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Nodulation and Root Traits in Four Grasspea (*Lathyrus sativus*) Ecotypes under Root-Zone Temperatures

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Abstract: In order to study the effect of four Root-Zone Temperatures (RZT) (5, 10, 15 and 25°C) on nodulation and nitrogen percent of four grasspea ecotypes (ardabil, zanjan, mashhad and sharkord), an experiment was conducted in a controlled-environmental chamber in 2005. There were differences (p<0.01) among ecotypes, RZT and ecotypes*RZT for root length, forage dry matter, root dry matter, nodule dry weight, nodule number, nodule cluster number, nodule cluster diameter, nodule diameter, nodule distribution (root length that has nodule) and plant nitrogen percent. Mashad and ardabil ecotypes produced the most and least nodule number at 25 and 5°C, respectively. The maximum and minimum nodule cluster number were observed in ardabil ecotype under 25 and 5°C RZT, respectively. Root distribution was the most and the least in mashhad and ardabil ecotypes under 25 and 5°C RZT, respectively. Ardabil produced the highest dry nodule weight at 25°C RZT. The least dry nodule weight was belonged to ardabil ecotype under 5°C RZT. Plant nitrogen percent was the highest in ardabile ecotype at 15°C RZT and the lowest in mashhad ecotype under 5°C RZT. This experiment showed that at low RZT (i.e., 5 and 10°C) none of ecotypes had preferred on other ecotypes in point of view measured traits except nodule diameter. Ardabile and mashhad ecotypes were better than other ecotypes at 15 and 25°C RZT respectively for most traits.

Key words: Grasspea (*Lathyrus sativus*), root zone temperature, nitrogen percent, nodulation

INTRODUCTION

Soil temperature has direct dramatic effects on microbial growth and development, organic matter decay, seed germination, root development and water and nutrient absorption by root (Decoteau, 1998). It is generally admitted that temperate legumes nodulate and fix N in a temperature range 10-30°C and the tropical ones in the temperature range 15-35°C. The temperature range for functioning the symbiosis is narrower than that of the plant supplied with combined nitrogen. Nodule initiation is particularly sensitive to low temperature, but nodules formed at a favorable temperature maintain nitrogen fixation when transferred to lower temperature, even 2°C (Dart and Day, 1971). Kalinina *et al.* (1982) reported that in the presence of a low-temperature stress, the involvement of roots and shoot in nitrogen assimilation was redistributed. The low temperature limit of the nitrogen-fixing symbiosis is largely due to sensitivity on the part of the host plant but can be modified by the strain of bacterial symbiont used (Lie, 1981).

Many studies have shown that sub-optimal temperatures affect the competitiveness of rhizobia for nodulation (Hardarson and Jones, 1979), delay root infection and inhibit nodule development and nitrogenase activity (Roughley and Date, 1986). In general, rhizobia have a poor growth at temperatures below 10°C, but they are tolerant to 4°C (Graham, 1992). The production and excretion of nod metabolites by *Rhizobium leguminosarum* bv. trifolii are reduced by lowering the temperature (McKay and Djordjevic, 1993). Consequently, growth of legumes can be significantly reduced as reported with alfalfa (Rice and Olsen, 1988) and soybean (Lynch and Smith, 1993) under cold conditions in Canada. In many studies, cold-adapted rhizobia isolated from arctic or sub-arctic regions showed the capacity to improve symbiotic nitrogen fixation and yield of legumes under low temperature conditions (Prèvost *et al.*, 1999).

The cold shock response is one of the well-characterized mechanisms which describes the expression of specific genes producing cold shock proteins (CSPs) after an abrupt shift to low temperature. Among other factors, low temperature is detrimental to nitrogen fixation by affecting photosynthesis and root nodulation (Prosperi, 1993). According to the literature, the most critical steps of nitrogen fixation under low temperature (8-10°C) are the penetration of the rhizobium into, the root and the formation of bacteroidal tissue, whereas

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nitrogenase can work at lower temperatures (Lynch and Smith, 1993). While climatic conditions can not be changed, the selection of cultivars (Papastilyanou, 1987), Rhizobium strains (Castillo *et al.*, 1999) or both (Webber, 1993), that it tolerate unfavorable conditions remains the best approach. Seven degree Celsius was shown as a low temperature that delays or inhibits infection for the establishment of symbiosis and affects nodulation (Gibson, 1971). Low temperatures alter N_2 fixation through different processes: The formation of nodules and/or the early stages of the infection processes and biochemistry of N_2 fixation (Zhang and Smith, 1994). An improved N_2 fixation combined with a growth more tolerant to low temperature during winter and early spring contribute to reduce the shortage of forage for livestock in areas characterized by poor soil conditions (Christophe *et al.*, 2005).

Grasspea (*Lathyrus sativus* L.) is an annual pulse crop belonging to the family Fabaceae and the tribe Vicieae. It is extremely drought tolerant, capable of yielding well even under the most adverse conditions and well adapted to cool season (winter) production in warm temperate and sub-tropical areas (Mediterranean basin, Bangladesh, India and Pakistan) (Cocks *et al.*, 2000). Grasspea has an important role as a legume crop in crop rotations, reportedly adding around 67 kg ha^{-1} of nitrogen to the soil in a single season and conferring yield and protein benefits on the subsequent non-legume crop (Muehlbauer and Tullu, 1997; Wang *et al.*, 2000). It thrives best in areas with 10-25°C (Muehlbauer and Tullu, 1997). The influence of high temperature on symbiosis is well documented but there is less information about the influence of cold on nodulation and nitrogen fixation spatially for grasspea (*Lathyrus sativus* L.).

MATERIALS AND METHODS

Seeds of the four grasspea (*Lathyrus sativus* L.) ecotypes included zanjan, sharkord, ardabil and mashhad were surface sterilized in 95% ethanol (for 5 s) and sodium hypochlorite (2% solution for 3 min) (Dodds and Roberts, 1995). Then seeds were rinsed thoroughly with distilled water and planted in trays containing sterilized sand. Nine-day-old seedlings, at the cotyledon stage, were transplanted into sterilized 15 cm plastic pots containing the same medium, in a growth chamber. The growth chambers light (300 µmol m^{-2} s^{-1}) was provided by cool white fluorescent tubes. Light intensity across the growth chambers was measured several times during the experiment and was always uniform. The photoperiod was 16/8 h (day/night). This work examined the influenced of four Root-Zone Temperatures (RZT) (5, 10, 15 and

25±0.2°C) and air temperature was held at 25/15°C day/night. In each growth chamber root-zone temperature was controlled by circulating cold water around large pots with 12 pots in each tank. Twelve medium sized pots containing plants were put into each of the large pots. A hole was drilled in the bottom of each medium sized pot to allow these pots to drain large pots. After being transplanted into the pots, the plants were acclimatized for 24 h prior to inoculation. The inoculum was produced by culturing *Rizobium leguminosarum* on a yeast mannitol agar medium at 28°C for 4 day. Then strains from previous culture was cultivated in 250 mL flasks contain yeast mannitol broth and shaken 125 rpm for 4 days at room temperature. Each inoculated plant received 1 mL of a 4 day old (log phase) culture which was adjusted with distilled water to OD_{620}= 0.08 (approximately 10^8 cells mL^{-1}) (Bhuvaneswari *et al.*, 1980). The inoculum was cooled to the corresponding RZT and applied by pipette to the root area. Plants were watered with a nitrogen-free Broughton and Dillworth solution (Beck *et al.*, 1993). Prior to each watering the temperature was adjusted to the treatment RZT.

The experimental design was a 4 by 4 factorial with treatments organized following a randomized complete block design with 3 replications and a total of 4 RZT and 4 ecotypes. Plants were harvested at 51 days after inoculation and the following data were collected: Root length, forage dry matter, root dry matter, nodule dry weight, nodule number, nodule cluster number, nodule cluster diameter, nodule diameter, nodule distribution along the root (root length that has nodule) and plant nitrogen percent (Kjeltec system, which includes digestion system 20 and a 1002 distilling unit, Tecator AB, Hoganas, Sweden). Results were statistically analyzed for variance using the SAS system (SAS Institute Inc. 1997). When analysis of variance showed significant treatment effects, Duncan's multiple range test was applied to compare the means at p<0.05.

RESULTS AND DISCUSSION

There were difference (p<0.01) among ecotypes, root zone temperatures and their interactions for nodule number and cluster. The maximum and minimum nodule numbers were observed in mashhad and sharkord ecotypes, respectively. Mashhad and sharkord ecotypes had similar nodule cluster, but ardabil and zanjan ecotypes had the highest and lowest nodule cluster respectively. The highest and lowest nodule number and nodule cluster were observed at 15 and 5°C, respectively. Mashhad ecotype produced the most nodule number at 25°C RZT and Sharkord ecotype had the least nodule

number at 5°C RZT. The maximum and minimum nodule clusters were observed in ardabil ecotype at 15 and 5°C RZT respectively (Table 1). Low temperatures retard root hair infection more than nodule initiation, nodule development, or N assimilation. Studies on subtropical legume crops have been concluded that low RZT decrease both nodulation and nodule function. Matthews and Hayes (1982) show that decreasing RZT from 25 to 10°C results in decrease nodule number and growth. This was attributed to inhibition of infection and nodule initiation by bacteria. Lynch and Smith (1993) reported that an RZT of 15°C restricted both infection and nodule development after inoculation. Nodulation ceased in plants at 10°C RZT (Matthews and Hayes, 1982; Peltzer *et al.*, 2002). With temperate legumes such as *Trifolium parviflorum* and *T. glomeratum*, Kumarasinghe and Nutman (1979) found that the onset of infection and rate of infection thread development in root hairs varied greatly with soil temperature. They found that at optimal soil temperatures (from 18 to 30°C) infections were initiated earlier and in larger numbers than at low (6 to 12°C) or moderately high (36°C) temperatures. Suboptimal RZTs suppress or delay root infection and nodulation (Walsh and Layzell, 1986). The respiratory capacity of a nodule is also likely to be lower at low temperatures (Earnshaw, 1981).

There were differences (p<0.01) among ecotypes, RZTs and interaction between ecotype and RZT for nodule distribution along the root. Mashhad ecotype had the most nodule distribution along the root and the least mentioned trait was belonged to ardabil and sharkord ecotypes. The maximum and minimum nodule distribution along the root produced at 25 and 5°C RZTs respectively. Mashhad ecotype had the highest nodule distribution

along the root at 25°C RZT and the lowest one was belonged to zanjan ecotype at 5°C RZT (Table 1). At 25°C RZT, Mashhad and zanjan ecotypes produced the most and least nodule distribution along the root respectively. At this temperature, ardabil and sharkord ecotypes had the same nodule distribution along the root. The most nodule distribution was observed in zanjan ecotype at 15°C RZT and the lowest nodule distribution was belonged to sharkord ecotype at the same teperature. Ardabil and mashhad ecotypes had the same nodule distribution along the root at 15°C RZT. At 10°C RZT, sharkord and ardabil had the most and least nodule distribution along the root respectively. At 5°C RZT, zanjan ecotype had the most mentioned trait and other ecotypes had the same nodule distribution (Table 1). Infection and early nodule development processes are most sensitive to low RZT (Lynch and Smith, 1993; Zhang and Smith, 1994). All of the stages of symbiotic establishment investigated to date (root hair curling, infection thread formation and penetration and nodule development and function) are inhibited by suboptimal RZT (Zhang and Smith, 1994). Roughley and Date (1986) reported that the aggregated area of bacteroid tissue volume is %79 in red clover grown under optimal temperature conditions.

RZT and an ecotype*RZT interaction had significantly effect on nodule and nodule cluster diameter (p<0.01). Ecotypes had different nodule cluster diameter and the same nodule diameter. Zanjan, ardabil and mashhad ecotypes had the most nodule cluster diameter and sharkord ecotype had the least mentioned trait. The highest and lowest nodule and nodule cluster diameter were produced at 15 and 5°C RZT, respectively. At 10°C RZT, ardabil, sharkord and mashhad ecotypes produced

Table 1: Mean comparisons of interaction effects of grasspea ecotypes and root-zone temperatures on various plant characters

RZT (°C)	Cultivar	Nodule No.	Nodule cluster No.	Nodule Distribution	Nodule diameter (mm)	Nodule cluster diameter (mm)	Nodule fresh weight (g)	Nodule dry weight (g)	Root length (cm)	Root dry matter (g)	Forage dry mater (g)	Nitrogen (%)
5	Zanjan	3.06ef	0.67h	1.27i	0.67efg	0.82f	0.058g	0.0004h	20.21ed	0.57cd	0.92d	2.84ef
	Ardabil	0.23g	0.1i	0.10j	0.33i	0.13g	0.000i	0.0000h	25.35bc	0.50d	0.90d	2.63f
	Sharkord	0.20g	0.17i	0.13j	0.13j	0.29g	0.001i	0.0001h	23.32cde	0.51d	1.30bc	2.84ef
	Mashhad	0.73g	0.20i	0.27j	0.50ghi	0.33g	0.061g	0.0023g	26.23bc	0.44d	1.20bc	2.63f
10	Zanjan	3.06ef	1.00gh	3.23g	0.47hi	0.92f	0.008i	0.0043f	19.63e	0.92a	1.33bc	2.98def
	Ardabil	2.93f	0.93gh	2.13h	1.07abc	1.79e	0.033h	0.0046f	28.46b	0.82ab	1.40bc	3.16def
	Sharkord	4.13def	1.07gh	5.60e	1.13a	1.11f	0.037gh	0.0043f	26.03bc	0.73cd	1.90a	3.07def
	Mashhad	4.20de	1.60f	4.40f	1.10ab	2.12cd	0.197c	0.0043f	24.74bc	0.87ab	1.85a	3.05def
15	Zanjan	8.67b	4.27b	8.50b	1.17a	2.57ab	0.170d	0.0166b	19.68e	0.61cd	0.83d	3.98ab
	Ardabil	10.73a	6.27a	7.10cd	1.17a	2.69a	0.116ef	0.0122c	24.06dc	0.62cd	1.26bc	4.10a
	Sharkord	3.86def	1.73f	4.53f	0.90cd	1.62e	0.131e	0.0107d	27.07bc	0.57cd	1.29bc	3.23def
	Mashhad	6.33c	1.40fg	7.43cd	0.93bcd	2.44ab	0.049gh	0.0082e	22.93cde	0.54d	1.46b	3.33cde
25	Zanjan	4.37d	2.40de	6.77d	0.70efg	2.44ab	0.116ef	0.0102d	19.51e	0.55cd	0.80d	3.43bcde
	Ardabil	4.20de	2.33e	8.27b	0.57fgh	2.27bc	0.280a	0.0250a	20.40ed	0.47d	0.89d	3.49bcd
	Sharkord	4.86d	2.80c	7.77bc	0.77de	1.89de	0.220b	0.0121c	25.12bc	0.54d	1.17c	3.33cde
	Mashhad	10.93a	2.87c	11.07a	0.63efgh	1.81e	0.100f	0.0108d	46.10a	0.58cd	1.38bc	3.84abc

Different letter(s) indicate significant difference between the values in the column (Duncan's multiple comparison test, p<0.05)

the maximum nodule diameter. Zanjan and Ardabil had the most nodule diameter at 15°C RZT. The least nodule diameter was belonged to sharkord ecotype at 5°C RZT. The highest nodule cluster diameter was belonged to ardabil ecotype at 15°C RZT, but this trait in zanjan and mashhad ecotype at 15°C RZT and zanjan ecotype at 25°C RZT were not significantly difference with nodule cluster diameter in ardabil at 15°C RZT. The minimum nodule cluster diameter was observed in ardabil ecotype at 5°C RZT that was not different with this trait in sharkord and mashhad ecotypes at the same temperature (Table 1). This result is similar to a previous report by Zhang *et al.* (1995) showing decreased nodule initiation, nodule development and nitrogen assimilation in soybean plants as RZT decreased to 17°C. Peltzer *et al.* (2002) showed that at the lowest RZT (7°C), nodulation was almost completely inhibited.

There were significantly differences (p<0.01) among ecotype, RZT and ecotype*RZT interaction for wet and dry nodule weights. Ardabil, sharkord and mashhad ecotypes had the most wet nodule weight and the least it was belonged to zanjan ecotype. Ardabil ecotype also had the highest nodule dry weight but this trait was similar in other ecotypes. The maximum and minimum wet and dry nodule weight were observed at 25 and 5°C, respectively. Ardabil ecotype produced the highest wet and dry nodule weights at 25°C RZT and the lowest it was in the same ecotype at 5°C RZT.

There were not significantly differences between ardabil and sharkord ecotypes at 5°C RZT for wet nodule weights. Ardabil, zanjan, sharkord ecotypes had the same dry nodule weight at 5°C RZT. This result is similar to a previous report by Peltzer *et al.* (2002) that showed the effect of low RZT on nodulation of lupin. They found that low temperature (7 and 12°C compared with 25°C) reduced the growth inoculated plants and at lower temperatures nodulation was severely inhibited. In their study the most sensitive stage to low root-zone temperature was nodule initiation and there appeared to be a critical temperature between 7 and 12°C at which initiation did not take place. the same result has been shown in this study. Gibson (1967) concluded that low root-zone temperature had greater effect on nodule initiation than on infection but that subsequent nodule development was also affected. Zhang and Smith (1994) showed that below 17°C, the steps involved in both infection and initiation of nodule on soybean (*Glycine max*) were severely inhibited, but once nodules were initiated, nodule number were only slightly reduced and the weight of each nodule was unaffected.

There were differences (p<0.01) among ecotype, RZT and ecotype*RZT interaction for root length. Mashhad ecotype had the most root length but other ecotypes had the same root length. The most and least

root lengths were obtained at 25 and 5°C RZT, respectively. The highest and lowest root lengths were observed in mashhad ecotype at 25 and 5°C RZT, respectively. Sharkord and ardabil ecotypes had the same root length with mashhad ecotype at 5°C (Table 1). This result is similar to a previous report by Evans and Savage (1959) showing increased meristem cell size of *Vicia faba* as RZT increased to 25°C. Crawford and Huxter (1977) showed that a temperature increase from 2 to 14°C RZT resulted in an interaction for root development among Pea varieties (*Pisum sativum*).

The result demonstrated that the ecotypes were different for forage dry matter. RZT also affected on this trait (p<0.01). The maximum and minimum shoot dry matters were observed in ardabil and mashhad ecotypes respectively. Mashhad and zanjan ecotypes had the same forage dry matter (Table 2). The most shoot dry weight was observed at 15 and 25°C but the least it was obtained at 5°C (Table 2). Ardabil ecotype produced the most shoot dry matter at 15 and 25°C. The lowest forage dry matter was produced by mashhad ecotype at 5°C. Sharkord, zanjan and mashhad ecotypes at 5°C and zanjan and mashhad ecotypes at 10°C produced the same forage dry weight (Table 1). The results are similar to those reported for other legumes (Evans and Savage, 1959; Walsh and Lazell, 1986; Crawford and Huxter 1977; Zhang *et al.*, 1995). Previous researched have shown that low RZT decreased plant weight, shoot dry weight, leaf number and area, yield and yield component values of annual alfalfa and soybean cultivars (Zhang *et al.*, 1995; Amini Dehaghi and Modarres Sanavy, 2003).

There were significantly effects among ecotypes for root dry matter but effect of RZT and ecotype*RZT on this trait were not significant (p<0.01). Root dry matter was similar at different RZTs. Ardabil ecotype had the most root dry weight and zanjan, sharkord and mashhad ecotypes had similar it (Table 2). The maximum root dry matter was observed in ardabil ecotype at 5°C RZT and the minimum it was obtained from zanjan ecotype at 25°C RZT. Sharkord and zanjan ecotypes at 25°C and mashhad

Table 2: Mean comparisons of main effects of grasspea ecotypes and root-zone temperatures on two plant characters

Rzt (°C)	Root dry matter (g)	Forage dry matter (g)
5	0.66a	0.97c
10	0.60a	1.11b
15	0.59a	1.42a
25	0.60a	1.47a
Ecotypes		
Zanjan	0.50b	1.08c
Ardabil	0.83a	1.62a
Sharkord	0.59b	1.21b
Mashhad	0.53b	1.06c

Different letter(s) indicate significant difference between the values in the column (Duncan's multiple comparison test, p<0.05)

and zanjan ecotypes at 10 and 15°C RZT had similar root dry weight, respectively. Low temperature reduced the growth rate of root and shoot of grasspea. Shoot fresh weight was more sensitive than root fresh weight. Peltzer *et al.* (2002) reported that root growth was less sensitive to lowered temperature compared with shoot growth but the initiation and development of nodules was very sensitive, especially at the lowest RZT used (7°C). Low RZT also severely inhibited the formation of root cap border cells (Peltzer *et al.*, 2002).

There were significantly differences (p<0.01) among ecotypes, RZT and ecotype*RZT interaction for plant nitrogen percent. Sharkord ecotype had the most nitrogen percent. Ardabil and mashhad ecotypes had the highest and lowest nitrogen percent at 15 and 5°C RZT, respectively. There were not differences among ecotypes for forage nitrogen percent at 5 and 10°C RZTs. Ardabil and mashhad ecotypes had the most nitrogen percent at 15 and 20°C, respectively (Table 1). High nitrogen percent in these two ecotypes is due to more nodule and nodule cluster production. Studies of the effects of suboptimal RZTs on soybean concluded that these conditions decrease N fixation activity by the nitrogenase enzyme complex (Layzell *et al.*, 1984) and suppress and/or delay root infection and nodulation (Walsh and Layzell, 1986). The effects of low temperature on the function of N_2-fixing nodules may be due, in part, to the changes in nodule oxygen permeability (Walsh and Layzell, 1986). Low temperature is known to increase O_2 solubility and decrease its diffusion coefficient for the oxygenated form of Leghemoglobin (Moll, 1968) and a change in the affinity of leg hemoglobin for O_2 and for other substrate (Stevens, 1982). The respiratory capacity of a nodule is also likely to be lower at low temperatures (Earnshaw, 1981). Legume plants export the fixed N in the nodule in form of ureide. The solubility of ureide is low and decreases sharply as temperature declines, therefore low temperature may also limit the rate of export of fixed N

from the nodule (Sprent, 1982). In turn, higher N concentrations inside the nodule may inhibit N2 fixation. Temperature decreasing results in progressively less bacteroid tissue (Lie, 1981).

The effect of low temperature on soybean nodulation and N fixation and assimilation may also be mediated via effects on plant physiology and growth. For grasspea, decreased aerial temperature resulted in decreased relative growth rate, stomatal conductance, net CO_2 exchange rate, leaf assimilate export rates and leaf elongation rate. Many adverse effects of low RZT on chilling-sensitive plants can be attributed to low temperature-induced membrane phase transitions which decrease the activity of membrane-bound enzymes (Duke *et al.*, 1978). The effect of low temperature on N_2 fixation and NO_3-N assimilation may also be mediated via effects on photosynthesis or translocation, as has been demonstrated in the case of limitations to nitrogenase activity. Decreased shoot and root respiration and increased carbon levels (Partly as starch) in mature leaves and stems at low RZTs (Walsh and Layzell, 1986) reduced transportation of energy to nodules and decreased nodule function.

Pearson correlation coefficients among traits showed that nodule number was positively correlated with nodule distribution along the root, nodule diameter, nodule cluster diameter and root length. There was positive correlation between root length and nodule distribution along the root. Correlations of nodule cluster diameter with wet and dry nodule weight were positive (Table 3). There were no correlations among nitrogen percent and nodule number and diameter (Table 3). The enhancement of nodule number and/or size by plant growth promoting rhizobacteria was not always related to increasing nitrogen fixation. Zhang *et al.* (1996) reported that strain S. proteamaculans1-102 did not increase nodule number at 15°C RZT, but did increase nodule size and plant nitrogen concentration.

Table 3: Correlation coefficients for annual medic traits under different RZT

Traits	Nodule No.	Nodule cluster No.	Nodule distribution	Nodule diameter	Nodule cluster diameter	Nodule fresh weight	Nodule dry weight	Root dry matter	Root length	Forage dry matter
Nodule cluster number	0.86**									
Nodule distribution	0.85**	0.72**								
Nodule diameter	0.58*	0.56*	0.46^{ns}							
Nodule cluster diameter	0.76**	0.78*	081**	0.70**						
Nodule fresh weight	0.37^{ns}	0.51*	0.61**	0.32^{ns}	0.64**					
Nodule dry weight	0.49^{ns}	0.63^{ns}	0.70**	0.25^{ns}	0.64**	0.83**				
Root length	0.65**	0.33^{ns}	0.63**	0.13^{ns}	0.34^{ns}	0.06^{ns}	0.26^{ns}			
Root dry matter	-0.17^{ns}	-0.05^{ns}	-0.20^{ns}	-0.15^{ns}	-0.17^{ns}	-0.06^{ns}	0.10^{ns}	-0.03^{ns}		
Forage dry matter	0.55*	0.68**	0.58*	0.35^{ns}	0.57^{ns}	0.48^{ns}	0.67**	0.34^{ns}	0.57*	
Nitrogen percent	0.12^{ns}	-0.12^{ns}	0.28^{ns}	0.01^{ns}	-0.39^{ns}	0.22^{ns}	-0.34^{ns}	-0.18^{ns}	-0.04^{ns}	-0.21^{ns}

ns, * and **: no significant and significant at 1 and 5% probability level respectively

CONCLUSIONS

The result showed that RZT below 15°C can reduces traits average related to nitrogen fixation in grasspea. Ardabil and mashhad ecotypes had better traits than other ecotypes at 15 and 25°C RZT. As a result, cultivation grasspea at zones that soil temperature reached to 10°C or below, maybe not success, but at higher soil temperature grasspea can remarkably form nodule and fixation nitrogen. The best temperature for nodule initiation and nitrogen fixation in grasspea is 15°C RZT.

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