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Effects of NaCl on Meristen Size and Proximity of Root Hairs to the Root Tips In *Secale cereale* (cv. K2) and *Triticum aestivum* (Cv. Chinese spring)

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Abstract
A 8 day exposure to NaCl reduced the length of root apical meristem in *Secale cereale* (cv. K2) to a greater extent than *Triticum aestivum* (cv. Chinese Spring). NaCl induced also root hair formation much closer to the root cap boundary than in the control roots. Contrasting cultivars were differentially sensitive by NaCl in respect of distance of the first root hair from the root cap boundary in root meristem and this may be used as a character for screening genotypes at the seedling stage for variability in NaCl tolerance.

Keywords: NaCl, Screening meristem size, *Secale cereale*, *Triticum aestivum*

Introduction

The "rooting" test was first used by Wilkins (1957) and its use was reviewed by Wilkins (1978). Full nutrient solution lacking phosphate and simple calcium salt solutions have been frequently used as background solutions for the rooting tests. Increasing concentrations of metals inhibit root growth and caused a decline in the index of tolerance in both tolerant and non-tolerant populations. This is however, much more marked in non tolerant populations (Wilkins, 1957).

Salt tolerance of contrasting species of natural populations and crop cultivars can be assessed by the rooting test and can then be transformed to the index of salt tolerance values as below:

$$\frac{\text{Mean root length of individuals in NaCl treatment solution}}{\text{Mean root length of individuals in a control solution}} \times 100$$

Hannon and Bradshaw (1968) measured NaCl tolerance in this way and also assessed changes in values for total yield and root shoot ratio. The rooting test proved a reliable method for comparing the salt tolerance of different populations of *Festuca* but other workers (e.g. Tiku and Snaydon, 1971) have found that measures for salt tolerance obtained by rooting test do not correlate with measures based on plant yield.

The present study was conducted on cereal crops comprising species of rye and wheat because of the wide spread intraspecific variation with respect to the salt tolerance.

Materials and Methods

The seeds were obtained from Plant Breeding International, Cambridge, U.K. Seeds of uniform size of *Secale cereale* cv. K2 (2n = 2x = 14) and *Triticum aestivum* cv. Chinese Spring (2n = 6x = 42) were sown on individual rafts (75 mm diameter) consisting of fibre glass tissue stretched across and glued with cow gum to a ring of expanded polystyrene. Rafts were floated on 1 dm^3 of nutrient solution containing 0, 15, 30, 45 or 60 mM NaCl supplied in a background of tenth strength Rorison's nutrient solution in plastic boxes (210 mm x 140 mm x 80 mm). The solution was continuously and gently aerated by bubbling air through diffusing stones using aquarium pumps. There were six rafts in each plastic box but the number of seeds sown on each raft were 10. The experiments were carried out in a growth chamber at a constant temperature of 20 ± 0.5°C with illumination provided for 16 hours per day by white fluorescent tubes. Seedlings were harvested randomly on day 8th and were immediately fixed in 3:1 v/v ethanol:glacial acetic acid mixture and stored at 4°C.

Prior to measurement of the meristem length and the distance of the first root hair from the root cap boundary to the proximal boundary, the roots were Feulgen-stained. The staining procedure was as follows:

Roots were rinsed with distilled water to remove fixative for 2x5 min. Roots were hydrolysed in 5M HCI for 25 min at 25°C. They were then rinsed with ice cold distilled water for 2 x 5 min and ice crystals were added to stop hydrolysis quickly. Roots were then stained for two to three hours in Feulgen reagents at 25°C. The roots were then transferred to 45 % glacial acetic acid.

The meristem size was determined as describes below:

The Feulgen-stained roots were mounted on a microscope slide in 45 % glacial acetic acid under a cover slip but without any squashing. Using an eyepiece graticule, the distance from the root cap junction to the proximal boundary of the meristem in the epidermal region (delimited by the region of intense staining) was measured (Powell *et al*., 1988). Similarly, the distance from the root cap boundary to the position of the 1st root hair was measured on six Feulgen stained roots per cultivar per treatment.

These measurements cannot be definitive measures of the internal extent of the meristem, but they may provide an approximation for comparative screening of the effects of

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externally supplied NaCl in the cultivars (Powell *et al.,* 1988).

Results

Meristem Length

The NaCl term and species term were highly significant (P < 0.001). However, the species x NaCl concentrations interaction were not significant (P > 0.05).

The meristem length of both cultivars was markedly reduced by increasing NaCl concentration (Fig. 1). The meristem length of *T. aestivum* cv. Chinese Spring was greater than *S. cereale* (cv. K2 in the control and at all NaCl concentration except 60 mM). The meristem length of *S. cereale* cv. K2 was 94.9 % of the control (0 mM NaCl) at 15 mM CaCl; this declined further to 79.2 % at 30 mM and 66.5 % at 45 mM and was finally reduced to 58.6 % of the control at 60 mM NaCl. The corresponding values for *T. aestivum* cv. Chinese Spring were 83.3 %, 82.6 %, 77.6 % and 50.4 % of the control treatment.

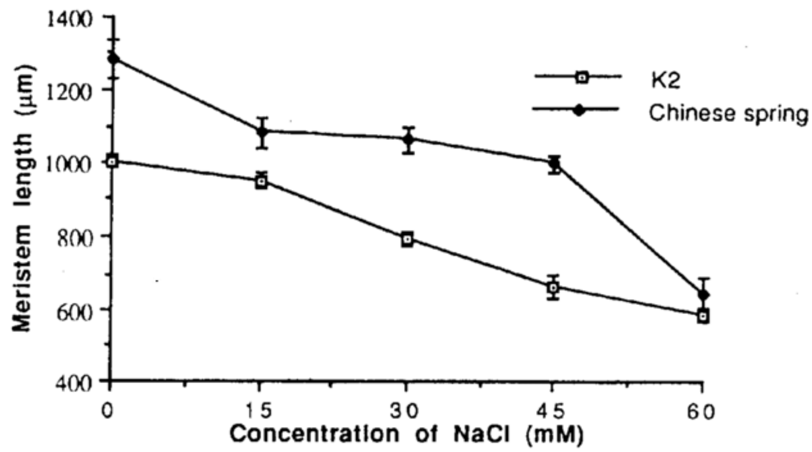

Fig. 1: The meristem length (µm) in seedlings of *Secale cereale* cv. K2 and *Triticum aestivum* cv. Chinese spring on experimental day 8, when grown in solution containg 0, 15, 30, 45 and 60 mM NaCl supplied in a background of 0.1 strength Rorison's nutrient solution. The vertical bars represent ± SE.

The mean distance of the first root hair from the root cap boundary

Although the NaCl term in analysis of variance was highly significant (P < 0.000), the species term was not. However, the species x NaCl concentration interaction was highly significant (P < 0.001).

There was marked difference between the species in the position of the first root hair in relation to the root tip in the control (0 NaCl). For example in *T. aestivum* (cv. Chinese Spring) root hairs did not form until a distance of 4700 µm proximal to the root cap boundary was reached; in *S. cereale* (cv. K2) this position was 3395 µm. The distance of first root hair from the root cap boundary showed a marked decline in both cultivars with increasing NaCl concentration. NaCl induced reduction was more pronounced in *T. aestivum* cv. Chinese Spring than in *S. cereale* cv. K2 (Fig. 2). This distance between the 1st root hair from the root cap boundary was reduced to 95.9 % and 58.9 % of the control at 15 mM NaCl in rye and Chinese Spring respectively; the corresponding figure at 30 mM was 84.7 % and 53.5 % respectively declining to 68.8 % and 51.1 % at 45 mM. There was a further reduction to 46.9% and 30.9% of the control at 60 mM NaCl in *S. cereale* cv. K2 and *T. aestivum* cv. Chinese Spring respectively.

Discussion

Root growth is extremely sensitive to increasing concentration of NaCl in rooting medium (Hannon and Bradshaw, 1968; Tiku and Snaydon, 1971) and has been used to quantify the tolerance of genotypes to NaCl in a similar way as was used to characterize the metal tolerance of plants (Wilkins, 1978). However, little is known about the mechanisms by which NaCl produces this inhibition of root growth. In particular, research is lacking on the effect of NaCl on the fundamental source of new cells for extension-cell division in the root apical meristem. One of the primary aims of this work was to gain information on the effect of NaCl on this aspect of root growth.

Meristem Length

Increasing NaCl concentrations markedly reduced the length of root apical meristem is *Secale* and *Triticum*. There were also differences between the cultivars of rye and wheat in the length of root apical meristem in the control (0 NaCl). The shortening of the apical meristem which was brought about by NaCl in both wheat and rye indicated that NaCl changed the balance that normally exists between cell division and the onset of elongation and differentiation. Cells immediately proximal to the boundary of the meristems generally show a dramatic increase in size. Ivanov (1981) pointed out that this sharp increase in cell length is due to a rapid increase in the rate of cell growth. Barlow (1976) suggested that the derivatives of initial or founder cells in a root meristem habe determinate reproductive life span. For example, in the root meristem of *Allium cepa*, cortical and potential metaxylem cells complete six to seven and five divisions, respectively before elongating (Gonzalez-Fernandez *et al*., 1966). The reduction in meristem length at the higher NaCl treatments reported here indicates that NaCl reduced the reproductive life span of cells in the meristem and leads to premature cell elongation. This phenomenon seems to be widespread in the response to stress factors in roots. For example, Ivanov (1981) showed that X-ray treatment of roots of corn caused premature cell elongation with shortening of meristem length. Powell *et al*. (1988) showed that zinc treatment reduced the length of apical meristem in the roots of *F. rubra*, the zinc-induced reduction was much more pronounced in a zinc sensitive cultivars (S59) than in a zinc tolerant cultivar (Merlin). Davies *et al*. (1991) shoed that this Zn-induced shortening of the root apical meristem was evident within twelve hours after transfer of roots of S59 to Zn. This is an extremely rapid response to the external environment.

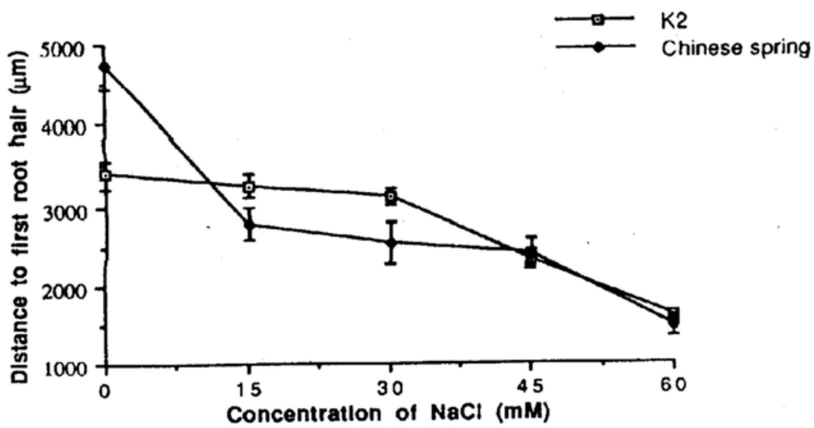

Fig. 2: The distance (µm) from the root cap boundary to the first root hair in seedling of *Secale cereale* cv. K2 and *Triticum aestivum* cv. Chinese spring om experimental day 8, when grown in solutions containing 0, 15, 30, 45 and 60 mM NaCl supplied in a background of 0.1 strength Rorison's nutrient solution. The vertical bars represent ± SE

Thomas (1992) showed that treatment with manganese reduced meristem length in roots of *Epilobium hirsutum* and *Chamerion angustifolium* and that the length of apical meristen of manganese treated roots prog-ressively increased in size, following transfer to Mn-free solution, eventually to the level of control roots. Similar recovery from a stress-induced reduction in meristem size occurs following alleviation of cold stress. For example, Barlow and Rathfedler (1985) showed that meristem length of roots of *Zea mays* treated at 5°C increased following transfer to 20°C; this increase was accompanied by a return to normal rates for cell division and by stimulation of cell proliferation in the quiescent centre. Moreover, the degree of meristem shortening and the time required for complete restoration to its original length was related to the duration of the treatment at 5°C (Barlow and Adam, 1989). The precise mechanisms by which these stress factors reduce meristem length is not clear. It may be related to their effect in inhibiting cell division (Powell *et al.*, 1986a, b; Thomas, 1992), although Ivanov (1981) showed that the suppression of cell division per se by using a variety of anti-mitotic drugs did not affect the transition of cells to elongation. Ivanov suggested that in normal roots, the cessation of division and the beginning of elongation regulated by different mechanisms.

NaCl reduced meristem length, the cultivar x NaCl concen-trations interaction was not significant for this character and thus meristem length was not a discriminated character between the species in term of their NaCl tolerance.

Distance of the First Root Hair

There were marked differences between the cultivars in the position of the first root hair in relation to the root tip in the control (0 NaCl). For example in *T. aestivum* (cv. Chinese Spring root hairs did not form until a distance of approximately 4700 µm proximal to the root cap boundary was reached.

This implies that the onset of cell differentiation (as measured by root hair formation) may have different positional controls in the cultivars. However, no other measures of cell differentiation e.g. position of first xylogenesis were made in this investigation. However, Powell *et al*. (1988) have shown that an essentially similar pattern of change in relative position of the most distal root hair and the most distal xylem element occurs in the root of *F. rubra* exposed to zinc. Increasing NaCl concentration in culture solution resulted in the most distal root hair being formed progressively closer to the root tip in the species/cultivars. There was nearly 2-fold shortening of the distance to the most distal root hair over the range of NaCl concentration used (0-60 mM) in Chinese Spring. The lowest reduction (2-fold) occurred in rye. There was an indication that the cultivars under study were differentially affected by NaCl-induced reduction.

The generally accepted idea of root growth is that cells in files act independently of their neighbours (Webster and MacLeod, 1980; Allan and Trewavas 1986; Barlow 1984). Transition points for cell formation, cell transition to elongation and finally termination of growth leading to maturation are believed to operate within cell files (Ivanov, 1973). Rost and Baum (1988) demonstrated that these transition points were variable and depended on growth conditions. For example, they found that root manipulation, particularly treatments that inhibited the root growth rate, resulted in xylem being formed close to the root tip than in control plants. This effect has now been confirmed in other studies where root growth is inhibited by toxic metals such as zinc (Powell *et al*., 1988) and Mn (Thomas, 1992).

The present study indicates that NaCl treatment can similarly alter these transition points leading to precocious root hair formation. The fact that contrasting cultivars differently affected by NaCl in respect of this character may make it suitable as a character for screening genotypes at the seedling stage for variability in NaCl tolerance.

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