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## Effects of NaCl Induced Stress on the Mitotic Cell Cycle and Growth Fraction in the Root Meristems of Root Tip Cells in Two Lines of Barley

M. Hanif and M.S. Davies

School of Pure and Applied Biology, University of Wales, Cardiff, UK

### Abstract

The seeds of *Hordeum sativum* lines B-88079 and B-jou87 were grown in aerated 0.1 Rorison nutrient solution for 72 hours. Then the kinetics of cell cycle in the root meristem were determined by a pulse labelling experiment with low specific activity tritiated thymidine following a 70 hours exposures to three concentrations of NaCl (0, 50 and 100 mM NaCl). *Hordeum sativum* lines B-jou87 had a longer cell cycle duration than that of line B-88079. The mitotic cell cycle in line B-88079 remained unaffected at 50 and 100 mM NaCl concentrations but that of B-jou87 was decreased. The growth fraction (refers to percentage of meristematic cells which are actively cycling as opposed to these that are not cycling in the meristem) was not determined due to non labelling of any cells in continuous labelling experiment.

### Introduction

Plants owe most of their increment to vegetative body due to mitotic activities within their meristems whether it may be simple proliferation of cells or differentiation of various tissues/organs mitosis plays her pivot role. The importance of the measurement of the rate and extent of division in a root meristem is important as it is the determinant of growth trends of the other vegetative and reproductive parts and any change in the cell cycle of the root meristem may affect the whole life pattern of the plant.

Various stages of cell cycle had already been identified which includes S (DNA synthesis), G1 (presynthetic interphase stage), G2 (postsynthetic interphase) and M (mitosis phases). There are different formulae/methods in the literature to measure various aspects/stages of cell cycle (Quastler and Sherman, 1959; Scadeng and MacLeod, 1976).

The cell cycle can be affected profoundly by environmental factors such as temperature, light and toxicity of metal (e.g. Van't Hof and Ying, 1964; Francis and Barlow, 1988) and salinity (Nabil, 1996; Hanif and Davies, 1998). Often some strains or varieties perform better than others in coping with stress whether induced artificially or naturally. Predictably, the cell cycle lengthens in response to stress resulting in lesser number of cells under stress conditions.

The aims of the present work is to determine the effects of varying NaCl concentrations in the nutrient solution on the length of mitotic cell cycle and its component phases and growth fraction in the root meristems of two lines of barley which will unveil their relative growth performance under NaCl induced stress.

### Materials and Methods

Seeds of two Pakistani barley lines *Hordeum sativum* (2x = 2x = 14) B-88079 and B-jou87 obtained from Ayub Agriculture Research Institute, Faisalabad - Pakistan were sown under the standard conditions (Hanif and Davies,

1998) in a growth room at a constant temperature of  $20 \pm 0.5^\circ\text{C}$  with illumination provided by white fluorescent tubes at a photon fluence rate of  $153 (\text{mol m}^{-2} \text{s}^{-1})$  for 16 hours per day. Seeds were imbibed for 24 hours in sandwich boxes containing  $1 \text{ dm}^3$  0.1 strength Rorison's nutrient solution before sowing. Ungerminated seeds were then removed and 15 seeds per cultivar were transferred to each of 6 rafts per sandwich box and were allowed to grow for a further 48 hours in Rorison's nutrient solution. The rafts were then transferred into the various NaCl treatment solutions. The NaCl treatments for these barley were 0, 50 and 100 mM NaCl supplied in background of 0.1 strength Rorison's nutrient solution. The different NaCl treatments were selected on sensitivity of roots of these lines to NaCl. There were two replicate sandwich boxes with six rafts per box per line NaCl concentration.

After 70 hr exposure to the NaCl treatments, the seedlings were transferred into an aqueous solution of radioactive thymidine  $^3\text{H-TdR}$  (concentration  $37 \text{ kBq cm}^{-3}$ , specific activity  $185 \text{ GBq m mol}^{-1}$ ). After 1 hour the rafts were transferred to "cold" -non radioactive thymidine (concentration  $10^5 \text{ M}$ ) for 1 hr. and then rinsed in deionised water before putting back into the original NaCl treatment. All the solutions were equilibrated to the growth room temperature before transfer. Five seedlings per cultivar per NaCl treatment were sampled at each time point. The first sample was taken 1 hr. after start of the pulse label with  $^3\text{H-TdR}$  and then at two hourly intervals thereafter, up to 36 hr. Samples were fixed in 3:1 v/v ethanol: glacial acetic acid and stored at  $4^\circ\text{C}$ .

Five roots at each sampling time, for each cultivar per treatment were Feulgen stained and squash preparations were made of the apical root meristem as outlined previously (Hanif and Davies, 1998). Permanent autoradiographs were made at a room temperature of  $18^\circ\text{C}$ . Ilford K2 photographic emulsion was mixed with an equal volume of distilled water, a drop of glycerol was added and the mixture was maintained at  $40^\circ\text{C}$  in a water

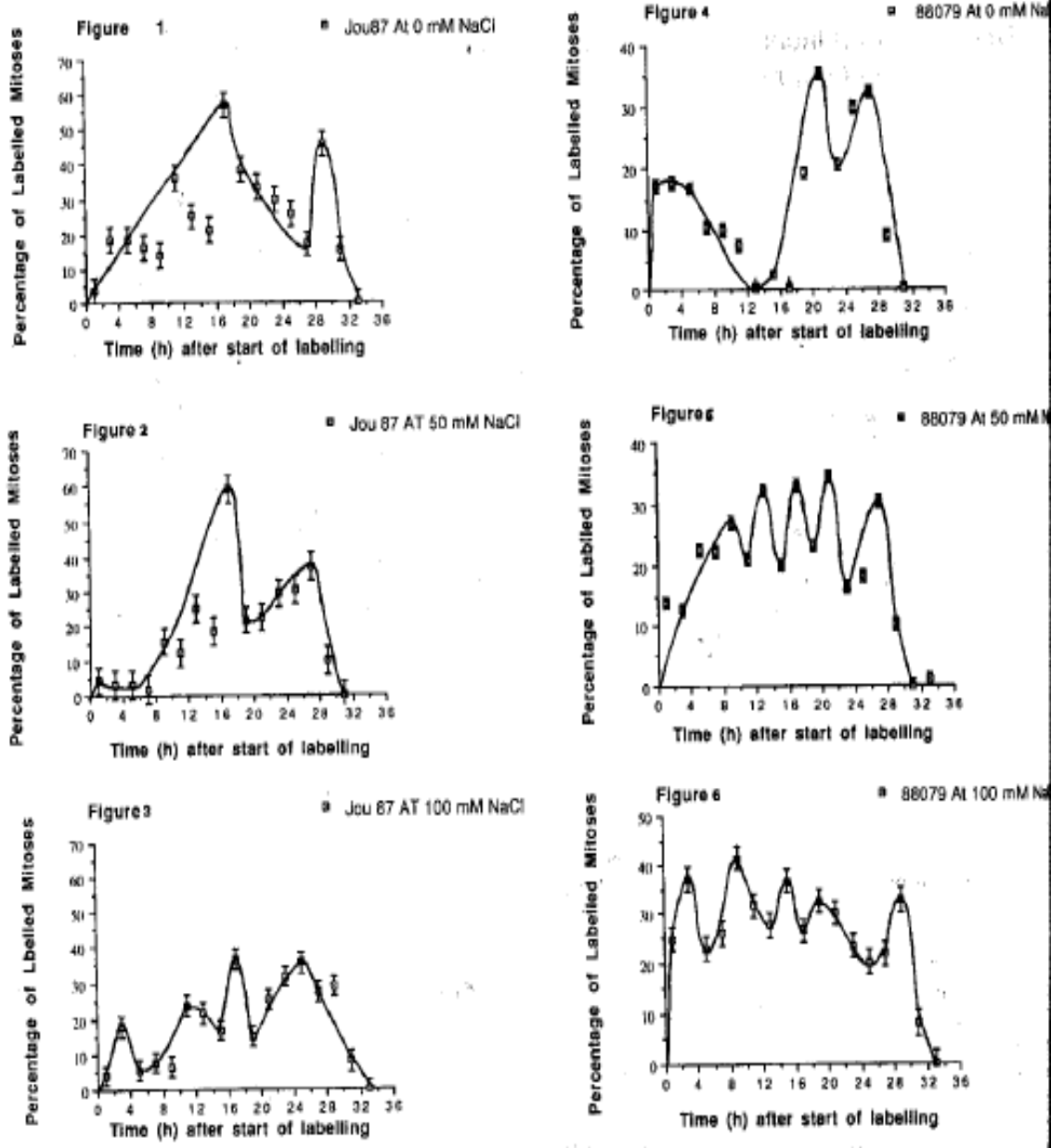


Fig. 1-6: The relationship between the percentage labelled mitoses ( $\pm$ SE) and time elapsed after the start of 1 hr puls label with 3bH-thymidine, in squash preparations of root meristems of *Hordeum sativum* lines B-88079 and B-jou8 7 following a 70 hr. exposure to solutions containing 0, 50 and 100 mM NaCl supplied in a background of 0.1 strength Rorison's nutrient solution

bath. Slides from each treatment were dipped in the emulsion to drain off under conditions of 40 percent relative humidity and at a room temperature of 25°C under Ilford F904 filters. The slides were stored in light-proof boxes in the presence of a desiccant (silica gel) in the dark for 14 days at 4°C.

Autoradiographs were developed in Ilford Phenisol develop for 6 mins precisely, rinsed for approximately 30 second in distilled water and fixed in Ilford Hypam fixer at 20°C for 6 mins and were then raised in distilled water. The slide were then dehydrated in an ethanol series (30, 50, 70, 80 and 2x100 percent for 5 mins at room temperature

**Hanif and Davies:** Cell cycle, *Hordeum sativum*, mitotic index, growth fraction, labelling, Labelling index.

Slides were then cleared in xylene for 10 mins and mounted in DPX. On each of the developed autoradiographs, the percentage of mitoses which were labelled was determined. One thousand nuclei per slide were scored for these PLM determinations. The mitotic index (M.I) and labelling index were also measured on each slide. Labelling index (L.I) is the percentage of cells which had incorporated  $^3\text{H-TdR}$  expressed as a percentage of total cells scored. The M.I was calculated as the percentage of cells in mitosis expressed as a percentage of total cells scored. For each time point the relationship between the percentage of labelled mitoses and time elapsed since the start of pulse labelling (up to 37 hr.) was plotted and the duration of the cell cycle and the component phases were calculated.

For growth fraction the above programme was repeated but after 70 hr. exposure to NaCl treatments, the seedlings were transferred to the same treatment solution described above but containing  $^3\text{H-TdR}$  (concentration  $9.25 \text{ kBq cm}^{-3}$ , specific activity  $74 \text{ GBq m mol}^{-1}$ ). The seedlings were harvested at the end of 26 hr. continuous exposure to the  $^3\text{H-TdR}$ , rinsed with water and were then fixed in 3:1 (v/v) ethanol:glacial acetic acid. Permanent Feulgen stained autoradiographs were made as described above. Surprisingly, there was a complete absence of labelled cells on all slides. In case the absence of labelling was due to problem with the autoradiographs, further autoradiographs were made after removal of the photographic emulsion (Bianchi *et al.*, 1964). However, again no apparent incorporation of label into any of the cells of the root meristems occurred.

## Results and Discussion

The plots of percentage labelled mitoses against time elapsed since the start of labelling are shown in Fig. 1-6. The PLM curves in *Hordeum* lines B-88079 and B-jou87 were a typical. There were multiple peaks with the second peak often being larger than the first. Hence only the peak to peak distance (TC) were calculated for these lines. In the diploid *Hordeum* the mitotic cell cycle was much shorter than hexaploid wheat cultivars Lyallpur 73 and Lu-26-S (Hanif and Davies, 1998a). The duration of cell cycle in the meristem of two lines of *Hordeum sativum* measured as the interval between the successive peaks of the PLM plots in the control (0 NaCl) treatment were 6 hours (21-27 hr.) in B-88079 and 12 hr. (17-29 hr.) in B-jou87, (Fig 1-6). However the cell cycle duration of line B-88079 was not affected at 50 mM i.e., 6 hr. but that of line B-jou87 was decreased to 10 hr. respectively. The cell cycle duration was also unaffected at 100 mM NaCl in B-88079 but that of line B-jou87 was decreased to 8 hr. (17-25 hr.) duration at 100 mM NaCl respectively. The PLM curves of two barley cultivars were a typical and thus no attempt was made to determine the component phases of cell cycle. The effect of NaCl on the duration of cell cycle were not consistent with its effect on root growth and mitotic index (Hanif and Davies, 1998b) in these cultivars but cell cycle

remain unaffected in B-88079 but shorten the cycle duration of B-jou87.

As there was not labelling of any cells of the cultivars investigated, perhaps a prolonged exposure of roots to a radioactive thymidine in continuous labelling experiment was more liable to perturb normal cell division activity than a short exposure used in pulse labelling experiments although no firm evidence for this exists. However the complete absence of labelled cells argues against a minor perturbation. It is more likely that, due to unknown factors, no cells were cycling in the root meristem of the lines. The growth fraction can be calculated by dividing the cell cycle duration by the respective cell doubling time estimates expressing as a percentage value. The cell doubling times of the lines of barley were not determined and hence growth fraction was not calculated. Another method of determining growth fraction was from the PLM curve (Clowes, 1976) was not attempted here since it was felt that curves were "a typical" and would produce spurious results.

The finding is in contrast to other work of the effects of stress on cell division in roots. For example, the zinc induced reduction in root length in contrasting cultivars of *Festuca rubra* (Powell *et al.*, 1986) was accompanied by corresponding increases in the duration of mitotic cycle in the root meristem, through an increase in the duration of G1. Thomas (1992) found that inhibition of root growth by manganese was accompanied by corresponding increases in the duration of cell cycle mediated largely through an increase in G1. Creber *et al.* (1993) had also demonstrated that low temperature increases in cell cycle duration in *Dactylis glomerata* were mediated mainly through an increase in G1. It is possible that NaCl treatment reduced root growth not primarily through an effect on the cell cycle but by reducing proportion of meristematic cells which were cycling (Growth fraction).

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## References

- Bianchi, N., A. Lima-de-Faria and H. Jaworska, 1964. A technique for removing silver grains and gelatin from tritium autoradiographs of human chromosomes. *Hereditas*, 51: 207-211.
- Clowes, F.A.L., 1976. Estimation of growth fractions in meristems of *Zea mays* L. *Ann. Bot.*, 40: 933-938.
- Creber, H.M.C., M.S. Davies and D. Francis, 1993. Effects of temperature on cell division in root meristems of natural populations of *Dactylis glomerata* of contrasting latitudinal origins. *Environ. Exp. Bot.*, 33: 433-442.
- Francis, D. and P.W. Barlow, 1988. Temperature and the Cell Cycle. In: *Plants and Temperature*, Long, S.P. and F.I. Woodward (Eds.). Society of Experimental Biology, Cambridge, UK., ISBN-13: 9780948601200, pp: 181-201.

**Hanif and Davies:** Cell cycle, *Hordeum sativum*, mitotic index, growth fraction, labelling, Labelling index.

- Hanif, M. and M.S. Davies, 1998. Effects of NaCl on meristem size and proximity of root hairs to the root tips in *Secale cereale* (cv. K2) and *Triticum aestivum* (cv. Chinese spring). Pak. J. Biol. Sci., 1: 15-18.
- Hanif, M. and M.S. Davies, 1998a. Mitotic activity in the root apical meristems of *Secale cereale* (cv. K2) and *Triticum aestivum* (cvs. Chinese spring, Lyallpur 73, Pak 81, Lu-26-S and Sandal) under elevated NaCl treatment. Pak. J. Biol. Sci., 1: 163-166.
- Hanif, M. and M.S. Davies, 1998b. The influence of NaCl on the Cell cycle and growth fraction in the root meristems of *Triticum aestivum* cvs. Lyallpur 73 and Lu-26-S. Pak. J. Biol. Sci., 1: 252-255.
- Nabil, M., 1996. Effects of salt on the cellular aspects of root growth in contrasting rice cultivars. Ph.D. Thesis, University of Wales, Cardiff, UK.
- Powell, M.J., M.S. Davies and D. Francis, 1986. 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.
- Quastler, H. and F.G. Sherman, 1959. Cell population kinetics in the intestinal epithelium of the mouse. Exp. Cell Res., 17: 420-438.
- Scadeng, D.W.F. and R.D. MacLeod, 1976. The effect of sucrose concentration on cell proliferation and quiescence in the apical meristem of excised roots of *Pisum sativum* L. Ann. Bot., 40: 947-955.
- Thomas, H.C., 1992. Effect of manganese and phosphorus on cellular aspects of root growth in contrasting species. Ph.D. Thesis, University of Wales, Cardiff, UK.
- Van't Hof, J. and H.K. Ying, 1964. Relationship between the duration of the mitotic cycle, the rate of cell production and the rate of growth of *Pisum* roots at different temperatures. Cytologia, 29: 399-406.