INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 7, p. 241-248, 2014

RESEARCH PAPER
OPEN ACCESS

The effects of drought stress and zeolites on the protein and mineral nutrients of *Lathyrus sativus*

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Key words: *Lathyrus sativus*, water stress, yield, zeolite.

http://dx.doi.org/10.12692/ijb/4.7.241-248

Abstract

The objective of this study was to determine the effect of different rates of zeolites and drought stress on the protein and mineral nutrients (nitrogen, phosphorus and potassium) of *Lathyrus sativus*. Factors were considered as two levels of irrigation (irrigation at field capacity (FC) and 50 % FC) were applied in two-leaf stage and four levels of zeolites (0, 10, 20, 30 ton/ha). Before planting, zeolite was added to the soil in deepness of root development. Results of ANOVA showed the significant effect of irrigation on the leaf nitrogen, leaf phosphorus, leaf potassium and leaf protein content, and however significant effect of zeolite on the leaf nitrogen, leaf phosphorus, leaf protein content and yield. The results indicated that water deficit stress significantly decreased leaf nitrogen, leaf phosphorus, leaf potassium and leaf protein content, whereas the application of zeolite compensated the negative effect of drought stress, especially in high rates of polymer application (30 ton/ha). The highest leaf nitrogen (2.17 %), leaf phosphorus (0.2 %), the percent (14.2 %) and yield of leaf protein (0.07 g/plant) were obtained from application of 30 ton/ha zeolite. These findings strongly suggested that the irrigation intervals of *Lathyrus* could be increased by application of zeolite.

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Introduction

Food lack and malnutrition, as one of the most disturbing problems of human society, is the lack of dietary protein, this is the biggest terms of physical and mental harm to human life. Legumes (Fabaceae family plants) with a lot of protein play an important role in solving these problems (Mortimore *et al.,* 1997; Van *et al.,* 1997). *Lathyrus sativus* L. belongs to the Fabaceae family and it has 20-32% proteins (Ramachandran *et al.,* 2005). In many regions of the world like Iran, drought stress is one of the most important factors that decrease agricultural crop production. Flowering, pollination and seed filling are sensitive stages to drought stress in plants (Thomas *et al.,* 2004). Improving the efficiency of water use in agriculture is associated with increasing the fraction of the available water resources that is transpired because of the unavoidable association between yield and water use (Jaleel *et al.,* 2007).

In regions where water scarcity is the principal limiting factor for cultivation, farmers are interested in using some methods to deduce injurious effects of water deficiency. One possible approach to reducing the effect of drought on plant productivity is through the addition of zeolite to soil (Manivannan *et al.,* 2007). Cultivation of short season plants could be a suitable strategy for second cultivation in arid regions (Wang *et al.,* 2003). However, some materials such as crop residuals, mulch plants, waste, litter, straw, stubble, and other synthetic materials like hydro plus zeolites could be used to save soil moisture (Silberbush *et al.,* 1993). Zeolites are highly hydrophilic due to low cross-links in their structure (Huang and Petrovic, 1994). Zeolites may have great potential in restoration and reclamation of soil and storing water available for plant growth and production (Zhang *et al.,* 2007).

Chemical treatment and agronomical crop management practices have been tried to reduce the drought effects (Manivannan *et al.,* 2007), but the application of zeolite to discharged plants attracted little attention. There are more than 50 known naturally occurring zeolites (Çoruh, 2008). Natural zeolites are hydrated aluminosilicates with comprising silica and aluminum tetrahedral which result in a stable three-dimensional framework. This honeycomb structure is generally very open, containing channels and cavities, which are filled with cations and water molecules (Karapinar, 2009). The cations are bound by weaker electrostatic bonds, increasing their mobility and the capability of being exchanged with cations present in solution (Farkas *et al.,* 2004; Maranon *et al.,* 2006). Gholizadeh *et al.* (2010) showed that the increasing of zeolite and water stress have a significant effect on most of measured growth parameters.

Because of important role of zeolite to compensate water deficit-induced reduction of yield, the main purpose of this study is to determine whether zeolite increases *Lathyrus sativus* drought tolerance by uptake of mineral nutrients and yield of protein.

Materials and methods

Experimental design and materials

This study was conducted as a factorial experiment based on randomized complete block design (RCBD) with 8 replications. The experiment was carried out at Agricultural Research Farm of Urmia University, Iran, (latitude 37.53° N, 45.08°E and 1320 m above sea level) in 2012. Application of drought stress was started at two-leaf stage. Irrigation treatments including irrigation at field capacity and permanent wilting point were considered as first factor according to Table 1.

According to soil analysis, chemical fertilizers and zeolite were distributed on the soil surface and incorporated with the soil in depth of 30 cm. Four levels of zeolite including 0, 10, 20 and 30 ton/ha were considered as second factor. The plots were consisted 100 cm long and 50 cm width. The grass pea seeds were disinfected and sown on 31 July in five lines (10 cm inter and 5 cm intra row space). The pots were put at enough distances from each other that triggered no competition for light absorption. Crops harvested on 23 September.

Measurements

To measure leaf phosphorus, dried leaves were milled, digested, and analyzed as described in Watanabe and Olsen (1965) and Onishi *et al.* (1975). The method described for P involves drying, homogenization, and combustion (4 h at 500 °C) of leaf sample. The plant ashes (5 mg) are digested in concentrated hydrochloric acid (1 mL of conc. HCl). The samples are then filtered and total P is quantified as phosphate (PO_4^-) using the ascorbic acid method (Watanabe and Olsen, 1965). The amount of phosphate in solution was determined colorimetrically at 882 nm (Graca *et al.,* 2005).

Leaf nitrogen contentwas determined by the micro-Kjeldahl method (Jackson *et al.,* 1973). About 25 mg of samples were transferred to a micro-digestion tube and digested with 1 mL of low nitrogen concentrated H_2SO_4 and a few mg of 3: 1 $CuSO_4$-K_2So_4 mixture (Stuart, 1936). Seed protein content was calculated by multiplying total nitrogen content with factor 6.25.
Protein yield was calculated by (biomass × protein percentage).

Concentrations of elements were measured by Flame spectrometer. Standards of 0, 0.5, 1.25, 2.5, 5, 7.5 and 10 ppm were used for obtaining standard curve.

Statistical analysis

Data analysis was done by using SAS 9.1 software, and mean comparison carried out with Student-Neuman-Keul›s Test (SNK) at $P \leq 0.05$.

Results and Discussion

Leaf nitrogen (%), phosphor (%) and potassium (%) content

The results showed the significant effect of zeolite and water deficit stress on the leaf nitrogen, phosphorus and potassium content (Table 2). Application of zeolite increased leaf nitrogen, phosphorus and potassium content, significantly. The minimum of leaf nitrogen and phosphorus content belonged to control treatment, but the higher amounts of zeolite caused to significant increase in leaf nitrogen and phosphorus content. So the maximum leaf nitrogen (2.17 %) and phosphorus (0.2 %) were observed from application of 30 ton/ha zeolite (Figs 1A and 2A). Zeolite application increased N and P content by prevention from nitrogen leaching. This result was similar to findings of researchers (Tohidi-Moghadam *et al.,* 2009). Short irrigation interval resulted increase in leaf nitrogen, phosphorus and potassium content (Figs 1B, 2B and 3). So, the highest (1.98, 0.18 and 0.77 % for nitrogen, phosphorus and potassium, respectively) were occurred in irrigation at FC (well watered plants) and the lowest values (1.58, 0.11 and 0.39 % for nitrogen, phosphorus and potassium, respectively) were observed in irrigation at 50% FC (stressed plants) (Figs 1B, 2B and 3).

Table 1. Irrigation for FC and 50 % FC.

Date	29-Aug	31-Aug	2-Sep	4-Sep	7-Sep	11-Sep	14-Sep	18-Sep
Evaporation	8.1	6.2	7.9	6.2	8.5	7	4.9	4.8
FC	√	√	√	√	√	√	√	√
50% FC		√		√		√		√

Table 2. Analysis of variance of some Nutrient uptake and protein content of *Lathyrus sativus* affected by irrigation (water deficit stem) and zeolite.

Source of variation	df	Leaf nitrogen	Leaf phosphor	Leaf potassium	Biological yield	Protein percentage	Protein yield
Replication	8	0.0541^{ns}	0.0011^{*}	0.0034^{ns}	0.0074^{ns}	2.3403^{ns}	0.0009^{ns}
Zeolite	3	0.7929^{**}	0.0184^{**}	0.0039^{ns}	1.0280^{*}	33.7071^{**}	0.0028^{**}
Irrigation	1	0.9801^{**}	0.0301^{**}	0.0554^{**}	1.0126^{ns}	41.6646^{**}	0.0012^{ns}
Zeolite×Irrigation	3	0.04169^{ns}	0.0005^{ns}	0.0075^{ns}	0.0171^{ns}	1.7723^{ns}	0.0005^{ns}
Error	56	0.0476	0.0002	0.0042	0.0064	2.0276	0.0005

“ns”: non-significant, *: significant at $p<0.05$, **: significant at $p \leq 0.01$.

Zeolites improve nutrient use efficiency by increasing P availability from phosphate rocks, improving the use of NH_4^+ and NO_3^-, reducing leaching losses of exchangeable cations, especially k^+ and also acting as slow-release fertilizer (Barbarick *et al.,* 1990; Bernardi *et al.,* 2008). According to Leggo (2000), due to the high affinity of zeolites for nutrients, these minerals may be used in growth media to improve plant yields.

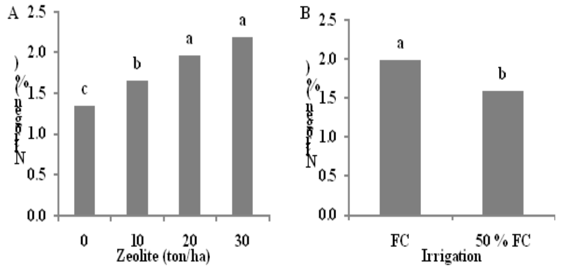

Fig. 1. Mean comparison of leaf nitrogen under different amounts of zeolite (A) at irrigation regimes (B). The same letters show non-significant difference at P≤0.05.

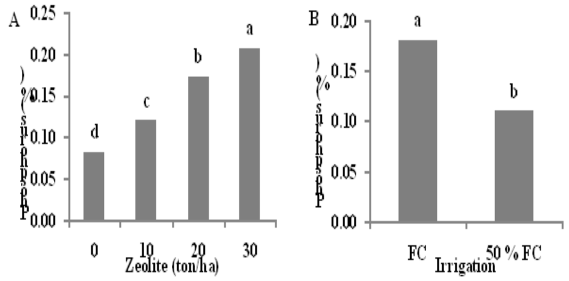

Fig. 2. Mean comparison of leaf phosphorus under different amounts of zeolite (A) at irrigation regimes (B). The same letters show non-significant difference at P≤0.05.

Biological yield

The lowest biological yield (0.64 g/plant) belonged to application 10 ton/ha zeolite and highest biological yield (1.29 g/plant) belonged to 30 ton/ha zeolite, while the lower rates of zeolite (10 and 20 ton/ha) did not increase biological yield as well as control treatment (Fig 4A). Limited irrigation decreased significantly biological yield (Fig 4B). This reduction was due to decrease in yield components such as leaf number, leaf and stem size, leaf and stem weight and length stem. There was significant decrease in biological yield due to decrease of vegetative growth and plant height. It's known that, decrease in plant height is due to decrease in cell division and assimilates transport. There are many reports about decrease of vegetative growth and plant height under conditions of drought stress. Shoot parts are sector from biological yield such as leaves, stem height. Increase of plant height is related two phenomena, increase of nod numbers and increase of inter nods length and these are strongly affected by drought stress (Wright *et al.,* 1995).

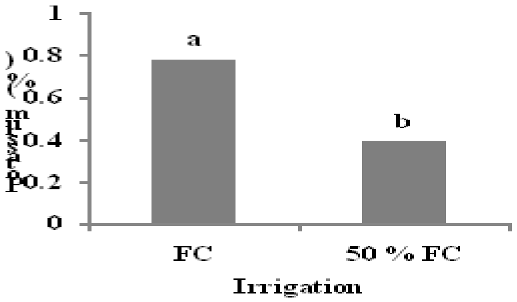

Fig. 3. Mean comparison of leaf potassium at irrigation regimes. The same letters show non-significant difference at P≤0.05.

It has been reported that mixtures of zeolite with other substrates increased plant yield in many species such as in gerbera (Issa *et al.,* 2001), cucumber (Gül *et al.,* 2007), tomato (Al-Ajmi *et al.,* 2009). Zeolite in substrates mixtures may promote anion and cation exchange capacity (Issa *et al.,* 2001). Mixtures of zeolite and fertilizers also had positive effects on lettuce (Gül *et al.,* 2005) and tomato yields (Valente *et al.,* 1986). Koljajic *et al.* (2003) reported that increasing the amount of zeolite significantly increased the dry matter, protein and crude fiber contents in beet.

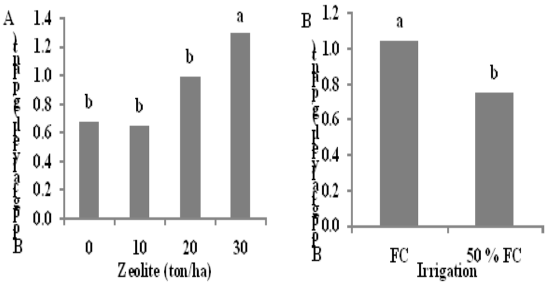

Fig. 4. Mean comparison of biological yield under different amounts of zeolite (A) at irrigation regimes (B). The same letters show non-significant difference at P≤0.05.

Leaf protein content and yield

Results showed that the minimum Leaf protein content (8.7 %) and yield (0.02) belonged to control treatment, but the higher amounts of zeolite caused to significant increase in this traits. So the maximum leaf protein content (14.02 %) and yield (0.07 g/plant) was obtained from 30 ton/ha zeolite application (Figs 5A and 6A). Drought stress decreased protein content and yield in leaves (Figs 5B and 6B). A maximum amount of protein was obtained from normal irrigation and water stress significantly decreased protein concentration in plant leaves.

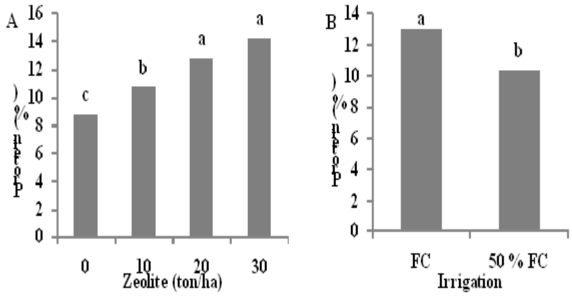

Fig. 5. Mean comparison of protein percentage under different amounts of zeolite (A) at irrigation regimes (B). The same letters show non-significant difference at P≤0.05.

Drought induced decrease in total soluble protein percent has also been reported in safflower (*Carthamus mareoticus* L.) and by Abdel-Nasser *et al.* (2002). Changes observed in protein, free amino acid and proline contents of several drought-stressed plant species have been attributed to a reduction in the rates of protein synthesis and an increase in photolytic activity, both of which tend to cause an increase in the total soluble nitrogen (Shen *et al.,* 1990). The decrease in protein contents might be due to increased photolytic activity. Proteins are hydrolyzed by proteases to release amino acids for storage and/or transport and for osmotic adjustment (like as proline) during drought stress in plant. Osmotic adjustment, protection of cellular macromolecules, storage form of nitrogen, maintaining cellular pH, detoxification of the cells and scavenging of free radicals are proposed functions of free amino acid accumulation (Parida *et al.,* 2007). Zeolites application had positive and enhancing effects on protein. Also, zeolite application in all irrigation treatments had desirable affects on protein, so that; Zeolite application decreased the adverse drought stress effects.

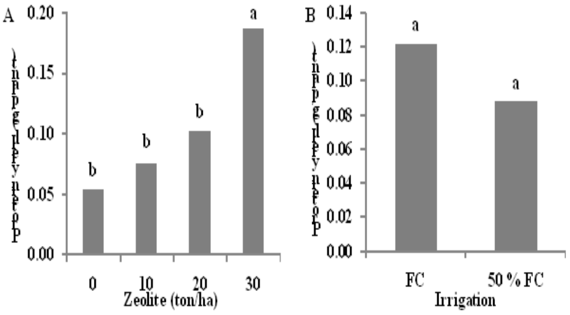

Fig. 6. Mean comparison of protein yield under different amounts of zeolite (A) at irrigation regimes (B). The same letters show non-significant difference at P≤0.05.

The changes in total soluble proteins under drought stress were consistent with the findings of (Riccardi *et al.,* 1998; Ge *et al.,* 2006) in maize, and Bensen *et al.* (1998) in soybean. Drought stress induced changes in protein synthesis in maize. The accumulation of dehydrin like proteins was detected in the roots and leaves of drought-stressed plants, which could protect plants from further dehydration damage (Mohammadkhani and Heidari, 2008). Water stress has a profound effect upon plant metabolism, and results in a reduction in protein synthesis. Several proteins were reduced by stress in maize mesocotyls (Bewley and Larsen, 1982). Dasgupta and Bewley (1984) pointed out water stress reduced protein synthesis in all regions of barley leaf.

Conclusions

In conclusion, it was observed that, under drought stress conditions, L*athyrus sativus* L. produced the lowest leaf nitrogen, leaf phosphorus, leaf potassium, biological yield, leaf protein content and yield. Whereas zeolite application in lands which are exposure to late season drought stress can keep soil water content and improve plant growth and produc-tion. In general, zeolite as a soil amendment that improved water retention capacity, soil cation exchangeable capacity led to higher yield under drought stress conditions, and then it can be suggested for these lands in arid and semi-arid regions.

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