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The effect of water stress on white clover (*Trifolium repens* L). 1. Role of potassium nutrition

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Abstract

The effect of water stress on the behaviour of white clover (*Trifolium repens* L., cv Crau) was studied in pot culture. The effect of potassium nutrition on behaviour under stress and in recovery on resumption of watering was also measured. Water deficit caused a large and progressive decrease in leaf water potential with rapid closure of the stomata. This was accompanied by decline in photosynthetic potential and symbiotic fixation of nitrogen. In the presence of potassium the diminution in N-fixation was significantly reduced. The favourable effect of potassium was also evident on rehydration of plants submitted to stress, the reestablishment of water potential and symbiotic N fixation being much improved. Potassium fertilization increased dry matter production in both stressed and unstressed plants. The results demonstrated the beneficial effect of K fertilizer in conferring drought toleration.

Introduction

Water is an essential growth factor. The fixation of atmospheric nitrogen by legumes depends on soil water availability (*Sprent [1972]; Obaton et al. [1982]*).

Potassium is also important in controlling water economy in the plant *(Mengel [1984])* and many workers have emphasised the important part played by potassium in improving drought toleration *(Raschke [1975]; Dhinndsa et al. [1975]; Zimmermann [1978]; Saxena [1985])*. K is involved in the early stages of CO_2 fixation in chlorophyllian organs through direct action on the osmotic mechanism of opening the stomata (*Humble and Raschke [1971]*).

Apart from information on stomatal regulation, there has been little work on the rôle of potassium in the development of nitrogenase activity in water-stressed perennial legumes but *Guckert and Laperrière [1987]* demonstrated a favourable effect of K on water potential and N-fixation by water stressed white clover.

The present study aimed to amplify these results and to measure the combined effect of the two factors: water supply to the plant and potassium nutrition. White clover was grown on a K deficient soil with and without added potassium to investigate effects on various physiological processes under water stress:

- water potential and stomatal resistance;
- photosynthesis;
- symbiotic nitrogen fixation.

The production of aerial biomass was also measured. The effects on these processes were also measured after the restoration of normal water supply to the plants.

Material and methods

Culture

The cultivar chosen for the experiment was Crau (a Ladino type) previously found *(Shamsun-Noor et al. [1989])* to be somewhat tolerant of water stress. Sown in 4 kg pots and thinned to 15 seedlings/pot, two lots without and with added potassium equivalent to 300 kg/ha K_2O with soil moisture maintained at 70% of field capacity (suitable for maximum N fixation) *(Laperrière [1984])* were grown in a greenhouse before transferring to the phytotron (temperature day/night, 21/18°C) one week before the start of the experiment. The soil was a K deficient loamy sand from northern Alsace (K content: 0.012% K_2O). Two water regimes: 70% field capacity (F.C.) (control) and 'deficit' (by suspension of watering) were established.

Methods

Leaf water potential was measured each day in the Schölander pressure chamber *(Schölander et al. [1965])*. Stomatal resistance was measured by porometer *(van Bavel et al. [1965])*, photosynthetic activity by measuring net assimilation of each pot *(Shamsun-Noor et al. [1989])* expressed as vpm (volume per million) $CO_2 \cdot h^{-1} \cdot pot^{-1}$ (3 replicates). N fixation (ARA) was indirectly measured by acetylene reduction (4 replicates) (*Koch and Evans [1966]*).

Statistical analysis

Variance analysis was used and means were compared according to Newman and Keul's test.

In the figures, standard errors of means are represented by vertical lines.

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Results

Water potential

Adding potassium had no effect on water potential in control plants. Water deficit had a highly significant effect ($P<0.001$) within two days of suspending watering and water potential fell drastically from the 4th day, though the effects were less severe in plants receiving K (Figure 1).

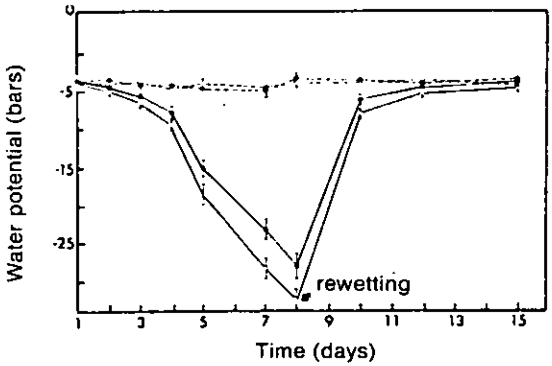

Figure 1 Development of water potential in white clover plants under stress (—) and non-stressed (---) at 2 levels of potassium supply (K_0: O and K_1: ●); K_1 equivalent to 300 kg/ha K_2O.

On resumption of watering, water potential was restored to normal after 5 days in the case of K_1 plants, but in K_0 plants only by the last day. The effect of K was negligible from day 12 to day 15.

After 8 days deprivation of water leaves of K_0 plants dried off while K_1 plants remained slightly turgid and green. They rapidly recovered their initial state on resumption of watering. In K_0 plants new leaves were formed to replace the wilted and necrosed.

Net photosynthesis and stomatal resistance

Water deficit significantly affected photosynthesis from the 3rd day (Table 1), the K_1 plants having somewhat higher values (though not significantly so at every stage). Following resumption of watering, photosynthetic activity was not completely restored and failed to attain the level of control plants, especially K_0 plants. This effect can be related to alteration of the photosynthetic apparatus by water stress.

Stomatal resistance of control plants was stable and low (0.8–2.8 $s \cdot cm^{-1}$) throughout (Figure 2). In contrast, water stressed plants showed a rapid increase in stomatal resistance (>200 $s \cdot cm^{-1}$). There was little difference between the curves for K_1 and K_0 (not significant at the 0.05 level).

On resuming watering, stomatal resistance declined rapidly, regaining the values for control faster than for water potential. Within 48 hours, the stomatal resistance declined from 200–220 to 2 $s \cdot cm^{-1}$.

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Table 1 Analysis of variance for net photosynthetic activity (mg $CO_2 \cdot h^{-1} \cdot pot^{-1}$) in white clover cv. Crau. NS: not significant at p=0.05, S=significant at P=0.05*, 0.01** and 0.001***.

Days	1	2	3	4	5	7	8	10	12	15
Effect of water stress	NS	NS	S***	S**	S***	S***	S***	S***	S*	S*
Control	45.86a	43.39a	44.78a	44.57a	44.46a	45.26a	44.07a	45.24a	45.33a	45.44a
Deficit	44.56a	40.40a	34.93b	31.00b	23.90b	8.73b	3.97b	23.08b	32.43b	38.15b
Interaction (Deficit × Potassium)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control – K_0	45.60a	42.63a	43.70a	44.89a	43.80a	45.59a	43.06a	45.86a	44.49a	45.18a
Control – K_1	46.13a	44.15a	45.87a	44.25a	45.12a	44.92a	45.08a	44.61a	46.17a	45.70a
Deficit – K_0	43.38a	39.66a	34.04b	29.78b	21.34b	7.06b	2.36b	19.89b	29.44b	35.46b
Deficit – K_1	45.74a	41.14a	35.83b	32.23b	26.46b	10.41b	5.59b	26.26b	35.42b	40.84b

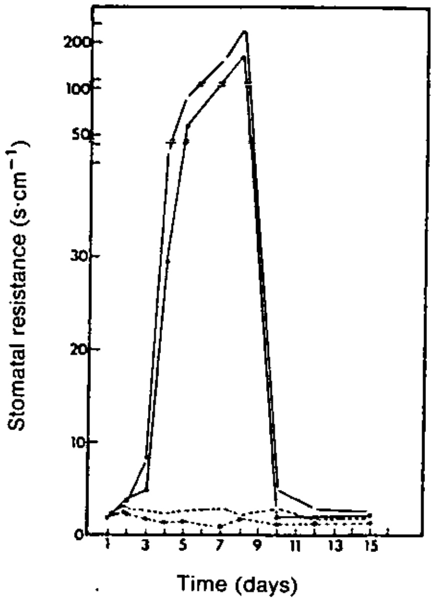


Figure 2 Development of stomatal resistance in stressed (—) and non-stressed (---) white clover plants at 2 levels of potassium supply (K_0: O and K_1: O); K_1 equivalent to 300 kg/ha K_2O.

Accumulation of aerial dry matter

Water deficit markedly reduced aerial (leaves, petioles and stolons) dry matter formation (Figure 3) from 5 days after watering ceased in both K_0 and K_1 plants. Restoring water had no effect. Applying K increased dry matter production by 17.5% in control and by 14.2% in stressed plants.

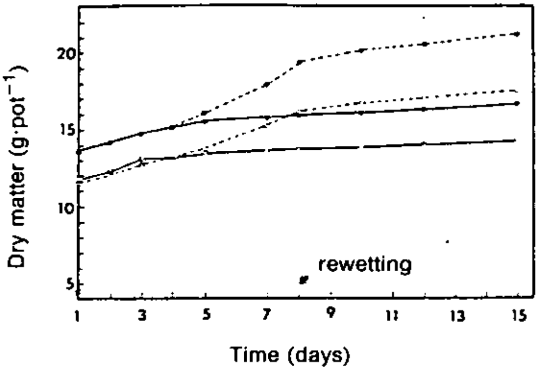


Figure 3 Aerial dry matter production in water-stressed (—) and unstressed (---) plants at 2 levels of potassium supply (K_0: O and K_1: ●); K_1 equivalent to 300 kg/ha K_2O.

Nitrogenase activity (ARA)

There was a significant effect (at the 0.01 level) of water deficit from the third day (Figure 4) and differences related to K supply appeared after 5 days. A higher ARA level was observed in stressed K supplied plants. Following resumption of watering, K supplied plants recovered to a higher level (60% of control value) than the K deficient (40%). In contrast to the other parameters, nitrogenase activity was not entirely restored by the end of the experiment.

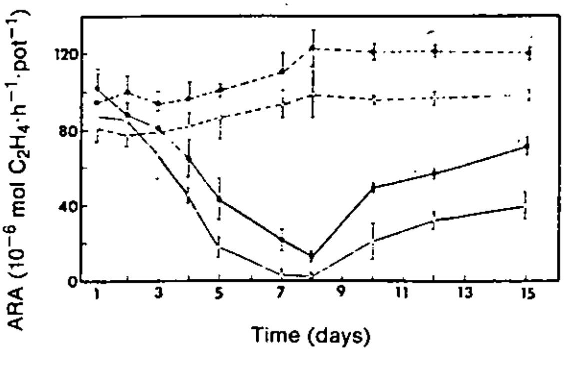
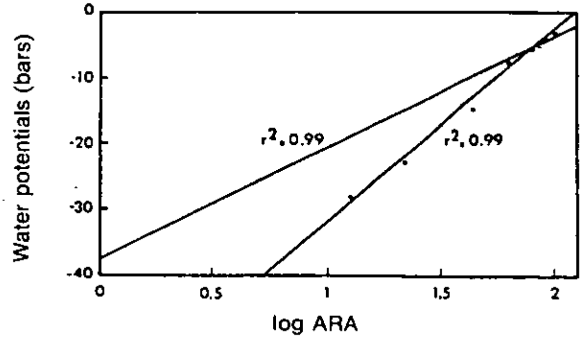


Figure 4 Development of nitrogen fixation (ARA) in stressed (—) and non-stressed (---) white clover plants at 2 levels of potassium supply (K_0: O and K_1: ●); K_1 equivalent to 300 kg/ha K_2O.



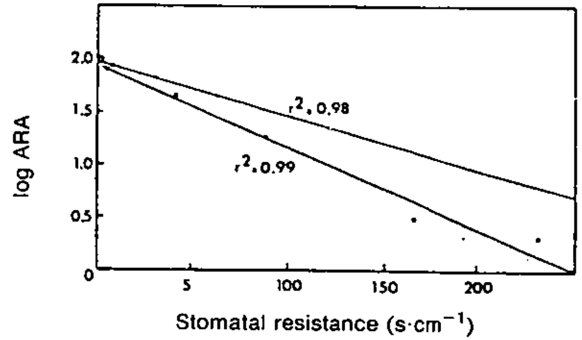


Figure 5 a. Relationship between ARA and water potential (K_0: O and K_1: ⊖); *b.* Relationship between ARA and stomatal resistance (K_0: O and K_1: ⊛).

Discussion and conclusions

Potassium has a favourable effect on dry matter accumulation by white clover. This applies to perennial legumes like lucerne *(Duke et al. [1980]; Barbarick [1985])* and to annuals like beans *(Mengel and Arneke [1982])* and peas *(Wahab [1984]).* Under our conditions water stressed plants receiving K gave the same dry matter production as unstressed plants without added K.

There was a large increase in stomatal resistance in water stress, though this experiment did not show any significant effect of potassium on stomatal regulation as observed by *Brag [1972]* in wheat and peas.

In this experiment K had a positive effect on photosynthetic activity in water stressed plants. *Peoples and Koch [1979]* demonstrated a positive effect of K on CO_2 assimilation in *Medicago sativa.* Potassium tends to increase the active photosynthetic area by stimulating leaf initiation and development with improved CO_2 fixation per unit area *(Cooper et al. [1967]).*

K also improves nitrogen fixation, and the bad effects of water stress are ameliorated by an adequate K supply. This confirms the earlier work of *Guckert and Laperrière [1987]* showing that K application makes it possible to maintain N fixation even under water stress.

N fixation does not fully recover on resuming watering possibly because of adverse effects on the nodules (*Albrecht et al. [1984]*). *Collins and Lang [1985]* showed that K increased N fixation in red clover and birdsfoot trefoil though nodule number was not affected. *Barta [1982]* suggested that the favourable effect of K on N fixation in lucerne regrowth was due to improved assimilate transport to the nodules with better utilisation of carbon chains in amino acid synthesis.

In this work it was interesting to find correlations between ARA and water potential and between ARA and stomatal resistance. These relationships (established from mean values) are exponential, and, in order to demonstrate the difference in behaviour between treatments mean values for ARA (expressed as 10^{-6} mol $[C_2H_4] \cdot h^{-1} \cdot pot^{-1}$) have been logarithmically transformed. The results are plotted in Figure 5 a and b. At a given level of water potential the ARA is higher in the presence of K. ARA decreases when stomatal resistance increases. At a given level of stomatal resistance ARA is higher in the absence of potassium.

White clover well supplied with potassium maintains a good level of N fixation and shows lesser diminution of dry matter production under water stress.

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