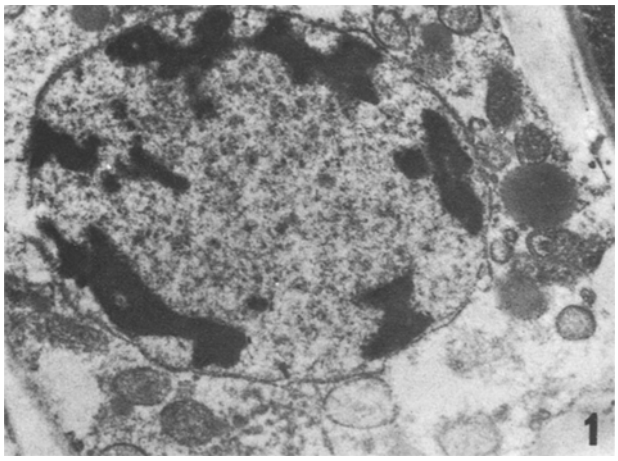

Fig. 1. The origin of protrusion from the nuclear membranes after the application of CTC. × 20,000.

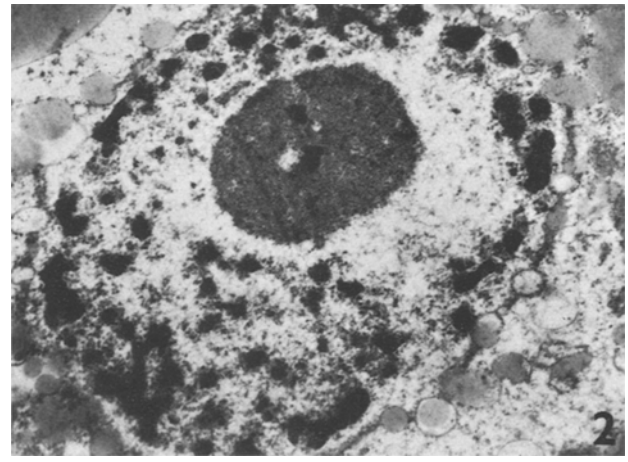

Fig. 2. Vacuolation of nuclear membrane after the treatment of CTC. × 30,000.

cide trifluraline on the cells of the root tips of the broad bean (*Vicia faba* L.)[12] and in the ageing of the mesophyll cells of tobacco leaves[13].

The changes described in the submicroscopic structure of the nucleus result from total degeneration of cells induced by the effect of CTC.

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Changes in IAA oxidase activity in rooting hypocotyl cuttings of *Phaseolus mungo* L.[1]

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Summary. IAA oxidase activity increased concomitant with root initiation during 16–52 h, in all cultures, thus showing the necessity of an active IAA oxidase for root initiation in *Phaseolus mungo* hypocotyl cuttings. The increase being very sharp in IAA alone, but less in all other cultures. The role of sucrose in root initiation via IAA oxidase activity has been discussed.

The allosteric nature of IAA oxidase was put forth in an earlier communication[2], to explain the dual behaviour of this enzyme [3–9]. Gurumurti et al.[10] implicated the oxidation products to explain the synergistic action of sodium metabisulfite with IAA in root initiation in *P. mungo* hypocotyl cuttings. To further strengthen this postulate, experiments were conducted to study the changes in IAA oxidase activity at periodic intervals to correlate it with root initiation in *P. mungo* hypocotyl cuttings. The results obtained show an interesting relationship between root initiation and IAA oxidase activity and constitute the subject matter of this paper.

Seedlings of *P. mungo* were raised as described earlier[10]. 1100 hypocotyl cuttings were divided into 4 equal groups of 275 cuttings each to be cultured in water, IAA (5 mg/l), sucrose (1%) and IAA (5 mg/l) + sucrose (1%), respectively. 50 hypocotyl cuttings at the beginning of the experiment (0 h) and later on from each group were removed from the culture media after 16, 28, 40, 52 and 64 h and 3.0-cm portions below the hypocotyl node were used for the assay of IAA oxidase as is described elsewhere[11]. The enzyme extract was prepared at pH 4.0. The reaction mixtures each time comprised of 4 ml of enzyme extract + 4 ml IAA solution + 1 ml H_2O_2 + 1 ml H_2O. The reaction mixtures were incubated for 30 min and the amount of IAA that was left unoxidized was estimated as described elsewhere[11]. The enzyme activity was expressed as µg IAA oxidized per mg protein and presented in the figure. Protein content was estimated by the method of Lowry et al.[12].

Records were also maintained of the time that was taken for initiation of roots. The number of rooted cuttings and the roots were also recorded after 7 days and presented in the table. The experiment was repeated 3 times with essentially similar results.

Results presented in the table show that all cuttings rooted in IAA, sucrose and IAA + sucrose as compared to 5 out of 10 in water. Sucrose as well as IAA increased the number of roots produced per cutting, the effect of the two together being more pronounced. It may also be noted that the root emergence was also earlier in IAA + sucrose than in other cases.

The IAA oxidase activity that was low initially (figure) did not change much at 16 h except in water, where it was slightly lower than the control. The activity increased subsequently in all the cases and reached maximum at 52 h. It may, however, be noted that while the increase was gradual and did not differ from one another in water, sucrose and IAA + sucrose, it was very rapid in IAA alone and as a consequence of which the peak reached at 52 h was much higher in IAA alone than in other cases. The activity decreased in all cases at 64 h, the decrease being much steeper in IAA and sucrose than in IAA + sucrose.

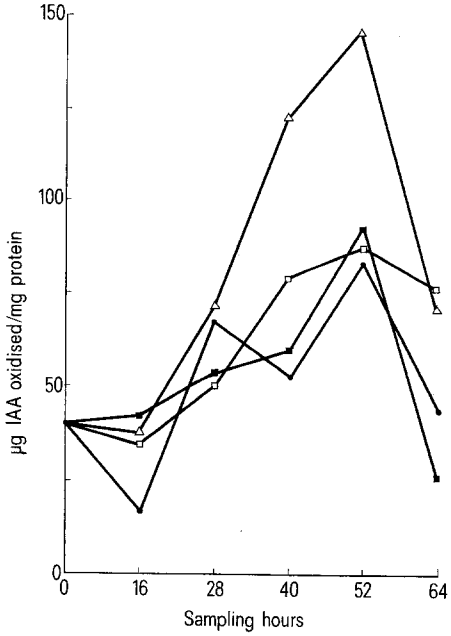
The results presented in this paper clearly show that an auxin nutrition balance is needed for root initiation as is reported earlier[13]. The increase in the activity of IAA oxidase, also lends support to the hypothesis that an active IAA oxidase is needed for root initiation. However, the intriguing part of the observations is that the increase in the activity of IAA oxidase is much more in IAA alone than in IAA + sucrose. This becomes more interesting in view of the fact that the specific activity of peroxidase enzyme remains higher in IAA as well as IAA + sucrose cultures[14], where as the activity of IAA oxidase is significantly lowered in the presence of sucrose even with IAA. Certain peroxidases are also known to oxidize IAA and are substrate induced[15-17]. This explains the high activity of IAA oxidase and peroxidase observed in cultures maintained in IAA alone. Where as if this IAA is present in combination with sucrose in the cultures the activity of the IAA oxidase does not increase, thus showing a differential behaviour. Infact, the activity of the enzyme remains almost the same as in sucrose alone (figure). This observation clearly supports that there may be 2 different species of the same enzyme or alternatively the IAA oxidase enzyme is an allosteric system with 2 active sites as postulated earlier[2]. Similar views regarding the nature of IAA oxidase enzyme have been put forth by other workers also[18,19]. This investigation also leads to another interesting observation that sucrose can modify the activity of IAA oxidase. This becomes more significant keeping in view that IAA oxidase has been reported to be a glycosylated enzyme[20]. The role of the carbohydrate moiety in the glycosylated enzyme has been reported to be the maintenance of the 3-dimensional structure of the enzyme[21]. Thus, sucrose in addition to acting as a carbon source in the process of root initiation[13] also helps in the maintenance of the 3-dimensional structure of the enzyme. This may be that it modifies the site 1, so that it becomes more active, leading to the more optimal production of oxidation products needed for root initiation, thus lending support to our earlier hypothesis[2,10].

Thus, the precise governance of the root initiation, by a very delicate balance between auxin and nutrition, becomes much more clear, with the role of sucrose in enzyme modification, especially those concerned with IAA-metabolism.

Rooting response of *Phaseolus mungo* hypocotyl cuttings after 7 days

Treatment	Number of cuttings rooted out of 10	Number of roots/rooted cutting*	Time of root initiation (h)
Control	5	4.2	52
IAA (5 mg/l)	10	7.2	52
Sucrose (1%)	10	19.7	52
IAA (5 mg/l) + sucrose (1%)	10	31.9	40

*Mean of 10 cuttings.



Periodic changes in IAA-oxidase activity in rooting hypocotyl cuttings of *Phaseolus mungo* when treated with water (●——●). IAA (5 mg/l; △——△), sucrose (1%; ■——■) and IAA (5 mg/l + sucrose 1%; □——□).

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