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ROOT REGENERATION IN VITRO BY SEEDLING CUTTINGS OF *PINUS NIGRA* ARN.

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Introduction

The regeneration of the adventitious root system by seedling or embryo cuttings has been investigated up to now only in two representatives of the genus *Pinus*.

In *Pinus lambertiana* Greenwood and Berlyn (1973) have stated that in embryos in which the rootlet was detached both sucrose and IAA act together on the regeneration of root meristems in the tissue of the transition zone between the hypocotyl and the rootlet. The interaction between the sugar and the auxin was evident as the presence of auxin increased the number of roots per cutting at any concentration of sucrose, although the root system was regenerated even without auxin.

In *Pinus radiata* Smith and Thorpe (1975a, b) have found that during the induction of the adventitious roots on the basis of the hypocotyl cuttings of seedlings there are there developmental sequences: the pre-initiative, the initiative and the post-initiative. In addition to that they have found that the presence of exogeneous growth regulators is directly connected with histological events at the site of the root formation. During the formation of the adventitious root the application of IBA was stimulative, while during the same time kinetin had an inhibitory effect, and the gibberelin G_3 — depending on the time of application — either a stimulative or an inhibitory one.

Following the resuls of the cited authors the present work examines the possibilites of root regeneration — in the tissue between the hypocotyl and the rootlet in seedling cuttings of the European Black Pine (*Pinus nigra* Arn.) — depending on the combined action of sugar (sucrose) and auxin (IBA).

Material and Methods

For the experiments seedling cuttings of *Pinus nigra* Arn. were used. They were obtained by detaching the rootlet from 8—10 day old seedlings, which were grown from seeds, germinating under sterile conditions. Such decapitated seedlings were transferred to a medium containing three basic components: Knop's mineral constituents modified by Nitsch (1951), sucrose added in concentrations of 20, 40, 60 and 80 g l^{-1} of medium and IBA, which was added in concentrations of 0.1, 1, 5 and 10 mg l^{-1} of medium. The control medium contained only the mineral components.

Four series of experiments were prepared, which differed in the concentration of sucrose. Each series contained 5 experiments each with 10 replications which differed in the concentration of the auxin in the medium.

The cultures were grown at a temperature of 26±1°C under artificial light (fluorescent tubes IPR 40 W, 220 V 6500° K) at an illlumination regimen of 16 hours light and 8 hours darkness daily (1250±250 lx).

Results

The observations during and after experiments were analysed and the percentage of rooted cuttings, the number of roots per cutting, the percentage of rooted cuttings with lateral branches, the mean length as well as the mean fresh weight were determined.

The percentage of rooted cuttings

The adventitious root system was induced and developed at the site of transition between the hypocotyl and the rootlet. The percentages are shown in Fig. 1. On media with 20 and 40 g l^{-1} sucrose and with 0.1,

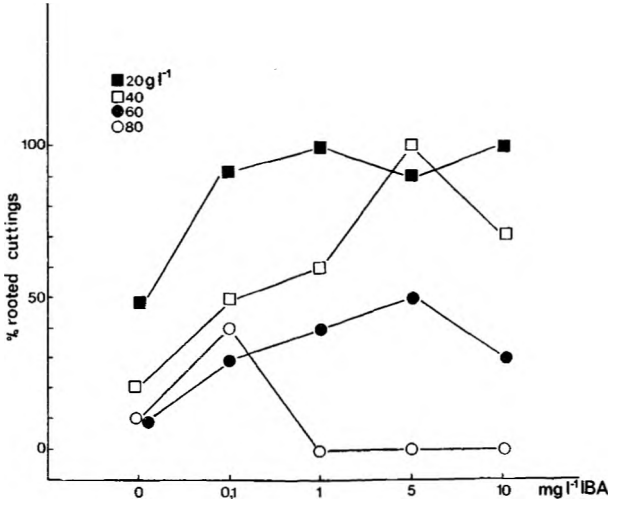

Fig. 1. Percentage of rooted cuttings depending on concentration of sucrose and IBA.

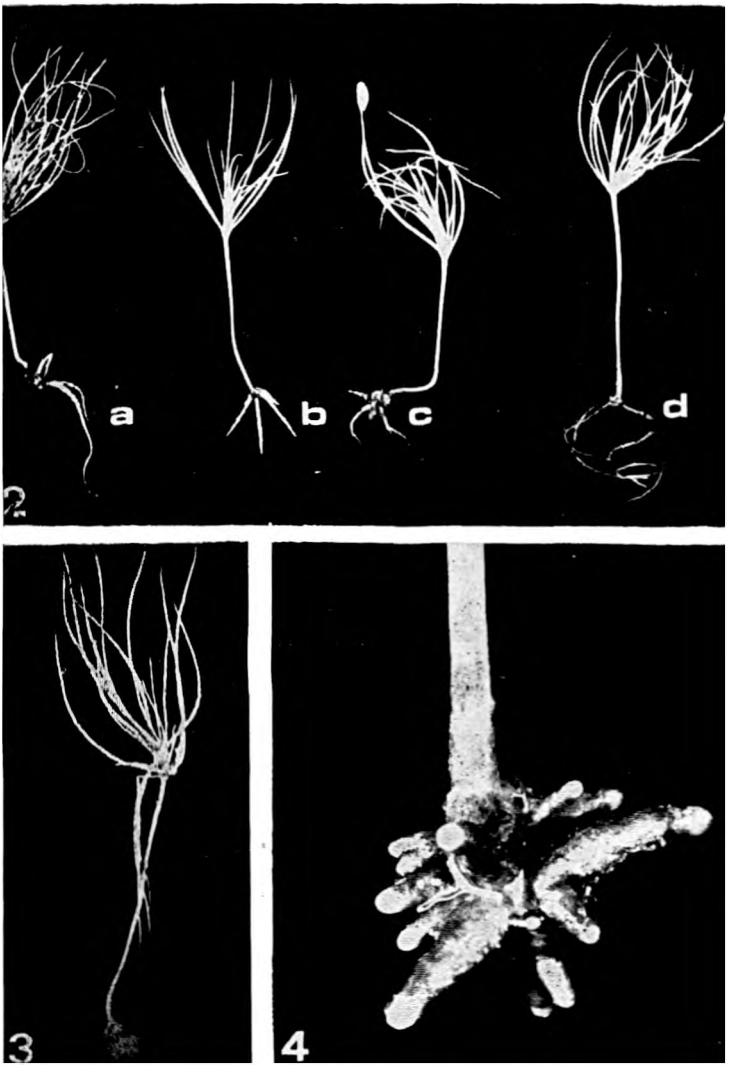

Fig. 2. Well developed root systems in cuttings (40 g l^{-1} sucrose and a — 0.1, b — 1, c — 5 and d — 10 mg l^{-1} IBA).

Fig. 3. Cutting with callus developed on the basis of hypocotyl (80 g l^{-1} sucrose and 5 mg l^{-1} IBA).

Fig. 4. Cutting with a very strong induction of adventitious rhizogenesis (20 g l^{-1} sucrose and 10 mg l^{-1} IBA).

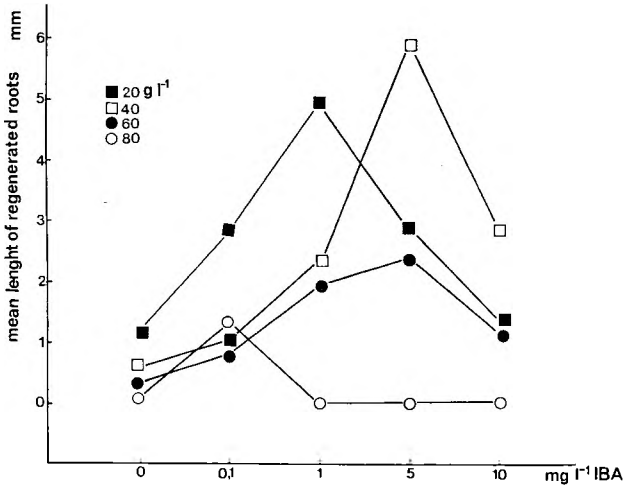

Fig. 5. Mean length of regenerated roots of cuttings depending on concentration of sucrose and IBA.

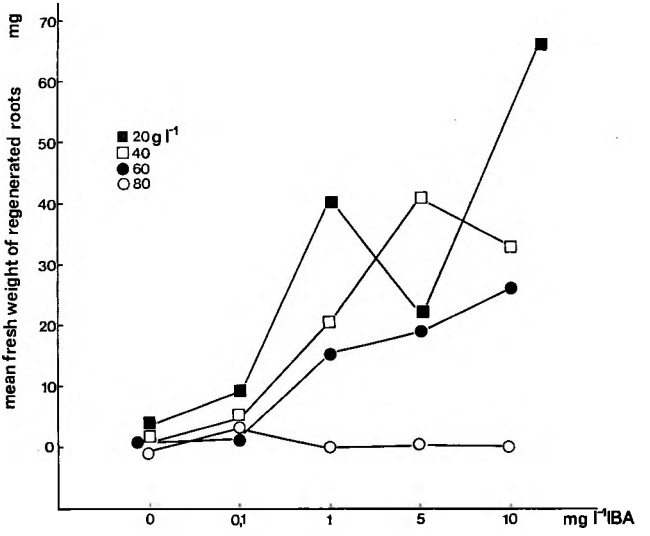

Fig. 6. Mean fresh weight of regenerated roots of cuttings depending on concentration of sucrose and IBA.

1, 5 and 10 mg l^{-1} IBA the adventitious rhizogenesis was induced in 50—100% of cuttings. The regenerated roots were normally developed (Fig. 2). The media containing 60 and 80 g l^{-1} of sucrose were unfavourable regardless of the concentration of IBA. On media with 80 g l^{-1} sucrose with 1, 5 and 10 mg l^{-1} IBA only the development of callus was induced (Fig. 3). It was also shown that the sucrose alone induced adventitious rhizogenesis. At the concentration of 20 g l^{-1} as many as 50% of cuttings developed roots, which were, however, weak and single. The inductive ability of the sucrose alone was lowered by increased concentration.

Number of roots per cutting and the percentage of cuttings with lateral roots

On media with 20 and 40 g l^{-1} sucrose and 0.1, 1, 5 and 10 mg l^{-1} IBA the adventitious root system was induced not only at a higher percentage, but was regularly also more vigorously developed, and later was composed of a higher number of roots. In the same series at a higher number the main root formed lateral ones of the 1st and 2nd order, as shown in Table 1. In the series with a higher percentage of sugar the number of roots in the root system, as well as the percentage of cuttings with lateral roots was lowered.

Table 1. Effect of sucrose and IBA on root regeneration of *Pinus nigra* seedling cuttings.

Concentration of sucrose	Concentration of IBA	The number of roots per cutting	% of rooted cuttings with lateral branches
20 g l^{-1}	0	1	—
	0.1	1—4	50%
	1	1—6	40%
	5	1—5	40%
	10	5—11	—
40 g l^{-1}	0	1	—
	0.1	1—3	10%
	1	1—6	20%
	5	1—6	20%
	10	1—4	40%
60 g l^{-1}	0	—	—
	0.1	1—2	20%
	1	1	—
	5	1—2	—
	10	1—4	—
80 g l^{-1}	0	1	—
	0.1	1—2	10%
	1	—	—
	5	—	—
	10	—	—

The mean length and weight of regenerated root systems

The highest value of mean length of all roots per rooted cutting was reached at 40 g l^{-1} sucrose and 5 mg l^{-1} IBA with 57 mm, and with only somewhat smaller value at 20 g l^{-1} sucrose and 1 mg l^{-1} IBA, namely 48 mm (Fig. 5).

The values of the mean fresh weight are in accordance with the upper results, as far as the experiments with 40 g l^{-1} sucrose and 5 mg l^{-1} IBA as well as 20 g l^{-1} sucrose and 1 mg l^{-1} IBA are concerned, being — for both experiments — 40 mg. The highest value for the mean fresh weight was found, however, in the experiment with 20 g l^{-1} sucrose and 10 mg l^{-1} IBA with 66.5 mg (Fig. 6). the mean lenght of all roots in this case was, however, small being only 1.35 mm. This disproportion was the consequence of a very strong induction of adventitious rhizogenesis so that the regenerated root systems consisted of 5—11 very vigorous and thickened roots (Fig. 4). Their development was however, already inhibited since the first two weeks from the beginning of the experiments.

On the control medium there was no rhizogenesis at all and the cuttings perished already during the first week of the experiment.

Discussion

The regeneration of root meristems in the tissue between the hypocotyl and the basis of the rootlet of the embryo was induced already in *Pinus lambertiana* (Greenwood and Berlyn 1965). According to the results of these authors 40% of such cuttings regenerated the roots without the presence of exogenous auxins. Due to the corresponding transport of auxins, they accumulated on the site of adventitious root formation (Greenwood and Goldsmith 1970). Exogeneous auxins had, however, an effect on the intensity of the induction. In this way IAA, added in very low concentrations (of 1 μM), did not only increase the percentage of rooted cuttings but also the number of roots developed per rooted cutting. This was happening at concentration of sucrose from 10—140 g l^{-1}, although the optimal concentration was 80 g l^{-1} of medium. Greenwood and Berlyn (1973) explain this fact by the interaction of sugar and auxin in the processe of root meristem regeneration in cuttings of *Pinus lambertiana.*

In the present investigations it has been also shown that in *Pinus nigra* Arn. even 50% of seedling cuttings regenerated roots without the presence of exogenous auxins. However, by addition of IBA in concentrations of 0.1—10 mg l^{-1} of medium the percentage of the rooted cuttings increased as well as the number of roots per rooted cutting, and consequently also the mean lenght and the mean fresh weight of all roots of the root system per cutting. In addition to that, due to the effect of IBA, a great number of rooted cuttings developed root branches of the 1st and 2nd order, a fact which was hitherto not known in *Pinus* species. Therefore it is possible to say that in *Pinus nigra* Arn. in the regeneration processes of root on seedling cuttings there was certainly an effect of interaction of sugar and auxin. The optimal concentration of sucrose was 20—40 g l^{-1}. An earlier investigation of the author (Kolevska-Pletikapić 1972) has shown that it is possible to obtain root regeneration also in short shoots, so far they were pretreated by auxins.

In addition to auxins there are also other substances, which stimulate the root regeneration. Smith and Thorpe (1977) were able to state that the application of some aromatic aminoacids increases the percentage of rooted seedling cuttings of *Pinus radiata*. These results were much depending on the time of their application to the site of adventitious root formation.

In the present work only IBA was applied. It is however possible that the application of other substances would give in *Pinus nigra* Arn. even more interesting results.

Conclusion

Root regneration has been studied in European Black Pine *(Pinus nigra* Arn.) on cuttings of seedlings under in vitro conditions. It has been stated that — in the tissue between the hypocotyl and the rootlet — sugar and auxin are equally important in regeneration processes of root meristems. Thus sucrose (20 g l^{-1}), alone, induced adventitious rhizogenesis at 50% of cuttings, but this induction was fully increased only in combination with IBA. At favourable concentrations of sucrose (20—40 g l^{-1}) and IBA (0.1—10 mg l^{-1}) the percentage of rooted cutting was in this way increased from 50 to 100%; the number of roots per cutting — the main root developed the lateral ones — and in this way the mean lenght and the mean fresh weight of the roots in the regenerated root system were also increased.

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SAŽETAK

REGENERACIJA KORIJENA IN VITRO KOD REZNICA MLADIH BILJAKA CRNOG BORA (*PINUS NIGRA* ARN.)

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Studirana je regeneracija korijena kod crnog bora (*Pinus nigra* Arn.) u in vitro uvjetima na reznicama mladih biljaka. Utvrđeno je da u procesima regeneracije korijenskih meristema u tkivu na prijelazu između hipokotila i korijenka podjednako važnu ulogu imaju šećer i auksin. Naime, sama saharoza inducirala je adventivnu rizogenezu kod 50% reznica, ali u kombinaciji s IBA ova indukcija postajala je intenzivnija. Tako se kod povoljnih koncentracija saharoze (20—40 g l^{-1}) i IBA (0.1—5mg l^{-1}) povećao postotak zakorijenjenih reznica od 50 na 100%, ali i broj korijena po zakorijenjenoj reznici; došlo je do bočnog grananja glavnog korijena na ogranke I. i II. reda, a s tim u vezi povećala se i prosječna dužina i težina korijena u novonastalom korijenovom sistemu.

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