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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

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Abstract
Eight day exposure to NaCl reduced the Mitotic indices in the root meristems of all the cultivars in aerated solution culture. There were large differences between the cuitivars in the control solutions. The relative frequency of prophase and metaphase in Mitotic cells decreased with increasing NaCl concentration while the relative frequency of anaphase and telophase increased. Mitotic index in the root meristem of Chinese spring was reduced to a greater extent than in K: cultivar.

Key words: *Triticum aestivum*, cultivar, Mitotic index, Root meristem

Introduction
The root meristem is a steady-state system which maintains its growth pattern over a period of time. The constant meristems size cell number, rate of cell production within the meristem and cell loss from the meristem are important features of such a system.

The information obtained by comparing the Mitotic indek (M.I.) being defined as the number of cells in mitosis expressed in percentage of total cells scored. An increase in the M.I. means either a relatively greater slowing down of mitotic phase as compared with the intermitotic phase, or a speeding up of the rate of progress through the intermitotic phase, whilst the mitotic phase stays constant or slows down. In other words, an increase in the M.I. value could be associated with either an increase or decrease in the rate of passage of cells thrbugh the mitotic cycle. Similarly a decrease in the M.I. can also be interpreted in two ways. Mitotic index counts are thus insufficient in themselves for determining changes in mitotic rate and should only be used in conjunction with one or other known factors, such as the rate of production of new cells, the time taken to pass through mitosis, or time spent in interphase.

It is known that continued root growth is dependent on the continuos production of new daughter cells in the root apical meristem. However comparatively little is known about the effects of NaCl on the mitotic rate in the root meristem. The aim is to investigate the effect of changes in the mitotic indices induced by varying concentrations of NaCl in root meristem of these cultivars.

Materials and Methods
The seeds of uniform size were sown on 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 or 0.1 strength Rorison's nutrient solution in plastic boxes ($210 \times 140 \times 80$ mm). The solution was continuously and gently aerated by bubbling air through diffusing stones using aquarium pumps. In general there were six rafts in each plastic box and ten seeds were sown on each raft. The experiments were carried out in a growth chamber a constant temperature of $20 \pm 0.5°C$ with illumination provided for 16 hours per day by white fluorescent tubes. Seedlings were harvested on day 8th and were immediately) fixed in 3:1 v/v ethanol:glacial acetic acid mixture and stored at 4°C.

Prior to the measurement of mitotic index and relative frequency of stages of mitosis, the roots were Feulgen-stained. The staining procedure was as follows: Roots were rinsed with distilled water to remove fixative for 2×5 min. Roots were hydrolysed in 5 M HCl for 25 min a 25°C. These were then rinsed with ice cold distilled water for 25 min and ice crystals were added to stop hydrolysis quickly. Roots were then stained for 2-3 hrs in Feulger reagents at 25°C. The roots were then transferred to 45 percent glacial acetic acid.

For determining the mitotic index the following procedure was undertaken:

Squash preparations were made of six Feulgen stained rool tips per cultivar per treatment. The apical region (2 mm) stained roots was cut off on a slide in a drop of 45,percent acetic acid. The meristem was then dissected using fine needles. A cover slip was placed on the slide and tapped gently to produce a monolayer of cells (squash preparation). The shape and size of cells were not distorted (Bansal and Davidson, 1978). The slides were then placed on dry ice and, when frozen, the cover slip was removed (Conger and Fairchild, 1953). Then slides were air dried overnight and passed through 45 percent glacial acetic acid solution. SO_2, water, alcohol, xylene and cover slips were mounted in DEX.

The mitotic index is the percentage of cells in the meristem which are in visible stage of mitosis (prophase, metaphase, anaphase or telophase) expressed in percentage of the total cells scored. The mitotic index were measured on a sample of 1000 cells in a series of random transects on each three slides per cultivar per treatment. Where mitotic cells were encountered, the stage of mitosis was noted and the percentage of mitotic cells in each visible stage was then recorded for each slide.

Results

***Secale cereale* (cv. K2) and *Triticum aestivum* (cv. Chinese Spring) (0-60 mM NaCl):** Increasing NaCl concentration had a significant negative effect on the mitotic index in both *S. cereale* cv. K2 and *T. aestivum* cv. Chinese spring (Fig. 1). The mitotic index of S. cereale (cv. K2 was generally less affected by NaCl than that of *T. aestivum* cv. Chinese spring).

The NaCl term in the analysis of the variance was highly significant ($p<0.001$) but the species term was not significant ($p>0.05$). The species x NaCl concentration interaction was not significant ($p>0.05$).

***Triticum aestivum* (cvs. Lyallpur 73, Pak 81 and Lu-26-S) (0-60 mM NaCl):** The NaCl term in the analysis of the variance was highly significant ($p<0.001$) but the species term was not ($p<0.05$). The species x NaCl concentration interaction was not significant ($p<0.05$).

Increasing NaCl concentration resulted in a marked reduction in the mitotic index in all the three cultivar (Fig. 2). *T. aestivum* cv. Lyallpur 73 had a higher mitotic index than the other two cultivars at all NaCl concentrations. However, the mitotic index of these cultivars was lower than that of *T. aestivum* cv. Chinese Spring. The mitotic indices of the cultivar Lyallpur 73 were 1.0 percent in the controlled treatment (0 mM NaCl) compared with 6.3 percent in wheat cv. Chinese Spring. This declined to 0.8 percent at 15 mM NaCl and a further decline to 0.7 percent occurred at 30 mM culminating in a mitotic index of 0.4 percent at 60 mM NaCl. The mitotic index of the cultivar Lu-26-S was proportionally less affected by increasing NaCl than Lyallpur 73 and Pak. 81; all the three cultivars had the same mitotic index at 60 mM NaCl.

***Triticum aestivum* (cv. Sandal) (0-60 mM NaCl):** There was a progressive decline in the mean mitotic index of the cultivar *Triticum aestivum* cv. Sandal with increasing NaC1 concentration (Fig. 3) The mitotic index of the cultivar was greater than the other cultivars (9.3% in the control treatment).

The NaCl term in the analysis of variance was significant ($p<0.05$).

Relative Frequency of Each Stage Of Mitosis

***Secale cereale* (cv. K2) and *Triticum aestivum* (cv. Chinese Spring) (0-60 mM NaCl):** In the control treatment, in both the species, prophase was the most frequent stage of mitosis (43%; *S. cereale* cv. K2, 71 %; *T. aestivum* cv. Chinese Spring) followed by metaphase (36% rye, 27% wheat). Increasing NaCl concentratior resulted in a decline in the relative frequency of prophase and metaphase and an increase in the relative frequency of anaphase and telophase, In *S. cereals* cv. K2 the frequency of prophase declined from 43 percent (0 mM NaCl) to 22 percent at 60 mM NaCl; anaphase frequency increased from 17 percent (0 mM NaCl) to 37% (60 mM) while telophase frequency increased from 4.3 percent (0 NaCl) to 26.3% at (60 mM NaCl). In *T. aestivum* cv. Chinese Spring similar dramatic NaCl-induced shifts in the frequency of various stages occurred: prophase frequency declined from 71 percent (0 mM NaCl) to 13.7 percent (60 mM NaCl) while the telophase frequency increased from 4.3 percent (0 NaCl) to 26.3 percent at (60 mM NaCl).

***Triticum aestivum* (cvs. Lyallpur 73, Pak 81, Lu-26-S) (0-60 mM NaCl):** In the control treatment, in the three Pakistani wheat cultivars, prophase was the most frequent stage of mitosis (47% in Lyallpur 73, 43% in Pak 81, 37% in Lu-26-S) followed by metaphase (37%; Lyallpur 73, 43%; Pak 81, 32%; Lu-26-S). Increasing NaCl concentration resulted in a decline in the relative frequency of prophase and metaphase and an increase in the relative frequency of anaphase and telophase. In Lyallpur 73 the frequency of prophase declined from 47 percent (0 mM NaCl) to 0 percent at 60 percent mM NaCl, anaphase frequency increased from 16% (0 NaCl) to 73 percent at 60 mM NaCl while telophase frequency from 0 percent (0 mM NaCl) to 28 percent at 60 mM NaCl. In cultivar Pak 81 similar dramatic NaCl induced shifts in the frequency of various stages occurred; prophase frequency declined from 46% (0 mM NaCl) to 0 percent (60 mM NaCl) while telophase frequency increased from 0 percent (0 mM NaCl) to 28% (60 mM NaCl).

In the cultivar Lu-26-5 similar NaCl-induced shifts in the frequency of various stages occurred; prophase frequency declined from 37% (0 mM NaCl) to 11% (60 mM NaCl) while the telophase frequency increased from 10% (0 mM NaCl) to 37% (60 mM NaCl).

***Triticum aestivum* (cv. Sandal) (0-60 mM NaCl):** In the control treatment, in *T. aestivum* cv. Sandal, prophase was the most frequent stage of mitosis (37%) followed by metaphase (15%). Increasing NaCl concentration resulted in a decline in the relative frequency of prophase and metaphase and an increase in the relative frequency of anaphase and telophase. The frequency of prophase dropped from 37 percent (0 mM NaCl) to 3.1 percent at 60 mM NaCl; anaphase frequency increased from 4.3% (0 mM NaCl) to 21.3 percent (60 mM NaCl) while telophase frequency increased from 0 per cent (0 mM NaCl) to 9 percent at 60 mM NaCl.

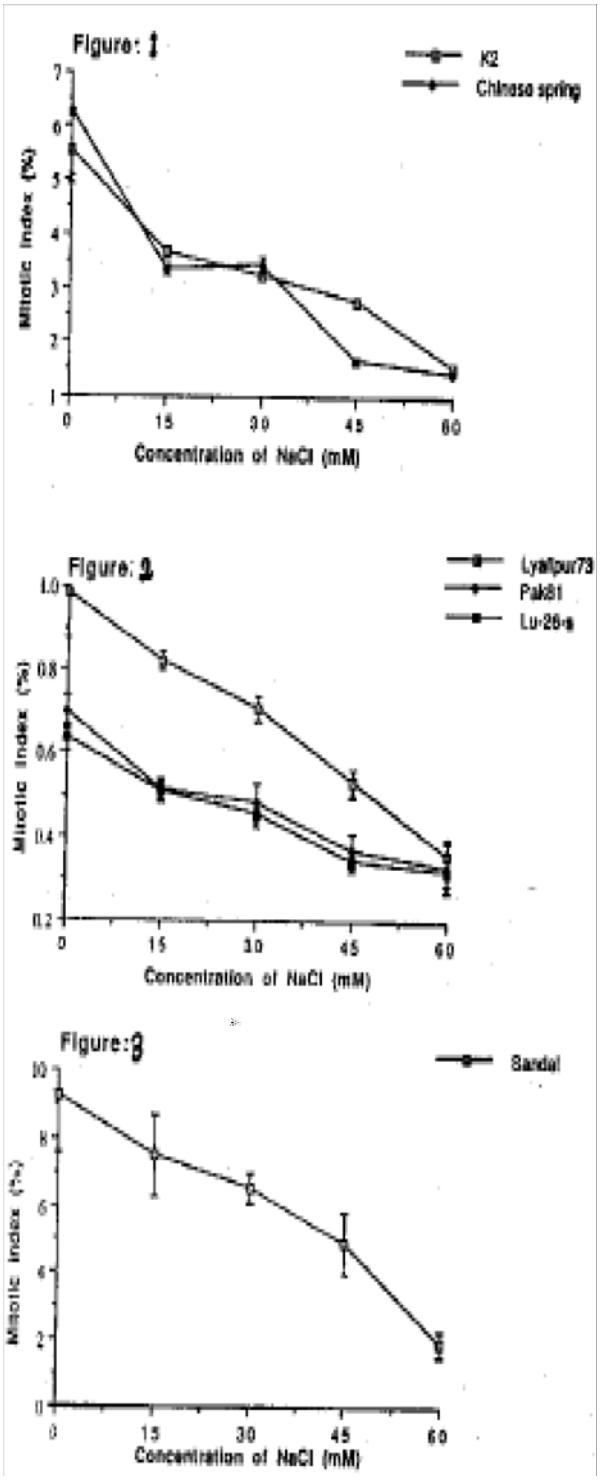

Fig. 1-3: The mitotic index in the root apical meristems of Secale cereale cv .K2, *Triticum aestivum* cvs. Chinese spring, Lyallpur 73, Pak 81, Lu-26-s and sandal on experimental day 8, when grown in solutions containing 0-60 mM NaCl supplied in a background of 0.1 strength Rorison's solution. The vertical bars represents ±SE

Discussion

Mitotic index in the root meristern was found to be very sensitive to NaCI. There were differences between the cultivars in mitotic index in the control (0 NaCI). Wheat cultivar Chinese.Spring had a mitotic index of 6.3% in the control solution; and Pakistani wheat cultivars ranged from 1.0% to 0.6% in the control. In contrast, the Pakistani wheat cultivar Sandal has a MI of >9 percent. Thus, as well as having much smaller root meristems than Chinese Spring and Sandal these three wheat cultivars (Lyallpur 73, Pak 81 and Lu-26-S) had a much smaller proportion of meristematic cells in visible stage of mitosis. Nevertheless, despite the smaller meristem length and mitotic index in these three cultivars in the control solution , the length of the longest root in this treatment was approximately the same in all the wheat cultivars.

Despite the differences in absolute values of mitotic index in the control treatment, the cultivars showed a near linear decline in MI range increasing NaCI concentration. This NaCI induced decline MI parallels that has been reported for toxic metals such as zinc (Swieboda, 1976; Kocik *et al*., 1982; Powell *et al*., 1986), aluminium (Clarkson, 1965; Morimura *et al*., 1978; Horst *et al*., 1983), nickel (Robertson and Meakin, 1980) and manganeSe (Thomas, 1992).

However, in many of the above studies the toxic metal has lesser effect in reducing mitotic index in tolerant populations than in the susceptibles e.g. Zinc resulted in less inhibitor of mitotic index in the zinc-tolerant population of *F. rubra* (Merlin) than in the zinc sensitive population (S 59) (Powell *et al*., 1986).

In the present 'study, however, the MI was reduced proportionally more by increased NaCI concentration in Lyallpur 73 than in Lu-26-S and Pak 81.

Treatment with NaCI also produced a marked shift in the relative frequency of various stages of mitosis in mitotic cells. In general prophase was the most frequent stage found in the, control treatment whereas increasing NaCI concentration resulted in a relative decrease in the frequency of prophase and metaphase and an increase in the proportion of mitotic cells in anaphase and telophase-this shift was such that there was a complete absence of prophase and metaphase in mitotic cells of some cultivars at 60 mM NaCI (Lyallpur 73). However, the MI index also showed a marked decline at these concentrations and given also the very low MI in the control solution (<1%) in these Pakistani wheat cultivars. The proportional calculations at high NaCI concentrations are based upon very few mitotic cells and must therefore be treated with caution. Nevertheless the trend towards a declining frequency of prophase/metaphase and increase of anaphase/telophase with increasing salt 'occurs both in *Secale* and a range of *Triticum* cultivars and is obviously worthy to further investigations. It is possible that at higher NaCI concentration the time cell spend in anaphase and

telophase is proportionally longer than the duration of prophase and metaphase and thus these later stages are represented more frequently in "instantaneous sampling". These data are in contrast to the effect of NaCl on mitotic index in the salt tolerant cultivar of *Festuca rubra* (Hawk) reported by Davies (1991). In Hawk, while root length was reduced by increasing NaCl concentration (0-150 mM) the mitotic index in the root meristerm increased. This doubling of MI over the range 0-50 mM NaCl was associated with a doubling of proportion of cells in metaphase and a corresponding decline in the proportion of mitotic cells in other stages of mitosis. Davies (1991) postulated that the NaCl-induced increase in MI was not a reflection of increased mitotic activity but may have resulted from either the duration of mitosis increasing proportionally more than other phases of the mitotic cell cycle or may have been due to cells arresting partially or totally, in metaphase, as would occur following treatment with an inhibit of the mitotic spindle such as colchicine. In our experiment, however, salt resulted in a marked decline in mitotic index and thus is unlikely to be acting as a spindle inhibitor in these wheat and rye cultivars.

The above discussion points to the dangers inherent in using mitotic index as the sole measure of cell division activity. Earlier studies (Powell *et al*., 1986; Thomas 1992) have shown that a stress-induced decline in the mitotic index is associated with an increase in cell doubling time and an increase in the duration of the cell cycle. However, such studies are rare and most workers have used MI alone as a measure of cell division. Lyndon (1967) and Clowes (1981) have stressed the dangers in this approach. Only when it is established that the duration of mitosis remains constant and that increase in cell cycle duration occurs via lengthening of other phases, can mitotic index be related to cell doubling time. If this scenario were true, a spurious increase in MI would occur if cells arrest to varying degree in particular stages of mitosis. Thus it is essential that measures are made to the effect of the stress factor, (in this case NaCl) on the duration of the mitotic cell cycle and its component phases by more critical techniques.

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