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Variation in Salt Tolerance and Ion Accumulation among Subterranean Clover Cultivars

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ABSTRACT

Increased productivity of forage crops under saline conditions is a desired characteristic in irrigated areas in both the USA and Australia. Clover *(Trifolium)* species are often used in single or mixed stands. Studies were conducted to determine physiological mechanisms and the extent of variability for salt tolerance among cultivars of subterranean clover. Six cultivars of subterranean clover (*T. subterraneum* L.) belonging to three different subspecies, *brachycalycinum (cv.* Clare and Wenijup), *subterraneum (cv.* Bacchus Marsh and Tallarook), and *yanninicum (cv.* Meteora and Trikkala), were tested for salt tolerance during germination and emergence and at the vegetative stage of growth in sand cultures salinized with 20 to 80 mM NaCl. Cultivars differed in final emergence, growth rates, salt tolerance, and ion accumulation. When salinity was applied at the time of seeding, the cultivars Clare and Tallarook were the most tolerant. When plants were salinized after the three- to four-trifoliate leaf stage, Clare had the highest relative salt tolerance as defined by shoot dry weight yield reduction as a percent of the unsalinized controls. Relative salt tolerance followed the order Clare > Bacchus Marsh = Trikkala = Tallarook > Meteora = Wenijup. Meteora had the highest productivity as defined by total dry weight production under high NaCl treatment. Productivity of clover under saline conditions requires high growth potential and low reduction in yield with increasing salinity. High productivity under salinity stress was positively correlated with restricted Na^+ uptake in the shoot and the maintenance of high K^+/Na^+ ratios.

SALINITY in soils and ground waters has become a major environmental issue (Shannon et al. 1994). In irrigated areas where decreasing pasture yields due to salinity is a major concern, there is a need for clover species with greater salt tolerance than either *T. repens* L. or *T. pratense* L. (Rumbaugh et al., 1993; Mizra and Tariq, 1993). Subterranean clover is an important crop in many areas of Australia (Quinlivan and Francis, 1976) and could have potential in the USA as well (Noble and Shannon, 1988). Previously, West and Taylor (1981) measured germination and early growth in the presence of NaCl in 15 cultivars of subterranean clover belonging to three subspecies, *brachycalycinum, subterraneum,* and *yunninicum.* In their studies, germination tests conducted on filter paper showed no cultivar differences after 6 d but the growth of cultivars were different under NaCl treatments in studies conducted in soil-filled pots. Salt-tolerance differences among cultivars were difficult to analyze in their studies because salt accumulation in the pots of plants with high growth rates was greater than in those with low growth rates. Additional studies were needed to separate the effects of salt and water stresses among cultivars and to determine if differences in salt tolerance existed as measured by emergence and at later growth stages. This information would be useful in situations in which nonsaline water could be used for plant establishment. Finally, information was needed to identify cultivar differences in ion uptake among subterranean clover cultivars as a possible selection tool for improved salt tolerance (Shannon, 1985; Munns and Termaat, 1986; Noble and Shannon, 1988).

Our objectives were to determine if salt tolerance differences existed among subterranean clover cultivars as measured by high seedling emergence and plant growth rates compared with nonsalinzed controls. In addition, we investigated ion accumulation under salinity as a potential physiological reason for differences in tolerance. We used frequently-irrigated sand cultures to avoid interactions between salt and water use, and used cultivars that had previously shown differences in vegetative growth response under saline conditions (West and Taylor, 1981). Ion accumulation in leaf, root, and shoot tissues was analyzed to determine possible mechanisms contributing to salt tolerance differences among cultivars of subterranean clover.

MATERIALS AND METHODS

In an attempt to examine a wide range of genetic variability, six cultivars of subterranean clover were selected from among three subspecies (ssp. *brachycalycinum:* Clare and Wenijup; ssp. *subterraneum:* Bacchus Marsh and Tallarook; and ssp. *yunninicum:* Meteora and Trikkala). The experiments were conducted in a greenhouse in 20 sand-culture tanks filled to a depth of 0.2 m with medium-textured sand. A randomized complete block design with four replications and four plants per replication was used, with salt (five levels) as the main factor and cultivars as a subfactor. Each block consisted of five sand tanks (salinity levels) and plants of each cultivar were planted into every tank. A nutrient solution composed of 4 mM $Ca(NO_3)_2$, 3 mM KNO_3, 1 mM $MgSO_4$, 0.5 mM $NH_4H_2PO_4$, 0.25 mM FeEDTA, 46 μM H_3BO_3 ,9 μM $MnCl_2$, 0.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, and 0.1 μM H_2MoO_2 was used to irrigate the tanks six-times daily for 20-min intervals during the daylight hours. The nutrient solution covered the sand surface for at least 10 min during each irrigation and prevented residual salt build-up. Overflow tubes prevented over-filling and reduced salt exposure to leaf surfaces. On 26

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Abbreviations: DAS, days after sowing.

Table 1. Salt effects on emergence of six subterranean clover cultivars 10 d after sowing.

Cultivar	Salinity (mM NaCl)			
	20	40	60	80
	Percent emergence			
Clare	100†	100	100	100
Wenijup	100	96	84	67
Bacchus Marsh	88	88	88	61
Tallarook	88	88	88	88
Meteora	100	100	91	75
Trikkala	100	96	93	43

† Values given as percentage of total number of seeds planted.

September, 12 to 16 seeds per replication were sown for each cultivar. Small, hard, and abnormal seeds were removed prior to sowing. Emerged seedlings were thinned to four uniform plants per replication and shoot samples were taken when seedlings had developed to the two- to three-trifoliate stage (18 d after sowing, DAS). Additions of 20 mM NaCl per day were made to the nutrient solutions. In this manner, treatments 0,20,40,60, and 80 mM NaCl were established over a 4-d period. Electrical conductivities of the treatment solutions averaged over the course of the experiment were 2.0, 4.5, 6.8, 8.9, and 10.9 dS m^{-1}, respectively. Solutions were replaced every week throughout the study and pH was maintained between 5.5 and 6.5. Daily maximum and minimum temperatures during this study averaged 35.2 and 23.3°C, respectively. Day/night ratio was approximately 1 l/13 h during the experiments.

On the day that the final treatment salinities were established, four to six seeds of each cultivar were sown in a randomized complete block design within another area of the same sand tanks used in the first study. The second experiment was initiated to examine the effects of NaCl on seedlings that had been stressed during the germination and emergence phase and to compare their growth responses to seedlings that had been established prior to salination. In the second study, Emergence counts were made daily for 10 d and plant samples were harvested 13, 17, and 22 DAS.

The central leaflet of the youngest, fully-expanded trifoliate leaf from each plant was sampled at intervals of 6, 11, 16,and 21 d after final salination. All plants were harvested on 7 November (42 DAS). Fresh and dry weights of shoots and roots were measured. All tissue samples were rinsed in distilled water, oven-dried at 60°C, and ground for mineral analyses. Ground tissue samples were ashed at 460°C for 15 h and analyzed by inductive coupled plasma spectrophotometry for Na+, K+, Mg^{+2}, and Ca^{+2} and for Cl^{-} by potentiometric titration (Cotlove, 1963). Statistical analysis was conducted using SAS-GLM (SAS Institute Inc., 1987) procedures. Significance of results was determined to be when $P \leq 0.05$ unless otherwise determined. Plant-to-plant variability within treatments was small due to the uniform environment maintained with sand cultures and the genetic uniformity inherent to a self-pollinated species.

RESULTS AND DISCUSSION

Salinity Stress Applied during Emergence

Salinity applied at the time of seeding in the second experiment reduced growth rates of emergent seedlings as a function of its concentration. Salinity decreased emergence, as measured by protrusion of the cotyledon above the sand surface, in all cultivars except Clare and Tallarook, and contributed to seedling death after emergence in Meteora and Trikkala (Table 1). Emergence in Clare reached 97 to 100% within 1 wk, whereas maximum emergence was 88% in Tallarook. In these two cultivars, emergence at 10 DAS was 100% of the control values in all salt treatments. Emergence of Wenijup, Trikkala and Meteora was significantly delayed by NaCl treatments higher than 40 mM NaCl, and emergence percentages of the latter two cultivars actually decreased between the seventh and tenth day because of seedling death.

Average seedling dry weight of Clare under nonsaline conditions was approximately 0.17 g plant-’ (22 DAS), which was significantly greater than the other cultivars. Under salt stress, at all salinity levels except the highest, Clare also produced significantly more dry matter than the other cultivars (Fig. 1). Seedlings emerging under nonsaline or 20 mM NaCl had three or four trifoliate

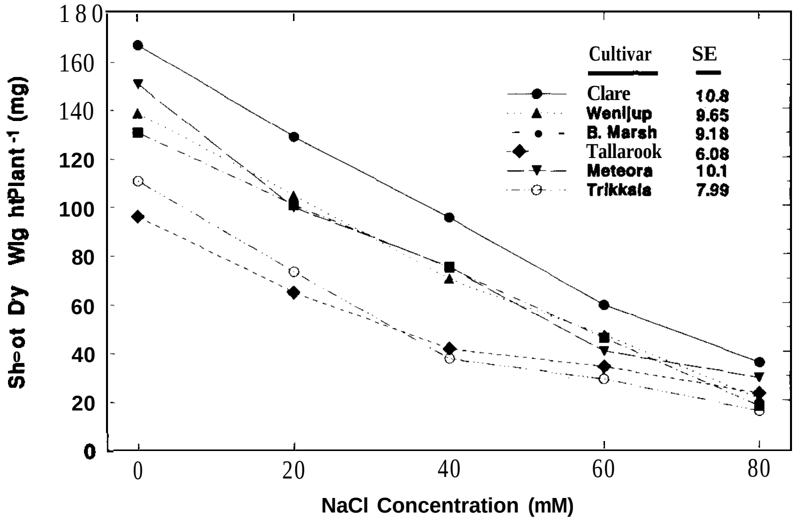

Fig. 1. Effects of NaCl applied at the time of sowing on the dry weight of emergent seedlings of six cultivars of subterranean clover, 22 d after sowing. Each value represents the combined shoot dry weight of 28 to 33 seedlings divided by the seedling number. Pooled standard errors of the mean by NaCl treatment are given for each cultivar.

leaves; whereas, those emerging in 60 to 80 mM NaCl had two or three leaves; cultivar differences could not be detected. At treatments less than 60 mM NaCl, absolute dry weights of the cultivars followed the order Clare > Meteora = Wenijup = Bacchus Marsh > Trikkala = Tallarook.

West and Taylor (1981) found no differences in salt tolerance among 15 cultivars of subterranean clover based on germination tests on filter paper at a maximum salt concentration equivalent to an osmotic potential of -330 kPa In our studies, the approximate osmotic potential of the Ca^{2+}-enriched nutrient solution at 80 mM NaCl was -420 kPa and cultivar differences were significant. However, West and Taylor (1981) found no relationship between salt tolerance measured as a percentage of germinated seeds on filter papers and tolerance at seedling stage as measured by seedling weights. Due to these factors, we decided to look at the effects of salinity applied at later growth stages.

Salinity Effects after Plant Establishment

Under saline conditions, shoot dry weights were reduced in proportion to increased NaCl concentrations in the irrigation solutions (Fig. 2). Salt treatment with 20 mM NaCl caused significant reductions in shoot weight in all cultivars and was above the salinity threshold of 1.6 dS m^{-1} described by Maas (1986) for a number of other clover species. At the time of final harvest, 42 DAS, Meteora grown under nonsaline conditions had significantly higher average shoot dry weight (4.14 g plant-‘) than the other cultivars (Fig. 2). Shoot dry weight averaged over all cultivars was about 2.6 g plant-‘, and the lowest dry weights were recorded for Clare and Trikkala (1.91 and 1.71 g plant-‘, respectively). Final productivity was not due to high growth rates prior to salination. Prior to salination Clare had the highest shoot dry weight and Tallarook had the lowest. Average shoot dry weight of seedlings at 18 DAS was 0.048 g and ranged between 0.027 and 0.065 g for the different cultivars (data not shown). Root dry weights over all cultivars averaged 0.008 g plant-’ at the beginning of salination.

Root dry weights over all cultivars averaged 0.305 g plant-’ in the nonsaline control treatment at harvest. Root dry weights were not significantly reduced by NaCl up to 40 mM, but thereafter, dry weight decreases among cultivars were proportional and almost identical in ranking to dry weights of the shoots (data not shown). Average root/shoot ratio prior to salination was 0.161 and decreased during the experiment to 0.118 in all treatments. Root/shoot ratios were similar for all cultivars and despite a few differences, could not be well correlated to differences in productivity or salt tolerance among cultivars. Fresh/dry weight ratios also differed slightly with cultivar but were not clearly correlated to differences in salt tolerance (data not shown).

We found that Clare and Meteora had significantly different slopes and intercepts in their evaluation for salt tolerance based upon absolute growth rates when salts were applied during the vegetative growth stage (Fig. 2). Linear regressions of shoot dry weight across salinities described the data well and correlation coefficients based on linear analysis ranged between -0.84 and -0.94 (df = 18). For this reason further analysis such as suggested previously (Maas 1986; van Genuchten and Hoffman, 1984) was not conducted. Based upon estimates of slope, Meteora (b = -0.025 ± 0.003) and Wenijup (b = -0.023 ± 0.003) were the most salt-sensitive cultivars and Clare (b = -0.008 ± 0.001) was the most tolerant. The high growth rate of Meteora was accompanied by approximately a 12% yield reduction at each level of increase in salinity; whereas, the more tolerant Clare suffered only an 8.5 % yield reduction at each higher salinity treatment level. The inverse relationship between high growth rates and salt sensitivity has been previously noted (Shannon, 1985; Shannon and Noble, 1990).

When cultivars were compared based on growth under saline conditions as a percent of maximum growth under nonsaline control conditions or relative salt tolerance, it

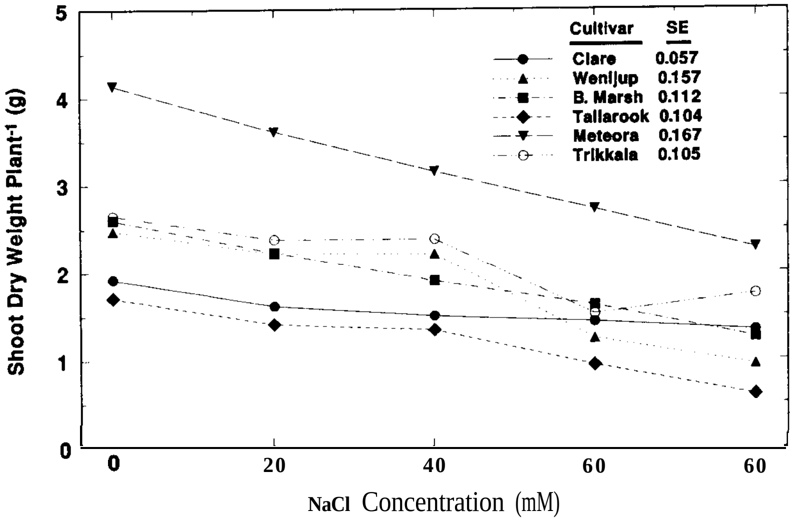

Fig. 2. Effects of NaCl applied at the two- to three-trifoliate growth stage on shoot dry weights of six cultivars of subterranean clover 42 d after sowing. Pooled standard errors of the mean by NaCl treatment are given for each cultivar.

was found that Clare was the most tolerant cultivar in both of the two studies. Meteora was the most productive cultivar across all salinities as a result of its high growth rate, but was also the most effected by salt, i.e., relative yield reduction per unit salinity increase was the greatest. Clare and Tallarook had comparatively low yields under nonsaline and saline conditions, but Clare had the least relative reduction in yield with increasing salinity. At the 80 mM NaCl treatment, only Meteora had significantly higher shoot weight than Clare. Due to rapid early growth under nonstressed conditions, plants salinized after establishment had higher relative yields than those salinized from the time of planting when compared to their respective controls (Fig. 3). Dry weight reduction per unit salinity usually was not different among cultivars up to 40 mM NaC1 treatment, thus, differences among cultivars at the two lower salinities could be attributed to differences in relative growth rates rather than differences in salt tolerance. Slopes plotted on the mean of the pooled cultivars were similar across the three highest NaCl treatments and the relative effect of early application of salt stress was observable at the lowest NaCl treatment.

Under nonsaline conditions, shoot dry weight of Meteora was 56% higher than the next most productive cultivar (Fig. 2). Despite the low relative salt tolerance of this cultivar, its high growth rate resulted in the highest net productivity at all NaCl concentrations. Thus, it would seem that vigor may be a more important plant characteristic for salt-affected growing regions than salt tolerance per se (i.e., a low relative growth reduction index with increasing salinity). This selection strategy has been proposed previously and its theoretical precepts explored (Richards, 1983; Shannon, 1985). However, the importance of improving both salt tolerance and high yield need to be recognized in any selection and breeding program. In tall wheatgrass [*Thinopyrum elongatum* (Host) D. R. Dewey], both vigor and salt tolerance were improved in a hybrid line between salt tolerant parents (Weimburg and Shannon, 1988).

Ion Accumulation in Tissues

At 42 DAS, concentrations of Na+ and Cl- in the root were positively correlated with NaCl treatment concentrations. Mean root Na+ averaged over cultivars increased from 80 to 1290 mmol kg-' across treatments. Similarly, root Cl-concentrations were about 45 and 490 mmol kg^{-1} dry weight in nonsaline and 80 M NaCl treatments, respectively. Root K+ did not decrease from that of the control (15 10 mmol kg^{-1}) until NaCl in the irrigation media reached 60 mM. Root concentrations of Ca^{2+} and Mg^{2+} did not change significantly from control values over the range of the salinity treatments and each averaged about 120 mmol kg-' dry weight. The coefficients of variations for the divalent cations were $\leq 5\%$ *in* all treatments.

Changes in shoot ion concentrations with salinity could be observed by analysis of tissues from the various treatments at final harvest, and by analysis of the fully expanded leaflet at periodic intervals throughout the experiment. Increases in Na+ and Cl- and a decrease in K+ in the shoot and root occurred as a result of the increasing NaCl concentration treatments. When salinity was applied at the time of seeding, shoot Cl- concentrations of seedlings increased with NaCl treatment. However, at 40 mM NaCl treatments and higher, shoot Cl- concentrations in Trikkala and Tallarook were higher than in other cultivars (Table 2).

At final harvest, mean shoot *Na+* across cultivars increased from 70 to 1860 mmol kg^{-1} dry weight as NaCl in the irrigation solution increased from 0 to 80 mM and mean shoot Cl-averaged over all cultivars increased from about 120 to 990 mmol kg-' dry weight over this same range of treatments. Shoot K+ decreased as Na+ increased at all treatment salinities, a result different from that found in the root, in which K+ remained high at NaCl treatment levels less than 60 mM. As with roots, Na+ concentrations in the shoot increased to a larger proportion than Cl-. Ca^{2+} and Mg^{2+} concentrations in shoots decreased significantly from those of the controls

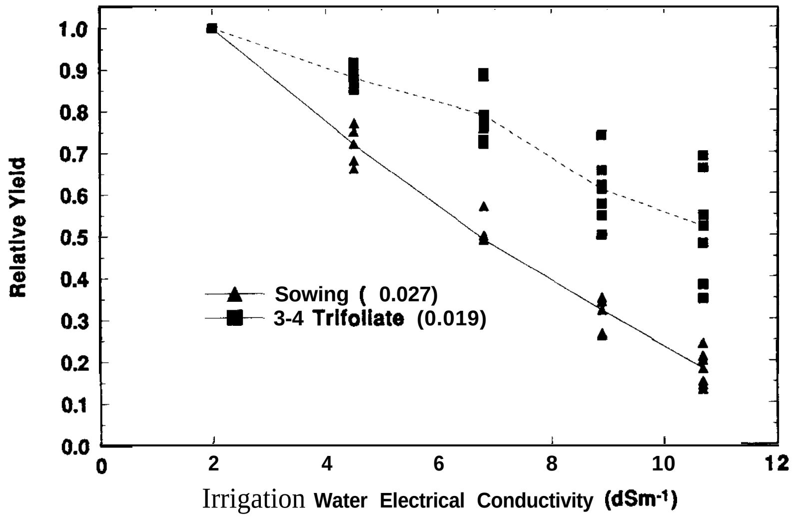

Fig. 3. Relative salt tolerance of six cultivars of subterranean clover when salts were applied at the time of sowing (▲) or at the two- or three-trifoliate growth stage (■).

Table 2. The influence of NaCl applied at the time of seeding on shoot chloride concentrations in six cultivars of subterranean clover (22 d after sowing).

Cultivar	Salinity (mM NaCl)				
	0	20	40	60	80
	Cl^-, mmol kg^{-1} dry weight				
Clare	70b†	465a	1082b	1755ab	2326 ab
Wenijup	72b	451a	1040b	1919ab	2476 ab
Bacchus Marsh	135a	424a	999b	1581b	2446 ab
Tallarook	111ab	539a	1501a	2012a	2575 a
Meteora	73b	515a	970b	1752ab	1923 b
Trikkala	74b	527a	1372a	1722ab	2581 a

† Numbers within columns that are significantly different at the a = 0.05 level as determined by Duncan's multiple range analysis are represented by different letters.

at NaCl concentrations above 40 mM. Ca^{2+} concentrations in control plants averaged 317 (±30) mmol kg-’ dry weight and decreased to 185 (±30) mmol kg-’ dry weight in shoots of plants grown under 80 mM NaCl. Shoot Mg^{2+} decreased from 98 (± 12) to 86 (± 18) mmol kg-’ dry weight, respectively.

Ion Accumulation Differences among Cultivars

Significant differences were observed among cultivars in the accumulation of Na+and Cl- in the shoots at final harvest (Table 3). Clare and Wenijup had the highest shoot Na+ and Cl- concentrations at the two highest NaCl treatments, whereas Meteora, Trikkala, and Bacchus Marsh had the lowest. Meteora was particularly efficient at maintaining low levels of shoot Cl-. Under 80 mM NaCl treatment, Meteora, which produced the highest shoot weight across all salt concentrations, had lower levels of shoot Na+ and Cl- than the other cultivars, but the highest concentrations of root Na+ and Cl- (1630 and 575 mmol kg-’ dry weight, respectively). The ability of Meteora to maintain low shoot Cl- may be a critical factor in high productivity under salt stress. Clare, the most salt-tolerant but least salt-productive cultivar, had one of the highest shoot Na+ and Cl- concentrations, but had the lowest levels of root Na+ and Cl- (870 and 410 mmol kg-’ dry weight, respectively). In seedlings salinized to 40 mM NaCl at the time of seeding, slow growth rates in Trikkala and Tallarook were accompanied by higher concentrations of treatment ions (Na+ data not shown) in the shoot than found in the other cultivars. Generally, increases in shoot ion concentrations were not clearly related to differences in growth rates (Fig. 1, Table 2).

The Na+ and Cl- concentrations of the youngest, fully-expanded, leaflets increased with salinity treatment and, as with the whole shoot, the extent of increase was dependent upon cultivar, but the relative changes were similar. For brevity, the changes in Na+ and Cl- concentrations of leaflets between 22 and 37 DAS are reported only for Clare and final shoot concentrations are reported for Clare and Meteora at final harvest (Fig. 4). For all NaCl levels except the highest, leaflet tissue concentrations of both Na+ and Cl- reached near-maximum levels soon after salinization (22 DAS) with slight decreases noted through Day 37. The Cl- levels in Wenijup leaflets harvested from the two highest salinity treatments continued to increase through the last sampling period (data not shown). Meteora and Trikkala had the lowest leaflet

Table 3. Shoot ion contents of six subterranean clover cultivars 42 d after seeding (salinity was applied at the two- or three-trifoliate stage of growth).

Cultivar	NaCl Treatments, mM				
	0	20	40	60	80
	Na, mmol kg^{-1} dry weight				
Clare	70a†	726c	1061c	1735ab	2061 b
Wenijup	74a	796b	1383a	1809a	2283 a
Bacchus Marsh	62a	830ab	1287ab	1552cd	1661 d
Tallarook	66a	857a	1383a	1626bc	1835 c
Meteora	67a	626e	1070c	1370e	1690 d
Trikkala	67a	665d	1152bc	1474de	1739 cd
	Cl-, mmol kg-’ dry weight				
Clare	77c	292bc	445c	1012ab	1279 ab
Wenijup	84c	428a	647a	1051a	1483 a
Bacchus Marsh	166a	318b	540b	744cd	832 c
Tallarook	151a	383a	507b	807c	1103 b
Meteora	137ab	282bc	347d	387e	453 d
Trikkala	116b	275c	390cd	637d	803 c
	K, mmol kg-’ dry weight				
Clare	1487a	988bc	600b	448b	324 b
Wenijup	1534a	1117a	687a	437b	316 b
Bacchus Marsh	1347b	957cd	672ab	494ab	412 a
Tallarook	1503a	1025b	735a	493ab	382 a
Meteora	1156c	911d	659ab	482ab	370 a
Trikkala	1306b	1020bc	703a	517a	414 a

† For each ion species, means within columns followed by different letters are significantly different by multiple range analysis at the 0.05 level of probability.

Cl- levels, and Wenijup, Clare, and Tallarook, the highest. These results were analogous to those found in whole shoots at the end of the study on Day 42. However, concentrations of Na+ and Cl- found in the leaflets of salinized plants were lower than those measured in whole shoots.

When salinity was applied at the time of seeding, Na+ and Cl- concentrations in the shoot were not clearly related to differences in growth between cultivars (Fig. 1, Table 2). In most cultivars, the ion concentration of the shoot at a given salinity did not increase with time over the nine-day period. At 40 mM NaCl, for example, average shoot Cl- concentrations across all cultivars at samplingdates 13, 17,and 22 DAS were 1158, 1196,and 1128 mmol kg-’ dry weight, respectively. The pooled standard deviation of the mean was 173 mmol kg^{-1}. The consistency of both Na+ and Cl- concentrations on a dry tissue weight basis indicates a balance between growth rate and salt loading into the shoot. This is further supported by data obtained from plants salinized after seedling establishment where Na+ and Cl- concentrations in leaflets of these plants increased fairly rapidly to a plateau that was a function of cultivar and salinity, but not time (Fig. 4).

In plants salinized after seedling establishment, ion analysis of both the entire shoot at final harvest and periodic samples of the central, fully-expanded leaflet provided a clearer understanding of the dynamics of the ion status within the shoot. At equivalent salinity treatments, Na+ and Cl- concentrations in whole shoots were higher than in individual leaflets (Fig. 4), and Na+ concentrations in whole shoots accumulated to levels two to four times higher than those of Cl- in both leaflets and shoots. Ratios of Na+/Cl- in the shoots of all cultivars except Meteora decreased as NaCl concentrations increased in the irrigation solution. For example, as NaCl treatment increased from 20 to 80 mM, Na+/Cl- ratios in Meteora shoots increased from 2.22 to 3.73; whereas, the average Na+/Cl- ratios over all other cultivars decreased from 2.32 to 1.79 over this same salinity range. Thus, as salinity increased, the physiological processes influencing Na+ and Cl- transport to the shoot were not consistent among cultivars.

Evidence for the compartmentation of ions throughout

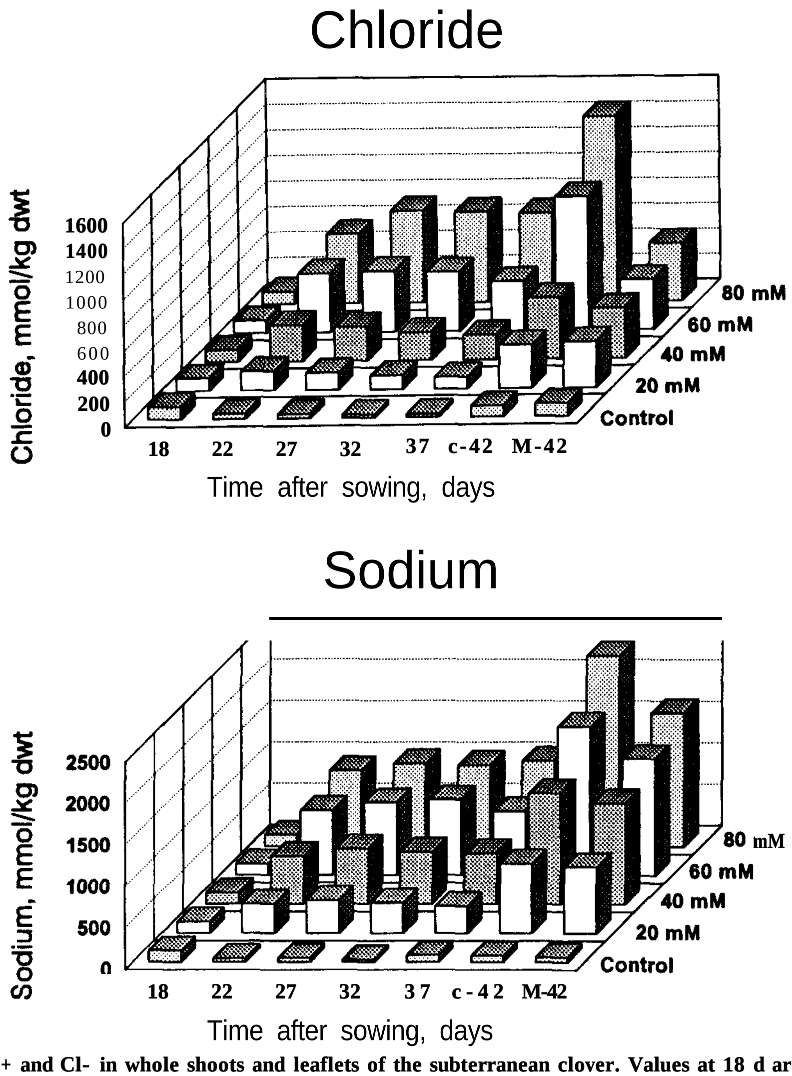

Fig. 4. Concentrations of Na+ and Cl- in whole shoots and leaflets of the subterranean clover. Values at 18 d are from whole shoots of Clare before salinization; values at 22 to 42 d are from the youngest, fully-expanded leaflet of Clare; values at C-42 and M-42 days are from the whole shoots of Clare and Meteora, respectively, at final harvest.

shoots has been presented in both dicots and monocots and has been proposed as a mechanism for salt tolerance (Munns and Termaat, 1986). What is yet unclear is the physiological basis for the mechanism. Ions may accumulate in leaves as simple physical functions of the amount of water transpired and their dilution by the growth of the tissues (Dalton and Poss, 1990), or plant genotype may impose a significant constraint on ion accumulation via mechanisms other than vigorous growth rate and membrane reflection coefficients. The high productivity of Meteora may be attributed to its ability to restrict Cl- movement into the shoot more effectively than the other cultivars. Thus, the concentrations of potentially harmful Cl- ions would be lower in the photosynthetically active tissues. As salinity increases, selectivity decreases in relation to that of the other cultivars as evidenced by changes in the Na+/Cl- ratios. This factor could explain the greater relative growth decreases in Meteora.

Shoot weight was negatively correlated with NaCl treatment concentration ($r = -0.97$) and accumulation of Na+ ($r = -0.94$) and Cl- ($r = -0.97$) in shoots at harvest. Shoot dry weights among cultivars were positively correlated with the retension of shoot K+($r = 0.94$). Differences in regression values among cultivars were relatively small (± 0.04). The correlation between the accumulation of shoot Na+ and growth was not significantly different from the correlation between shoot Cl- and growth, even though shoot Cl- concentrations were lower than shoot Na+ concentrations (Table 3 and Fig. 2). Exclusion mechanisms at the root may be responsible for reduced transport into the shoot.

Meteora had higher root NaCl concentrations than many of the other cultivars, so it is possible that exclusion mechanisms at the root may be responsible for reduced transport into the shoot. The relative sensitivity of Meteora to salinity could be a result of high energetic costs associated with control of Na+concentrations in both root and shoot. Penning de Vries (1975) has estimated that as much as 10 mg g^{-1} dry weight per day in leaves can be utilized by ion transport mechanisms within the plant. Under saline conditions, this cost could be substantially higher due to compartmentalization of ions against higher gradients and leakage (Yeo, 1983). Clare had higher shoot and leaflet ion concentrations and a lower growth rate, but also had a higher relative salt tolerance. However, the lower growth rate of Clare would make it an unattractive alternative to Meteora under conditions of low to medium salt stress (e.g., in instances where moderately saline water is available for irrigation).

Variability in salt tolerance exists in subterranean clover and there is probably potential for its improvement through selection. Clearly, the ability to exclude Cl- from the shoot and maintain a high growth rate is an important criterion in subterranean clover for high productivity under saline conditions. The maintainance of high K+/Na+ selectivity may also be important. These characters, in association with each other, may be useful as physiological markers in a breeding program.

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