RADIOLABELLED (Me-^{3}H) THYMIDINE INCORPORATION INTO DNA IN ROOTS OF COTTON SEEDLING

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Abstract

Cotton (*Gossypium hirsutum* L.) Cv. MNH-93 was tested to see the differences in rates of cell division between slow-growing and fast-growing roots by maesuring the (Me-^{3}H) thymidine incorporation into DNA. At early 24 and 36 h germination times, the incorporation of (Me-^{3}H) thymidine into DNA was more in long than in short roots. At germination times after 36h, the incorporation was almost the same in the tips of roots of all lengths. Thymidine incorporation values were markedly higher in younger (24-48h) roots than in older (60-96h) roots.

Introduction

Root growth consists of two basic processes of cell division and cell elongation. An integral part of the first process is the synthesis of DNA (*Maksymowych et al.*, 1986). In primary roots, DNA synthesis and cell division are restricted to the meristematic regions. In plants, cell division is a primary determinant for many aspects of development. The patterns of cell division within the root are central in determining its future structure (Barlow, 1987) and root growth ultimately depends on the rate of cell proliferation in the apical meristem. The number of cell divisions occurring in the meristem is considerably high. Cell production per hour is about 6,500 for sunflower, 11,200 for broad bean, 17,500 for corn between 10,900 and 13,700 for pea (Van't Hof, 1967). One intriguing aspect of plant cell division is that it is often asymmetric (Davidson, 1991). The cytoplasmic environment of each daughter cell is often quantitatively different and they do not continue to cycle at the same rate. In pea, 90% of daughter cells have different cycle times (Webster, 1979). At the margin of the meristem, such asymmetric division may result in cells which have different levels of competence to respond to transportable morphogens (hormones, etc) from other parts of the plant.

There are reports that thymidine serves specifically as a precursor of DNA, and it presumably is incorporated only by cells which are synthesizing DNA during the S phase (Amano *et al.*, 1959). The incorporation of radiolabelled thymidine into DNA provides a highly specific method for measuring the rate of cell division. In the present study, radiolabelled (Me-^{3}H) thymidine was applied to cotton seedling roots to measure its incorporation into DNA in order to characterise any changes in cell division during the growth of cotton seedling roots.

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Materials and Methods

Cotton seed Cv. MNH-93 obtained from the Pakistan Central Cotton Committee Research Institute, Multan, Pakistan were used. The seeds after delinting with concentrated H_2SO_4 were surface disinfected with 1.5% Sodium hypochlorite solution then thoroughly washed with sterilized distilled water before use.

Selection of seedling roots: A set of 100 seeds were germinated for 24, 36, 48, 60, 72, 84 and 96 hours. At the end of each period, the seedlings were harvested, ungerminated seeds were discarded and the remainder were arranged according to root length. Ten seedlings were selected from the centre of the root length distribution i.e., median roots. Two sets of 10 seedlings were then selected, one of which shared a longer root length and the other a shorter root length. The roots were designated as long, medium and short roots.

Incorporation of (Me-³H) thymidine: The method described by Baiza *et al.*, (1989) was used to measure the incorporation of (Me-³H) thymidine. Each set of 10 whole roots was washed separately for 1 minute in 200 ml 0.5% Na hypochlorite followed by four washes with sterile distilled water. The roots were placed in upright positions in a vial containing a solution of 5 ml 50 mM Tris-HCl buffer pH 7.6, 50 mM KCl, 10 mM $MgCl_2$, 2% sucrose, 10 μg/ml chloramphenicol and 1 μCi (Me-³H) thymidine. Water-saturated air was bubbled into the vials from a fish tank pump and the roots were incubated for 4 hours at 25°C in a reciprocating water bath. At the end of this radiolabelling period, the medium was removed from the roots by vacuum filtration through a sintered-glass funnel. The roots were then washed three times with distilled water then with 10 ml of 1% Na citrate followed by 10 ml of 80% ethanol (both solutions containing 200 μg/ml non-radioactive thymidine). The washed roots were dissected to produce 1 cm tip segments. The segments from the same set of the roots were bulked together and each group of segment was homogenized for 1 minute in 1.5 ml of 80% ethanol containing 200 μg/ml non-radioactive thymidine. The homogenate was centrifuged for 10 minutes at 3000 rpm in a MSE-Microcentaur centrifuge. The pellet was resuspended in 0.5 ml 1M NaOH and heated for 3 minutes in a water bath at 90°C. After cooling, 2 ml of 10% TCA were added and the resulting suspension was put in ice for 2 hours. It was then filtered through a 2.5 cm Whatman GF/A glass microfibre filter placed in a sintered-glass filter funnel. The filter with the retained particulate material was washed with 5 ml of 5% TCA followed by 10 ml of 95% ethanol. The filter paper and its contents were then dried and placed in glass scintillation vial with 10 ml of scintillation fluid (Cocktail- T). Uptake of radioactivity into the tissue was determined from the sum of the radioactivity in aliquots taken after the centrifugation (supernatant), filtration (filtrate) and in the particulate material collected in the filter. The incorporation of radioactivity into the DNA was determined as the radioactivity contained in the particulate material on the glass micro fibre filter. The radioactivity was determined by scintillation spectrometry. Raw counting data were converted from counts per minute (cpm) to disintegration per minute (dpm) by the spectrometer using the Crompton "end effect".

Results and Discussion

At 24 h the uptake of (Me-^{3}H) thymidine was 30.3, 21.9 and 21.3 x 10^3 dpm (10 tips)$^{-1}$ in long, medium and short roots, respectively (Fig.1A). The highest uptake was measured at 24 h germination period which reduced quickly to reach only 7.2, 8.1 and 8.2 x 10^3 dpm (10 tips)$^{-1}$ in the long, medium and short roots, respectively at 96 h germination indicating that root age had large and negative effect on uptake. At 24 h the seedling groups with long roots apparently took more radioactivity than those with short roots. At 36 h and beyond, however, there was a small but negative effect of root length on uptake. Further statistical analysis by ANOVA confirmed that uptake was slightly dependent upon seedling group (P = 0.05) and that the effect of germination time was highly significant (P < 0.001). The interaction between seedling group and germination time (P < 0.001) reflected the differences in the effect of seedling group on uptake in relation to germination time.

The corresponding results for the incorporation of (Me-^{3}H) thymidine into DNA showed a similar trend to that for the uptake (Fig.1B). At 24 h germination period, incorporation was 3962, 2204 and 1910 dpm (10 tips)$^{-1}$ in long, medium and short roots, respectively. The highest incorporation was measured at 24 h germination which quickly reduced to 410, 372 and 399 dpm (10 tips)$^{-1}$ in long, medium and short roots respectively, at 96 h germination. The results for uptake showed that root age had a large effect on incorporation. Root length had a much smaller and inconsistent effect except at 24 and 36 h where the seedling group with long roots incorporated more (Me-^{3}H) thymidine than the short ones. Statistical analysis showed that the parameter was highly dependent upon both seedling group (P = 0.003) and germination time (P < 0.001). The significant interaction between seedling group and germination time (P = 0.001) confirms that the effect of seedling group changed with germination time.

In order to get a more accurate assessment of the true incorporation, it was decided to calculate incorporation/uptake ratios. The trend was similar to that for the straight incorporation values. At 24 h germination, the I/U ratio was 0.130, 0.101 and 0.089 in long, medium and short roots, respectively (Fig.1C). The highest ratio measured at 36 h germination was 0.250, 0.180 and 0.130 in long, medium and short roots respectively which quickly declined again to reach only 0.057, 0.047 and 0.049 respectively at 96 h germination indicating that root age had a large effect on the I/U ratio, while root length had a much smaller effect except at 24 and 36 h germination. Statistical analysis of the data showed that the I/U ratio was highly dependent upon both seedling group (P = 0.005) and germination time (P < 0.001). There was no significant interaction between seedling group and germination time (P = 0.17).

Since incorporation of ^{3}H-thymidine was almost the same in the tips of roots of all lengths at later germination times, it can be assumed that the rate of cell division was similar in long and short roots. Such similar reports have been made by Gifford & Kurth (1982), who found that apical cells of *Equisetum scirpoides* Michx., are as active mitotically in long roots as in short roots. The difference in incorporation values between long and short roots at 24 and 36 h indicated that the rate of cell division during early germination was greater in long roots as compared to short root. This difference is probably not a significant direct contributor to the differences in root
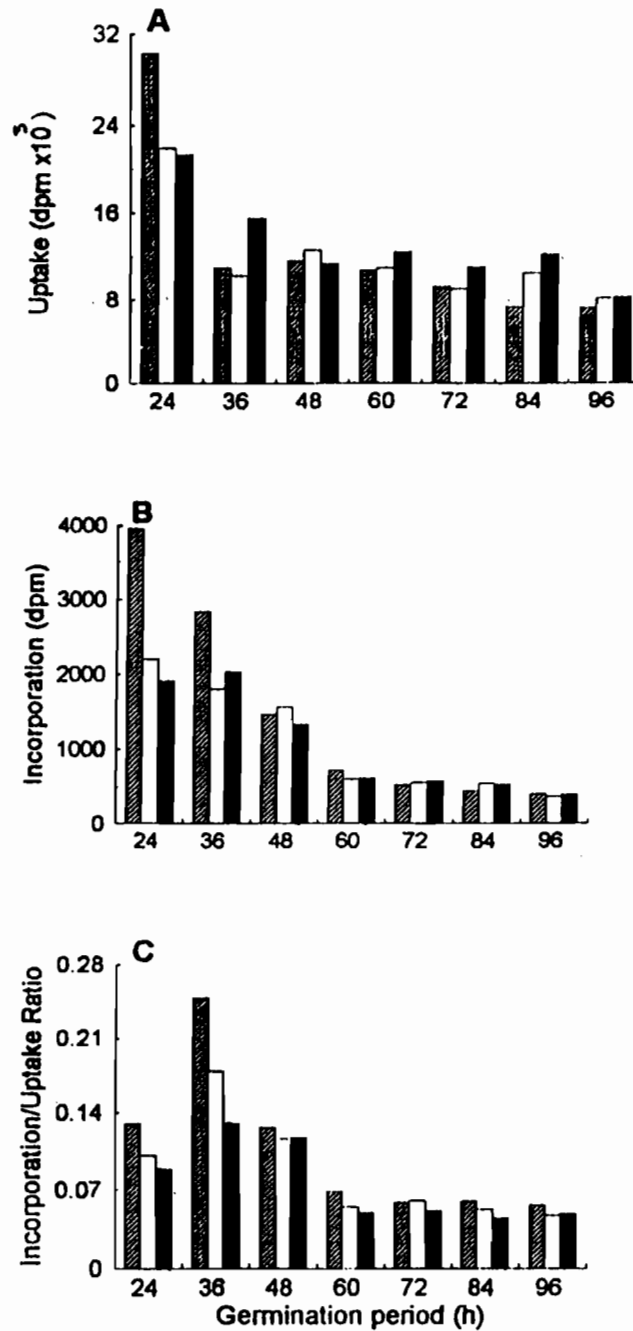
Fig.1. (Me-H) thymidine uptake and incorporation at different germination times. A, uptake; B, incorporation; C, incorporation/uptake ratio; §, long roots; [] medium roots; [] short roots.

elongation, however, because short roots contained slightly more DNA than long ones did (Chachar, 1995). Our results also show that incorporation values were higher in younger (24-48h) roots than in older (60-96h) roots. These results are in agreement with the results of Hecker *et al.*, (1979), who reported that cell division in the *Agrostemma githago* L., seedling root tips was mainly confined to the earliest part of germination.

At early (24 and 36 h) germination times, the incorporation of (Me-^{3}H) thymidine into DNA was more in long than in short roots, indicating that the rate of cell division during early germination was greater in long roots than in short roots. At later germination times beyond 36 h the incorporation was almost the same in the tips of roots of all lengths. It can be assumed from this observation that the rates of DNA synthesis and cell division at the later times were similar in long and short roots. The thymidine incorporation values were higher in younger than in older roots. The rate of cell division declines quickly as germination and early root growth proceeds. It was also observed that older (beyond 48h) roots showed signs of browning of the tip which could be one reason for the decline in the rate of cell division. It was probably not a major reason however, because thymidine incorporation was decreasing well before any tip damage could be seen. The most significant decrease was observed between 24 and 36 h. Seedling roots from 24 hours germination incorporated much more thymidine than those from 36 h even though the short roots at 24 h were half of the length of those at 36 h. This suggests that cell division starts in the radicle of the seed immediately following water imbibition and before germination. It then decreased progressively as germination proceeded. Since the difference between slow-growing and fast-growing roots before 24 h was not recorded measurements of (Me-^{3}H) thymidine incorporation could not be carried out at these times. The observed higher rate of DNA synthesis in long roots compared with short roots during this early germination period suggests that a faster rate of cell division might contribute to the faster growth of the long roots. The histological data for cell dimensions and cell flux through the elongation processes indicate that this is however not the case (Chachar, 1995). The differences between the rates of cell division thus appear to be unimportant.

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