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XVIII IGC (1997) Manitoba & Saskatchewan

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# WATER RELATIONS IN BERSEEM CULTIVARS UNDER DROUGHT STRESS

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## ABSTRACT

Berseem clover (*Trifolium alexandrinum* L.) is a crop of particular interest in semi-arid regions, used for herbage and hay productions. However, its seed yield is reduced by drought stress that occurs during seed filling. In this study five cultivars (Axi, Big Bee, Lilibeo, Sacromonte and Saniros) subjected to four irrigation frequencies in controlled environment, were compared for their physiological responses to subsequent water stress period (3 days). Some parameters (leaf water potential ( $\Psi$ ), osmotic potential ( $\Psi_p$ ), relative water content (RWC) and gravimetric soil water content (GSWC)) related to plant water status were recorded. Plants subjected to previous periods of water stress were less sensitive to final drought stress than plants never stressed (control). Among cultivars examined, Lilibeo showed significantly higher values of  $\Psi$ ,  $\Psi_p$  and RWC. Therefore, it seems to be the most tolerant cultivar for its ability to maintain high water status under water stress conditions.

## KEYWORDS

Berseem, preconditioning, water potential, osmotic potential, relative water content

## INTRODUCTION

Berseem clover (*Trifolium alexandrinum* L.) is a very interesting annual leguminous crop in the Mediterranean environments. This species produces high quantities of hay when cultivated in pure stand or in mixtures with graminaceous (Stringi et al., 1983); however, seed yield is reduced by drought events that often occur during seed formation and filling. Martiniello and Ciola (1995) found consistent differences among berseem cultivars for forage and seed yield in field trials; but few data are available for this species concerning the physiological responses to soil drying and genotypic variability for drought stress tolerance. A better knowledge of the plant responses to water deficit is useful for agronomical management improvement and for drought resistance breeding programs. Plant responses to water deficit depend on the environmental conditions in which it develops but also on duration and intensity of soil water depletion (Dale, 1988). The current study was undertaken to analyse water relations of some berseem cultivars subjected to water stress following an initial growth period during which the plants were exposed to four irrigation treatments.

## MATERIALS AND METHODS

Five seeds of berseem cultivars, Axi, Big Bee, Lilibeo, Sacromonte, Saniros, were sown on 14 April 1995 in pots (17 cm diameter and 14 cm deep) containing 1000 g of air-dry medium-textured soil, 333 g of sand and 100 g of peat. The experiment was performed in a growth chamber with day/night temperatures of 23°C/18°C, 16 h day-length, PAR 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (400-700 nm) and a mean relative humidity of 50%, during both the preconditioning and water stress periods. A randomized complete block design with two replications and four treatments was used. Each replication consisted in one pot with five plants for each treatment and cultivar. Plants were kept well watered (daily restoring field capacity (36%) of the soil), until 69 days after sowing; then, during the preconditioning period, were divided into four groups and watered every 1, 2, 3 and 4 days, referred as T1 (control), T2, T3 and T4 treatments, respectively. When the plants were 81 days old, at the end of the preconditioning period,

they all were well watered for 7 days. Water was thereafter withheld from all treatments and plant measurements were made during a drying phase (3 days). Leaf water potential ( $\Psi$ ) was measured with a pressure chamber, osmotic potential ( $\Psi_p$ ) was measured cryoscopically with a micro-osmometer and relative water content (RWC) was determined gravimetrically weighing fresh, turgid and dry weights of the leaves. All leaf water relations were measured at midday using the youngest fully expanded leaves. Gravimetric soil water content (GSWC) was determined by pot weight. The data were subjected to ANOVA and LSD at P= 0.05 was used to detect differences between cultivars and treatments.

## RESULTS AND DISCUSSION

Withholding water caused a clear reduction of  $\Psi$ ,  $\Psi_p$  and RWC of the youngest fully expanded leaves and also a gradual decrease of soil humidity for all the treatments (Fig. 1). However, plants showed quantitatively different responses for each treatments. Plants in which drought stress intensity during the preconditioning period was higher (T3 and T4), showed less negative values of  $\Psi$ ,  $\Psi_p$  and RWC during the final drought stress period. Plants subjected to pretreatment T2 showed similar responses to that of pretreatment T1; they developed water stress more rapidly. After 3 days from stress imposition, treatment T1 showed values of  $\Psi$ ,  $\Psi_p$ , RWC and GSWC lower than those of treatment T4 (41%, 49%, 31% and 14%, respectively). After the first day of stress, differences in leaf water potential were already evident among cultivars, but as water stress increased, these variations decreased (Table 1). Whereas, osmotic potential and RWC values were not significantly different among cultivars at the initial stress period. Among the cultivars examined, Lilibeo showed higher values of  $\Psi$ ,  $\Psi_p$  and RWC. In fact, at the end of the stress period its values were 9%, 14% and 20%, respectively higher than the mean of the other cultivars. Also for the soil humidity, expressed as GSWC, Lilibeo had higher value. Concluding, the lowering of leaf water potential, osmotic potential, RWC and soil water content values after water stress imposition was significantly reduced in all cultivars as a result of preconditioning treatments. Value reductions were proportional to water stress severity that plants have previously experienced. Thus stress-conditioned plants are less sensitive to tissue water deficits. Our results may suggest that there are two drought responses among berseem genotypes tested. The cultivar Lilibeo showed higher  $\Psi$ ,  $\Psi_p$  and RWC values. Experimental data from other species (Matin et al., 1988; Levitt, 1972; Schonfeld et al., 1988) indicated that drought resistant cultivars had higher total leaf water potentials and RWC. If the ability to maintain high water status can be considered as an indication of drought tolerance, Lilibeo is the most drought tolerant cultivar among those evaluated in the experiment.

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**Table 1**

Means of water potential, osmotic potential, relative water content (RWC) and gravimetric soil water content (GSWC) calculated over treatments of five cultivars of berseem during 3 days after withholding water

Cultivar	Day		
	1	2	3
Water potential (-MPa)			
Axi	1.68ab	2.63a	3.78a
Big Bee	1.82a	2.71a	3.64a
Lilibeo	1.29c	2.21b	3.41b
Sacromonte	1.55b	2.59a	3.73a
Saniros	1.65ab	2.53a	3.66a
Osmotic potential (-MPa)			
Axi	1.48a	2.47a	3.67a
Big Bee	1.31a	2.11b	3.45a
Lilibeo	1.57a	1.99b	3.15b
Sacromonte	1.40a	2.12b	3.68a
Saniros	1.51a	1.98b	3.51a
RWC (%)			
Axi	83.95a	59.74c	42.56b
Big Bee	79.61a	56.63c	42.46b
Lilibeo	85.17a	67.93a	50.67a
Sacromonte	82.38a	60.50bc	41.08b
Saniros	83.90a	65.79ab	43.26b
Gravimetric soil water content (Kg/Kg)			
Axi	0.24b	0.18b	0.16b
Big Bee	0.24b	0.18b	0.16b
Lilibeo	0.26a	0.20a	0.17a
Sacromonte	0.24b	0.18b	0.16b
Saniros	0.24b	0.19ab	0.16b

Means with the same letters in each column do not differ significantly at 5% level.

**Figure 1**

Leaf water potential, osmotic potential, relative water content (RWC) and gravimetric soil water content (GSWC) of four preconditioning treatments (T1, T2, T3 and T4) during the final drought period (3 days). Each point is the average of 2 replicates and 5 cultivars. Vertical bars are  $\pm$  standard error of the mean.

