http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 1 (1): 1-4, 1998

Effects of NaCl on Meristem Size and Proximity of Root Hairs to the Root Tips in *Triticum aestivum* (cvs. Lyallpur-73, Pak-81 and Lu-26-S)

M. Hanif[♪] and M.S. Davies
Department of Pure And Applied Biology, University of Wales College of Cardiff-U.K.

Abstract
Seedings of *Triticum aestivum* cvs. Lyallpur-73, Pak-81 and Lu-26-S were grown in solution culture at varying concentration of NaCl for 8 days. Increasing concentrations of NaCl reduced the size of apical meristem and caused the most distal root hair to form closer to the root tip in all the cultivars. Although the position of the first root hair from the root cap boundary was reduced by salinity, yet there were comparatively little differences between these Pakistani Wheat cultivars in response to NaCl, thus in these studies the relative positions of root hairs is not a good discriminating character for assessing the salt tolerance.

Keywords: *Triticum aestivum*, cultivar, NaCl, Root hair, Root cap

Introduction

An inhibitory effect of toxic metals such as zine on root growth is well documented (Woolhouse, 1983) indeed comparisons of root growth in solutions containing various concentrations of metals with that in control solutions is a long-established method for determining the metal tolerance of plants (Wilkins, 1978) and both interspecific and intraspecific variation in zinc tolerance has been demonstrated. There is however, very little published information on the physiological basis of the inhibition of root growth by zinc.

Considerable work have been done on different aspects of salinity yet limited information is available on specific affects of varying salt concentration on plant physiological processes. Root growth which is an integrated process involving cell division, cell extension and cell differentiation, in particular, we know little about the effects of salinity in the root meristem.

As an understanding of physiology of the salt tolerance of plants is important for an effective approach to the salinity problems. To understand the mechanisms of salt tolerance control of Pakistani indigenous cultivars would be extremely valuable to overcome the salinity problem in Pakistan, by the development of salt resistance crops. Considerable work has already been done on the chosen Pakistani cultivars, instead of selecting altogether new cultivars, it was considered more useful to investigate these available Pakistani wheat cultivars. Furthermore, the wide genotypic variations between salt tolerant and salt sensitive cultivars, in respect to a number of pertinent physiological and biochemical parameters, have drawn attention to gain insight of the mechanism of salt tolerance by provision of interspecific variability for salt tolerance. Here some effects of NaCl on the size of meristem and relative positions of the first visible signs of the formation of roots hairs are reported.

Materials and Methods

The wheat seeds were obtained from Punjab Agriculture Research Institute Faisalabad, Pakistan. Seeds of uniform size of Pakistani wheat cultivars *Triticum aestivum* cvs. Lyallpur-73, Pak-81 and Lu-26-S (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 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 hydrolyzed 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 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. This method was used by Powell *et al.* (1988). The measurements cannot be definitive measures of the internal extent of the meristem, but they may provide an approximation for comparative screening of the

[♪]Present Address: Government College, Faisalabad, Pakistan

effects of externally supplied NaCl in cultivars (Powell *et at*., 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.

Results

Meristem length

Although the NaCl concentration and the cultivar terms in the analysis of variance were highly significant ($P < 0.001$), the cultivar x NaCl concentration interaction was non significant ($P > 0.05$).

The length of the root apical meristem in wheat cultivars was markedly reduced by increasing NaCl concentration (Fig. 1). The meristem lengh of *T. aestivum* cv. Lyallpur-73 was greater, followed by Lu-26-s while Pak-81 had the shortest meristem length. The meristem length of cultivars Lyallpur-73, Pak-81 and Lu-26-s were 283.3, 195.0 and 256.0 (µm) respectively in the control (0 mM NaCl), treatment. Cultivars showed a similar response to NaCl, the meristem length of the wheat cultivars i.e., Lyallpur 73, Pak 81 and Lu-26-s at 60 mM NaCl concentration was 60.6%, 62.1% and 59.2% of the control meristem length respectively.

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

Although the NaCl term was highly significant ($P < 0.001$) as was cultivar term ($P < 0.001$). However, the cultivar x NaCl concentration interaction was not significant ($P > 0.05$). The wheat cultivars (Lyallpur 73, Pak-81 and Lu-26-s) formed their first root hair much closer to the root top (570-650µm). The pattern of decline in the distance of the first root hair from the root cap boundary in these cultivars was similar to that of root meristem length (Fig. 2). The distance to the first root hair declined linearly with increasing NaCl concentration in all three cultivars. The distance to the first root hair was 661.6, 568.3, 651.0 µm in the cultivrs Lyallpur 73, Pak 81 and Lu-26-s, respectively in the control treatment (0 mM NaCl). The distance to the first root hair in Lyallpur 73 was 89.2 % of the control at 15 mM NaCl; this declined to 73.1 % at 30 mM NaCl and a further decline to 5939 % occurred at 45 mM culminating in the distance to the first root hair being 52.9 % of the control distance at 60 mM NaCl. The pattern of decline was similar in all these cultivars.

Discussion

Increasing NaCl concentration markedly reduced the length of root apical meristem in all the cultivars under study. There were also differences between the cultivars of wheat in the length of root apical meristem in the control (0 NaCl). Lu-26-s cultivar has more meristem size than Pak-81 while Lyallpur 73 has more meristem length than Lu-26-s in the control (0 NaCl). The shortening of the apical meristem which was brought about by NaCl in all the cultivars indicated that NaCl changed the balance that normally existed 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 was due to a rapid increase in the rate of cell growth. Barlow (1976) suggested that the derivatives of initial of founder cells in a root meristem had 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 indicates that NaCl reduces 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. lvanov (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) indicated that the Zn-induced shortening of the root apical meristem was evident within twelve hours after transfer of roots of S59 to Zn. This was extremely rapid response to the external environment.

Thomas (1992) observed that treatment with manganese reduced meristem length in roots of *Epilobium hirsutum* and *Chamerion angustifolium* and that the length of apical meristem of manganese treated roots progressively 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 occurred following alleviation of cold stress. 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 reduced meristem length was 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 were regulated by different mechanisms. However, although NaCl reduced meristem length, the cultivar x NaCl concentration 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 Lyallpur 73 root hairs did not form until a distance of approximately 661.6 µm proximal to the root cap boundary was reached. While in Pak 81 it was at 568.3 µm and in cultivar Lu-26-s it was at a distance of 651 µm.

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) had shown that an essentially similar pattern of change in the 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 concentrations in the culture solution resulted in the most distal root hair being formed progressively closer to the root tip in all the cultivars. There was a indication that the Pakistani wheat cultivar were differentially sensitive to the extent of this NaCl-induced reduction. For example, the NaCl tolerant cultivar Lyallpur 73 showed 2-fold reduction over the range 0-60 mM NaCl while the sensitive cultivar Pak 81 showed a 3-fold reduction. Data indicated that NaCl as well as perturbing root extension, perturbs the normal positional controls of cell elongation and differentiation, leading to precocious differentiation.

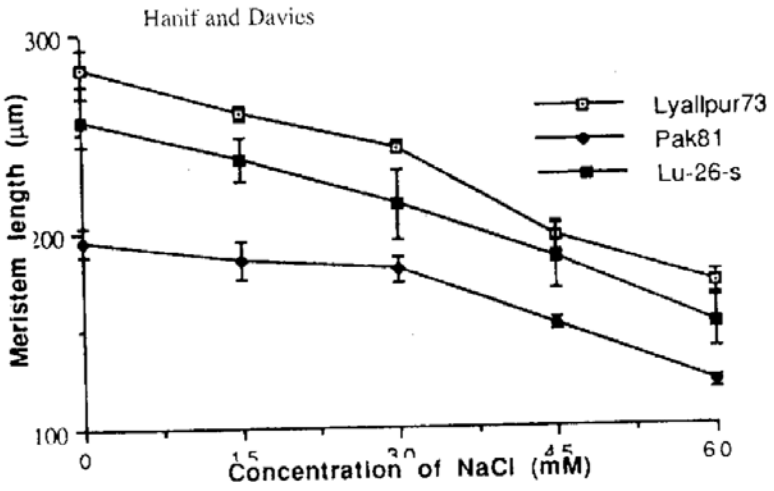

Fig. 1: The Meristem length (µm) in seedlings of *Triticum aestivum* cvs. Lyallpur 73, Pak 81 and LU-26-S on experimental day 8, when grown in solutions containing 0, 15, 30, 45 and 60 mM NaCl supplied in a background of 0.1 strength Rorison's nutrient solution. The vertical bars represent±SE

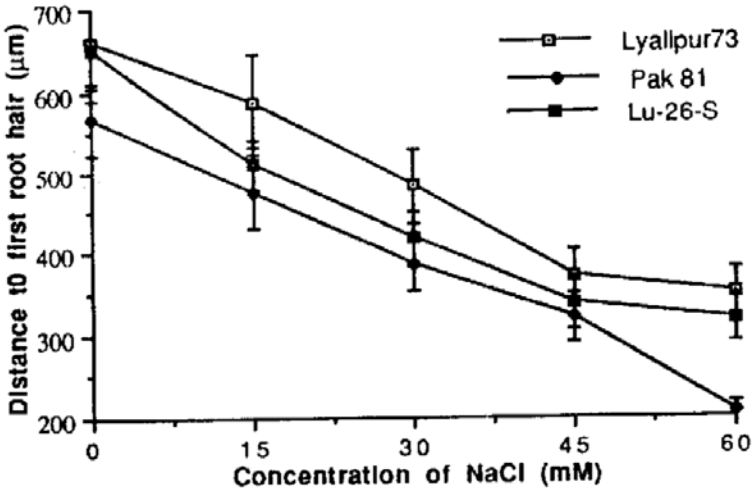

Fig. 2: The distance (µm) from the root cap boundary to the first root hair in seedlings of *Triticum aestivum* cvs. Lyallpur 73, Pak 81 and LU-26-S on experimental day 8, when grown in solutions containing 0, 15, 30, 45 and 60 mM NaCl supplied in a background of 0.1 strength Rorison's nutrient solution. The vertical bars represent±SE

The generally accepted idea of root growth in 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. 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.

Although the position of the first root hair from the root cap

boundary was reduced by salinity, yet there were comparatively little differences between these Pakistani Wheat cultivars in response to NaCl, thus in these studies the relative positions of root hairs is not a reliable selection character for assessing the salt tolerance.

References

Allan, E.F. and A. Trewavas, 1986. Tissue-dependent heterogeneity of cell growth in the root apex of *Pisum sativum*. Bot. Gazette, 147: 258-269.

Barlow, P.W. and E.L. Rathfelder, 1985. Cell division and regeneration in primary root meristems of *Zea mays* recovering from cold treatment. Environ. Exp. Bot., 25: 303-314.

Barlow, P.W. and J.S. Adam, 1989. The response of the primary root meristem of *Zea mays* L. to various periods of cold. J. Exp. Bot., 40: 81-88.

Barlow, P.W., 1976. Towards an understanding of the behaviour of root meristems. J. Theoret. Biol., 57: 433-451.

Barlow, P.W., 1984. Positional Controls in Root Development. In: Positional Controls in Plant Development, Barlow, P.W. and D.J. Carr (Eds.). Cambridge University Press, Cambridge, UK., ISBN-13: 9780521254069, pp: 281-318.

Davies, M.S., D. Francis and J.D. Thomas, 1991. Rapidity of cellular changes induced by zinc in a zinc tolerant and non-tolerant cultivar of *Festuca rubra* L. New Phytol., 117: 103-108.

Gonzalez-Fernandez, A., J.F. Lopez-Saez and G. Gimenez-Martin, 1966. Duration of the division cycle in binucleate and mononucleate cells. Exp. Cell Res., 43: 255-267.

Ivanov, V.B., 1973. Growth and Reproduction of Cells in Rots. In: Physiology of Roots, Brucheva, N.Y.O. (Ed.). U.N.I.T.I. Publishers, Moscow, Russia, pp: 1-40.

Ivanov, V.B., 1981. Cellular basis of root growth. Soviet Scient. Rev., 2: 365-392.

Powell, M.J., M.S. Davies and D. Francis, 1986a. The influence of zinc on the cell cycle in the root meristem of a zinc-tolerant and a non-tolerant cultivar of *Festuca rubra* L. New Phytol., 102: 419-428.

Powell, M.J., M.S. Davies and D. Francis, 1986b. Effects of zinc on cell, nuclear and nucleolar size and on RNA and protein content in the root meristem of a zinc-tolerant and a non-tolerant cultivar of *Festuca rubra* L. New Phytol., 104: 671-679.

Powell, M.J., M.S. Davies and D. Francis, 1988. Effects of zinc on meristem size and proximity of root hairs and xylem elements to the root tip in a zinc-tolerant and a non-tolerant cultivar of *Festuca rubra* L. Ann. Bot., 61: 723-726

Rost, T.L. and S. Baum, 1988. On the correlation of primary root length, meristem size and protoxylem tracheary element position in pea seedlings. Am. J. Bot., 75: 414-424.

Thomas, H.C., 1992. Effects of manganese and phosphorus on cellular aspects of root growth in *Contrasting* species. Ph.D. Thesis, University of Wales, UK.

Webster, P.L. and R.D. MacLeod, 1980. Characteristics of root apical meristem cell population kinetics: A review of analyses and concepts. Environ. Exp. Bot., 20: 335-358.

Wilkins, D.A., 1978. The measurement of tolerance to edaphic factors by means of root growth. New Phytol., 80: 623-633.

Woolhouse, H.W., 1983. Toxicity and Tolerance in the Responses of Plants to Metals. In: Physiological Plant Ecology III: Responses to the Chemical and Biological Environment (Encyclopedia of Plant Physiology, New Series Volume 12, Part C), Lange, O.L., P.S. Nobel, C.B. Osmond and H. Ziegler (Eds.). Chapter 7, Springer, Berlin, Germany, ISBN: 978-3-642-68155-4, pp: 245-300.