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Effects of Different Rates of Potassium on Nitrogen Fixation and Agronomic Traits of Three *Medicago sative* Varieties

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Abstract: An experiment was conducted to evaluate the effects of K (0, 50 and 100 kg K ha^{-1}) on nitrogen fixation and dry matter production of three medicago (*Medicago sativa* L.) varieties (Hamadani, Sacoeal and Gharayoungeh), in the agricultural research station of Urmia University, West Azarbijan province. The performance management was under irrigating and cutting regime in a small-plot trial (1 m^2) with low K soil. The experimental layout was a randomized complete block split-plot design with four replicates. Three plants from each plot at initial flowering stage were taken for measuring N_2-fixation by using the natural abundance of ^{15}N, dry weight of leaves, stems, nodules and nutrients (K, C) of the shoots. Nodule weights were estimated from root systems of plants prior to third harvest. The highest rate of nitrogen fixed from atmosphere in shoots of Gharayoungeh (122 kg ha^{-1}) was obtained with the application of 100 kg K ha^{-1}, whilst the lowest yield (83 kg ha^{-1}) belonged to Sacoel in unfertilized plots. Both dry matter yield of stems and nodule weight were significantly increased by K fertilization. Dry matter yield of leaves in plants was reduced by K fertilization. K application increased the K, carbon contents of shoots of overall plant varieties by more than 50 kg K ha^{-1}. These evaluations demonstrated that varieties responded to different levels of K fertilizer. Increased yield of stems and nodules, in contrast, reduction in leaf yield by K application indicated that carbon from photosynthesis is preferably used for stem production and nodule formation.

Key words: Potassium, *Medicago sativa* varieties, N_2 fixation, nodule

INTRODUCTION

Multi-year Lucerne culture as long-term no-till management has resulted in significant vertical soil potassium (K) stratification (Howard *et al*., 1999). This may reduce plant K uptake and thus induce K deficiency in crop tissues as well as yield loss and reduction in N_2 fixation ability during growing seasons. The risks of reduction in plant K uptake by drought or low temperature in no-till fields become severe when soil K concentrations in deeper layers are low to optimize plant K uptake.

Potassium is of special importance with heavy crops. In particular, medicago, contain large amounts of K under high cropping intensity and improved carbohydrate transport from shoot to roots (Collins and Duke, 1981).

Alleviating K deficiency in N_2 fixing legume plants may alter the N_2-fixation process in the legume plant, as it has been reported that K can improve nodule weight and specific nitrogenase activity of *Trifolium vesiculosum* (Lynd *et al*., 1984). Improved K nutrition increased nodule number and acetylene reduction activity in alfalfa (Collins *et al*., 1986). The mineral nutrition of legumes is somewhat more complex than that of other plant species because of the special symbiotic relationship existing between the legume host and the associated rhizobial bacteria. Particular nutritional requirements are necessary for this extra physiological process to operate efficiently. These affect survival and growth of the rhizobia in the soil, infection and nodulation of the host root and functioning of nitrogen fixation reactions within the nodule. It is important to recognize that both the types of mineral elements and the concentration required for these additional functions may not be the same as those required for normal growth of the host plant itself. A qualitative requirement for K has been demonstrated for some rhizobia (Sherwood, 1970; Vincent, 1977). *R. trifolii* and *R. meliloti* show restricted growth when K is omitted from a defined medium and a linear response in cell yield up to 0.006 mM was obtained in batch culture (Vincent, 1977).

The amount of N_2 fixed is therefore closely related to legume dry-matter yield. However, levels of other nutrients may limit the percentage of N derived from the atmosphere (Peoples *et al*., 2001).

The rate and range of depletion for potassium in plant rhizosphere depends on the soil types and plants species (Shi *et al*., 2004). Although it has been well reported that there is a genotypic difference in the capacity and efficiency of mineral nutrients uptake, transport and utilization in plants (Yin and Vyn, 2003).

Subsurface placement of K fertilizer, therefore, may improve applied K availability, ability to Biological Nitrogen Fixation (BNF) and reduce soil K stratification in no-till systems. Pinkerton and Randall (1993) in glasshouse experiments examined and compared the internal K requirements of 7 legume species. Plants were supplied with 6 rates of K and these investigators found different K requirements among the species.

The objectives of the study were to 1) to quantify the above-ground dry matter yield of three-year-old *Midicago sativa* varieties and 2) to assess N_2 fixation under K fertilization. These findings would have important implications for the evaluation of legume performance in the field under conditions of K limitation.

MATERIALS AND METHODS

The experiment was carried out on the farm of Urmia University, where the experimental plot had been under continuous Lucerne study, for 3 years. The soil of the experimental field was relatively low in available K.

The Lucernes *Medicago sativa* L. cv. Hamadani, Sacoeal and Gharayoungeh, Azarbijanian commercial Lucerne were used. Seeds were inoculated with indigenous *Medicag sativa* rhizobia (*Rhizobium meliloti*), mixed with barley grass (*Hordeum violaceum*) seeds, hand spread and troweled into a prepared seedbed in April 2001. Seeding rate was 20 kg ha^{-1}. The inoculants was prepared from nodules of indigenous Lucerne plant, which were isolated on Yeast Mannitol Agar (YMA) containing 0.5 g K_2HPO_4, 0.2 g $MgSO_4$ $7H_2O$, 2.5 g yeast extract (Difco) and 10.0 g mannitol in distilled water, solidified with 1.5% agar (Difco). The purity of the isolates was checked on YMA containing Congo red and peptone-glucose agar (Vincent, 1970).

Inoculation was applied from the inoculants made up to a slurry with 10% sugar solution thoroughly mixed through the seed lots. A high rate of inoculum was used to ensure adequate bacterial numbers. Plots were spray-irrigated with 2 cm of water after sowing and required thereafter to limit moisture stress.

The experiment was based on a randomized complete block split-plot design with four replicants having nine treatments including three *medicago sativa* varieties (Hamadani, Sacoeal and Gharayoungeh) in the main plots and three levels of K (0, 50, 100 kg K ha^{-1}) in the subplots (1 m^2). Potassium fertilizer was applied in the form of potassium sulfate (K_2SO_4) in early spring in 2004. Soil physical-chemical properties were determined for pH (0.01 M $CaCl_2$) and other properties (Table 1).

Plant samples were taken on three cuttings at first flowering stage. At each harvest, the plants were cut at ground level. Leaves and stems were oven-dried at 65°C for 48 h and weighed separately. Leaves and stems from three periods of regrowth during growing season were determined for shoot dry matter production and sub-samples milled to a fine uniform powder for ^{15}N measurement. Roots were sampled at the third cutting down around the plot center with a circular cutter (30 sq cm^2), to a depth of 30 cm and removed the cylinder of soil and loose earth with a small shovel. This volume of the root system was found to represent the major proportion of the total root and nodule weight per plant. The roots were washed free of soil in a large sieving box. The root system and free nodules were taken to the laboratory and nodules removed washed, oven-dried and weighed. Only active nodules (in pink color) were taken and any decayed and broken nodules were rejected.

The stem and leaf tissue from three harvests (total three cutting during growing season) were ground and analyzed for N, K and C. Dried plant samples were ashed at 550°C in a furnace and plant K concentration was measured by flame photometer and oxidable carbon determine by the walkly- Block method (Blakemore *et al*., 1981; Rayment and Higginson, 1992).

The three medicago varieties and barley grass materials were analyzed for ^{15}N by mass spectrometry. Then the natural abundance technique was used to estimate N_2 fixation during growing seasons. The requirement of the technique is that legume and grass (reference plant) assimilate the indigenous soil N with the same ^{15}N content during growth (Ledgard *et al*., 1985). The proportion of Lucerne's nitrogen fixed from atmospheric N_2 was calculated from the equation (Peoples *et al*., 1989).

$$\text{P fix} = 100\ \frac{\delta^{15}\text{N grass} - \delta^{15}\text{N Legume}}{\delta^{15}\text{N grass} - \text{B}}$$

Where P fix is the proportion of Lucerne nitrogen, which originated from atmospheric N_2, $\delta^{15}N$ Legume and $\delta^{15}N$ grass are the δ values of N_2-fixing Lucerne and non-N_2 fixing barley grass, respectively grew together n the experimental area. The value of B is the $\delta^{15}N$ value of total N accumulated by nodulated lucernes in N-free

Table 1: Some physicochemical properties of the soil studied before further fertilization

Dept	Sand (%)	Silt (%)	Clag	Texture	pH	K av (mg kg^{-1})	Total N (%)	P av (mg kg^{-1})
0-30	34	26	40	Silty loam	7.4	106	0.12	19.8
30-60	32	26	42	Silty loam	7.4	96	0.11	20.2

nutrient solution in a controlled N-free environment (Bergersen *et al*., 1988). The amount of N_2 fixed by Lucerne was calculated by multiplying P fix by the shoot N content at the time of sampling.

The data were analyzed using MSTATS software for analysis of variance. Mean values were separated by Duncan's test.

RESULTS

Differences were observed among three varieties in the agronomic traits tested. Application of K fertilizer resulted in an increase in stem dry matter. The increase was significant in Sacoeal and Gharayoungeh at 100 kg K ha^{-1} when compared with that of same unfertilized varieties. There was a variety × fertilization interaction for stem dry matter ($p = 0.01$). In overall varieties, during the growing season, stem dry matter collection was highest at level of 100 kg K ha^{-1} and lowest in unfertilized plots (Fig. 1).

The K fertilizer affected leaf dry matter only in Gharayoungeh at 100 kg K ha^{-1} as compared with the unfertilized control plot (Fig. 2). Main effects of varieties differences only occurred between Hamadani and Sacoeal during growing season.

Weight of active nodules per plot was enhanced by supplying K in three medicago varieties although the difference was not significant in each of the varieties. In contrast, the main effect of K fertilization was significantly increased at both rates of 50 and 100 kg K ha^{-1} (Fig. 3).

The different K fertilizer rates resulted in similar K accumulation in plants for the three varieties. Main effects of K fertilization for K concentration were different between the varieties. The amount of K accumulation for Hamadani was higher than that of Sacoel and Gharayoungeh (Fig. 4). The K content differences in plant tissues between varieties were not significant with K application (data not presented). As shown in Table 2, the estimated P fixation was higher in potassium fertilized plants, which was significant for Sacoeal and Garayoungeh. The degree of the response was different among the varieties as shown by the varieties × K fertilizer interaction.

The three medicago varieties varied considerably in their fixed N levels with changes in K fertilization. Hamadani responded only slightly to K application. Fixed N in Gharayoungeh increased at high K levels (100 kg ha^{-1}), while Sacoel increased with both low (50 kg ha^{-1}) and high (100 kg ha^{-1}) K levels (Fig. 5).

There were statistical differences between K application rate for C content in Sacoel and Gharayoungeh. The Sacoel had lower content at 50 kg ha^{-1}, while Gharayoungeh had a lower amount at

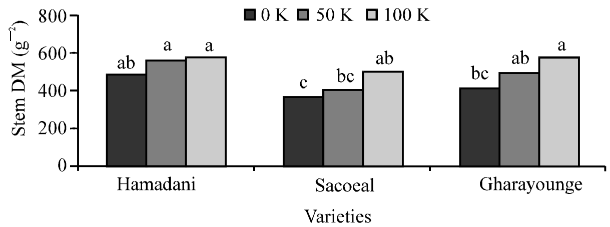

Fig. 1: Effects of potassium application on stem yield of *Medicago sativa* varieties

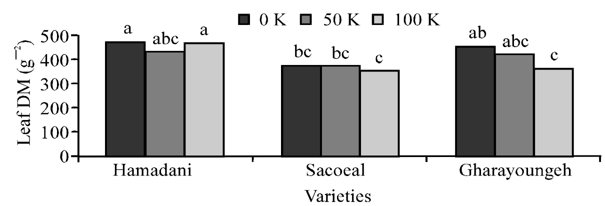

Fig. 2: Effects of potassium levels on leaf yield of *Medicago sativa* varieties

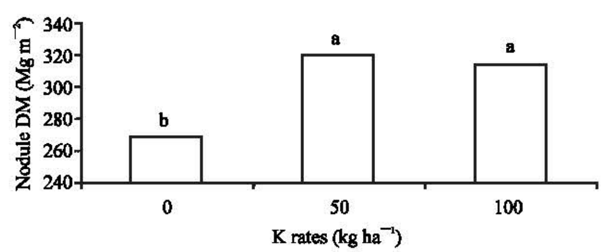

Fig. 3: Main effects of K fertilization on nodule weight of *Medicago sativa* varieties prior to third harvest

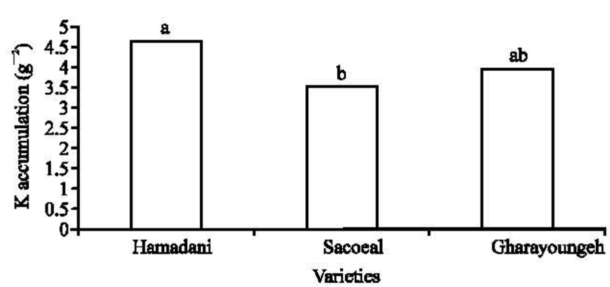

Fig. 4: Main effects of varieties on K uptake in plant tops

Table 2: Effects of potassium fertilizer on P fix (%) of shoots of three *Mmedicago sativa* varieties. (Total of three periods of regrowth)

Treatments	Varieties		
Kg K ha^{-1}	Hamadani	Sacoeal	Gharayoungeh
0	66	53	62
50	67	61	65
100	69	65	68
Varieties × K Interaction	NS	**	*

* Significant; **Highly significant, NS: Non Significant

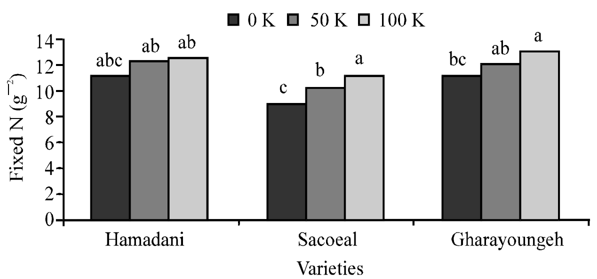

Fig. 5: Effects of potassium fertilizer on fixed N in shoots of three *Medicago sativa* varieties during growing seasons

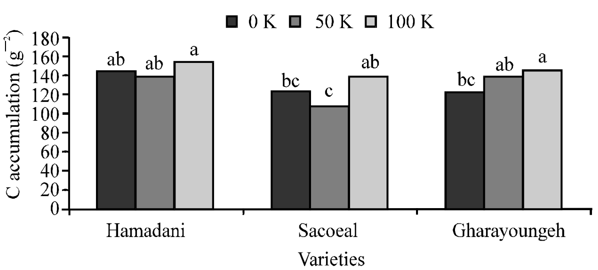

Fig. 6: Content of C in dry matter as affected by K application rates

unfertilized control plots (Fig. 6). The main effects of changing K rates on varieties, in plant tissues only occurred at 100 kg ha^{-1}, which was 50 kg ha^{-1}higher than in unfertilized plants.

DISCUSSION

There was an increase in agronomic traits and nutrient status of plants grown at higher K levels. According to Pfluger and Cassier (1977), the increase in agronomic traits, by K application indicated the role of K in higher photosynthetic rates is due to carbon supply. In this study, stem dry matter increased with increases of K fertilizer rate. In contrast, leaf dry matter was lower with higher rates of K treatment. According to Ruiz *et al.* (2000) increased K rates applied jointly with N reduced the leaf biomass in the capsicum plant. This could be explained as a result of translocation of carbohydrate from leaf to stem and nodules on the root system.

Varieties of Sacoeal and Gharayoungeh showed significant differences in dry matter production of shoot top as affected by K application rates, while in the case of Hamadani, the difference was negligible. This could be attributed to different varieties of constituents as well as differences in comparative efficiency to utilize K to meet requirements for growth and development (Yang *et al.*, 2004). Different K concentrations in plant tissues of three varieties showed that plant species and even varieties within species, differed in the efficiency with which they acquired and utilized potassium (Shi *et al.*, 2004).

When K fertilizer was applied there were significant differences among two of the three Lucerne varieties in P fixation (Table 2). Cadisch *et al.* (1989) evaluated the effects of K fertilizer on N_2 fixation of a number of forage-legumes and reported that, with K fertilizer all legumes derived at least 70% of their N from symbiosis, whereas without K, both lower values and larger differences in P fix between species were observed. Higher increase in P fix with K supply could often be assumed to improved N_2 fixation, although % N was similar in overall plant varieties (data not presented). Fixed N (kg N m^{-2}) and its response to K differed markedly between varieties and ranged from 83 kg N ha^{-1} per year in unfertilized control in Sacoeal to 122 kg N ha^{-1} per year at 100 kg N ha^{-1} in Gharayoungeh. The results of this experiment, therefore, appear to be similar to other estimates which showed relatively a large range of fixed N in a number of forage legumes (from 11-49 to 25-115 kg N ha^{-1}) due to P, K fertilizer (Cadisch *et al.*, 1989).

In this experiment only active nodules (pink color) were collected. Ineffective nodules (old, black or empty) mostly do not have potential for N_2-fixation. A similar result was reported by Cadisch *et al.* (1989) who found that the number of effective nodules per plant was enhanced by K fertilization in four out of five legumes tested. Neither the number of inactive nodules nor their size was affected significantly by the K treatment. The ranking of varieties related to the K total N concentration had same pattern from the ranking related to nodule weight.

Patterns of shoot C concentration and nodule weight were similar to those obtained with shoot N_2 fixed. The results agree with those of Atkins *et al.* (1978) and Gja *et al.* (1979), who reported that symbiotic N_2-fixation in legumes requires significant impact of C substrates to provide energy for nitrogen reduction and accepter molecules for subsequent transport of reduced N. It has been shown that legumes dependent upon N_2-fixation as their source of reduced N, require more energy per unit of N incorporated, than plants grown on combined N (Mahon and Child, 1979). Thus due to this large carbon requirement, symbiotic N_2-fixation has often been said to be closely coupled to photosynthate production (Hardy and Havelka, 1976).

The results of this study suggest that the K supply is important in increasing net photosynthate and availability to the nodule for N_2-fixation.

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