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Effect of potassium on the tolerance to PEG-induced water stress of two white clover varieties (*Trifolium repens* L.)

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Abstract

The response to water stress was studied on white clover grown hydroponically. Two varieties (Crau and Huia) were both subjected to a moderate and a more-severe stress, induced by polyethylene glycol (10 and 20% respectively), in the presence of a nutrient solution poor in potassium ($K_1 = 0.005 \text{ mM}$), or abundantly supplied ($K_2 = 5 \text{ mM}$). Dawn water potential and nitrogen fixation (acetylene reduction activity) decreased with the increasing stress. Conversely, the stomatal resistance increased when *osmoticum* was added. Crau had a lower stomatal resistance to the deficit, than did Huia. In relation with the K supply, treatment K_2 confirmed the superiority of Crau. Crau also showed greater nodule mass and number than Huia. The data show relationships between dawn water potential, stomatal resistance and nitrogen fixation activity.

Introduction

Water stress in plants influences many metabolic processes, and the extent of its effects depends on drought severity (Hsiao, 1973; Turner, 1986). Drought stress also results in a decrease in nitrogen uptake. For numerous legumes, nitrogen fixation has been shown to be sensitive to a reduction in the soil water. Drought could directly inhibit nitrogen fixation, which has been found more sensitive than photosynthesis (Sprent, 1972), although this loss of activity could be due also to an inhibition of the photosynthesis (Huang *et al.*, 1975a). In temperate regions, legumes such as white clover are often subjected to drought. Moreover, the root nodules are mostly confined to the upper 50 to 150 mm of soil which often dries out during spells of drought. The inhibition of symbiotic nitrogen fixation due to water stress has been studied especially in Rhizobium-nodulated plants (Sprent, 1976) and in actinorhizal-nodulated tree (Sundström and Huss-

Danell, 1987). It has been well-established that potassium plays an important role in many physiological processes associated directly with nitrogen fixation and photosynthesis (People and Koch, 1979). However, little information is available about drought tolerance in conjunction with K-fertilization in white clover (Guckert and Laperriere, 1987).

In this work we studied under artificial conditions, the time-course of the inhibition of nitrogenase activity by water stress in white clover. The two factors considered were potassium fertilization and clover variety. Crau (Ladino) and Huia (Hollandicum) are two varieties that have been previously studied (Shamsun-noor *et al.*, 1989) for their tolerance to water deficit.

The effect of water stress was assessed here by measuring the leaf water potential and the stomatal resistance. The number, water content and mass of the nodules during the stress experiment were also determined.

Materials and methods

Plant material and growth conditions

Seeds of two white clover varieties (*Trifolium repens* L.) cv Crau (Ladino) and cv Huia (Hollandicum) were incubated for 48 h, on a filter paper and sterile vermiculite, to allow their germination. The unifoliated clover seedlings were transferred to a hydroponic system and inoculated with *Rhizobium trifolii* U.S.D.A. 2063. When the seedlings reached the 3-leaf stage, 120 plants were transplanted to a 45 L PVC box containing 20 L of a Wood-Cooper-Holding (WCH) nutrient solution (Wood *et al.*, 1983). The nodules in the upper part of the roots remained just above the solution, which ensured their sufficient humidification. Two rates of potassium were applied: 0.005 (K_1) and 5 mM KCl (K_2). Plants were maintained in growth chambers under day/night temperatures of 23/18°C, and relative humidity of 75% under a light intensity of about $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ on the canopy delivered during 14 h of daylight using 20 HQI 400W mercury vapor lamps.

Water stress induction

A polyethylene glycol solution (purified PEG 6000 (no 600) Merck, molecular weight 5000–7000) in WCH was used as osmoticum to induce moderate (10% PEG w/v) or severe stress (20% PEG w/v). The osmotic pressure of the control solution and each PEG solution was determined by a Beckman cryoscope. Two-months-old plants of each variety were selected according to their size. Water stress was then induced by dipping their root systems in the appropriate PEG solutions. Pre-dawn leaf water potential (Ψ) was then determined at the end of the dark period, first, after 22 hours of the PEG treatment and subsequently daily. Measurements were made with a Schölander-type pressure bomb (Schölander *et al.*, 1965).

The stomatal resistance was measured at the middle of the photoperiod on fully-expanded leaves of control and stressed plants (0 and 20% PEG; 10% not determined), using a van Bavel diffusion porometer (van Bavel *et al.*, 1965).

Plant dry matter, nodule number and nodule water content were measured and expressed as a percentage of the control.

The nitrogenase activity of the plants was estimated with the ARA method (Balandreau and Dommergues, 1973) as previously described (Shamsun-noor *et al.*, 1989). The ethylene concentration was measured by gas chromatography using a steel column with 10% Na_3PO_4 on Spherosil XOB 075. The nitrogenase activity was expressed as nmoles $\text{C}_2\text{H}_4\cdot\text{g}^{-1}$ nodule fresh weight per hour of incubation.

Statistical analysis

Data were analyzed using the analysis of variance procedure. Means were classified (statitc program) according to the Newman-Keuls test ($p = 0.05$).

Results

Pre-dawn leaf water potential

The leaf-water potential was measured every two days during the first five days of the treatment (Fig. 1). The potential of stressed plants decreased with the time and the decrease was larger between day 1

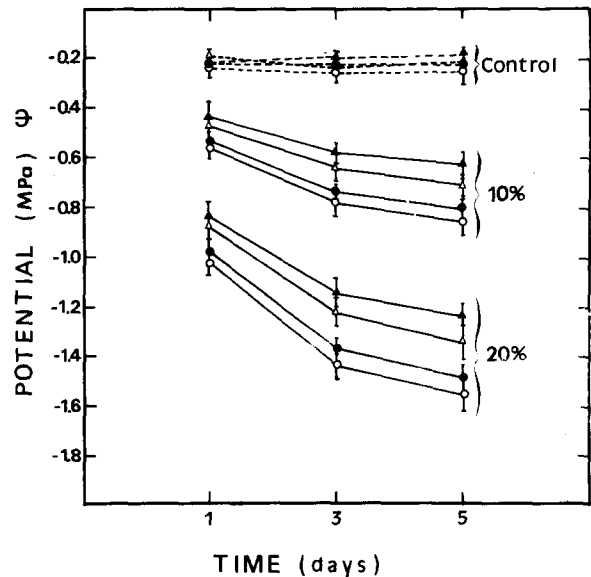


Fig. 1. Dawn water potentials (bars) of the 2 varieties of white clover (Crau and Huia) during the osmotic stress induced by PEG (0, 10 and 20%) under 2 levels of K: $K_1 = 0.005$ and $K_2 = 5 \text{ mM}$. \blacktriangle Crau K_2 , \triangle Crau K_1 ; \bullet Huia K_2 ; \circ Huia K_1 .

and day 3 than between day 3 and day 5. Differences between the PEG treatments were large and obviously statistically significant.

The Crau variety maintained a significantly higher dawn water potential than the Huia variety, and a K effect with variety factor was clear.

Stomatal resistance

Figure 2 gives the stomatal resistance of some of the plants which remained constant for the control plants. However, PEG induced an increased in stomatal resistance and a significant difference was observed between the two cultivars subjected to a severe stress (20% PEG). However, no significant "variety-PEG-Potassium" interaction was detected in general, but Crau seemed to have the best resistance, as shown by the significant "PEG-K" interaction at the end of the experiment.

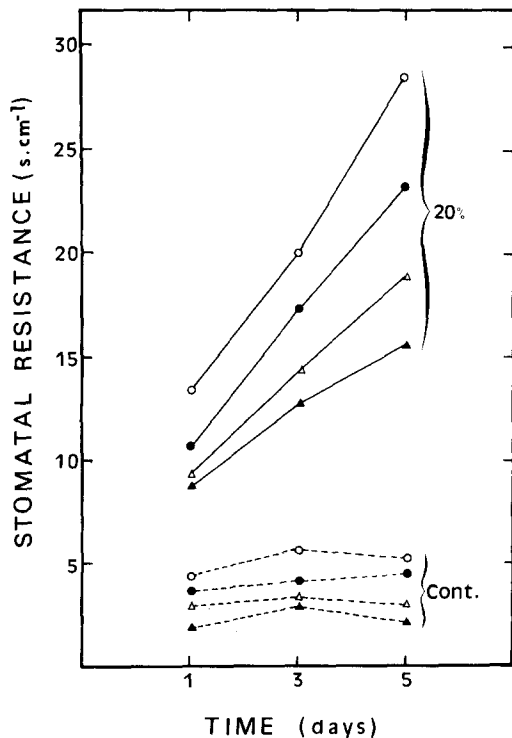


Fig. 2. Stomatal resistance of the 2 varieties during the osmotic stress induced by PEG (0 and 20%) under 2 levels of K: K₁ = 0.005 and K₂ = 5 mM. ▲ Crau K₂, △ Crau K₁; ● Huia K₂; ○ Huia K₁.

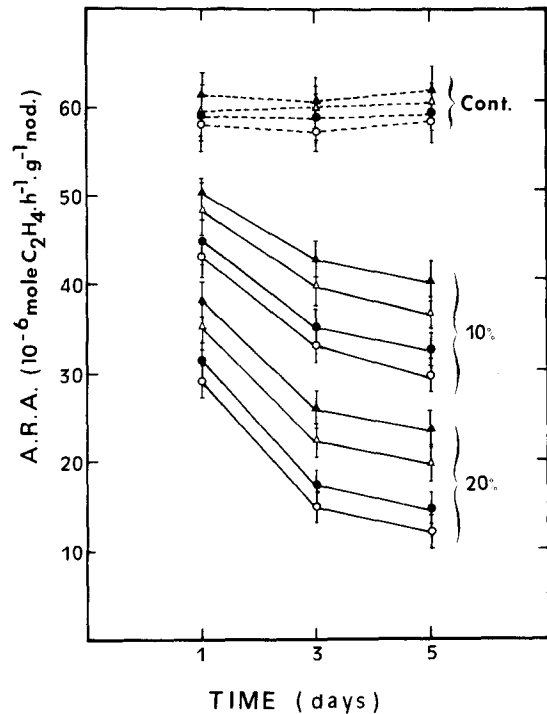


Fig. 3. Nitrogen fixation N₂ (Acetylene reduction activity) of the 2 varieties during the osmotic stress induced by PEG (0, 10 and 20%) under 2 levels of K: K₁ = 0.005 and K₂ = 5 mM. ▲ Crau K₂, △ Crau K₁; ● Huia K₂; ○ Huia K₁.

Acetylene Reduction Activity

The nitrogen fixation capacity was estimated by ARA measurements (Fig. 3). The non-stressed plants showed no significant differences between varieties and treatments. For the PEG-treated plants the ARA declined rapidly towards the end of the experiment. The decline was less pronounced after 3 days. The inhibition of the nitrogenase activity was more severe with 20% PEG.

Significant differences were observed between the varieties, Crau exhibiting the highest ARA values. Furthermore the K₂ treatment increased ARA more than the K₁ treatment.

A significant "cultivar-PEG" interaction was also observed, showing a differential effect of PEG on the varieties. Significant correlations also existed between ARA and water potential (0.86 < r < 0.92) and between ARA and stomatal conductance (-0.85 < r < -0.89).

Table 1. Nodule mass ($\text{g} \cdot \text{plant}^{-1}$), nodule number (per plant) and nodule water content (90% of the controls)

Variety	K level	Control			10% PEG			20% PEG		
		Nodule		Water content (%)	Nodule		Water content	Nodule		Water content
		number	f. wt (mg)		number	f. wt (mg)		number	f. wt (mg)	
<i>1st day</i>										
CRAU	K1	33	22	100	32	19	90	33	17	84
	K2	45	25	100	41	18	90	42	17	86
HUIA	K1	30	17	100	27	8	79	29	12	74
	K2	43	19	100	38	15	80	37	14	75
<i>3rd day</i>										
CRAU	K1	39	18	100	35	17	82	35	23	76
	K2	51	21	100	45	29	84	45	32	78
HUIA	K1	32	17	100	30	10	68	29	16	61
	K2	43	18	100	40	14	69	39	25	63
<i>5th day</i>										
CRAU	K1	33	20	100	40	28	79	37	28	73
	K2	52	35	100	49	39	80	45	30	71
HUIA	K1	34	16	100	33	15	65	33	20	58
	K2	45	22	100	41	22	68	40	29	60

Modifications of the N-fixing system

Table 1 shows the nodule number per plant, mass and water content of the nodules during the five days of treatment. Crau showed the highest values for each parameter and this variety also had better leaf and root development. The mass of the nodules (FW) was decreased by the osmoticum, but increased by the high K concentration. The number of nodules increased with time and was systematically higher in the controls than in the treated plants. "Variety" and "K" were significant treatment effects. The water content of the nodules decreased with the osmoticum concentration. This effect was lower in the presence of K.

Discussion and conclusions

Potassium is a major element influencing the leaf-water status (Mengel, 1984) and osmotic processes in plants. The movement of stomatal guard cells is dependent on the potassium movements in these cells (Humble and Raschke, 1971). The leaf-water status can be described by the leaf-water potential and the stomatal resistance. Previous research has clearly demonstrated that severe plant-water deficits are associated with in-

creasing stomatal resistance (Bennett *et al.*, 1987; O'Toole *et al.*, 1977), at low leaf-water potentials. In alfalfa, a severe K deficiency generally brings on an increase in the stomatal closure (People and Koch, 1979). A low potassium concentration in the nutrient solution produced plants with a high transpiration rate (Brag, 1972). In our work we have used a hydroponic system to study the response of white clover to a stress induced by an osmotic agent.

The results show clearly that potassium is an essential factor determining the resistance to water stress and the water balance of white clover. The water potential decreases in relation to the potassium content of the culture medium.

The stomatal resistance is a sensitive indicator of plant-water stress (Finn and Brun, 1980). This parameter has long been recognized as a key factor influencing the leaf gas exchanges by regulating the diffusion of water vapor and CO_2 (Stiles, 1970). Further, carbohydrate availability is limited when the leaf-water potential and the photosynthetic activity decrease (Engin and Sprent, 1973). In addition, a decreasing K^+ supply generally reduces the assimilate translocation (Mengel and Kirkby, 1982). Water stress may induce indirectly an inhibition of the nitrogenase activity of plants, by reducing the photosynthetic activity (Huang *et al.*,

1975a; 1975b). In this work we have observed that the osmotic stress resulted in a rapid decrease in nitrogen fixation. Moreover, a potassium deficiency increased the severity of the nitrogenase inhibition. This inhibitory effect can be explained by direct processes. Water stress might modify the membrane permeability of the nodules, thereby inducing a direct effect on nodule morphology and physiology (Engin and Sprent, 1973). In this work, the osmotic stress of white clover directly affected the number, water content and weight of nodules. Water stress can increase the resistance to oxygen transport (Pankhurst and Sprent, 1975), with severe water deficit damaging membrane-bound oxidases (Sprent, 1976).

Differences in water-stress resistance have been observed between white clover varieties (Shamsun-noor *et al.*, 1989). Our work confirmed these results: Crau (Ladino) was more tolerant of the deficit than Huia (Hollandicum).

The Crau variety showed better nitrogen fixation under stress. This resistance to water stress was reinforced by a high supply of potassium. Potassium uptake by plants decreases under water stress conditions and consequently reduces the root absorbing power (Hsiao, 1973). Here Crau maintained N-fixation, therefore its ability to resist a water stress was probably due to a better water use and nutrient uptake efficiency. In conclusion, under these controlled conditions, potassium plays an important role in the tolerance to the water deficit of the white clover, and the tolerance depends partly on the variety.

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