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IN VITRO STUDIES OF CALLUS GROWTH OF *PINUS NIGRA* ARN. 2. GROWTH REGULATORS REQUIREMENTS AND THEIR EFFECTS

BRANKA KOLEVSKA-PLETIKAPIĆ

(Department of Botany, Faculty of Science, University of Zagreb)

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The reaction of the callus strain K of *Pinus nigra* Arn. to the contents of growth regulators was an uneven growth. The strain K grew normally without cytokinin (kinetin) only on a medium with higher concentrations of 2,4-D. Along with kinetin, except in combination with 2,4-D, only NAA affected the callus growth. IAA and IBA were completely inefficacious with or without kinetin.

Introduction

Quantitative growth analyses of the callus strain K of *Pinus nigra* Arn. (Kolevska-Pletikapić 1978, 1982) have shown that this species reacts in some ways specificially to some substances in the medium. In an indirect way these investigations have also shown that a differentiation of organogenic structures cannot be induced easily in the callus of this strain. Besides xylogenesis, in which mineral salts have been shown to be the critical factor, it has not been possible to provoke any other morphogenetic effects. This is, however, in accordance with the generally accepted view that it is much more difficult to induce morphogenesis than to initiate the origin of callus and to maintain its growth indefinitely by subculturing. There are many reasons for this. One of them is, according to Bonga (1977), the fact that the high concentration of synthetic auxins, which have a higher inductive activity and are therefore used for the induction and growth of callus, breaks down the precise balance between the auxins and cytokinins, which is otherways a conditio sine qua non in the induction of morphogenesis.

For this reason, the present study is concerned with the activity of growth regulators. For this purpose the kind of auxins (synthetic as well as natural) and their concentrations were varied.

Material and Methods

After being succesfully used in studies of the activity of medium composition on growth, the callus strain K was chosen for the present work. It was induced and subcultivated on an induction medium — modified MS-medium adapted to the needs of *Pinus nigra* Arn. (Kolevska-Pletikapić 1974). After the 12th subculture the callus strain K was transfered to media in which the content of auxins and kinetin had been varied in many ways.

The conditions of culturing were the same as described previously (Kolevska-Pletikapić 1982).

Experiments and Results

The induction medium contained, as described previously (Kolevska-Pletikapić 1982), the growth substances: synthetic auxin 2,4-D (5 mg l^{-1}) and cytokinine kinetin (0,5 mg l^{-1}).

In the present work in addition to the activity of 2,4-D, the effects of synthetic auxins NAA and IBA, as well as natural auxin IAA were investigated. Each of the auxins was used in many concentration either without or in combination with kinetin which was also used in many concentrations. In this way 28 kinds of media were obtained (each with 10 replications) which were divided into 8 groups:

1. MS-medium 2,4-D — 0.1; 5.0; 10 mg l^{-1} instead of the addition of 5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} kinetin of the induction medium.

2. MS-medium NAA — 0.1; 5.0; 10 mg l^{-1} instead of the addition of 5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} kinetin of the induction medium.

3. MS-medium IAA — 0.1; 5.0; 10 mg l^{-1} instead of the addition of 5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} kinetin of the induction medium.

4. MS-medium IBA — 0.1; 5.0; 10 mg l^{-1} instead of the addition of 5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} kinetin of the induction medium.

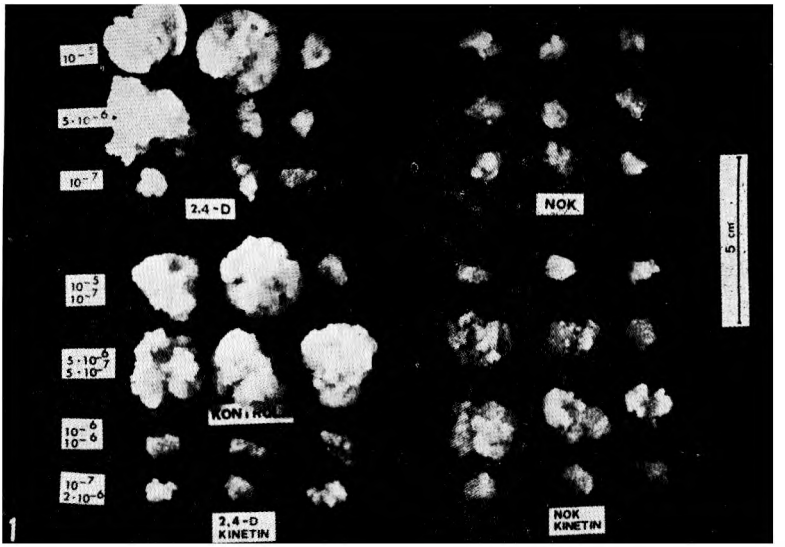
5. MS-medium 2,4-D + kinetin — 0.1 + 2; 1 + 1; 5 + 0.5 (= control = induction medium); 10 + 0.1 mg l^{-1} instead of the addition of 5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} kinetin of the induction medium.

6. MS-medium NAA + kinetin — 0.1 + 2; 1 + 1; 5 + 0.5; 10 + 0.1 mg l^{-1} instead of the addition of 5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} kinetin of the induction medium.

7. MS-medium IAA + kinetin — 0.1 + 2; 1 +1; 5 + 0.5; 10 + 0.1 mg l^{-1} insteaed of the addition of 5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} kinetin of the induction medium.

8. MS-medium IBA + kinetin — 0,1 + 2; 1 + 1; 5 + 0.5; 10 + 0.1 mg l^{-1} instead of the addition of 5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} kinetin of the induction medium.

After 6 weeks culturing the callus strain K, grown on each of the 28 media, was compared and the differences in growth analyzed. In general it is possible to say that NAA, IAA and IBA both alone, and combined with kinetin stimulated the growth of the callus strain K (Figs. 1 and 2) better than 2,4-D. From the data about the average increase in fresh and dry weight on media with auxins only (Figs. 3 and 4) and with auxins in combination with kinetin (Figs. 5 and 6) one can see that the K strain



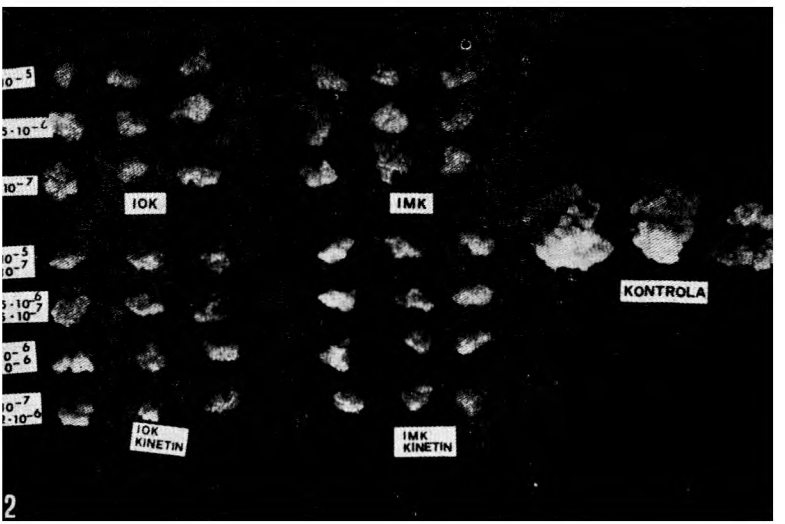


Fig. 1. Callus strain K grown on media with 10, 5 and 0.5 mg l^{-1} 2,4-D and NAA (= NOK) and also with 10 + 0,1; 5 + 0.5; 1 + 1 and 0.1 + 2 mg l^{-1} 2,4-D and kinetin and NAA (= NOK) and kinetin. Control = induction medium.

Fig. 2. Callus strain K grown on media with 10, 5 and 0.1 mg l^{-1} IAA (= IOK) and IBA (= IMK) and also with 10 + 0.1; 5 + 0.5; 1 + 1 and 0.1 + 2 mg l^{-1} IAA (= IOK) and kinetin and IBA (= IMK) and kinetin. Control = = induction medium.

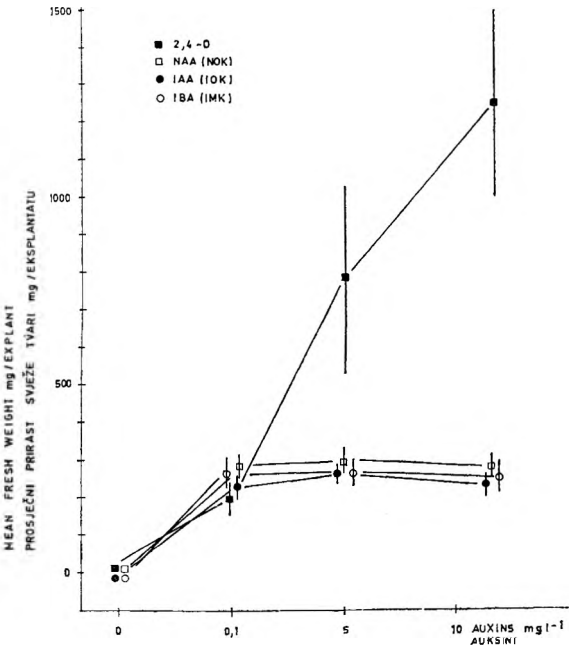


Fig. 3. Callus growth (averge increase in fresh weight in mg per explant) on media with different concetrations of auxies (2,4-D, NAA, IAA and IBA).

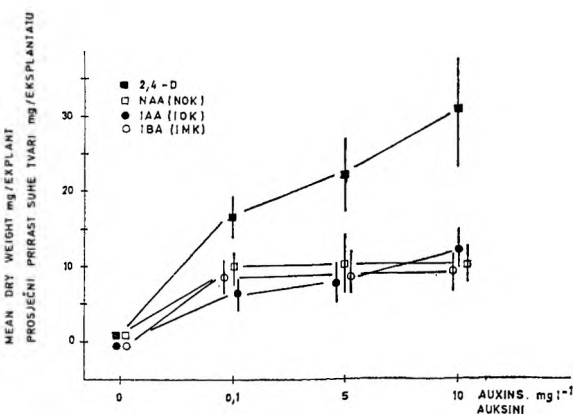


Fig. 4. Callus growth (average increase in dry weight in mg per explant) on media with different concentrations of auxins (2,4-D, NAA, IAA and IBA).

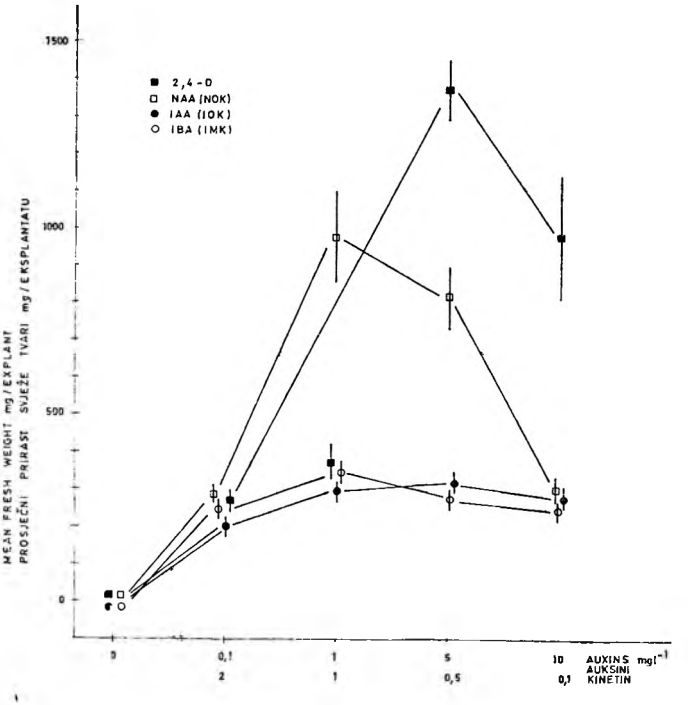


Fig. 5. Callus growth (average increase in fresh weight in mg per explant) on media with different concentration of auxins (2,4-D, NAA, IAA and IBA) and kinetin.

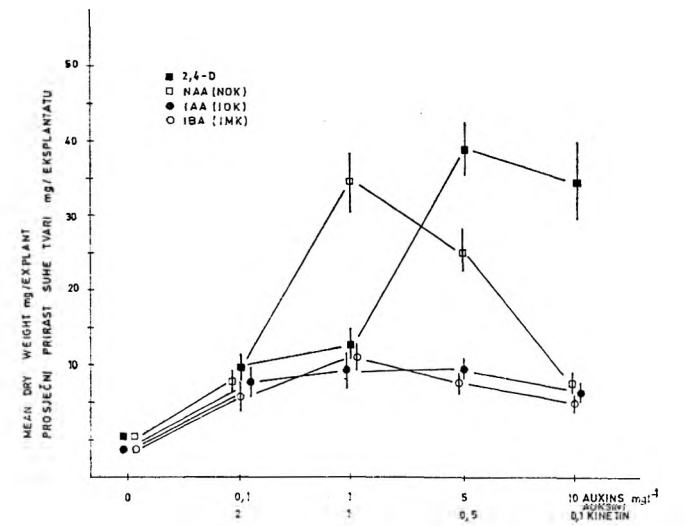


Fig. 6. Callus growth (average increase in dry weight in mg per explant) on media with different concentrations of auxins (2,4-D, NAA, IAA and IBA) and kinetin.

grew best on the induction medium (control = MS) which contained 5 mg l^{-1} 2,4-D and 0.5 mg l^{-1} kinetin. On this medium the average increase in fresh weight was 1338 ± 68 mg, and dry weight 38 ± 4.8 mg, per explant. Less than on this medium, but still well, the strain K grew on media with 5 or 10 mg l^{-1} 2,4-D alone, as well as on media with 5 + 0.5 and 1 + 1 mg l^{-1} NAA and kinetin. On all other media the callus of the strain K grew only slightly.

Discussion

It has been shown that the growth regulators are a factor of the medium considerably influencing the callus growth of the strain K. The results of the investigations of this component are in accordance with the results of other authors.

Kinetin stimulated the callus growth of this strain, while the most effecitve auxin was 2,4-D, as also stated by other authors (Harvey 1967, Rogozinska 1970, Konar 1974 and others). However, the results of the present work have also shown, that the changes in the content of this component alone do not affect the exhibition of morphogenetic effects in the callus investigated, as could have been expected. Neither the reduced concentrations of synthetic auxins, nor the natural auxin as IAA have influenced the induction of differentiation in the callus of the strain K. More over, natural auxin has been shown to be thoroughly inactive even in the growth of this strain. It follows from this fact that the role of many other factors is not less important. Some of them are known from literature. Some authors, such as Winton (1971), Chalupa (1974), Campbell and Durzan (1975) prefer cytokinin BAP to kinetin if the induction of morphogenesis is in question. Many other chemical factors are known. Jacquiot (1966) has shown that the formation of buds in *Ulmus campestris* depends on the existence of a certain balance between auxin and meso-inositol, while Ishikawa (1974) has demonstrated the doubtless effect of abscisic acid, otherwise a natural inhibitor, in the shoot induction in *Cryptomeria japonica*. The choice of explants, used for callus initiation, is also considered to be of great importance. So it could be shown by Mehra and Mehra (1974) that in *Prunus amygdalus* the morphogenetic potential of callus from various origin varied regardless of the fact that there were no differences in its growth. It is generally considered, as Bonga (1977) points out, that in some species it is very difficult to achieve a total cell-dedifferentiation, which is a conditio sine qua non for the exhibition of the cell-totipotence. This is especially the case in the secondary tissues, where it is necessary — according to this author — to take care in chosing the tissue for initial explants for callus culture. In the work with *Pinus nigra* some of these data have been considered (Jelaska, Kolevska-Pletikapić, Vidaković 1979). Very young receptive female cones were used as the initial explants in which the initial callus was grown. After that 81 media with cytokinin BAP and other positively acting substances were tested by the method of De Fossard (1976). However, morphogenetic reactions could still not be achieved, a fact which indicates that in *Pinus nigra* these processes are controlled by very complex mechanisms and therefore cannot be easily induced.

Conclusion

The callus of *Pinus nigra* Arn. — strain K — was grown on 28 media which differed in the kind and concentration of auxin, as well as in the auxin in the combination with kinetin, again in various concentrations. The results have shown the following:

1. The callus strain K reacts to the composition of growth regulators by a very unbalanced growth. Without kinetin it grows only on media with high concentrations of 2,4-D. In combination with kinetin in addition to 2,4-D only NAA has some effect on the growth of this strain. IAA and IBA are thoroughly ineffective, both with and without kinetin.

2. Changes in the composition of growth-regulators alone cannot induce any morphogenetic effects in the strain K.

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SAŽETAK

IN-VITRO-ISTRAŽIVANJA RASTA KALUSA VRSTE *PINUS NIGRA* ARN. 2. POTREBNI REGULATORI RASTA I NJIHOVO DJELOVANJE

Branka Kolevska-Pletikapić

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Na sastav regulatora rasta kalusna loza K crnog bora (*Pinus nigra* Arn.) reagirala je veoma neujednačenim rastom. Bez citokinina (kinetin) rasla je normalno jedino na podlozi s višim koncentracijama 2,4-D. S kinetinom, osim 2,4-D samo je još NAA utjecala na rast te loze. IAA i IBA bile su potpuno nedjelotvorne, kako s kinetinom tako i bez njega.

Dr. Branka Kolevska-Pletikapić
Botanički zavod (IV)
Prirodoslovno-matematički fakultet
Rooseveltov trg 6/III p.p. 933
YU-41001 Zagreb (Jugoslavija)