IAA oxidase activity in relation to adventitious root formation on stem cuttings of some forest tree species[1]

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Summary. IAA oxidase activity was very high in stem cuttings of *Salix tetrasperma* and *Populus robusta,* which rooted profusely, less in stem cuttings of *Hibiscus rosa sinensis* which rooted less, and insignificant in those of *Eucalyptus citriodora* which did not root at all. Protein(s) extracted from the stem cuttings of *E. citriodora* inhibited the activity of IAA oxidase as well as root formation on hypocotyl cuttings of *Phaseolus mungo.* The possibility of involvement of IAA oxidase activity in the process of adventitious root formation is discussed.

A close relationship between IAA oxidase activity and adventitious root formation has been reported by some workers[3-7]. It was considered to be of interest to compare IAA oxidase activity of the cuttings of some easy-to-root species with that of difficult-to-root ones to check its relationship with their rooting ability. Stem cuttings of *Salix tetrasperma* Roxb., *Populus robusta* Schneid., and *Hibiscus rosa sinensis* Linn. were selected as representative of the easy-to-root, and *Eucalyptus citriodora* Hook. of the difficult-to-root types of plant species.

Healthy 1-year-old branches of each of the above-mentioned plant species were made into 15-cm-long cuttings after excising their apical parts and leaves. These were planted in sand in earthenware pots under the natural conditions of temperature and relative humidity prevailing at Chandigarh (temperature: max. 35 ± 3 °C, min. 22 ± 4 °C, relative humidity 40%). Observations on rooting were recorded after 30 days and IAA oxidase activity was determined after 120 h. For determining IAA oxidase activity, stem cuttings were frozen overnight, sliced and then ground for 30 min in ice-cold water. From this crude water extract, protein(s) were precipitated by adding chilled acetone. The details of the precipitation of proteins and enzyme assay are given elsewhere[7]. Protein content was determined by the method of Lowry et al.[8].

The results presented in table 1 show that while all the stem cuttings of *S. tetrasperma* and *P. robusta* rooted with 18.9 and 16.5 as the average number of roots produced per cutting, respectively, only 3 out of 30 cuttings of *H. rosa sinensis* rooted with an average of 0.13 roots per cutting and none of the *E. citriodora* cuttings rooted at all. IAA oxidase activity was very high in the stem cuttings of *S. tetrasperma* and *P. robusta,* low in those of *H. rosa sinensis* and negligible in those of *E. citriodora.* These results show that the differences in rooting potential of these plant species bear a direct relationship to their IAA oxidase activity.

In another experiment, the protein(s) extracted from the stem cuttings of *E. citriodora* were used, after denaturation by boiling, to study their effect on the rooting of hypocotyl cuttings of etiolated *Phaseolus mungo* L. seedlings and their IAA oxidase and phenolase activities. The method of preparation of cuttings, culture and assay of IAA oxidase activity were as described elsewhere[9]. Phenolase activity was studied according to the method of Taneja and Sachar[10]. Observations of the number of cuttings rooted and roots produced were recorded after 7 days. The experiment was repeated 3 times with similar trends of results.

The results, together with the treatments presented in table 2, show that all cuttings rooted in water (control) and 5 µg/ml IAA. IAA-treatment increased the number of roots per cutting. Protein(s) extracted from *E. citriodora* inhibited rooting, the effect decreasing with decreasing concentration of the extract. Further, *E. citriodora* protein(s) inhibited in vitro activity of IAA oxidase (table 3). It may be noted that the *E. citriodora* protein(s) inhibited the in vitro activity of phenolase also (table 3). These results show that inhibitor(s) of root formation present in the protein(s) extracted from the cuttings of *E. citriodora* inhibit IAA oxidase activity also, further supporting the view that a positive correlation exists between IAA oxidase activity and the process of adventitious root formation. IAA oxidase causes detoxification of excessive IAA[11,12]. According to this postulate, the inhibition of this enzyme should inhibit rooting when the level of endogenous IAA is supra-optimal but promote it when it is sub-optimal. Table 2 shows that rooting is inhibited when IAA oxidase activity is suppressed and this happens even though the endogenous level of IAA is not supra-optimal, as is apparent from the fact that an exoge-

Table 1. Rooting response and IAA oxidase activity (expressed as µg IAA oxidized/mg protein/h) of stem cuttings. The data are the average of 3 experiments, with 30 replicate cuttings per treatment and are given with 95% confidence intervals

Plant	Number of cuttings that rooted out of 30	Average number of roots per cutting	µg IAA oxidized/mg protein/h
Salix tetrasperma	30	18.93 ± 3.71	152.3 ± 5.9
Populus robusta	30	16.67 ± 2.23	127.1 ± 4.8
Hibiscus rosa sinensis	3	0.13 ± 0.09	36.2 ± 4.7
Eucalyptus citriodora	0	0	1.2 ± 1.2

Table 2. Effect of IAA and the protein extract of *Eucalyptus citriodora* on rooting cuttings of *Phaseolus mungo* seedlings. Data are average of 3 experiments, with 10 cuttings per treatment, and are given with 95% confidence intervals

Treatment	Amount(µg/ml)	Number of cuttings that rooted out of 10	Average number of roots/cutting
Water (control)	0	10	4.2 ± 0.3
IAA	5	10	8.1 ± 0.5
Protein extract	300	0	0
Protein extract	30	7	1.3 ± 0.2
Protein extract	3	10	3.9 ± 0.4

Table 3. Effect of protein extract of *Eucalyptus citriodora* on in vitro activities of IAA oxidase and phenolase enzymes. Conditions as in table 2

Treatment	Amount(µg/ml)	IAA oxidase (µg IAA oxidized/mg protein/h)	Phenolase (enzyme units)
Control	0	64.9 ± 1.6	2.27 ± 0.13
Protein extract	60.0	9.8 ± 0.4	0.42 ± 0.01
Protein extract	6.0	19.5 ± 0.7	1.12 ± 0.03
Protein extract	0.6	52.8 ± 1.2	1.94 ± 0.04

nous supply of IAA promotes root formation. Thus IAA oxidase does not appear to play merely a detoxifying role. It may be that IAA oxidase enzyme acts on IAA to produce some oxidative products which are biologically more active than IAA[13-16]; however, there are many reports contrary to this postulate[17-19]. The 3rd possibility is that IAA oxidase and phenolase enzymes act on IAA and some 'phenolic co-factors' respectively modifying them in such a way as to form 'auxin-phenolic conjugates', which cause/promote the formation of adventitious roots[20-22]. That inhibition of activity is caused by the inhibitor(s) present in the protein extract of *E. citriodora* which also inhibits the formation of roots on cuttings of *Phaseolus mungo* is rather interesting. However, more work is needed to check on this point.

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3 J.M. Moinar and L.J. Lacroix, Can. J. Bot. *50,* 315 (1972).
4 G. Dhaliwal, N.C. Bhattacharya and K.K. Nanda, Indian J. Pl. Physiol. *17,* 73 (1974).
5 C. Frenkel and C.E. Hess, Can. J. Bot. *52,* 295 (1974).
6 R.N. Chibbar, K. Gurumurti and K.K. Nanda, Experientia *35,* 202 (1979).
7 R.N. Chibbar, K. Gurumurti and K.K. Nanda, Biochem. Physiol. Pfl. *165,* 625 (1974).
8 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193,* 265 (1951).
9 K.K. Nanda and A.K. Dhawan, Experientia *32,* 1167 (1976).
10 S.R. Taneja and R.C. Sachar, Phytochemistry *13,* 1367 (1974).
11 A.W. Galston and P.J. Davies, Science *163,* 1288 (1969).
12 J. Raa, Physiologia Pl. *24,* 498 (1971).
13 W.J. Meudt, Ann. N.Y. Acad. Sci. *144,* 118 (1967).
14 P.S. Basu and V. Tuli, Pl. Physiol. *50,* 499 (1972).
15 K. Gurumurti, R.N. Chibbar and K.K. Nanda, Experientia *30,* 997 (1974).
16 C. Frenkel, Physiologia Pl. *55,* 480 (1975).
17 S.A. Andersen, I.B. Moller and J. Hansen, Physiologia Pl. *27,* 105 (1972).
18 M.L. Evans and P.M. Ray, Pl. Physiol. *52,* 186 (1973).
19 L.W. Roberts, J. exp. Bot. *25,* 761 (1974).
20 C.E. Hess, Proc. XV Easter School in Agricultural Science, p.42. Ed. W.J. Whittingon. University of Nottingham, Nottingham 1968.
21 B.E. Haissig, N.Z. J. For. Sci. *4,* 311 (1974).
22 H.T. Hartmann and D.E. Kester, Plant Propagation, Principles and Practices. 3rd edn. Prentice Hall Inc. Englewood Cliffs, N.J. 1975.

DNA sequence organisation in relation to genome size in birds[1]

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Summary. DNA sequence interspersion was investigated in pheasant and pelican nuclear DNA. As is typical for birds, these genomes are organized in a long period pattern. Altogether, 5 bird species with genome sizes between 1.6 and 1.9 pg DNA are compared with regard to the extent of repetitive and single copy sequence interspersion. The result indicates that the average length of interspersed repetitive sequences increases with genome size.

A consistent feature of eukaryotic nuclear DNA is the presence of repeated sequences[4]. In many genomes, short repetitive sequences of about 0.3 kilobases (kb) are regularly interspersed with unique sequences less than 2 kb in length[5]. This type of sequence organisation has been termed 'short period interspersion' pattern, or 'Xenopus-pattern', after the organism in which it was first described in detail[6]. The functional, or structural significance, if any, of this pattern is not known, but among others regulatory tasks have been ascribed to it[7]. However, in a wide range of organisms, this pattern appears to be absent, or reduced beyond detectability by the methods commonly used so far, i.e. DNA reassociation techniques[5]. Such genomes are said to be organized in a 'long period interspersion' pattern, that is, repetitive and unique sequences extend uninterrupted for several kb each, an example being the genome of *Drosophila*[8]. It has been noted that a correlation seems to exist between the presence of short period interspersion and genome size: if, among related species with differing nuclear DNA content, genomes with and without short period interspersion co-exist, it is usually the smaller genomes in which this pattern is not detectable[5,9,10]. It is conceivable that genomes organized in the long period fashion are derived from *Xenopus*-like genomes in the course of evolution, through loss and rearrangement of repeated sequences[11].

Among the vertebrates, the only group of species in which short period sequence interspersion is usually absent are birds[12,13]. Compared with their closest relatives, birds have smaller genomes: reptile genomic DNA contents range between 60 and 89% of the mammalian value, whereas bird genomes have 44–59%[14]. We have observed, however, that among birds there seems to be an inverse correlation between genome size and the amount of sequence interspersion on a given stretch of DNA[13]. This correlation was based on observations of 3 species only, chicken *(Gallus domesticus),* pigeon *(Columba livia domestica),* and duck *(Cairina domestica).* We have now investigated the mode of DNA sequence interspersion in 2 additional bird nuclear genomes, namely those of the pheasant *(Phasianus colchicus)* and the pelican *(Pelicanus occidentalis).* These results are reported here, and are discussed together with the previous findings.

Materials and methods. Pheasants were obtained from a local poultry hatchery. Pelican blood was obtained with the kind cooperation of Tierpark Hellabrunn (München) and Vogelpark Walsrode. DNA was extracted from blood cell nuclei as described before[13]. The mode of sequence interspersion in pheasant and pelican nuclear DNA was evaluated by comparing the reassociation of long and short fragments, using conventional techniques[15]. When using the hydroxyapatite assay to monitor reannealment as a function of incubation time, this method directly reveals the proximity of sequences with different repetition frequencies. In this assay DNA molecules which are fully or only partially double stranded become absorbed to HAP at low salt concentration. Thus, with a high degree of repetitive and unique sequence interspersion, more DNA is retained