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ALFALFA (*Medicago sativa* L.) FORAGE YIELD RESPONSES TO TRIPLE SUPER PHOSPHATE, PHOSPHATE SOLUBLIZING BACTERIA AND GIBBERLLIC ACID FOLIAR APPLICATION

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Abstract

Phosphorus (P) is an essential element as plant macronutrition. Approximately all the most important biochemical and assimilation processes relate to phosphorus availability. In the old alfalfa stands the phosphorus availability becomes lower and lower and, consequently, alfalfa forage yield decreases extensively. Thus, in order to study the effect of P on alfalfa recovering rate we compared the combined effect of P and plant growth regulator - gibberllic acid (GA) application in a 3-years-old alfalfa stand, cut three times in year. Sources of P were phosphorus solubilizing bacteria (PSB) biofertilizer and triple super phosphate (TSP) fertilizer. Effects on alfalfa were studied after each of the three cuts in a field experiment set up as a split-plot randomized complete block design in four replications in 2011 year in the region of Shazand, Arak, Iran. Phosphorus treatments at 3 levels were applied to the main plots and included P0: control, P1: PSB and P2: TSP. GA levels, applied to the sub-plots, were also in 3 levels; G0: control, G1: 35 ppm of GA and G2: 75 ppm of GA. The results showed that in the third cut, application of TSP had a significant effect on the increase of fresh and dry weight of alfalfa's stem and leaf. GA applied with biological and chemical fertilizers effectively increased forage yield of alfalfa as well. Application of GA in the G2 concentration significantly increased stem yield up to 5.37 t/ha.

Key words: *alfalfa, gibberllic acid, phosphorus solublizing bacteria, triples super phosphate.*

INTRODUCTION

Alfalfa has been grown as a forage crop since the beginning of recorded history and can now be found almost anywhere in the world. It is generally agreed that alfalfa originated in the vicinity of Iran and was first brought to North America by the European colonists in the early 1700s. The ideal alfalfa soil is deep and well drained. Alfalfa has a vigorous root system which enables it to obtain water and nutrients from a large volume of soil. This characteristic helps alfalfa produce high yields and survives through dry periods. Poor soil drainage restricts oxygen supply to the roots, increases winter heaving problems, causes more disease problems, and damages alfalfa's nitrogen-fixing bacteria. All these effects lead to low productivity or loss of the stand. Alfalfa removes large amounts of nutrients from the soil. A ton of alfalfa hay can remove 5.5 kg of phosphate from soil. Phosphate is the fertilizer needed in the greatest amounts to establish and grow alfalfa and should be applied before sowing. Fertilizer can be applied at any time of the year and in one application rate (Lacefield et al., 1997). Phosphorus is used in various plant processes, particularly in energy acquisition, storage and utilization (Epstein and Bloom, 2005). The deficiency of phosphorous supply and availability remains a severe limitation on nitrogen fixation and symbiotic interactions (Beck and Munns, 1985, Pereira and Bliss, 1989). Thus, phosphorus (P) fertilization is essential for alfalfa production and is one of the most common nutrient inputs for this crop. This nutrient is involved in many essential metabolic roles within the plant, and deficiencies result in slow growth, suppressed yields, and lost of income.

A brief review by Mikkelsen (2004) covers some of the recent work regarding P fertilizer management for achieving high alfalfa yields. Many sources of P fertilizer are successfully used for alfalfa production including both, solid and liquid forms. A number of comparisons have shown that most of P fertilizer sources are equivalent, when used properly (Cihacek, 1993; Reid et al., 2004). The phosphorus content per unit dry weight is usually considerably higher in the nodules than in the roots and shoots, particularly at low external phosphorus supply (Azcon et al., 1988; Adu-Gyamfi et al., 1989).

Nitrogen fixing plants have an increased requirement for P over that receiving direct nitrogen fertilization, probability due to need for nodule development and signal transduction, and to P-lipids in the large number of bacteria (Graham and Vance 2000). Also, capability of developing nodules to compete with other vegetative sinks (root and shoot meristems) for P at limited external supply may be different between legume species (Jakobsen 1985, Robson and Bottomley 1991). Gull et al. (2004) proved an increased growth of chickpea plants, when co-inoculated with phosphorus solublizing bacteria and rhizobial cultures.

The very old research showed, that the foliage of alfalfa treated with gibberellins solution before and after forage harvesting, had no visible effect on the elongation of leaves and stems of alfalfa. Treatments with 200 ppm of gibberellins had no distinguishable effect on weight of alfalfa (Corns, 1958). But it seems that the effect of gibberelline on alfalfa plants needs to be revised. In this work the effects of phosphorus (P) and gibberllic acid (GA) application on alfalfa forage yields in three-years-old alfalfa (*Medicago sativa*) stand were studied.

MATERIALS AND METHODS

In order to study the effects of phosphorus (P) and gibberllic acid (GA) on alfalfa forage yields in three-years-old alfalfa (*Medicago sativa* cv. Hamedani) stand in the low phosphorus soil conditions (6 ppm) a field experiment was set up in 2011 at Shazand, Arak, Iran, as a split-plot with randomized complete block design in four replications.

A comparison of the P solublizing bacteria (PSB) biofertilizer (PSB is a combination of *Pantoea agglomerans* strains P5 and *Pseudomonas putida strain* P13 registered as Barvar2®) and triple super phosphate (TSP) fertilizer and also the effects of gibberllic acid (GA) on alfalfa were studied. P was applied to the leaves (PSB) or to the soil (TSP) in the main plots, whereas the GA was foliar applied to the sub-plots. Plant growth regulator GA was applied promptly after each cut by a sprayer. The experiment included three cuts of alfalfa, from early spring to late summer of 2011 period. P treatments included 3 levels – P0: control, P1: PSB biofertilizer – 100g of PSB in 200 L of water per each hectare, P2: 250 kg ha^{-1} TSP fertilizer, as a broadcast method. GA solutions were applied also in 3 levels – G0: control, G1: 35.0 ppm of GA and G2: 75.0 ppm of GA. The measured plant characteristics were: plant height, leaf and stem dry and fresh weight.

Before each cuts the randomized quadrates were chosen in each of the plots of 25 X 25 cm area, where all alfalfa stem number was counted within quadrate. Plant height and forage fresh weight were weighed on farm and later all the samples were transfer to laboratory for drying in oven in 75°C over 48 h. Statistical analysis of data was performed for all the three cuts by MSTAT-C software and the means were compared based on Duncan multiple test at 5% level.

RESULTS AND DISCUSSIONS

For plant height, P and GA and interactions were significant (Table 1). It is shown that the soil was poor in available P content and alfalfa responded significantly to the P application. Moreover, the application of P had also a meaningful interaction with GA as for the stem height. The TSP fertilization was more effective in comparison with PSB biofertilizer, resulting in 6% increase in the alfalfa height. Table 1 shows that G2 application increased plant height by about 11 cm, as compared with control. Analysis of variance for P: GA interaction for plant high showed significant effects (Table 2). Survey of the interaction between P and GA showed the highest plant high was in P3G1 combination - 92.7 cm.

Table1. Summary of analysis of variance

S.O.V	D.F	Mean square Plant height	Fresh weight Stem	Fresh weight Leaf	Dry weight Stem	Dry weight Leaf
R	3	$4.3^{n.s}$	$0.02^{n.s}$	$0.01^{n.s}$	$0.02^{n.s}$	$0.02^{n.s}$
P	2	350.5^{**}	3.66^{**}	3.06^{**}	2.15^{**}	2.21^{**}
E1	6	1.36	0.03	0.01	0.02	0.01
GA	2	368.3^{**}	2.25^{**}	2.09^{**}	0.84^{**}	0.86^{**}
P:GA	4	18.2^{**}	0.15^{**}	0.1^{**}	0.04*	0.04^{**}
E	18	1.19	0.03	0.02	0.01	0.007
CV%		1.34	3.18	3.91	6.23	6.24

** Significant at P< 0.01 level;

* Significant at P< 0.05 level; ns: non-significant. R: replication, P: Phosphorus, GA: Gibberlic acid and E: error.

Table 2. Mean comparisons for alfalfa characteristics

Treatments	Means Plant high cm	fresh weight(g/stem) Stem	fresh weight(g/stem) Leaf	dry weight(g/stem) Stem	dry weight(g/stem) Leaf
Control (P1)	76.01c	4.43c	3.03c	1.18c	0.93 c
PSB (P2)	81.29b	5.01b	3.53b	1.60b	1.33b
TSP (P3)	86.81a	5.53a	4.04a	2.03a	1.79a
Control (G0)	75.29c	4.51c	3.12c	1.32c	1.07c
GA 35 ppm (G1)	86.13a	5.37a	3.95a	1.85a	1.60a
GA 75 ppm (G1)	82.69b	5.08b	3.54b	1.65b	1.38b
P1G0	72.62g	4.14f	2.63f	0.96e	0.77g
P1G1	79.40e	4.69de	3.40cd	1.36d	1.10e
P1G2	76.00f	4.45e	3.06e	1.24d	0.92f
P2G0	74.62f	4.59de	3.19de	1.35d	1.02ef
P2G1	86.25c	5.41b	4.07b	1.92b	1.61c
P2G2	83.00d	5.02c	3.35cd	1.55c	1.38d
P3G0	78.62e	4.81cd	3.53c	1.66c	1.41d
P3G1	92.75a	6.01a	4.38a	2.27a	2.10a
P3G2	89.07b	5.78a	4.21ab	2.17a	1.85b

Within a column, values followed by the same letter are not significantly different at 0.05 level of Duncan multiple test at P<0.05.

Unfertilized and without GA application alfalfa had significantly lower fresh and dry weight. It was showed that alfalfa is a plant of high sensitivity to P and GA treatments. Stem and leaf fresh and dry weight, as compared to those that did not received TSP and 35 ppm GA, were significantly lower as compared to the other combinations (Table 2). A maximum increase in dry leaf and stem weight was for P3G1 treatments, by about 2.1 and 2.27 g per plant unite, respectively. We observed significant differences between PSB and TSP treatments for leaf and stem fresh weight too (Table 2). Our findings confirm the results by other studies, that reported positive influence of P and GA on alfalfa yield (Cihacek, 1993; Reid et al, 2004, Graham and Vance 2000), but our findings don't support Corns (1958) research report about none effect of GA on alfalfa yield.

CONCLUSIONS

Phosphorus nutrient performs several functions and is responsible for various metabolic processes in the plant, such as maintenance of osmotic concentration of cells, electron transport systems and enzymatic activity. Biological P is one of the most important fertilizers that can improve and optimize the insufficiency of soil mineral P significantly. Regarding to current report the deficiency of phosphorous in soil could be effectively replaced by PSB plus GA application in alfalfa stands after each cutting.

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