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ORIGINAL ARTICLE

Influence of root-zone temperature on growth and nitrogen fixation in three Iranian grasspea landraces

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Abstract

In order to study the effects of different root-zone temperature (RZT) and two *Rhizobium leguminosarum* strains (originating from cold area and temperate area) on some grasspea (*Lathyrus sativus*) landraces (Ardabil, Shahrekord, and Zanjan) of Iran, an experiment was conducted in a controlled-environment chamber. In this experiment, four root-zone temperatures (5, 10, 15, and 25°C) and a constant air temperature were considered. Results showed that there were differences among the grasspea landraces for nodulation and nitrogen fixation, growth and development, and dry matter. Low RZT reduced growth, nodulation, and nitrogen fixation in grasspea landraces. Strains had different effects on landraces. Inoculated plants with strain originating from cold areas produced the most nodule number, and plant nitrogen concentration at 15°C RZT, whereas inoculated plants with strain originating from temperate area produced the most nodule number, and plant nitrogen concentration at 25°C RZT. Nodulation, nitrogen fixation, and nitrogenase activities of inoculated plants with a strain originating from cold areas in inoculated plants were higher at low temperature than in inoculated plants with a strain originating from temperate areas. This experiment also showed that grasspea production is not likely to be successful when RZT is below 10°C, especially during vegetative development. Therefore, in the zones where soil temperature is greater than 10°C RZT, grasspea landraces have normal growth and produce average yields.

Keywords: *Grasspea, low zone temperature, nitrogen fixation, nodulation, strain.*

Introduction

Grasspea (*Lathyrus sativus* L.) 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 (Wang et al., 2000). It thrives best in areas with air temperature of 10–25°C (Muehlbauer & Tullu, 1997). Those N-fixing plants represent a natural source of nitrogen and therefore allow a reduction of the costs of the crop and the ecological impact of input of N fertilizers. Biological nitrogen fixation is a complex process depending on the molecular signal exchanges between plant and bacteria and also dependent on plant–bacteria–environment interactions (Prell & Poole, 2006; Simoes-Araujo et al., 2008).

It is generally admitted that temperate legumes nodulate and fix N over the temperature range 10–30°C, and the tropical ones over the temperature range 15–35°C. The temperature range for functional symbiosis is narrower than that of the plant supplied with combined nitrogen. Kalinina et al. (1982) reported that, in the presence of a low-temperature stress, the involvement of roots and shoot in nitrogen assimilation was re-distributed. The low-temperature limit of 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; Robin et al., 2005). Temperate climates are characterized by short growing seasons, which are subjected to temperatures below the optimal for symbiotic nitrogen fixation. Consequently, growth of legumes can

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be significantly reduced as reported with alfalfa (Rice & Olsen, 1988) and soybean (Lynch & 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). It was demonstrated that poor N_2 fixation by native medics cultivars or accessions can be improved by the selection of more adapted *Sinorhizobium* strains (Papastilyanou, 1987). While climatic conditions cannot be changed, the selection of cultivars (Papastilyanou, 1987), *Rhizobium* strains (Castillo et al., 1999), or both (Webber, 1993) that tolerate unfavorable conditions remains the best approach. Roughley (1970) found that the strain TA1, isolated from a comparatively cold environment, nodulated and formed bacteroid on sub clover (*Trifolium subterraneum* L.) at 7°C root temperature, while strain SU97, isolated from a warmer environment, did not. There is variability among *Rhizobium leguminosarum* strains for ability to nodulate grasspea plants and increase grasspea growth under low root-zone temperatures, and strains of *Rhizobium leguminosarum* selected for growth at low temperatures have the ability to overcome low root-zone temperature inhibition of grasspea nodulation in the field in a short-season, cool spring area. Hence, the objective of this study was to evaluate selected strains of *Rhizobium leguminosarum* for their ability to improve grasspea landrace growth and nitrogen fixation in a controlled-environment chamber with different root-zone temperatures.

Materials and methods

Seeds of the three grasspea landraces were collected (September 2005) from some agricultural fields located in the provinces of Zanjan (36° 41′ N, 48° 29′ E; 1663 m elevation), Shahrekord (32° 20′ N, 50° 51′ E; 2061.4 m elevation), and Ardabil (38° 39′ N, 48° 17′ E; 1332 m elevation). According to the classification of Emberger (cited by Nahal, 1981), these regions have a semiarid climate with cold winters and dry summers.

Two strains of *Rhizobium leguminosarum* were isolated from soil of a cold region (Zanjan) and a temperate region (Tehran). Soil samples were collected from 0–15 cm depth under agricultural fields (cultivated with grasspea) in five different locations of Tehran and Zanjan. Plastic pots (500 ml) were separately filled with collected soil based on each province and some sterilized soil. The three seeds of grasspea were surface sterilized in 95% ethanol (for 5 s) and sodium hypochlorite and then were sown in pots. Grasspea seedlings established under controlled conditions. After 7 weeks, 20 plants were carefully dug up with the roots intact and transported to the laboratory. Rhizobia were isolated from fresh nodules by the Hotel isolation method (Vincent, 1970).

Single colonies were picked and checked for purity by repetitive streaking on yeast mannitol agar (YMA) medium. The inoculums were produced by culturing two strains on a YMA medium at 28°C for 4 days, and then previous culture was suspended in yeast mannitol broth in 250-ml flasks and shaken at 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 optical density 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.

Seeds of the 3 grasspea landraces (Zanjan, Shahrekord, and Ardabil), were surface sterilized in 95% ethanol (for 5 s) and sodium hypochlorite (2% solution for 3 min) (Dodds & Roberts, 1995). The seeds were then rinsed thoroughly with distilled water and planted in trays containing sterilized sand. Six-day-old seedlings, at the cotyledon stage, were transplanted into sterilized 15-cm plastic pots containing the same medium, in a growth chamber in 2006. Every pot contained three grasspea seedlings. The growth chamber light (300 μmol $m^{-2} s^{-1}$) was provided by cool-white fluorescent tubes. Light intensity across the growth chamber was measured several times during the experiment and was always uniform. The photoperiod was 16/8 h (day/night). As this work examined the influence of four RZTs (5, 10, 15, and 25±0.2°C), the air temperature (±0.2°C) was held at 25/15°C day/night. 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 into large pots. After being transplanted into the pots, the plants were acclimatized for 24 h prior to inoculation. Plants were watered with Broughton and Dillworth's solution; prior to each watering the temperature of the nutrient solution was adjusted to the treatment RZT.

The experimental design was a 3 (grasspea landraces) by 4 (root-zone temperatures) by 2 (*R. leguminosarum* strains) factorial with treatments organized following a randomized complete-block design, with 3 replications. The six plants were harvested at 40 days after inoculation from each treatment (involved 4 pots) and the following data were collected: plant height, leaf area (Delta-T area meter; Delta-T Devices Ltd, Cambridge, England), leaf number, root length, shoot dry matter, root dry weight, nodule dry weight, nodule number and diameter, plant nitrogen concentration (Kjeltec system, which includes digestion system 20 and a 1002 distilling unit, Tecator AB, Hoganas, Sweden), specific nodule weight (nodule weight/nodule number), and nitrogen fixation. Acetylene-reduction activity was used for measurement of nitrogenase activity. This method involved exposure of the detopped roots of plants in each pot to 10% acetylene in a sealed 1l mason jar. The jar was maintained at the treatment temperature for 24 h. A 0.5-ml gas aliquot was then extracted and analysed by gas chromatography (Hardy et al., 1968). Acetylene and ethylene concentrations were determined with a Unicam 4600 gas chromatograph equipped with a flame ionization detector (FID). The plot fused-silica capillary column (CP-Al_2O_3/Na_2SO_4) was 50 m long × 0.25 mm in diameter. The oven temperature was 160°C. Helium was used as the carrier gas with 2 psi pressure. Under these conditions the retentions for ethylene and acetylene were approximately 5 and 6 minutes, respectively. Results were statistically analysed for variance using the SAS system (SAS Institute, 1997). When analysis of variance showed significant treatment effects, Duncan's multiple-range test was applied to compare the means at $P < 0.05$ (Steel & Torrie, 1980).

Results

There were significant differences ($P < 0.01$) among landraces, RZTs, and strains for plant height and stem dry matter. There was also an RZT × strain interaction for both traits ($P < 0.01$). Ardabil landrace had the most plant height and stem dry matter. Plant height in Zanjan and Shahrekord landraces was the lowest, and values for the two were similar. Zanjan landrace had the lowest stem dry matter, which was statistically similar with stem dry matter in Shahrekord landrace. The maximum and minimum plant height and stem dry matter were obtained at 25 and 5°C RZT, respectively. Plant height and stem dry matter increased in inoculated plants with the strain originating from temperate areas (Table I). Mean comparisons showed that plant height increased in inoculated plants with the strain originating from temperate areas at 25°C RZT. The lowest plant height belonged to inoculated Shahrekord landrace with the strain originating from temperate areas at 5°C RZT, and maximum plant height belonged to inoculated Ardabil landrace with the strain originating from temperate areas at 25°C RZT. The maximum stem dry matter was observed in inoculated Zanjan landrace with the strain originating from temperate areas at 25°C RZT. Landraces inoculated with the strain originating from cold areas produced the most stem dry matter at 15°C RZT and they were inoculated with the strain originating from temperate areas to produce the most stem dry matter at 25°C RZT (Table II).

Leaf area showed significantly differences among landraces, RZTs, and strains. All interactions among treatments were significant for leaf area ($P < 0.01$). The highest and the lowest leaf area were observed at 25 and 10°C RZT, respectively. Leaf area of plants at 5 and 10°C RZT was similar. Inoculated plants of the strain originating from temperate areas produced more leaf area than did incubated plants of the strain originating from cold areas (Table I). Inoculated Ardabil and Zanjan landraces with the strain originating from temperate areas produced similar leaf area at 25°C RZT. Former landraces had the lowest leaf area at 10°C RZT when they were inoculated with the strain originating from cold areas. Shahrekord landrace inoculated with each strain produced the highest and lowest leaf area at 25 and 5°C, respectively (Table II).

There were differences among RZTs, strains, and their interaction effect for root length. Root dry weight was affected by landraces, strains, and their interaction effect. There was also a strain × RZT interaction for root dry weight ($P < 0.01$). Landraces had similar root length. Ardabil and Zanjan landraces had the highest and the lowest root dry weight, respectively. Root length was similar at 10, 15, and 25°C RZT. The shortest root length was observed at 5°C RZT. Root dry weight was similar in all RZTs. Inoculated plants with the strain originating from temperate areas increased root length and dry weight (Table I). Except for Zanjan, mean comparison showed that root length in inoculated landraces with the strain originating from temperate areas was the maximum at 10°C RZT. Root length was similar in inoculated landraces with the strain originating from temperate areas at 10 and 25°C RZT, and in inoculated landraces with the strain originating from cold areas at 15°C RZT. Inoculated landraces with the strain originating from cold areas had the lowest root length at 5°C RZT. Except for Ardabil, the highest root dry weights were observed in inoculated landraces with the strain originating from temperate areas at 25°C RZT, and inoculated landraces with the strain originating from cold areas did not have similar root length at all RZTs (Table II).

Nodule number was affected by RZTs, landraces, strains, and their interactions ($P < 0.01$). Zanjan and Ardabil landraces had similar nodule numbers. Shahrekord landrace produced the lowest nodule number. Inoculated plants with the strain originating from cold areas increased nodule number in

Table I. Mean comparisons of main effects of grasspea landraces, root-zone temperatures, and strains on various plant characteristics.

T	PH (cm)	SDM (g pot^{-1})	LA (cm^2 pot^{-1})	RL (cm)	RDW (g pot^{-1})	NN	ND (mm)	NDW (mg)	SNW (mg nodule^{-1})	SNC (mg g^{-1})	NF (µmol day^{-1} plant^{-1})
5	43.06d	0.93d	66.61c	15.96b	0.55a	0.98d	0.47d	0.5d	1.0c	29.46d	0.00c
10	46.58c	1.19c	61.94c	21.89a	0.57a	2.68c	0.99b	4.20c	1.90b	31.67c	0.00c
15	54.58b	1.56b	89.22b	22.48a	0.57a	6.70a	1.12a	11.10b	1.90b	38.37b	1439.22b
25	62.94a	1.93a	151.56a	22.34a	0.59a	5.33b	0.87c	12.90a	2.60a	41.82a	2874.72b
Landrace											
Ardabil	55.33a	1.51a	110.33a	20.76a	0.63a	4.40a	1.00a	9.0a	2.0a	37.73a	1275.28a
Zanjan	50.39b	1.30b	82.08b	20.72a	0.51c	4.45a	0.78b	6.0b	1.0b	34.69b	1089.74b
Shahrekord	49.66b	1.39b	84.58b	20.53a	0.57b	2.92b	0.80b	5.0c	1.0b	33.57b	870.44c
Strain											
ST	54.04a	1.50a	106.36a	22.48a	0.67a	3.51b	0.76b	7.0b	1.07b	36.56a	985.50b
SC	49.54b	1.30b	78.30b	18.86b	0.47b	4.33a	0.97a	7.30a	1.90a	34.00b	1171.47a

PH: Plant height; SDM: Stem dry matter; LA: Leaf area; RL: Root length; RDW: Root dry weight; NN: Nodule no.; ND: Nodule diameter; NDW: Nodule dry weight; SNW: Specific nodule weight; SNC: Shoot nitrogen concentration; NF: Nitrogen fixation. ST: strain originating from temperate areas; SC: strain originating from cold areas. Within columns, means followed by the same letter are not significantly different at $P < 0.05$ (Duncan's multiple-range test).

comparison with inoculated plants with the strain originating from temperate areas (Table I). Inoculated landraces with the strain originating from cold areas produced a higher nodule number at 15°C than at other RZTs. The lowest nodule number was observed in inoculated Zanjan and Shahrekord landraces with the strain originating from temperate areas at 5°C RZT (Table II).

There were differences among RZTs, landraces, strains, and their interactions for nodule diameter ($P < 0.01$). Ardabil landrace had the greatest nodule diameter. The maximum and the minimum nodule diameter were obtained at 15 and 5°C RZT, respectively. Inoculated plants with the strain originating from cold areas produced greater nodule diameter than did inoculated plants with the strain originating from temperate areas (Table I). Inoculated Ardabil landrace with the strain originating from cold and temperate areas had the greatest and the least nodule diameter at 10 and 5°C RZT, respectively. Inoculated Zanjan landrace with the strain originating from temperate areas produced its maximum nodule diameter at 15°C RZT. Shahrekord and Zanjan landraces had the greatest nodule diameter at 15 and 25°C RZT when inoculated with the strain originating from cold areas (Table II).

Significant effects of the landraces, RZTs, strains ($P < 0.05$), and their interactions ($P < 0.01$) were observed on nodule dry and specific nodule weight. Ardabil and Shahrekord landraces produced the highest and the lowest nodule dry and specific nodule weight, respectively. Specific nodule weight was similar in both Zanjan and Shahrekord landraces. The highest and the lowest nodule dry weight and specific nodule weight were obtained at 25 and 5°C, respectively. The strain originating from cold areas increased nodule dry weight and specific nodule weight in inoculated plants (Table I). Inoculated Ardabil and Shahrekord landraces with the strain originating from temperate areas produced the highest nodule dry weight at 25°C RZT. Nodule dry weight in inoculated plants with each strain was lowest at 5°C RZT. Among Zanjan landrace treatments, inoculated plants with the strain originating from cold areas produced the highest nodule dry weight at 15°C RZT. Inoculated Ardabil and Zanjan landraces with the strain originating from temperate areas produced the highest specific nodule weight at 25°C RZT. The lowest former trait belonged to inoculated Ardabil and Zanjan landraces with the strain originating from temperate areas at 5°C RZT. Among Shahrekord landrace treatments, inoculated plants with the strain originating from temperate areas had the highest specific nodule weight at 15°C RZT (Table II).

Table II. Mean comparisons of interaction effects of inoculated grasspea landraces with Zanjan and Tehran strains at root-zone temperatures on various plant characteristics.

RTZ (cm^2 pot^{-1})	Strain	Landrace	PH (cm)	SDM (g pot^{-1})	LA	RL (cm)	RDW (g pot^{-1})	NN	ND (mm)	NDW (mg)	SNW (mg $nodule^{-1}$)	SNC (mg g^{-1})	NF (μmol day^{-1} $plant^{-1}$)
5	ST	Ardabil	44.51ghij	0.73i	91.33de	19.63ef	0.92a	0.33lk	0.33gh	0 l	0 k	29.28ghi	0f
10			46.66fghij	1.10ghijk	87.00def	28.46a	0.82ab	2.93i	1.06bc	4.40h	1.50fghi	31.64fghi	0f
15			57.41cd	1.53defg	101.67d	18.80f	0.73bc	3.93efgh	1.17bc	12.10d	1.10hji	40.51dc	1534.6e
25			81.80a	2.23b	217.67a	24.74abc	0.87a	8.47b	0.59ef	25.00a	6.10a	57.41a	3044.9b
5	SC		46.00fghij	1.33fghi	71.67efgh	11.60g	0.40g	1.40j	0.75de	0.60kl	4.0jk	31.68fghi	0f
10			54.87cdef	1.40efgh	53.67hji	17.33f	0.43g	3.20ghi	1.85a	7.00g	2.20defg	35.47def	0f
15			60.53c	1.90bcd	134.67c	26.03abc	0.43g	10.73a	1.22b	13.80c	3.60b	40.95c	4057.5a
25			50.83defgh	1.85bcde	125.00c	19.47ef	0.43g	4.20ef	1.07bc	15.60b	1.80efgh	34.89efgh	1565.5e
5	ST	Zanjan	42.10gij	0.80kj	65.67fghi	20.21def	0.57def	0.20l	0.68de	0.40kl	0.10k	28.39i	0f
10			41.43gij	0.97hijk	48.33ji	25.35abc	0.50efg	1.53j	0.49efg	2.30ij	0.80ji	29.76fghi	0f
15			48.86defghi	1.50defg	74.67efgh	19.73ef	0.51efg	7.80b	1.18b	8.50f	1.90efg	39.82cde	1433.8e
25			76.33ab	2.80a	232.33a	26.23abc	0.60cdef	6.80b	0.68de	10.20e	2.30de	43.70bc	2611.8c
5	SC		44.50ghij	0.92ijk	53.00hij	12.63g	0.47fg	3.07hi	0.38fg	0.40kl	2.30de	29.17hi	0f
10			45.33fghij	0.90ijk	42.33j	18.03f	0.53efg	3.07hi	0.72de	5.00h	3.00bc	30.92fghi	0f
15			56.73cde	1.30fghi	77.33efg	23.32bcde	0.47fg	8.66b	1.05bc	16.60b	1.10hji	41.15c	3234.9b
25			47.81efghi	1.20ghij	63.00ghij	20.27def	0.44g	4.47def	1.05bc	9.70e	1.40ghi	34.31fghi	1437.5e
5	ST	Shahrekord	37.93j	1.00hijk	53.33hij	19.68ef	0.61cde	0.20l	0.13h	0.1l	0.50jk	28.95i	0f
10			50.24defgi	1.57defg	66.33fghi	24.06bcd	0.62cde	0.67jkl	1.13bc	4.30h	1.00ji	30.71fghi	0f
15			50.82defgh	1.70cdef	59.33ghji	19.93ef	0.57def	3.87fghi	0.90dc	4.80h	2.80dc	32.33fghi	1348.8e
25			70.33b	2.03bc	178.67b	22.93cde	0.70bcd	4.87de	0.76de	12.20d	2.50de	47.37b	1852.4d
5	SC		43.30hij	0.83jk	64.67ghi	12.03g	0.43g	1.20jk	0.57efg	1.40kj	2.40de	29.32ghi	0f
10			40.97ij	1.26fghij	74.00efgh	18.13f	0.50efg	4.13efg	0.70de	2.70i	2.20def	31.46fghi	0f
15			53.13cdefg	1.40efgh	87.67def	27.07ab	0.60cdef	5.20d	1.21b	10.70e	0.90ji	34.14defg	2447.0c
25			50.52defghi	1.35fghi	92.67de	20.40efd	0.54efg	3.20ghi	1.04bc	4.80h	1.50fghi	33.26fghi	1315.3e

PH: Plant height; SDM: Stem dry matter; LA: Leaf area; RL: Root length; RDW: Root dry weight; NN: Nodule no.; ND: Nodule diameter; NDW: Nodule dry weight; SNW: Specific nodule weight; SNC: Shoot nitrogen concentration; NF: Nitrogen fixation. ST: strain originating from temperate areas; SC: strain originating from cold areas. Within rows, means followed by the same letter are not significantly different at $P < 0.05$ (Duncan's-multiple-range test).

Shoot nitrogen concentration was affected by landraces, RZTs, strains ($P < 0.01$), and their interactions ($P < 0.05$). Ardabil landrace had more shoot nitrogen concentration than did other landraces. The maximum and the minimum shoot nitrogen concentration were observed at 25 and 5°C RZT, respectively. The strain originating temperate cold areas increased shoot nitrogen concentration in plants in comparison with the strain originating from cold areas (Table I). Inoculated Ardabil landrace with the strain originating from temperate areas produced the most shoot nitrogen concentration at 25°C RZT. The lowest shoot nitrogen concentration was produced by inoculated Zanjan and Shahrekord landraces at 5°C RZT. Among Zanjan and Shahrekord landraces, inoculated plants with the strain originating from temperate areas produced the greatest shoot nitrogen concentration at 25°C RZT (Table II).

There were significant differences among RZT, landraces, and strains for acetylene reduction ($P < 0.01$). There was also an RZT × strain interaction for acetylene reduction. Ardabil and Shahrekord landraces had the most and the least acetylene reduction (nitrogen fixation), respectively. At 25°C RZT, acetylene conversion into ethylene was more than other temperatures. No ethylene was produced at 5 or 10°C RZT. Inoculated landraces with the strain originating from cold areas produced more ethylene than did inoculated landraces with the strain originating from temperate areas (Table I). Mean comparison of RZT × strain × landrace interaction showed that inoculated landraces with the strain originating from temperate and cold areas produced the most ethylene at 25 and 15°C RZT, respectively. Strains did not produce any ethylene at 5 or 10°C RZT (Table II).

Discussion

Root-zone temperature and strain significantly affected stem dry matter and plant height. The maximum and the minimum stem dry matter for each landrace were produced at 25 and 5°C RZT, respectively. RZT enhancement increased stem dry matter in landraces due to shoot nitrogen concentration and leaf area increment. Lemaire et al. (2008) showed that leaf area and nitrogen content had an effect on radiation capture and radiation-use efficiency, respectively. Researches on soybean (Zhang et al., 1995) and annual medics (Amini Dehaghi & Modarres Sanavy, 2003) have shown that low RZT decreased root dry matter, leaf number, and leaf area. Poustini et al. (2005) reported that in the low RZT, plants had fewer leaves and smaller leaf area than did plants at a higher RZT.

RZT enhancement increased shoot nitrogen concentration and nitrogen fixation, due to greater nodule number and diameter and more dry weight at high RZTs than at low RZTs. Previous investigations showed that suboptimal RZT reduced soybean nodulation and nitrogen fixation. A study of the effects of suboptimal RZTs on soybean concluded that these conditions decrease N_2-fixation activity by directly decreasing the activity of the nitrogenase enzyme complex (Layzell et al., 1984). Low temperature is detrimental to nitrogen fixation, by affecting net C assimilation and root nodulation (Prosperi, 1993); for example, nodulation ceased in plants at 10°C RZT (Peltzer et al., 2002). Infection and early nodule-development processes are most sensitive to low RZT (Lynch & Smith, 1993; Zhang & Smith, 1994). The nodulation was significantly delayed at the 7°C temperature (Robin et al., 2005).

Strains had different effects on landraces. Inoculated plants with the strain originating from temperate areas resulted in plant height enhancement, increased leaf area and stem dry matter, and nitrogen concentration increment in plants. Inoculated landraces with the strain originating from temperate areas produced the highest nodule number and nitrogen concentration at 25°C RZT, while inoculated landraces with the strain originating from cold areas produced the highest nodule number and nitrogen concentration at 15°C RZT. Inoculated landraces with the strain originating from temperate areas produced the most ethylene at 25°C RZT. Rhizobium activity is high when more acetylene converts into ethylene. Nitrogen reduction increment in plant increases vegetative growth as that yield increases. Plant nitrogen fixation with the strain isolated from temperate areas was increased by temperature increment. This nitrogen-fixation enhancement can be the result of increasing nodule number, nodule dry matter, and plant nitrogen concentration, while the strain originating from cold areas decreased nitrogen fixation in inoculated plants due to a reduction in nodule number, nodule dry matter, and nitrogen concentration at 25°C. The maximum mentioned traits were obtained in inoculated plants at below 15°C. Millhollon & Williams (1986) reported that the effect of low temperature on N_2 fixation and NO_3-nitrogen assimilation might be mediated via effects on photosynthesis or translocation, as has been demonstrated through photosynthetic limitation of nitrogenase activity (Sloger et al., 1975).

The selection of bacterial strain adapted to prevailing conditions should minimize adverse environmental effect on legume nodulation and N fixation (Gibson, 1971). The lower temperature limit of an N-fixation symbiosis is largely due to sensitivity on the part of the plant but can be modified by the strain of bacterial symbiont used. Nodulation and N fixation at a given temperature vary with bacterial strain. A number of studies have attempted to correlate the environmental origins and growth in culture of rhizobial strains with their symbiotic effectiveness under low-temperature stress. Ek-Jandér & Fåhraeus (1971) found that the performance of rhizobial strains at low temperatures was influenced by their geographical origin. Prèvost et al. (1987) reported that the nitrogenase activities of rhizobia isolated from cold areas were higher at low temperature than were those from warm areas. Roughley (1970) also found that a strain isolated from a cold environment caused nodulation and formed bacteroids under low temperatures while a strain isolated from a warmer environment did not.

Many authors found that *R. leguminosarum* strains isolated from saline soils or from high-temperature soils survive upon being exposed to such stresses, but lose their infectivity (Raman & Prasad, 1983; Roughley & Date, 1986; Moschetti et al., 2005). These results confirm that the evolution of rhizobial strains can be influenced by environmental conditions such as biological barriers to gene exchange, geographical isolation, soil type, and genotype of the host plant (Demezas et al., 1995).

Generally, landraces at low RZTs had lower vegetative growth, nodulation, and nitrogen fixation than did landraces at high RZTs. Therefore, in the zones where soil temperature is greater than 15 °C, grasspea can establish a symbiotic relationship with rhizobial bacteria and fix nitrogen. Most of the inoculated landraces with the strain originating from cold and temperate areas produced the highest nodule number and nitrogen concentration at 15 and 25 °C RZT, respectively. Consequently, our results showed that both the strain-originating area and the zone temperature should be taken into consideration for effective agronomic management.

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