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RESPONSE OF *TRIFOLIUM SUBTERRANEUM* TO SODIUM CHLORIDE UNDER DIFFERENT NUTRIENT CONDITIONS

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Abstract

Salt tolerance and interaction of NaCl with nutrient ions of the growth medium was studied in *Trifolium subterraneum* (cv. Wenigup). Growth of this species was reduced at 50 m-equiv/l NaCl indicating that it is a salt sensitive species. Such growth reduction was due to higher sodium and chloride uptake and lower potassium content of the plant tissues. Adverse effects of NaCl on growth of this species were more pronounced when the plants were grown in low than in high nutrient solution.

In studying the interaction of NaCl at four different nutrient concentrations, the results showed an improvement in growth linearly as the strength of nutrient concentration increased. It is concluded that NaCl at low nutrient reduces growth by ion unbalance which is mainly due to low calcium status of the nutrient medium.

Introduction

Little information is available on the salt tolerance of *Trifolium subterraneum* L. commonly known as sub-clover.

Knowledge of the salt tolerance of legumes is important particularly in the management of saline soils both under natural rainfall and under irrigation. They are important on saline lands because of their role in nitrogen fixation, improvement of soil structure resulting in an increase in fertility and checking erosion.

Many legumes are more salt sensitive than most cereal crops. Toniolo and Poli (1958) have for example shown that salt tolerance decreased in the order barley > rye > wheat > oats > beans > peas > lucerne. The purpose of the present research was to determine the salt tolerance of sub-clover and to study in the same species the interaction of NaCl with the nutrient ions of the growth medium.

Materials and Methods

In the present experiment water culture technique was used. The seeds of sub-clover (cv. Wenigup) were germinated in sand in the glass house. When cotyledons had fully emerged they were transferred to culture dishes of 1 lit capacity containing nutrient solution (Hoagland and Arnon 1938). The nutrient solution was aerated and changed frequently. Seedlings of sub-clover thus raised were used in three experiments. Expt. 1 was carried out to determine the salt tolerance of sub-clover and in experiments 2 and 3, the interaction of NaCl with the nutrient ions of the growth medium was studied. For each experiment there were 5-7 replications. Further details of the experiments were as under:

Experiment 1

For determining the salt tolerance, the seedlings of sub-clover were grown in the saline solutions containing 0, 5, 25, 40 and 100 m-equiv/l NaCl for 13 days. At harvest, the plant parts separated were (a) young leaves, (b) old leaves, (c) rest of shoots and (d) roots.

Experiment 2

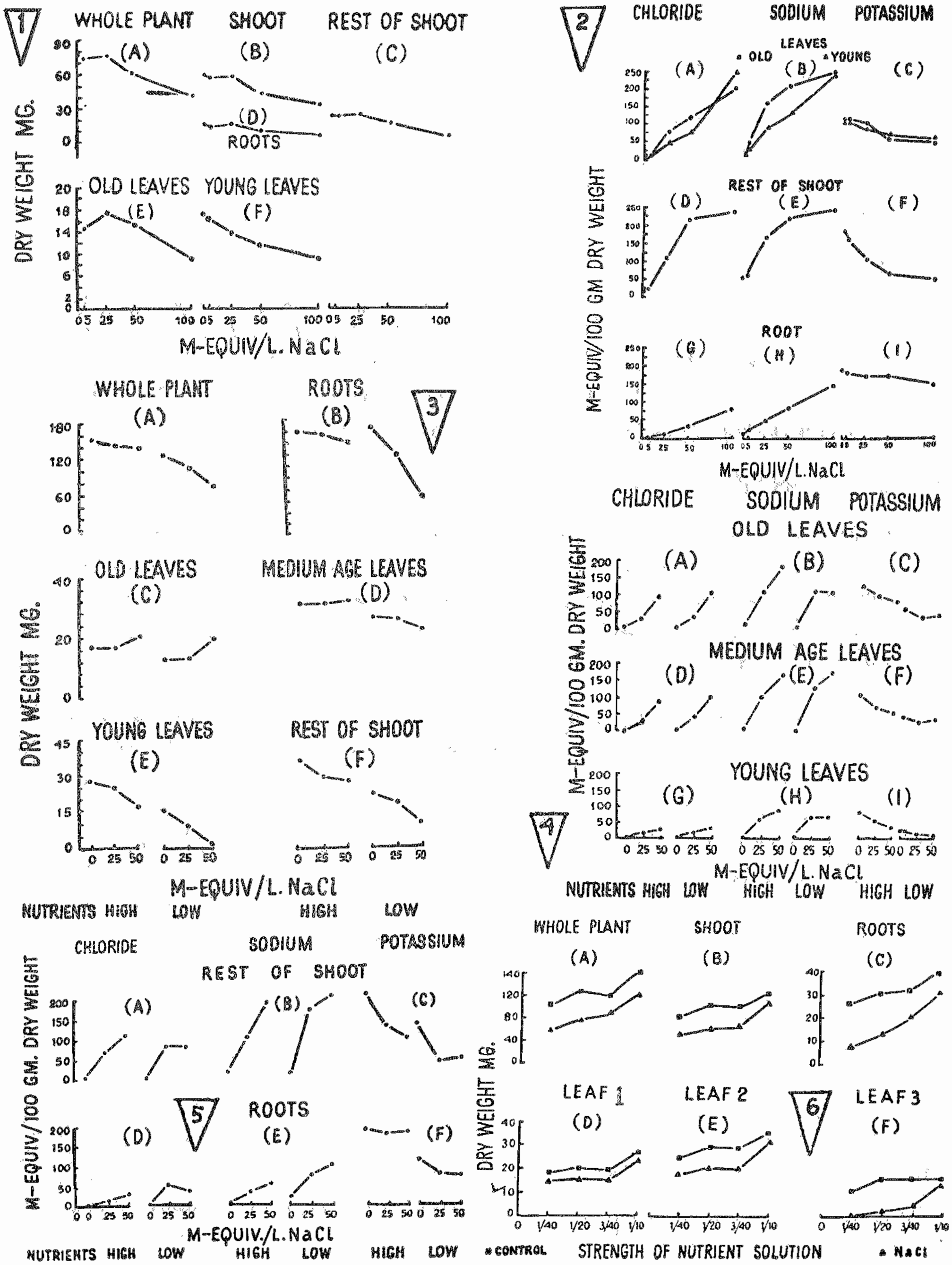
Interaction of NaCl with other nutrient ions of the growth medium was first studied by growing sub-clover seedlings at 1/40 dilution and at full strength of the Hoagland solution and also in solutions containing NaCl at concentration of 0, 25 and 50 m-equiv/l at the above nutrient levels. In the text the two nutrient levels i.e. 1/40 dilution and full strength Hoagland solution have been referred to as low and high nutrient respectively. The duration of the experiment was eleven days. At harvest the following plant parts were separated:

1. Old leaves (L_1)
2. Medium age leaves (L_2)
3. Young leaves (L_3)
4. Rest of shoots
5. Roots

Experiment 3

In this experiment the interaction of NaCl with other nutrient ions of the growth medium was studied over a wide range of dilutions of Hoagland solution. These dilutions were 1/40, 1/20, 3/40, 1/10 and full strength of Hoagland solution. Sub-clover seedlings were grown for eleven days in these diluted solutions. Saline treatments were imposed by adding NaCl at concentration of 0 and 50 m-equiv/l to the above dilutions of Hoagland solution. At harvest the plant parts separated were the same as in Expt. 2.

In all the three experiments the plants were transferred for harvest from glass house to the laboratory before sunrise. Roots were rinsed in distilled water. All the plant parts separated were dried at 80 C for chemical analysis and dry wt determination. Chloride was determined by the electro-titrimetric method of Best (1950) and sodium and potassium by flame photometer.



Effect of various concentrations of NaCl on the dry wt (Figs. 1 and 3) and ion concentration (Figs. 2, 4 and 5) of the whole plant and its individual parts. Effect of NaCl (50 m-equiv./l) on the dry wt of whole plant and its individual parts over a range of nutrient strength (Fig. 6).

Results

(a) *Effects of different NaCl concentrations* (*Expt. 1*)

Dry weight: For the whole plant, the first adverse effect of NaCl was found at 50 m-equiv/1 with a more pronounced decrease in growth at 100 m-equiv/1 lit. A similar response was found for all individual parts except roots. The growth of young leaves was reduced even at 25 m-equiv/1 NaCl in the medium (Fig. 1).

Ion concentration in plant tissues: Chloride and sodium concentrations in both control and 5 m-equiv/1 NaCl were low. Above 5 m-equiv/1 NaCl, chloride and sodium usually increased markedly with an increase of NaCl concentration of the medium. Such increase was more pronounced in old leaves and rest of the shoot (Fig. 2). Chloride and sodium uptake in young leaves were smaller than in old leaves in all the saline treatments except at 100 m-equiv/1 NaCl.

(b) *Effects of NaCl at low and high nutrient strength* (*Expt. 2*)

Dry weight: NaCl at both 25 and 50 m-equiv/1 reduced growth of the whole plant and such effects were more pronounced at low than at high nutrients. These trends were particularly pronounced in the younger leaves and roots (Fig. 3). The second and third trifoliate leaves (L_2 and L_3) were reduced only at 50 m-equiv/1 of NaCl in low nutrient.

Ion concentration: Sodium and chloride. At high nutrient, chloride and sodium concentrations rose with increasing concentrations of the medium and the increase were close to linearity (Figs. 4 & 5). Similarly chloride concentration in the roots at low nutrients did not increase between 25 and 50 m-equiv/1 (Fig. 5D). At 25 m-equiv/1 NaCl in the medium sodium concentrations of roots and rest of shoot were higher at low than at high nutrient and the same was found for chloride in the roots. This rather complicated situation is best interpreted by assuming that low nutrient induces two opposing effects on chloride and sodium uptake.

(i) An increase due to less competetion by other ions.

(ii) A decrease due to reduced health of the plants.

It is assumed that at 25 m-equiv/1 NaCl the first tendency of increased uptake would be still apparent in some cases, but at 50 m-equiv/1 the decreased health would become the dominant factor, with the result that the chloride and sodium concentrations in tissues are not much higher at 50 m-equiv/1 low nutrient than at 50 m-equiv/1 high nutrient.

Potassium concentration:

Addition of NaCl to the medium decreased potassium content of all shoot organs a large decrease was found particularly in shoots and roots of low nutrient treatment (Figs. 4 & 5). At high nutrient NaCl treatment reduced potassium of the shoot but had little effect on root content. A similar situation is found in barley at high NaCl (Greenway 1963). Though at lower sodium and chloride in the medium, selectivity of shoot is usually most pronounced (Sutcliffe 1957, Pitman 1965).

(c) *NaCl effects over a range of nutrient strength (Expt. 3)*

Dry weight: NaCl reduced the dry wt of the whole plant and these effects of NaCl became progressively more pronounced as the nutrient strength of the culture solution decreased (Fig. 6). NaCl reduced root growth more than shoot growth and the adverse effects on roots were particularly pronounced again at low nutrient. In shoot the strong effect of NaCl at low nutrient was found in the development of youngest leaf (L_3) (Table 1).

TABLE 1

Growth (dry weight) in NaCl (50 m-equiv/1) as a percentage of the growth in corresponding nutrient with no NaCl

Nutrient strength	1/40	1/20	3/40	1/10	Full
L_1	112	117	112	114	116
L_2	66	72	84	96	104
L_3	6	12	27	90	63
Rest of shoot	61	58	67	80	82
Roots	25	40	67	67	80
Whole plant	55	59	72	86	89

Discussion

The results of the present experiments have shown that growth of sub-clover was reduced at 50 m-equiv/1 NaCl and that of the youngest leaves of this plant at 25 m-equiv/1 NaCl, thus indicating that it is a sensitive species. The present data do not permit to draw a definite conclusion on the cause of reduced growth; however, ionic balance in plant tissues was changed strongly at higher NaCl level i.e. at 50 m-equiv/1 NaCl most parts of the shoot contained more than 150 m-equiv/100 gms dry weight of sodium and chloride and had very low potassium contents. Greenway (1962) has also concluded that growth of a salt sensitive variety of *Hordeum* was reduced due to high chloride and sodium and low potassium uptake.

Under saline conditions the behaviour of sub-clover with respect to ion concentrations in leaves of different ages is similar to those observed by others, for example, chloride content of older leaves were found to be higher than younger leaves in beets and onions (Yankovitch 1949) and in strawberries (Ehlig and Bernstein 1958). This difference in old and young leaves is probably due to the fact that older leaves regulate their sodium and chloride contents by limited uptake whereas young leaves do it by their high relative growth rate (Greenway and Thomas 1965). Further, retranslocation from old leaves is small so that sodium and chloride concentrations increase with time.

At low nutrient, addition of 25 m-equiv/1 NaCl (Expt. 2) i.e. an increase of only 1 atmosphere in osmotic pressure decreased growth of the youngest leaf (Fig. 1). This emphasises the importance of nutrient strength of the medium in salinity studies. Under this low nutrient high NaCl level roots became brown first and then appeared collapsed at the end of the experiment indicating that growth as well as cell organisation was disturbed.

In studying the interaction of NaCl at 4 different nutrient concentrations (Expt. 3, Table 1), the results showed a linear relationship between nutrient strength of the medium and growth in NaCl solution. Between 3/40 and 1/10 nutrient solution this might be due to improved growth even in the control treatments. However, the youngest leaf and roots, the most sensitive indicators of potential growth in a short experiment showed a strong improvement in growth between 3/40 and 1/10 nutrient in NaCl treatment but not in the control treatment. At 1/10 nutrient NaCl reduced growth to a small extent showing that this nutrient concentration was antagonizing nearly all the specific unbalance effects observed at low nutrient levels. In Table 1 growth, (dry weight) in NaCl treatment is expressed as percentage of the corresponding nutrient with no NaCl.

Growth reduction under low nutrient high sodium observed in the present study is probably due to ion unbalance created by high proportion of sodium and chloride ions compared to other essential elements. This ion unbalance is mainly due to low calcium status of the nutrient medium because rapid recovery in growth was noted when plants grown first in 1/40 nutrient (0.2 m-equiv/1 Ca) containing high sodium chloride transferred to the same solution containing additional 0.6 m-equiv/1 of Ca (Hyder and Greenway 1965).

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