Nitrate Uptake Kinetics of Ineffectively and Effectively Nodulated Alfalfa

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Introduction

Alfalfa is one of the most important legume crops in temperate climates. It has high herbage yield and symbiotic N_2 fixation potential, is deeply rooted and provides high economic returns per unit land area. Efforts are under way to develop alfalfa for specific environmental quality goals, such as improved uptake of subsoil NO_3. Alfalfa forms a symbiosis with *Rhizobium* bacteria, which fix N_2 from the atmosphere, but alfalfa also absorbs inorganic N from the soil solution.

Because symbiotic N_2 fixation requires more energy than absorption and reduction of NO_3, the general understanding has been that legumes absorb inorganic N in preference to fixing N_2 gas. Research by our group has shown that alfalfa continues to fix atmospheric N_2, even with high rates of N fertilizer. Moreover, field experiments have demonstrated that ineffectively nodulated alfalfa, which cannot fix N_2 gas, absorbs NO_3 more efficiently than alfalfa with effective nodules. Greater NO_3 absorption occurred even when herbage yields of the ineffectively nodulated alfalfa were smaller than those of the effective types. The objective of this study was to determine whether NO_3 uptake kinetics could explain the disparity between these alfalfa types.

Materials and Methods

Plants of two effectively nodulated alfalfa germplasms, Saranac and Agate, and two ineffectively nodulated alfalfa germplasms, Ineffective Saranac and Ineffective Agate, were grown from seed for seven weeks in sand culture in the greenhouse, shoots were harvested about 5 cm above the crown, and plants selected for uniformity were transplanted individually into pots (PVC; 7.5 cm diam. by 30 cm long) filled with approx. 1200 mL nutrient solution containing 2 mg NO_3-N L^{-1}. The nutrient solution was aerated constantly and exchanged every 3 d. The plants were inoculated with *Rhizobium meliloti* strain 102F51. The plants were transferred to a growth chamber one week before NO_3 uptake kinetics were measured with day/night cycles of 14h/10h at constant 25° C and a relative humidity of ~ 90%. Photosynthetic photon flux during the day cycle was 550 µmol photons m^{-2} s^{-1} measured at the top of the canopy.

Two experiments were conducted. In the first, measurements were made on plants that received 2 mg NO_3-N L^{-1} every 3 d. Under such conditions the ineffective alfalfa germplasms were severely N deficient. To assay plants subjected to less N stress, a second experiment was conducted with plants receiving 2 mg NO_3-N L^{-1} daily for 2 wk before the assay period. Two hours after onset of the light period, the nutrient solution in the pots was exchanged with solution containing 0.6 mg NO_3-N L^{-1}, the weight of the pots was recorded, and starting 1 h later the solution was sampled (2 mL) every 10 min for the next 7 h, after which the weight of the pot was determined. Net decline in pot weight provided an estimate of H_2O transpiration. The following morning the nutrient solution was sampled again and the plant was harvested. Nitrate in the nutrient solution samples was measured spectrophotometrically, and total plant dry mass, Kjeldahl N concentration, and pot weight were determined. The time course of NO_3 disappearance from the nutrient solution was fit to a Lineweaver-Burke plot to determine the parameters of apparent Michaelis-Menten nitrate uptake kinetics.

Results and Discussion

In both experiment 1 and experiment 2 (Table 1), Saranac had the highest shoot yield and N content. Shoot yield and N content of Agate were intermediate. Shoot yield and N content of the two ineffectively nodulated, non-N_2-fixing germplasms were lowest and not different from one another. The ineffective germplasms were N limited in both cases, resulting in 70% less shoot yield and 85% less shoot N content in experiment 1 and 67% less shoot yield and 82% less shoot N content in experiment 2, respectively, as compared to Saranac. However, the ineffective germplasms showed higher affinity for uptake of NO_3. In experiment 1, C_{min}, the minimal concentration of NO_3 below which there was no net uptake of NO_3, of the ineffective germplasms was one half that of Saranac and in experiment 2 C_{min} remained 22% lower than that of the N_2-fixing germplasms. The term I_{max} is the maximal uptake of NO_3 from nutrient solution by the plant when NO_3 concentration is nonlimiting (approx. concentration of NO_3 in the nutrient solution > 2 K_m). The ineffective germplasms had 126% higher I_{max} in experiment 1 and 171% higher I_{max} in experiment 2, respectively, than the effective germplasms. Additionally, we found differences in the water use efficiency between the effective and ineffective germplasms. The ineffective germplasms transpired 37% more water per unit shoot weight than the effective germplasms (2.60 vs. 1.95 g H_2O g shoot DM^{-1} h^{-1}).

Conclusion

Results of this study clarify the underlying physiological differences that lead to higher NO_3 uptake efficiency of ineffective alfalfa than N_2-fixing alfalfa we observed in the field. Ineffective alfalfa can deplete the nutrient solution to a lower NO_3 concentration and has a higher maximal NO_3 uptake capacity than effective alfalfa. Moreover, ineffective alfalfa transpired more water per unit shoot weight than effective alfalfa regardless of N nutrition. Higher water use in the field will cause more NO_3 to move to the roots, thereby increasing NO_3 availability for uptake.

Table 1. Shoot yield, shoot N content, and NO_3 uptake parameters of effectively and ineffectively nodulated alfalfa germplasms. Plants received 2 mg NO_3-N L^{-1} in nutrient solution every 3 d (Exp. 1) or every d (Exp. 2), respectively, before the uptake measurements. (n = 6).

Germplasm	Shoot dry weight g shoot^{-1}		Shoot N content mg N shoot^{-1}		C_{min} μM		K_m μM		I_{max} µg N min^{-1} plant^{-1}	
	Exp		Exp		Exp		Exp		Exp	
	1	2	1	2	1	2	1	2	1	2
Saranac	5.6	7.3	120	240	19	19	22	24	1.5	2.0
Agate	4.9	5.4	100	160	16	20	19	24	2.2	2.1
Ineffective Saranac	1.8	2.7	20	50	11	15	15	21	4.2	5.8
Ineffective Agate	1.6	2.1	20	40	9	16	13	21	4.2	5.2
$LSD_{0.05}$	0.7	1.4	30	30	4	2	3	2	1	2