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A Comparison Of The Effect Of NaCl On The Cell Doubling Time Of The Root Apical Meristem In *Triticum aestivum* (cvs. Lyallpur 73, Pak 81 and Lu-26-S)

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Abstract
The Pakistani wheat cultivars under investigation exhibited different cell doubling times not only in the control but also differed in response to increasing the external NaCl concentration. The rates of metaphase accumulation of the cultivars were very slow and thus cell doubling times were very long. This may be a reflection of very slow mitotic activity in root meristems.

Key words: Root meristem, Cultivar, Cell doubling time, Metaphase

Introduction
The cell doubling time (CDT) is defined as the time taken for a population of cells to double. The alkaloid drug colchicine has been used by many researchers to determine the CDT (Lyndon, 1970). In a meristem which is in a steady rate and in which divisions are asynchronous, the rate of entry of cells into metaphaie is equal to the rate of cell division and thus the rate of artificially induced accumulation of metaphase is proportional to the rate of cell division.

Colchicine disrupts the formation of the mitotic spindle and hence chromosomes are arrested when they reach metaphase (Barber and Callan, 1943) and the proportion of cells in metaphase increases with increase in time of exposure to colchicine. The kinetics of movement of cells from late interphase to metaphase following treatment with colchicine is used to estimate the cell doubling time (Evans, *et al*., 1957; Lyndon, 1970; Webster and MacLeod, 1980). However, colchicine is a highly cytotoxic drug and may itself perturb the passage of cell through the cell cycle (Webster and MacLeod, 1980).

Colchicine arrest method has been used by several workers to investigate the effect of environmental stress factors on cell doubling time in root meristems, e.g. Zinc (Powell *et al*., 1986) and manganese (Thomas, 1992).

In this experiment the objectives were to determine the effects of varying NaCl concentration on cell doubling time in three cultivars of Pakistani wheat, using colchicine-induced metaphase accumulation method.

Materials and Methods
Seeds of *Triticum aestivum* cultivars of Lyallpur 73, Pak 81 and Lu-26-S (2n = 6x =42) were obtained from Punjab Agriculture Research Institute, Faisalabad-Pakistan. Sixteen seeds of uniform size per raft of these cultivars were grown under standard conditions (Hanif and Davies, 1998) in boxes containing 1 dm^3 of 0.1 strength Rorison's nutrient solution. The seedlings were grown in a growth room at constant temperature of 20 ± 1°C with, illumination provided by white fluorescent tubes at a rate of 200 μ mol m-2 s-1 for 16 hours per day.

After severity two hrs. of sowing, ungermirrated seeds were removed and the rafts were transferred to the NaCl treatment solutions (0, 30 and 70 mM NaCl) supplied in a background of 0.1 strength Rorison's nutrient solution for further 70 hrs. Rafts were transferred to the same NaCl treatment solution containing 0.1% w/v colchicine. Seedlings were sampled at 0,2,3,4,5,6,7,8,9 or 10 hours after transfer to the colchicine solutions. Six seedlings per cultivars were sampled at each time point and fixed in 3:1 v/v ethanol alcohol: glacial acetic acid and stored at 4°C. Permanent Feulgen-stained squash preparations of root meristem were made for three roots per cultivars per treatment at each sampling point (t0 to t10) (Powell *et al*., 1986).

Squash preparations were made following Feuigen stained root tips per cultivar per treatment was as follows:

The apical region (2 mm) of stained roots was cut off on a slide in a drop of 45% 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 then passed through 45% glacial acetic acid, SO_2 water, alcohol, xylene and then cover slips were mounted in DPX. In the t0 to t10 samples, the mitotic index, frequency of prophases, metaphases, anaphases and telophases were recorded (Powell *et al*., 1986).

Results
The pattern of accumulation of percentage of cells in metaphase over time characteristically had three phases. First from tO and t2 following exposure, there was only a

slight rise (Fig. 1-3), following this there was a steady rise in metaphase frequency although this departed from linearity in some cultivars/treatments and finally the frequency of metaphase plateaued. The percentage of metaphase was regressed upon time following exposure to coichicine and linear regression coefficient was calculated. The regression coefficient was calculated over the most linear region of the plot of % metaphase against time. However, in many cases the relationship was non linear and thus, it was decided to calculate the regression over the range before a decline in metaphase occurred. The following intervals were selected:

Lyallpur 73 at 0 mM	0-8h (Fig. 1)
Lyallpur 73 at 30 mM	0-6h (Fig. 1)
Lyallpur 73 at 70 mM	0-5h (Fig. 1)
Pak 81 at 0 mM	0-8h (Fig. 2)
Pak 8i at 0 mM	0-3h (Fig. 2)
Pak 81 at 0 mM	0-7h (Fig. 2)
Lu-26-S at 0-mM	0-8h (Fig. 3)
Lu-26-S at 30-mM	0-7h (Fig. 3)
Lu-26-S at 70-mM	0-5h (Fig. 3)

The formula for calculating the cell doubling time which is a modification of the formula of Evans *et al*. (1957) used by Lyndon (1970) is given below.

$$T = \frac{100 \log e 2}{\wedge}$$

Where T = Cell doubling time (h) and
^ = Rate of accumulation of metaphases over one hour (regression coefficient)

The rate of metaphase accumulation in *T. aestivum* cv. Lyallpur 73, Pak 81 and Lu-26-S was very low (Fig. 1-3) and consequently, the calculated cell doubling time was very long.

The Pakistani wheat cultivars exhibited different cell doubling times not only in control (0 mM NaCl) but also differed in their response to increasing NaCl concentration. The cell doubling time of cultivars Lyallpur 73 and Lu-26-S was similar i.e. 175.9 hrs. and 177.4 hrs. in control (0 mM NaCl) while that of Pak 81 was greater (635.2 hrs.). At 30 mM, the cell doubling time of the Lyallpur 73 was slightly increased from that noticed in control (195.4 hrs.). Lu-26-S increased from 177 h (0 NaCl) to 383 h (30 mM NaCl). However, in Pak 81 there was a decrease in CDT from 635 h (0 NaCl) to 260.6 h (30 mM NaCl).

At 70 mM NaCl, the cell doubling time of Lyallpur 73 decreased to 90 h; that of Lu-26-S Increased to 484 h whereas that of Pak 81 showed a slight increase to 299 h. The pattern of change in cell doubling time of Lu-26-8 at 30 mM NaCl and the highest concentration (70 mM NaCl) was inversely related to the responses to NaCl in term of

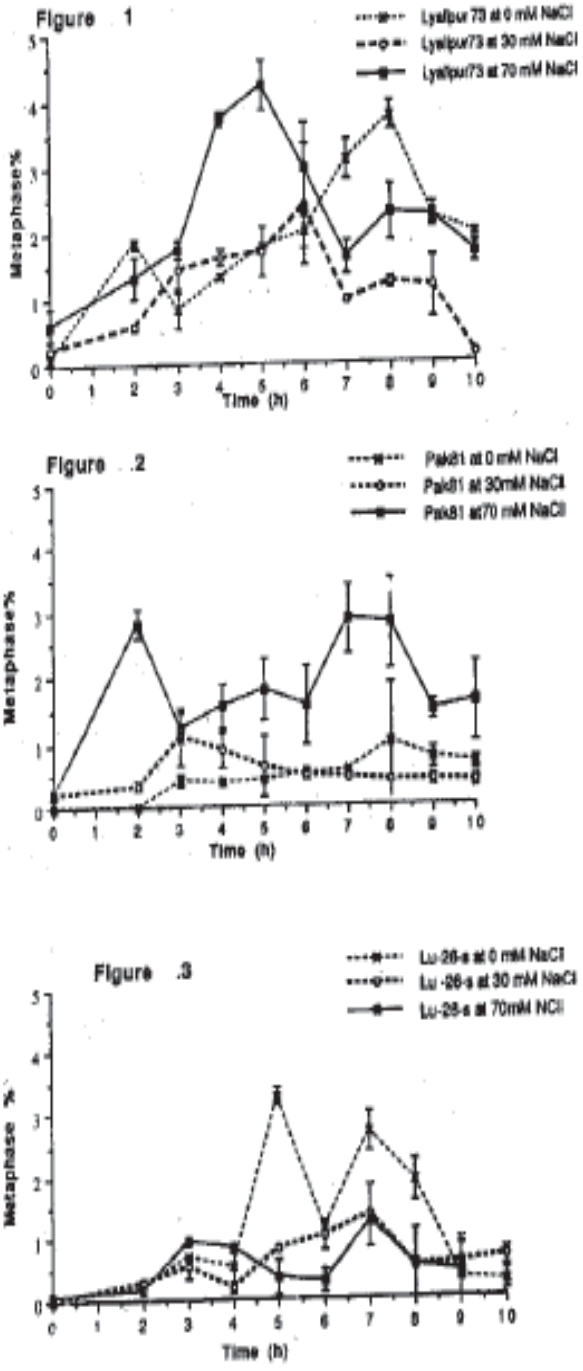

Fig. 1-3: Percentage frequency of meristematic cells in metaphase in the root mertstems of *Triticum aestivum* cvs, Lyallpur 73, Pak 81 and Lu-26-s on experimental day 5 when grown in solution containing 0, 30 and 70 mM NaCl supplied in a background of 0.1 strength Rorison's nutrient solution at various time following exposure to 0.1 percent Colchicine. The vertical bars represent ± SE

root extension and mitotic index. As the mitotic index of this cultivar decreased in response to increasing NaCl concentration, the cell doubling time was increased.

At 30 mM NaCl concentration in Lyallpur 73 there was small increase in CDT although the root extension and mitotic index of this cultivar had decreased over this range of NaCl. However, a reduction of cell doubling time occurred at 70 mM NaCl which was similar to the reduction of the root extension and mitotic index of this cultivar over that concentration.

In cultivar Pak 81, there was a reduction of cell doubling time at 30 mM and 70 mM NaCl which was a similar trend of reduction in the root extension and mitotic index of the cultivar at these concentrations.

Discussion

The use of colchicine has been widely used for estimating cell doubling time in plant meristems. This chemical blocks the anaphase separation of chromatids by inhibiting the formation of the mitotic spindle and thus cells are arrested in metaphase (Barber and Callan, 1943). The proportion of cell in metaphase increases with duration of exposure to colchicine as cells in the meristem enter mitosis and are arrested at the metaphase stage. Thus the rate of accumulation of cells in metaphase with time of exposure to colchicine is proportional to the rate at which the cells in the meristem enter mitosis; this rate has been used to estimate cell doubling time (Evans *et al*., 1957; Lyndon 1970).

As colchicine is a highly cytotoxic drug and may itself perturb the passage of cell through the cell cycle. The drug has been shown to adversely affect the number of cells in prophase (Webster and Davidson, 1969), the passage of the cells through interphase (Nagl, 1972) and the duration of the cell cycle itself (Webster and Davidson, 1969; Macleod, 1971). Despite these drawbacks the use of rate of colchicine-induced metaphase accumulation to estimate cell doubling time is still a valuable tool for comparative studies of contrasting cultivars to varying concentration of NaCl.

In the present study, the rates of metaphase accumulation with time after treatment with colchicine were very low (Fig. 1-3) and in some instances the pattern of change was non-linear. The maximum percentage of metaphase was also very low (<5%). This meant that the calculated value for cell doubling times were very long, (between 90-484 hrs.). These values are not reflected in very long cell cycle times as estimated by the PLM method (Hanif, 1993). And thus it may be concluded that the colchicine accumulation method is not producing reliable estimates of cell doubling in this experimental material. The effect of NaCl on estimated cell doubling time did not correlate with its effect on measures of root growth and other cellular characters. In Lyallpur 73 the CDT was 176 h in control, 195 h at 30 mM NaCl and then dramatically declined to 90 h at 70 mM NaCl. The cultivar Pak 81 had a CDT of 635 h in the control, the declined to 260 h and 299 h at 30 and 70 mM NaCl respectively. The cultivar Lu-26-S had a CDT of 177 h in the control; then increased progressively to 382 h and 482 h at 30 mM and 70 mM NaCl, respectively.

It is possible that the concentration of colchicine used in this study (0.1%) was too low to effectively inhibit the formation of the mitotic spindle and allow metaphases to accumulate. Powell *et al*. (1986) used a colchicine concentration of 0.5% for estimating cell doubling time in *Festuca rubra.* D. Walker (personal communication) found that the optimum colchicine concentration for metaphase accumulation in *Dactylis glomerate* was 0.5% but for *Agrostis capillaris* it was 0.1%. The optimum colchicine concentration in both shoot and root meristems of *Lolium temulentum* was 0.1% (L. Moses pers. comm.). Thomas (1992) found that the optimum concentration for metaphase accumulation in *Epilobium hirsutum* and *Chamerion angustifolium* was 0.2%. The concentration of colchicine used here was chosen on the basis of experiments by Evans *et al*. (1957) who found that concentrations of colchicine of 0.1, 0.05 and 0.025% had similar capacities for metaphase accumulation over exposure period of 1 to 6 h duration in broad root meristem. With increased exposure the weaker concentrations were more efficient than the stronger ones in accumulation metaphases. The depression of the rate of metaphase accumulation which was found in exposure periods of more than 6 h duration was attributed to an inhibitory effect of colchicine which resulted in slowing the rate of entry of cells in to mitosis. A higher concentration might have resulted in more effective metaphase accumulation and hence estimates of cell doubling time that were more rapid. On the other hand, the mitotic indices in these cultivars at time 0 were very low (<1%) and the very low measure of cells doubling time may be a reflection of very low mitotic activity in the root meristems.

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