ROLE OF AUXINS, ANTIAUXIN AND PHENOL IN THE PRODUCTION AND DIFFERENTIATION OF CALLUS ON STEM CUTTINGS OF *POPULUS ROBUSTA*

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ABSTRACT

Stem cuttings of **Populus robusta** Schneid. were treated with 0, 10 and 100 mg/l each of indole-3-acetic acid (IAA), indole-butyric acid (IBA), tri-iodo-benzoic acid (TIBA) and carbolic acid (CA) at the apical and basal ends after 0, 7 or 14 days. Observations on rooting, callus formation and callus differentiation were recorded. Auxins increased rooting, the effect being more marked with IBA than IAA, and when applied on day 0 rather than after 7 or 14 days. Callus was formed at the apical end of the cuttings by treatment of that end on day 0 with water or any other regulatory substance. This also occurred when the basal end was treated with TIBA or CA. The amount of callus decreased with a delay in time of treatment. Callus developed on almost the whole length above the soil when cuttings were treated with 100 mg/l IBA at the apical end, and below soil level when cuttings were treated at the basal ends. In contrast to this, callus formed by IAA remained confined to a small part of the end to which it was applied. While callus formed at the apical end by IBA differentiated into roots, that produced by TIBA or CA differentiated into shoots. The number of differentiating cells increased when the treatment was repeated. It is considered that callus is produced at a time when differentiation of cambial derivatives is not able to keep pace with cell-divisional activity and that a proper balance between the supply of auxin and assimilates is needed for organogenesis. A high sucrose to auxin ratio leads to the production of phloem, and a low sucrose to auxin ratio to xylem, which is necessary for vascularization of root primordia. A schematic representation is proposed of the possible, self-catalysing and self-perpetuating mechanism that may be involved in callus formation and its differentiation into xylem and phloem, and thus to the production of roots or shoots.

INTRODUCTION

During investigations on the effect of auxins on rooting stem cuttings in relation to polarity, Nanda *et al.* (1969) showed that callus formation occurred at the upper end of *Populus* cuttings kept in the dark regardless of whether these were planted erect or inverted. The fate of the callus, however, depended on the manner of planting. On cuttings planted normally it differentiated into branches, but on those planted in an inverted manner it dried ultimately and did not develop. Lee and Skoog (1965, a, b) showed that high levels of auxin inhibited bud formation in tobacco callus. While

phenols interacted with auxin by speeding up or slowing down the rate of IAA inactivation, TIBA disturbed the polarity of callus cells (Skoog, 1954; Keitt and Skoog, 1959). This paper deals with the effect of auxins, TIBA and phenol on the production and differentiation of callus on stem cuttings of *Populus robusta* when planted in the dark.

MATERIAL AND METHODS

One-year-old branches of more or less uniform size were taken from trees growing on the University campus and were made into 20-cm cuttings after excising the apical parts and the leaves. A total of 1080 cuttings was divided into 4 treatments:

1. Hormone applied to the apex of the cutting
2. Hormone applied to the base of the cutting
3. Hormone applied initially to the apical end and subsequently (at 3 application times—see later) to the base
4. Hormone applied initially to the base and subsequently (at 3 application times—see later) to the apex

With each treatment, 4 sub-treatments (hormones) were applied: (1) Control (treated with water), (2) IAA 10 mg/l, (3) IAA 100 mg/l, (4) IBA 10 mg/l, (5) IBA 100 mg/l, (6) TIBA 10 mg/l, (7) TIBA 100 mg/l, (8) CA 10 mg/l, (9) CA 100 mg/l.

Within each sub-treatment 3 application times were tested: (a) at the time of severance, (b) 7 days after severance, (c) 14 days after severance. This provided a total of 108 groups with 10 cuttings per group. The period of treatment in all cases was 24 hours and the experiment was carried out in the dark.

RESULTS

These are presented in 4 tables, each corresponding to one of the four major treatments.

Rooting response

Tables 1 to 4 show that while 10 mg/l IAA increased only the number of rooted cuttings, 100 mg/l IAA and both concentrations of IBA increased both the number of rooted cuttings and the number of roots per cutting when applied at day 0, or after 7 days. The effect was independent of the end of the cutting that was treated. It was observed that the roots on cuttings treated at the apex with 100 mg/l IBA emerged throughout the length of the cutting, even if the basal end was subsequently treated. TIBA in general inhibited rooting while CA on the other hand increased both the number of rooted cuttings and the number of roots per cutting, provided that the treatment was repeated (Tables 3 & 4). Whether the initial treatment was given to the apical or to the basal end was not important.

Callus at the Apical End

1. *Amount* Callus was produced at the apical end of the cuttings by treatment at that end with water (Treatment 1 — Control). The amount of callus growth was greater when treatment was repeated at the alternate end (Treatments 3 & 4 — Control) and declined with a delay in subsequent treatment time. IAA & IBA increased callus growth most under the conditions that were most favourable for the water controls. TIBA & CA on the other hand increased callus regardless of whether they were applied apically or basally but again the repeat treatments (3 & 4)

with the least delay in treatment time were optimal for callus growth (Tables 3 & 4). When cuttings were treated at the apical end with 100 mg/l IBA callus developed on almost the whole length of the cutting above the ground.

2. *Differentiation* Callus which formed at the apical end of the cuttings treated with water, IAA or 10 mg/l IBA did not differentiate, and dried ultimately regardless of the end that was treated. Callus on 100 mg/l IBA-treated cuttings did differentiate into roots however, when the treatment was given initially to the apical end. The number of roots and rooted cuttings decreased with a delay in treatment (Table 1).

   In marked contrast, the callus produced on cuttings treated with TIBA or CA differentiated into shoots instead of roots. The number of sprouts increased considerably in Treatments 3 & 4, the effect increasing with a delay in the application of the repeat treatment (Tables 3 & 4).

TABLE 1 - Formation and differentiation of callus on stem cuttings of Populus robusta and their rooting response when treated with 0, 10 and 100 mg/l each of IAA, IBA, TIBA and CA at the apical end after 0, 7 or 14 days. The number of cuttings set in each case was 10.

Treatment (mg/l)	Days to treatment	No. of rooted cuttings (No. of roots/rooted cutting)	Callus at Apical end: No. of cuttings with callus (Amount of callus, based on simple numerical score)	Apical end: No. of cuttings with differentiated callus (No. of roots or shoots/cutting)	Basal end: No. of cuttings with callus (Amount of callus, based on simple numerical score)	Basal end: No. of cuttings with differentiated callus (No. of roots or shoots/cutting)
Control	0	5 (2)	9 (2)	-	6 (1)	-
	7	5 (2)	4 (2)	-	5 (2)	-
	14	3 (2)	5 (1)	-	8 (1)	-
IAA, 10	0	7 (2)	10 (3)	-	6 (3)	-
	7	2 (2)	5 (2)	-	8 (3)	-
	14	3 (2)	3 (2)	-	5 (7)	-
IAA, 100	0	5 (4)	4 (2)	-	10 (6)	-
	7	1 (7)	6 (2)	-	6 (3)	-
	14	2 (1)	2 (2)	-	6 (4)	-
IBA, 10	0	9 (4)	6 (2)	-	9 (5)	-
	7	7 (2)	7 (2)	-	4 (3)	-
	14	-	5 (2)	-	4 (5)	-
IBA, 100	0	10 (21)	10 (9)	10 (11)	7 (5)	-
	7	8 (3)	10 (4)	2 (2)	9 (3)	-
	14	-	5 (2)	-	4 (3)	-
TIBA, 10	0	6 (2)	8 (7)	6 (8)	7 (3)	-
	7	-	7 (3)	3 (4)	-	
	14	-	5 (2)	-	2 (3)	-
TIBA, 100	0	2 (1)	9 (6)	4 (4)	3 (3)	-
	7	2 (2)	8 (3)	-	4 (3)	-
	14	1 (2)	9 (2)	-	7 (4)	-
CA, 10	0	6 (2)	9 (8)	3 (2)	7 (4)	-
	7	-	9 (6)	4 (5)	2 (3)	-
	14	1 (1)	8 (9)	6 (6)	2 (4)	-
CA, 100	0	3 (2)	8 (8)	3 (7)	9 (4)	-
	7	2 (2)	6 (5)	-	3 (4)	-
	14	2 (1)	1 (1)	-	4 (3)	-

TABLE 2 - Formation and differentiation of callus on stem cuttings of Populus robusta and their rooting response when treated with 0, 10 and 100 mg/l each of IAA, IBA, TIBA and CA at the basal end after 0, 7 or 14 days. The number of cuttings set in each case was 10.

Treatment (mg/l)	Days to treatment	No. of rooted cuttings (No. of roots/rooted cutting)	Callus at Apical end: No. of cuttings with callus (Amount of callus, based on simple numerical score)	Callus at Apical end: No. of cuttings with differentiated callus (No. of roots or shoots/cutting)	Callus at Basal end: No. of cuttings with callus (Amount of callus, based on simple numerical score)	Callus at Basal end: No. of cuttings with differentiated callus (No. of roots or shoots/cutting)
Control	0	2 (1)	6 (2)	-	10 (1)	-
	7	3 (2)	2 (2)	-	8 (1)	-
	14	1 (1)	-	-	3 (1)	-
IAA, 10	0	4 (2)	9 (2)	-	10 (1)	-
	7	7 (1)	6 (3)	-	10 (1)	-
	14	4 (1)	1 (2)	-	3 (1)	-
IAA, 100	0	8 (2)	7 (1)	-	10 (2)	-
	7	6 (2)	4 (1)	-	10 (2)	-
	14	6 (3)	5 (2)	-	9 (1)	
IBA, 10	0	10 (7)	7 (2)	-	10 (3)	-
	7	8 (3)	7 (2)	-	10 (2)	-
	14	5 (4)	3 (2)	-	8 (1)	-
IBA, 100	0	10 (7)	6 (3)	-	10 (9)	-
	7	10 (14)	3 (1)	-	10 (8)	-
	14	9 (5)	-	-	10 (3)	-
TIBA, 10	0	-	6 (8)	3 (12)	9 (1)	
	7	3 (2)	8 (9)	-	9 (1)	-
	14	4 (1)	2 (2)	-	9 (1)	-
TIBA, 100	0	-	9 (6)	4 (10)	6 (1)	-
	7	1 (2)	8 (9)	-	6 (2)	-
	14	-	3 (2)	-	4 (1)	-
CA, 10	0	2 (1)	8 (2)	-	6 (1)	-
	7	7 (2)	6 (5)	-	6 (1)	-
	14	4 (2)	7 (4)	-	5 (1)	-
CA, 100	0	3 (2)	10 (4)	-	8 (1)	-
	7	4 (1)	7 (2)	-	6 (1)	-
	14	-	4 (5)	-	6 (1)	-

Callus at the Basal End

1. *Amount* Both IAA and IBA increased callus at the basal end even when the cuttings were treated at the apical end. Generally the amount decreased with a delay in the treatment. The number of cuttings callused and the amount of callus increased considerably when the initial treatment at the apex was repeated at the basal end, although the amount decreased with a delay in the timing of the treatment (Table 3).

   TIBA and CA also increased callus at the basal end when applied at the apex on day 0. This response did not change with time of application (Table 1) or when the treatment was repeated at the basal end (Table 3).

2. *Differentiation* Callus which formed at the basal end dried ultimately, without differentiation.

DISCUSSION

The results presented in this paper demonstrate that the behaviour of cambial derivatives is dependent on the rate with which different phases of cellular growth are affected by regulatory substances. One hundred mg/l IBA markedly increased cell divisional activity. The elongation of new cambial derivatives also occurred rapidly by utilization of available nutrients. As, however, the next facet of growth, the differentiation of these derivatives could not keep pace with the cell division and enlargement, undifferentiated cells accumulated in the form of callus. In marked contrast to this, a balance between cell division, cell enlargement and cell differentiation is maintained in cuttings treated with water and lower concentrations of auxin; and the cambial derivatives at the lower end of the cutting differentiate into roots with only a little callus.

An interesting point that emerges from this investigation is that the disorganised growth of cambial derivatives leading to callus formation may be caused even by TIBA and CA in which case callus is produced at the upper end of the cutting regardless

TABLE 3 - Formation and differentiation of callus on stem cuttings of Populus robusta and their rooting response when treated with 0, 10 and 100 mg/l each of IAA, IBA, TIBA and CA at the apical end initially and at the basal end after 0, 7 and 14 days. The number of cuttings set in each case was 10.

Treatment (mg/l)	Days to treatment	No. of rooted cuttings (No. of roots/rooted cutting)	Callus at Apical end: No. of cuttings with callus (Amount of callus, based on simple numerical score)	Apical end: No. of cuttings with differentiated callus (No. of roots or shoots/cutting)	Basal end: No. of cuttings with callus (Amount of callus, based on simple numerical score)	Basal end: No. of cuttings with differentiated callus (No. of roots or shoots/cutting)
Control	0	4 (2)	10 (4)	-	7 (1)	-
	7	5 (2)	8 (2)	-	9 (1)	-
	14	2 (3)	9 (3)	-	9 (1)	-
IAA, 10	0	7 (2)	10 (8)	-	7 (1)	-
	7	5 (2)	10 (3)	-	9 (1)	-
	14	3 (1)	9 (4)	-	8 (1)	-
IAA, 100	0	8 (11)	10 (8)	-	9 (8)	-
	7	9 (4)	9 (3)	-	9 (1)	-
	14	6 (2)	8 (2)	-	9 (1)	-
IBA, 10	0	10 (17)	10 (5)	-	10 (10)	-
	7	8 (7)	8 (2)	-	10 (8)	-
	14	9 (8)	7 (2)	-	10 (8)	-
IBA, 100	0	10 (6)	10 (8)	-	10 (15)	-
	7	10 (12)	10 (7)	9 (6)	10 (2)	-
	14	10 (27)	10 (7)	8 (4)	10 (3)	-
TIBA, 10	0	2 (2)	10 (12)	2 (3)	7 (2)	-
	7	7 (1)	8 (2)	2 (6)	3 (1)	-
	14	1 (3)	7 (7)	6 (11)	7 (1)	-
TIBA, 100	0	-	10 (13)	2 (2)	9 (3)	-
	7	3 (1)	10 (5)	4 (7)	10 (1)	-
	14	-	9 (3)	6 (12)	3 (1)	-
CA, 10	0	9 (4)	9 (7)	3 (4)	7 (3)	-
	7	8 (2)	9 (9)	4 (8)	10 (1)	-
	14	5 (3)	7 (6)	2 (15)	7 (1)	-
CA, 100	0	9 (4)	10 (9)	3 (3)	7 (2)	-
	7	6 (2)	8 (1)	-	10 (1)	
	14	4 (2)	10 (2)	2 (4)	10 (1)	

TABLE 4 - Formation and differentiation of callus on stem cuttings of Populus robusta and their rooting response when treated with 0, 10 and 100 mg/l each of IAA, IBA, TIBA and CA at the basal end initially and at the apical end after 0, 7 and 14 days. The number of cuttings set in each case was 10.

Treatment (mg/l)	Days to treatment	No. of rooted cuttings (No. of roots/rooted cutting)	Callus at Apical end: No. of cuttings with callus (Amount of callus, based on simple numerical score)	Callus at Apical end: No. of cuttings with differentiated callus (No. of roots or shoots/cutting)	Callus at Basal end: No. of cuttings with callus (Amount of callus, based on simple numerical score)	Callus at Basal end: No. of cuttings with differentiated callus (No. of roots or shoots/cutting)
Control	0	6 (2)	10 (4)	-	7 (1)	-
	7	2 (2)	2 (1)	-	6 (1)	-
	14	1 (2)	5 (1)	-	10 (1)	-
IAA, 10	0	7 (2)	10 (8)	-	7 (1)	-
	7	4 (2)	4 (2)	-	8 (1)	-
	14	2 (5)	5 (2)	-	9 (1)	-
IAA, 100	0	8 (11)	10 (8)	-	9 (8)	-
	7	8 (3)	6 (2)	-	10 (2)	-
	14	7 (5)	4 (2)	-	10 (3)	-
IBA, 10	0	10 (17)	10 (5)	-	10 (10)	-
	7	7 (2)	7 (2)	-	10 (2)	-
	14	7 (4)	6 (2)	-	10 (2)	-
IBA, 100	0	10 (6)	10 (8)	-	10 (15)	-
	7	8 (9)	8 (4)	-	9 (8)	-
	14	10 (4)	10 (3)	-	10 (6)	-
TIBA, 10	0	2 (2)	10 (12)	2 (3)	7 (2)	-
	7	2 (3)	9 (2)	3 (5)	9 (2)	-
	14	-	6 (5)	8 (8)	6 (1)	-
TIBA, 100	0	-	10 (3)	2 (2)	4 (3)	-
	7	-	9 (3)	3 (7)	5 (1)	-
	14	-	5 (6)	5 (4)	8 (1)	-
CA, 10	0	9 (4)	9 (7)	3 (4)	7 (3)	-
	7	4 (2)	9 (3)	8 (15)	4 (1)	-
	14	1 (3)	10 (3)	8 (19)	8 (1)	-
CA, 100	0	9 (4)	10 (9)	3 (3)	7 (2)	-
	7	2 (1)	9 (2)	-	8 (1)	-
	14	5 (2)	9 (2)	-	5 (1)	-

of which end was treated with the chemicals. Studies in progress in this laboratory have revealed that phenolic compounds if used in combination with sucrose closely resemble auxin in promoting the production of adventitious roots on hypocotyl cuttings of *Phaseolus mungo*. Without sugar, both IAA and phenolics are ineffective (Parmar, 1972). Finkle (1967) considers that there is some significant structural relationship between phenolic compounds and indole compounds. He also reported that the phenolics act as inhibitors of an enzyme system that oxidatively destroys IAA, and thus acts as a hormone. TIBA, on the other hand, is considered to affect growth by preventing the polar transport of endogenous auxins (Hay, 1956; Zwar and Rijivan, 1956; Blackman and Sargent, 1959; Winters and Musik, 1963). The formation of callus by treatment of the upper end of a cutting with phenol or TIBA is, therefore, understandable. But the point at issue is: how do these chemicals cause the production of callus at the upper end when they are applied at the basal end? An interesting observation was made in this connection. The axillary buds started developing on cuttings treated with water, TIBA

or CA but remained arrested in development on cuttings treated with 100 mg/l IBA. It would, therefore, appear that these developing axillary buds near the apex serve as a source of endogenous auxin as well as nutrients necessary to increase the division and growth of the cambial cells. The auxin level, however, is not adequate to cause differentiation and as a consequence of which the mass of cells remain disorganised resulting in the production of callus. The three growth regulatory substances, therefore, closely resemble one another in that all increase the divisional activity of the cambial cells.

The fate of the callus formed on the cuttings, however, varies with the nature of the regulatory substance. Thus, while the callus produced on cuttings treated with water or with lower concentrations of auxins remained undifferentiated, that produced by IAA or IBA differentiated into roots but that produced by phenol or TIBA differentiated into shoots. The considerable increase in the differentiation of callus into roots in the former and shoots in the latter, when the treatment is repeated, lends support to the fact that the morphogenetic changes caused by these regulatory substances differ completely.

Two questions that naturally arise are: (i) how is it that callus is formed at the upper end even on cuttings treated with water, particularly as the auxin content at this end must have decreased almost to nil due to polar transport? (ii) what is the mechanism involved in the differentiation of the same callus into roots by IBA and into shoots by phenol or TIBA? Sheldrake (1968) reported that auxin is produced by autolysing cells and that in higher plants it is produced as a consequence of the death of cells (Sheldrake and Northcote, 1968a, b, c, d). It would appear that autolysis of cells starts soon after the cuttings are made. The endogenous auxin so produced causes the division of cambial cells at the upper end. The cambial derivatives increase in size in the presence of available nutrients in the phloem, causing thereby some undifferentiated growth even on cuttings treated with water. However, due to a limited production of auxin, the callus does not increase much and dries ultimately.

The exogenous supply of a differentiating factor such as an auxin or a phenol or TIBA causes divisional activity resulting in an increase in the amount of callus. If the level of auxin increases either endogenously by autolysation of cells or by exogenous application, the cambial derivatives differentiate into xylem. Since the differentiation of cambial derivatives into xylem involves autolysation, the level of auxin increases further creating thereby a gradient of auxin from xylem to phloem across the cambium and thereby causing difference in the hormonal environment on the two sides of the cambium (Sheldrake, 1971). The phloem side has mature functional sieve elements, and thus the cambial derivatives in the lateral and longitudinal proximity of phloem are exposed to a relatively high concentration of sucrose and other translocated metabolites but to a low concentration of auxin. Those in the vicinity of xylem, on the other hand, are exposed to a high concentration of auxin and a low concentration of sucrose. Both sucrose and auxin are necessary for organised differentiation of cells. A high sucrose to low auxin ratio leads to the production of excessive phloem, while high auxin to low sucrose ratio stimulates the differentiation of cambial derivatives into xylem (Wetmore and Rier, 1963; Jeffs and Northcote, 1966; Rier and Beslow, 1967; Sheldrake, 1971; Shininger, 1971; Wodzicki, 1971). With exogenous application of IBA, therefore, the callus will be differentiated into xylem leading to the production of roots. With an exogenous application of TIBA or phenol, however, it will develop into phloem leading to the production of shoots, provided that there is an adequate supply of nutrients. The

necessity of a proper balance between nutrients and auxin for optimal production of roots on etiolated stem segments of *Populus nigra* and *Salix tetrasperma* and hypocotyl cuttings of *Phaseolus mungo* and *Impatiens balsamina* has already been shown in this laboratory (Nanda and Jain, 1971; Nanda *et al.*, 1971; Dhaliwal, 1971; Parmar, 1972). These results, thus, demonstrate that a proper balance between the two is needed not only for the production of roots but also for the production of shoots. The main difference is that while a high concentration of auxin balances with nutrition to produce roots (by differentiating the cambial derivatives into xylem necessary for vascularization of root primordia) shoot production requires differentiation of the cambial derivatives into phloem elements necessary for translocation of food.

A schematic representation of the potential mechanism that may be involved in callus formation and the differentiation of cambial derivatives into xylem and phloem leading to the production of roots or shoots is presented in Fig. 1. This seems to be a self-catalysing and self-perpetuating mechanism.

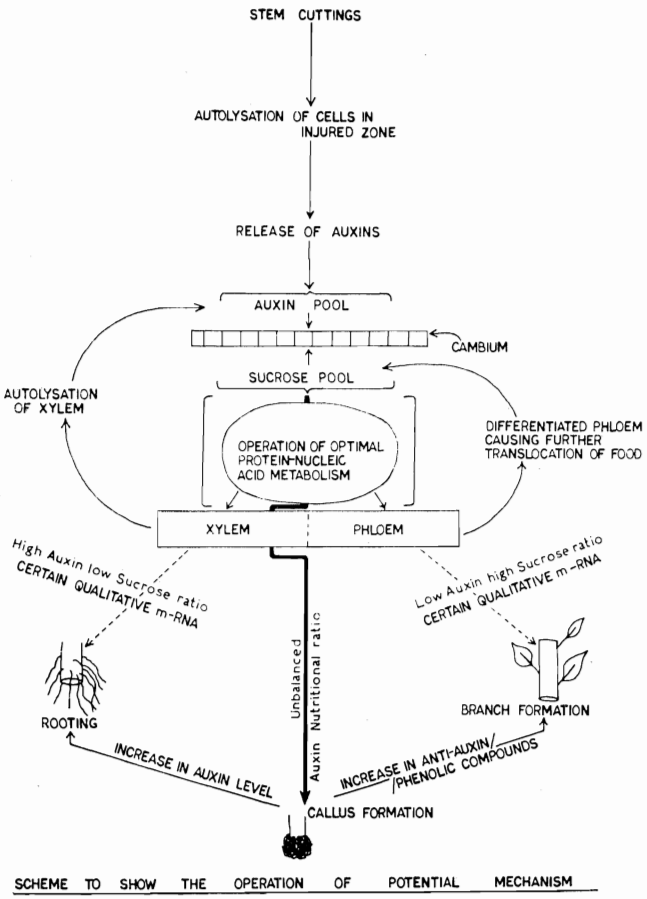

FIG. 1—Schematic representation of the mechanism proposed for the formation and differentiation of callus on stem cuttings of **Populus robusta.**

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