattern and plant density on plant yield and amino acid profile.

Methods: The experiment was carried out in a split-split plot design with three replications during 2017 and 2018. The main plot comprised planting pattern at two levels, including one-row plantation (PP1) and two-row plantation (PP2) and sub plot comprised plant density at three levels, including high plant density (HPD:50 plant/m^2), moderate plant density (MPD:40 plant/m^2) and low plant density (LPD: 30 plant/m^2). In both years, plants were harvested at the end of pod filling stage at October. Amino acid profiles were determined using a HPLC system.

Result: The results showed that in the first year and under PP2, MPD increased the biological yield by 19% and 16% compared to HPD and LPD, respectively. Increased pod yield was observed at MPD compared to HPD and LPD. In the second year under PP1, MPD improved grain yield by 75% and 65% in comparison to HPD and LPD, respectively. Increased protein and amino acid content were observed at MPD and PP2. The greatest amino acid content was Leu followed by Lys, Arg, Phe, Val, Ile, Thr, Tyr, His, Met, Trp and Cys.

Key words: Amino acid content, Grain yield, Medium plant density, Protein content.

INTRODUCTION

Grain legumes are a rich source of protein and microelements such as iron (Seetharamu *et al*. 2020). Tepary bean belongs to the genus *Phaseolus* with a genome size of 647 Mbp, which is slightly greater in comparison with common bean (637 Mbp) and Lima bean (622 Mbp) (Assefa *et al*. 2019). Tepary bean is tolerant to drought, salt and heat stresses; and resistance to biotic and abiotic stress factors (Mhlaba *et al*. 2018) and identified as a deal crop for cultivation in arid and semi-arid regions. Tepary bean is mainly grown for its dry grains containing high protein, essential minerals and fatty acids (Porch *et al.* 2017). It has a potential to sustain food and nutritional security, particularly protein contents (Mhlaba *et al*. 2018).

Plant density is a critical management factors affecting the plant growth, adjusting the capacity to capture radiation, water and nutrients (Jin *et al.* 2017). Optimal plant density is an important factor in achieving improved resource efficiency and higher productivity (Sangtam *et al*. 2017). It has been that the planting date, density and genotype, as well as their interactions had a significant effect on the yield and yield components of bean (Musavi Fakher *et al.* 2019). The crop spatial pattern is the agronomical important affecting grain yield and crop competitiveness against weeds and environmental stress (Olsen *et al*. 2012). The spatial distribution of crops with changing leaf area index significantly influences the sun light rate and soil moisture, which finally determines corresponding weeds and soil microorganisms (Mashingaidze *et al*. 2012).

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Iran has mostly an arid and semiarid climate, where annual precipitation averages 25 cm or less. The bean is an important grain crop cultivated in Iran in recent years. To our knowledge, the information on the plant density on tepary bean at row spacing is not available in the literature. The experiment were therefore carried out in a semi-arid area in Iran to determine the effect of planting pattern (one row and two rows) and plant density (30, 40 and 50 plants/m^2) on growth and yield and essential amino acid concentrations.

MATERIALS AND METHODS

Plant material and growth condition

A field experiment was carried out with three replications in the Research Station of Islamic Azad University, Arak Branch, in Iran during the period of June 2017 to October 2018. Fig 1 shows the ombrothermic diagram of the case study. Table 1 shows the selected physicochemical characteristics of the soil.

Treatment details

The experiment was conducted in a split-split plot design with three replications during 2017 and 2018. The main plot had two levels planting pattern, PP1 (one-row plantation) and PP2 (two-row plantation) and the sub plot had three plant density levels, including HPD (high density: 50 plant/m^2), MPD (moderate plant density: 40 plant/m^2) and LPD (low plant density: 30 plant/m^2). The seeds of tepary bean were obtained from the Pakan Bazr Company, Isfehan, Iran. No pesticide and chemical fertilizers were used and weeds were controlled manually. In both years, plants were harvested at October at the end of pod filling stage.

Fresh weight yield (FWY) and dry weight yield (DWY)

Biological yield was estimated in ton per ha with the total dry weight of plants in the harvest. Grain yield was estimated in tone per ha with a total weight of grains after threshing. The 100-grain weight was recorded in g of the weight of 100 randomly selected grains after threshing (Toker, 2004).

Protein estimation

Dried grains were ground using a blender (Moulinex, type AY46, Shenzhen, Guangdong, China) to obtain a fine powder. Samples were prepared according to the method of Kudre *et al*. (2013).

Amino acid analysis

An HPLC system was used to determine the amino acid profiles after samples were hydrolyzed with 6 M HCl as previously reported (Bidlingmeyer, 1984). The cysteine and methionine contents were determined after performic acid oxidation and tryptophan content was determined after alkaline hydrolysis (Landry and Delhaye, 1992).

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). When statistical significance ($p < 0.05$) was detected, the mean values subjected to Duncan's multiple range tests.

RESULTS AND DISCUSSION

Biological and pod yield

Two-row plantating (PP2) resulted in a higher biological yield compared to one-row plantating (PP1). In the first year under high plant density (HPD), it has been observed a 9% increase of biological yield under PP2 in comparison to PP1. MPD was suitable to meet the optimum biological yield. In the first year under PP2, MPD increased the biological yield by 19.4% and 16.2% compared to HPD and LPD, respectively (Table 2). Pod yield was influenced by plant density (Table 2). Increased pod yield was obtained at MPD. It has been observed a 50% and 34% improvement of pod yield at MPD compared to HPD and LPD, respectively, under PP2 during second year. Pod yield also decreased with increase in plant densities (Table 2). These results are consistent with Zhao *et al*. (2017) results. They reported that the highest pod yield, biological yield and grain yield in peanuts were obtained from medium density (225000 seed ha^{-1}). These traits decreased with increasing seed density. Rahman *et al.* (2011) found decreased pod yield of two soybean varieties under HPD. It might have corresponded to the fact that Per Plant Light Interception (PPLI) is less at HPD, which leads to lower carbon fixation that subsides crop's capability in carbon assimilation and translocation towards production of new branches and thus potentially reduces plant growth and developments. The MPD facilitates more light interception at early stages of crop growth in comparison with the more sparsely grown plants at lower densities. The widely spaced plants could not take advantage of the increased light interception at the early growth stages of the crop compared to HPD crops due to insufficient canopy closure.

Plant density affects grain yield in tepary bean 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. A marked response of plant yield to planting pattern is in agreement with results of Khalil *et al*. (2011) and Ferreira *et al.* (2016) who observed that there was a significant change in plant yield with altering row spacing. At LPD, nutrients availability for each plant increased so that more pods can be brought to maturity. Coupled with high temperature, HPDs tend to create high humidity which lead to more pod abortion. HPDs also created a canopy too dense to allow sufficient light for proper development of the pods. Regarding the significant effect of planning arrangement.

Grain yield and weight

The highest grain yield was observed at MPD followed by LPD and HPD. In the second year and under PP1, MPD improved grain yield by 75% and 65% in comparison to HPD and LPD (Table 2). The highest 100-grain weight was obtained in the second year under PP2 at MPD (9.57 g) (Table 1). In contrast, the lowest (7.23 g) 100-grain weight was observed in second year under PP2 at HPD (Table 2).

Table 1: The soil characteristics for cultivating the tepary bean grains.

Year	Depth	pH	EC (dS m^{-1})	OC (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Sand (%)	Silt (%)	Clay (%)
2017	0-20 cm	8.2	0.8	1.2	0.98	18	272	21	45	34
	20-40 cm	8.3	0.5	1.1	0.98	17	255	21	42	37
2018	0-20 cm	8.1	0.9	1.3	0.96	21	277	20	47	33
	20-40 cm	8.2	0.4	1.2	0.95	19	255	21	43	36

Table 2: The effect of planting pattern and plant density on plant yield.

Year (Y)	Planting pattern (PP)	Plant density (PD)	Biological yield (t ha^{-1})	Pod yield (t ha^{-1})	Grain yield (t ha^{-1})	100-grain weight (gr)
2017	PP1	LPD	3.08±0.2de	1.30±0.08b	1.10±0.09b	8.30±0.29b-e
		MPD	3.61±0.16ab	1.73±0.16a	1.68±0.13a	8.83±0.33a-c
		HPD	2.96±0.12de	1.10±0.08b	1.01±0.06b	7.60±0.16e-g
	PP2	LPD	3.33±0.07b-d	1.30±0.08b	1.10±0.08b	8.07±0.30c-e
		MPD	3.87±0.13a	1.80±0.07a	1.77±0.09a	8.93±0.28ab
		HPD	3.24±0.23b-e	1.20±0.08b	1.07±0.05b	7.77±0.20e-g
2018	PP1	LPD	3.01±0.22de	1.20±0.08b	1.09±0.08b	7.97±0.16d-f
		MPD	3.51±0.23a-c	1.67±0.12a	1.80±0.08a	8.70±0.32b-d
		HPD	2.88±0.18e	1.20±0.09b	1.03±0.05b	7.37±0.32fg
	PP2	LPD	3.24±0.05b-e	1.70±0.16a	1.17±0.013b	7.50±0.16fg
		MPD	3.89±0.19a	1.30±0.08b	1.70±0.16a	9.57±0.17a
		HPD	3.16±0.07c-e	1.17±0.05b	1.07±0.10b	7.23±0.38g
CD (P=0.05)						
Year (Y)			NS	NS	NS	NS
Planting pattern (PP)			**	NS	NS	NS
Y*PP			NS	NS	NS	NS
Plant density (PD)			**	**	**	**
Y*PD			NS	NS	NS	NS
PP*PD			NS	NS	NS	**
Y*PP*PD			NS	NS	NS	NS

** and * Significant at P≤0.01 and P≤0.05, respectively.

The high plant density may cause more lodging, less light penetration in the crop canopy and reduced photosynthetic efficiency that results in low grain yield (Soratto *et al*. 2017). Additionally, the grain yield decreased and weight at wider canopies. It can be due to the fact that at LPD, the sunlight easily reduces soil moisture thereby, decreases nutrient uptake by plants. Similar to our work, Khalil *et al.* (2011) observed decreased grain yield of faba bean at HPD. However, Mishra *et al*. (2009) reported with increasing plant density in pigeonpea, the grain yield increased significantly.

Protein content

As shown in Fig 2, in both 2017 and 2018, increased protein content was observed at MPD and PP2. There were no significant differences between first and second years. In the first year, protein content at MPD and PP2 was 251.3 mg g^{-1} protein. Similarly, this amount was found in second year also (Fig 2). There is a different correlation of protein content and plant density. We observed enhanced protein content at MPD and PP2. Bellaloui *et al.* (2015) showed a positive and strong relationship between plant density and protein content of soybean grains, which were not observed in the present study.

Amino acid contents and profile

Increased Essential Amino Acid (EA) was observed at MPD and PP2. The maximum EA was obtained at MPD and PP2 in second year was 186.87 mg/g protein (Table 3). Non-essential amino acid (NEA) was affected by plant density, planting pattern and the triple interaction of year, plant density and planting pattern (P≤0.05, Table 3). NEA at MPD and PP2 in second year (48.13 mg/g protein) was greater than other experimental treatments (Table 3). Total amino acid (TAA) at MPD and two-row plantation was the highest in second year (235 mg/g protein) (Table 3). The amino acid profile ranged from 4.7 to 37.54 mg/g protein (Fig 3 and 4). Leu was the greatest amino acid followed by Lys, Arg, Phe, Val, Ile, Thr, Tyr, His, Met, Trp and Cys.

The correlation between amino acid contents and the traits

Biological yield was positively correlated with grain yield (r=0.734), pod yield (r=0.634), protein content (r=0.820), EAA (r=0.740), NAA (r=0.694) and TAA (r=0.750). A significant correlation between grain yield with pod yield (r=0.908), 100-grain weight (r=0.769), protein content (r=0.918), EAA (r=0.733), NAA (r=0.44) and TAA (r=745). Pod yield was positively correlated with 100-grain weight (r=0.775), protein content (r=0.881), EAA (r=0.733), NAA (r=0.607) and TAA (r=0.775). Close correlation was also observed in 100-grain weight with protein content (r=0.852), EAA (r=0.758), NAA (r=0.602) and TAA (r=0.738) (Table 4). Similar slopes and correlation coefficients between grain protein supported the view that higher EAA, NAA and TAA amino acid contents can generally be achieved under higher protein content (Fig 5).

Table 3: The effect of planting pattern and plant density on amino acid content.

Year (Y)	Planting pattern (PP)	Plant density (PD)	EA (mg g protein^{-1})	NEA (mg g protein^{-1})	TAA (mg g protein^{-1})
2017	PP1	LPD	180.03±0.57d	46.61±0.91bc	226.64±0.40fg
		MPD	182.03±0.68c	46.99±0.51bc	229.02±0.29d
		HPD	174.42±1.44g	44.98±0.31e	219.33±1.81j
	PP2	LPD	181.20±0.39cd	46.71±0.30bc	227.91±0.11de
		MPD	184.51±0.38b	47.52±0.09ab	232.03±0.43b
		HPD	178.24±0.42fe	45.67±0.22de	224.58±0.85h
2018	PP1	LPD	180.69±0.33cd	46.88±0.46bc	227.57±0.12ef
		MPD	183.57±0.48b	46.85±0.18bc	230.42±0.65c
		HPD	176.97±0.66f	45.38±24e	223.30±0.27i
	PP2	LPD	181.35±0.43cd	46.92±0.39bc	228.30±0.10de
		MPD	186.87±0.53a	48.13±0.15a	235.00±0.67a
		HPD	178.54±0.15e	46.34±0.65cd	225.88±0.11g
CD (P=0.05)					
Year (Y)			*	NS	*
Planting pattern (PP)			**	**	**
Y*PP			NS	NS	NS
Plant density (PD)			**	**	**
Y*PD			NS	NS	**
PP*PD			*	NS	**
Y*PP*PD			NS	*	**

** and * Significant at P≤0.01 and P≤0.05, respectively.

Table 4: Correlations among experimental traits under planting patter and plant density.

	Biological yield	Grain yield	Pod yield	100-grain weight	Protein	EAA	NAA	TAA
Biological yield	1							
Grain yield	0.734**	1						
Pod yield	0.634**	0.908**	1					
100-grain weight	0.739**	0.769**	0.775**	1				
Protein	0.820**	0.918**	0.881**	0.852**	1			
EAA	0.740**	0.733**	0.773**	0.758**	0.889**	1		
NAA	0.694**	0.444*	0.607**	0.602**	0.729**	0.831**	1	
TAA	0.750**	0.745**	0.775**	0.738**	0.865**	0.988**	0.878**	1

** and * Significant at P≤0.01 and P≤0.05, respectively.

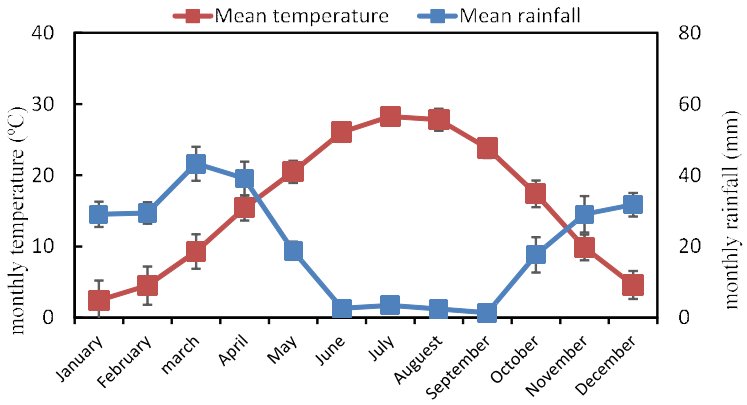

Fig 1: Mean temperature and rainfall in the case study.

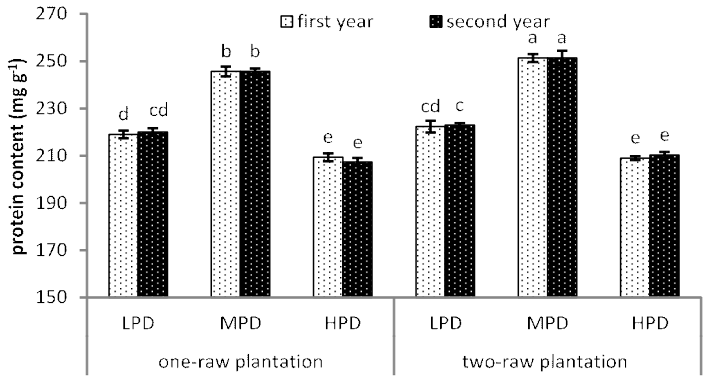

Fig 2: Protein content in tepary bean seeds under planting pattern and plant density. LPD: Low plant density, MPD: Medium plant density, HPD: High plant density.

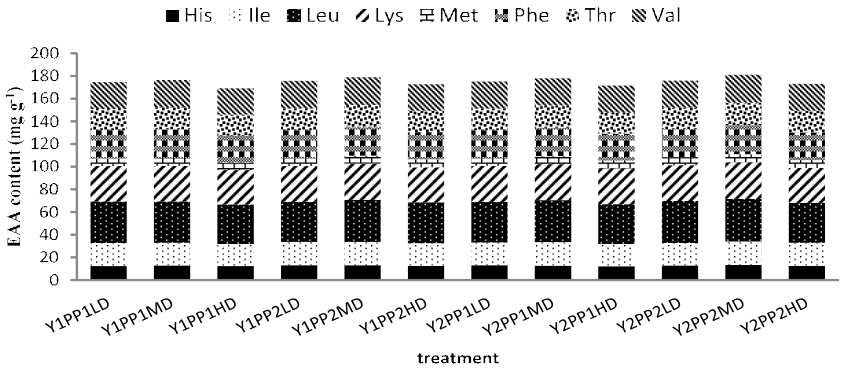

Fig 3: Essential amino acid content under planting pattern and plant density during 2017 and 2018. Y1: 2017, Y2: 2018, PP1: One-row planting, PP2: Two-row planting, LPD: Low plant density, MPD: Medium plant density, HPD: High plant density.

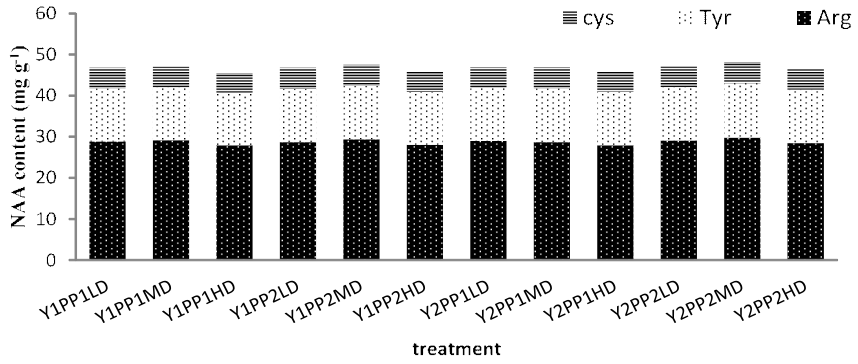

Fig 4: Non-essential amino acid content under planting pattern and plant density during 2017 and 2018. Y1: 2017, Y2: 2018, PP1: One-row planting, PP2: Two-row planting, LPD: Low plant density, MPD: Medium plant density, HPD: High plant density.

The planting pattern can change the plant yield and amino acid profile. The dry matter of plants at the same planting density, but in different patterns varies at the time of ripening (San-oh *et al*. 2006). The important factor corresponding to improved yield in PP2 was a consequence of the better light-intercepting characteristics of the canopy in plants of this planting pattern. These results revealed that the capacity of leaf photosynthesis was influenced by planting pattern, which results in change of plant growth. Specific trends under planting pattern for amino acid profile, however, could not be obtained. For example, Lysine, which is used for preventing and treating cold sores in PP1, was greater than PP2. The negative response of some amino acids to more desirable environmental conditions, such as greater water content and solar radiation, could be associated with the decline in protein concentration, is a result of the accumulation of relatively greater oil concentration in seeds. The positive correlation between high solar radiation and yield during the grain filling period is probably because of an increase in grain weight (Aguirrezabal *et al*. 2003).

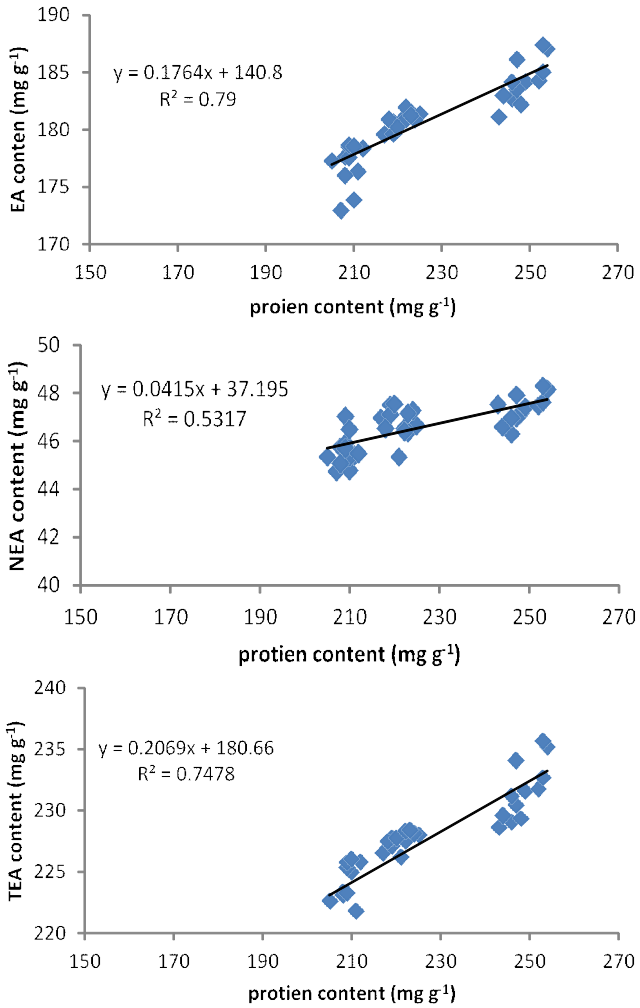

Fig 5: The relationship between amino acid and protein content.

CONCLUSION

We found that the PP2 has a higher yield and concentration of essential amino acid compared to PP1. In terms of plant density, we can obtain the optimum yield, protein content and TAA of tepary bean at MPD. Hence, we can prefer the use of PP2, when the seeds are sown on the MPD in semiarid conditions.

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