

Stimulation of Lateral Root Production and Bud-break with Growth Regulators in Hevea Budded Stumps

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Treatment of the tap roots of budded stumps with indolebutyric acid (IBA), α -naphthalene-acetic acid (α -NAA) and 2,3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate (Furadan) stimulated production of lateral roots. Several methods of application were tested for their effectiveness in inducing rooting in budded stumps. Among these, a modified powder method was found to be simple and effective. The method involves painting and dipping the tap root with a slurry containing IBA and other adjuvants incorporated in kaolin. The effect of spraying the tap root with α -NAA on bud-break was also investigated.

Examination of the stumps two months after application of the formulation containing IBA, potassium nitrate and N-trichloromethyl mercapto-4cyclohexene-1, 2-dicarboximide (Captan-50) showed several-fold increase in dry weight of lateral roots compared with the untreated stumps. These differences gradually decreased with time up to eleven months. At the end of the experiment, the treated stumps still gave slightly higher dry weight of roots. The growth regulators tested were most effective in promoting lateral roots especially during the initial two months after treatment. Treatment of IBA with fertilisers also enhanced root production.

A solution containing benzyladenine, Triton X-100 and dimethyl-sulfoxide (DMSO) sprayed on the bud patch of budded stumps indicated earlier bud-break compared with the controls. The treated stumps also tended to have bigger shoots than the untreated stumps.

In the early 1930s budded stumps were not transplanted directly after cutback. They were left undisturbed for ten days to allow the snag to heal and the grafted bud to be stimulated and swollen¹.

Ostendorf² studying the development of young *Hevea* buddings showed that retention of lateral roots improved the growth of buddings and reduced the mortality on transplanting. He reported that stumps transplanted with their lateral roots intact gave bud-break eight to ten days earlier than those whose lateral roots were pruned. However, he pointed out that it was difficult to transplant budded stumps with their lateral roots intact, especially on a large

scale. Morris¹ made similar studies as Ostendorf² on buddings of AVROS 50, Pilmoor A44 and Pilmoor B84 and showed that growth of these buddings transplanted with lateral roots was significantly better than growth of plants whose lateral roots were completely removed before transplanting.

Currently, budded stumps are transplanted after pruning part of the taproot and all the laterals. The success of transplanting of bare-root budded stumps and stumped buddings are largely dependent on good weather conditions. Under normal conditions bare-root budded stumps take about six to eight weeks for initiation of

lateral roots. The initial two months after transplanting are critical for the survival of the stumps and if the weather is unfavourable severe casualties could occur. Therefore, earlier initiation of lateral roots could aid survival and help to withstand adverse weather conditions for a longer period.

Growth substances which are auxenic in nature, promote initiation of roots^{3,4} and treatment of seedling roots with auxins will usually initiate a crop of laterals⁵. Romberg and Smith⁶ demonstrated that when toothpicks containing 4 mg of indolebutyric acid (IBA) were inserted into roots of five- to seven-year-old trees, there was an increase in root development compared with that of the controls. They also showed that ten-year-old nursery pecan trees can be transplanted successfully and economically when the roots are first treated with IBA-impregnated toothpicks. Baptist⁷ showed that treatment of *Hevea* seedling stumps with a proprietary growth substance in water at a dilution of 1:320 for 24 h, reduced the number of stumps lost through die-back, hastened the appearance of roots and shoots, increased the number and weight of roots and the length and weight of shoots. He also showed that the initial advantage of early establishment was maintained in subsequent growth of stumps for a period of six months after treatment.

Hafsah and Pakianathan⁸ screening a number of growth substances for root-promoting activity in *Hevea* showed that IBA, Furadan (2,3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate), α -naphthaleneacetic acid (α -NAA) and naphthalene acetamide (NAAm) were active in inducing lateral roots in budded stumps. In a more recent study⁹, it was shown that IBA formulated with kaolin, potassium nitrate and Captan-50 could be effectively used to induce rooting of budded stumps and stumped buddings.

Fungicides such as Ferbam^{10,11}, Phygon XL¹² and Captan^{13,14,15} have been shown to increase rooting and/or improve the quality of roots. In these studies, only the fungicide Captan-50 was tested for root production.

This paper reports the results of preliminary investigations on the following:

- Testing the effectiveness of various methods and formulations for induction of rooting in budded stumps
- Determining the optimum concentration of IBA for two girth classes of rootstocks
- Observing whether fertilisers further enhanced lateral root production after IBA treatment.

MATERIALS AND METHODS

All the experiments were carried out at the RRIM Experiment Station, Sungei Buloh. The stumps used in the experiments were from illegitimate seedling stocks of 6-7, 11-12 and 18-22 months old. They were either budded with RRIM 703 or RRIM 600. The budded stumps were cut back, uprooted and their lateral roots pruned. The tap root was cut at a slant at about 45 cm below the collar. Latex from the cut was peeled off before treatment. The lateral roots were trimmed to give a fresh open end to enable absorption of the growth regulators and other adjuvants. The stumps were randomised according to stock size.

Preparation and Application of Powder Formulation

With 50% commercial alcohol 1 kg refined kaolin obtained locally was made into a slurry. To give an overall concentration of 2000 p.p.m. 2 g IBA was dissolved in 10 ml absolute alcohol and added to the slurry. The alcohol ensured complete

solubility and adsorption of IBA by kaolin on drying. Acetone or other suitable organic solvents may be substituted for commercial alcohol. Appropriate amounts of potassium nitrate and Captan-50 were mixed in 10 ml of water and added to the slurry to give a concentration of 1% and 5% respectively. The slurry was stirred in a blender for 10 minutes. The mixture was then dried in an oven overnight at about 70°C. Formulations containing other IBA concentrations were also prepared by following the same procedure. The powdered formulation was made into a slurry by diluting it 2.5 times its weight with either 50% ethanol or water. The final concentrations of IBA in the slurry before treatment of stumps were 400, 800 and 1200 p.p.m. for the respective powdered formulations. In some of the experiments, the slurry was applied to the tap root using a paint brush. In other experiments, the tap roots were dipped into the slurry and the excess slurry allowed to drain off. The treated stumps were allowed to dry for 10 min and then planted into polybags containing a 1:1 mixture of Rengam series soil and sawdust. In one experiment, the stumps were planted into polybags containing only Rengam series soil.

Preparation of Growth Regulator Solutions and Method of Application

Solutions containing 2000 p.p.m. and 4000 p.p.m. of IBA were dissolved in 10 ml of absolute alcohol each and mixed with 30% solution of di-methyl-sulfoxide (Grade 1 Sigma). An equivalent of 0.1% Triton X-100 was added to the mixture to act as surfactant. The same procedure was followed to prepare 2000 p.p.m. and 4000 p.p.m. of α -NAA solution.

The tap root of each stump was sprayed with an equivalent of 2 ml of the test solution. The treated and untreated stumps were wrapped in moist gunny sacks and

kept in separate buckets at 25°C–27°C. The moisture of the gunny sacks was maintained by spraying water daily. When the root initials appeared after ten to fifteen days, the stumps were transferred into polythene bags containing 1:1 mixture of Rengam series soil and sawdust.

Application of Growth Regulator to Budpatch

To study the effect of growth regulators on bud-break, a mixture containing benzyl-adenine (Grade 1 Sigma) in 30% dimethyl-sulfoxide and containing 0.1% Triton X-100 was sprayed directly to the budpatch. Observations on bud-break were made daily for one month. Extension growth was measured at weekly intervals for three to four months.

RESULTS

Effect of Adjuvants on Lateral Root Production

An experiment was carried out to test whether Captan-50 and potassium nitrate enhanced the rooting response in the presence and absence of IBA applied at two concentration levels. The results are given in Table 1.

Dry weight measurements of roots at two months after treatment showed that application of Captan-50 alone did not induce as much growth of lateral roots as the formulation containing 2000 p.p.m. and 4000 p.p.m. IBA alone. However, in this experiment, the formulations containing IBA, Captan-50 and potassium nitrate enhanced root production to some extent.

Stimulation of Rooting in Young and Old Rootstocks with Indolebutyric Acid

This experiment was carried out to study the effect of three levels of IBA on the rooting response of two girth classes of stumps. Younger rootstocks were 6–7

TABLE 1. EFFECT OF CAPTAN-50 AND POTASSIUM NITRATE ON LATERAL ROOT PRODUCTION

Treatment	Mean dry weight of roots per plant (g)	Response (%)
Control	0.42	100
Captan-50 (5%)	0.43	102
IBA (2000 p.p.m.)	1.33	317
IBA (2000 p.p.m.) + Captan-50 (5%) + KNO ₃ (1%)	1.56	371
IBA (4000 p.p.m.)	1.60	381
IBA (4000 p.p.m.) + Captan-50 (5%) + KNO ₃ (1%)	1.64	390

Each treatment consisted of twenty-five stumps. Kaolin was used as the carrier for all the treatments.

Rootstocks were six to seven months old and were budded with RRIM 600.

months old and had a mean diameter of 1.25 ± 0.12 cm while older rootstocks were 20–22 months old and had a mean diameter of 5.0 ± 0.09 centimetres. The stumps were sampled for determination of dry weight of lateral roots at two and four weeks from initial planting. The results are given in Table 2.

Examination at four weeks after treatment showed that compared with the controls, all concentrations of IBA treatments irrespective of the age of stumps induced root production. In all treatments, maximum rooting response was observed four weeks after treatment and declined after eight weeks. In this experiment, variable responses to rooting were obtained in treatments containing potassium nitrate and Captan-50. Higher dry weight of roots was observed in the older rootstocks but the order of response was higher in the younger stumps examined at four weeks after treatment.

Effect of Indolebutyric Acid and Furadan on Rate of Root Production

The aims of this experiment were to test the root-inducing ability of IBA and Furadan and assess the relative activities of these compounds over a period of nine months. Both IBA and Furadan were applied to the tap roots of budded stumps at 1000, 2000 and 4000 p.p.m. in the absence and presence of potassium nitrate and Captan-50.

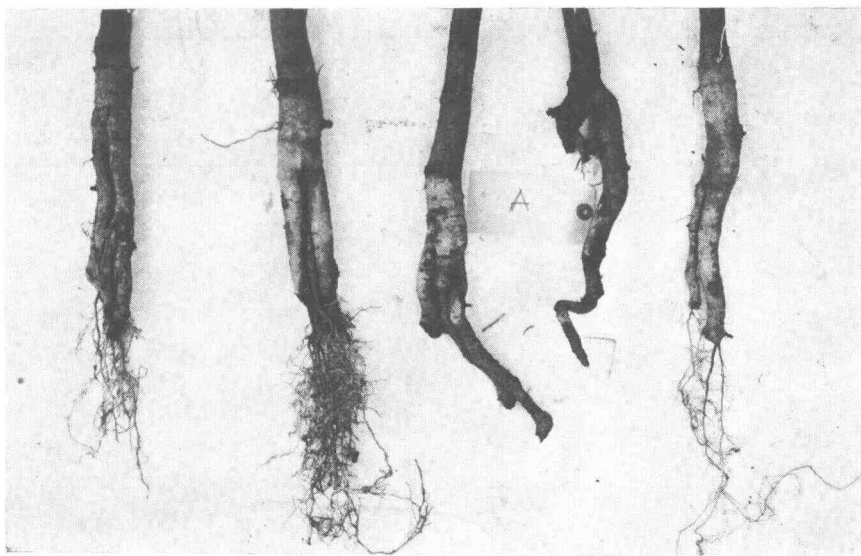
Examination of dry weights of roots two months after treatment (Table 3) indicated good rooting response with IBA at 2000 p.p.m. irrespective of the presence of potassium nitrate and Captan-50. Furadan gave positive rooting response except in one treatment when applied alone at 4000 p.p.m. it gave negative response.

Examination of root growth after five and nine months from initial application showed that the response to root production between the control and treated stumps gradually narrowed over this duration. The overall dry matter production of roots in the various treatments at the end of the experiment was marginally higher than that of the control, except in the case of treatment with 2000 p.p.m. IBA at nine months' harvest. This decrease in dry weight of roots to below the control level was due to dieback and hence smaller sample number.

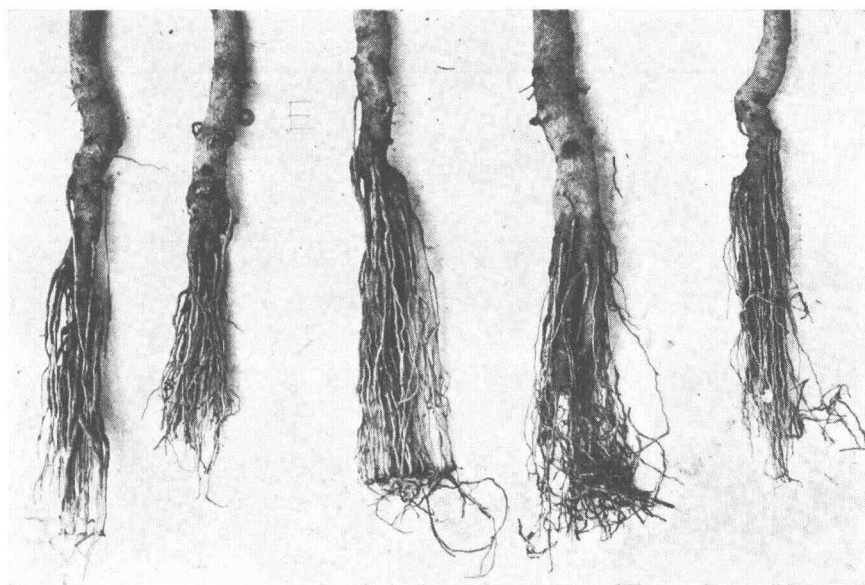
Treatment of stumps with Furadan also increased root production compared to control, but IBA was found to be more effective especially two months after application.

Effect of Spray Application of α -naphthalene-acetic Acid to Tap Root of Stumps

Figure 1 shows root development of control and α -NAA treated stumps examined five months after application. Spraying



Control



Treated with 4000 p.p.m. α -NAA

Figure 1. Effect of α -naphthaleneacetic acid and indolebutyric acid on rooting by spray method.

TABLE 2. STIMULATION OF LATERAL ROOTS IN YOUNG AND OLD ROOTSTOCKS OF BUDDED STUMPS WITH INDOLEBUTYRIC ACID

Treatment	4 weeks		8 weeks	
	Dry weight of root (g)	Response (%)	Dry weight of root (g)	Response (%)
6-7 month-old rootstocks ^a				
Control	0.3	100	7.8	100
IBA (2000 p.p.m.)	0.9	300	9.3	119
IBA (1000 p.p.m.) + KNO ₃ + Captan-50	0.9	300	8.4	108
IBA (2000 p.p.m.) + KNO ₃ + Captan-50	1.8	600	9.0	115
IBA (3000 p.p.m.) + KNO ₃ + Captan-50	2.4	800	10.2	131
20-22 month-old rootstocks ^b				
Control	1.2	100	10.1	100
IBA (2000 p.p.m.)	2.4	200	12.4	122
IBA (1000 p.p.m.) + KNO ₃ + Captan-50	2.7	225	11.2	107
IBA (2000 p.p.m.) + KNO ₃ + Captan-50	4.6	400	12.8	125
IBA (3000 p.p.m.) + KNO ₃ + Captan-50	6.2	450	13.5	132

^aEach dry weight value represents mean of fifteen plants. Mean diameter of these stumps taken 2 cm immediately below the bud-patch was 2.5 ± 0.05 centimetres.

^bEach dry weight value represents mean of fifteen plants. Mean diameter of these stumps taken 2 cm immediately below the bud-patch was 5 ± 0.25 centimetres.

TABLE 3. EFFECT OF INDOLEBUTYRIC ACID AND FURADAN ON RATE OF ROOT PRODUCTION

Treatment	Rate of root production					
	2 months		5 months		9 months	
	Mean dry wt. of roots (g)	Response (%)	Mean dry wt. of roots (g)	Response (%)	Mean dry wt. of roots (g)	Response (%)
Control	0.45	100	4.03	100	13.9	100
IBA (1000 p.p.m.)	1.42	316	4.70	117	20.1	145
IBA (2000 p.p.m.)	1.64	364	5.60	139	9.8	71
IBA (4000 p.p.m.)	1.44	320	7.60	189	13.9	100
IBA (1000 p.p.m.) + K + C	0.68	151	5.20	129	16.1	116
IBA (2000 p.p.m.) + K + C	2.38	529	5.20	129	16.1	116
IBA (4000 p.p.m.) + K + C	1.11	247	5.70	141	14.1	101
F (1000 p.p.m.)	0.66	147	5.75	143	14.8	107
F (2000 p.p.m.)	0.74	164	4.65	115	16.0	115
F (4000 p.p.m.)	0.34	76	4.30	107	14.9	107
F (1000 p.p.m.) + K + C	0.50	111	5.05	125	18.6	124
F (2000 p.p.m.) + K + C	0.62	138	3.60	89	17.3	125
F (4000 p.p.m.) + K + C	0.69	153	4.35	105	21.5	155

K = Potassium nitrate (1%)

C = Captan-50 (5%)

F = Furadan

Each sampling consisted of ten plants per treatment. Rootstocks were seven months old and budded with RRIM 703.

4000 p.p.m. of α -NAA resulted in a marked increase in the production of lateral roots, while the untreated stumps showed a large variation in root production.

In the same experiment, observations were made on the total number of root initials over a period of three weeks. The results are given in *Table 4*. It can be seen that both α -NAA and IBA induced root initiation within eleven days after treatment while the control did not induce rooting up till the twenty-first day. Both α -NAA and IBA enhanced earlier root initiation. It should also be noted that α -NAA at 4000 p.p.m. resulted in more uniform appearance of root initials *i.e.* root initials appeared in almost every stump, but the total number of root initials counted was higher with 4000 p.p.m. IBA.

Preliminary Investigations on the Effect of Indolebutyric Acid Singly and in Combination with Mag-X on Root Production

The aims of this experiment were firstly, to test whether the fertilisers initiated rooting, secondly, whether IBA-induced roots responded to application of these fertilisers and thirdly, if application influenced extension growth.

Ten grammes of Mag-X (mixed fertiliser consisting of N:P:K:Mg, 8.4:14.4:7.2:2.1)

were applied to every polythene bag. Observations were made on root and shoot production. The results of this experiment are given in *Table 5*.

The results showed that treatment of Mag-X alone did not increase the percentage of rooting of stumps. Indolebutyric acid applied at 2000 p.p.m. induced rooting in 72% of the stumps while treatment of IBA at 2000 p.p.m. plus Mag-X induced 80% rooting.

Application of Mag-X alone gave a positive response to extension growth compared with the control. Treatment of IBA alone gave an extension growth response of 266% of the control while combination of IBA and Mag-X gave a response of 352% of the control.

Effect of Christmas Island Rock Phosphate and Indolebutyric Acid on Root Production

This experiment was aimed to test if Christmas Island Rock Phosphate (CIRP) initiated rooting and/or enhanced root production of budded stumps. This fertiliser was applied at 7 g and 14 g per plant to the IBA treated and untreated stumps. Each treatment consisted of fifteen plants. The dry weights of roots of the various treatments were measured two months later. For comparison, one treatment with Mag-X

TABLE 4. EFFECT OF INDOLEBUTYRIC ACID AND α -NAPHTHALENEACETIC ACID ON ROOT INITIATION

Treatment	No. of roots		
	11 days	16 days	21 days
Control	Nil	Nil	Nil
α -NAA (2000 p.p.m.) + DMSO + Triton X-100	6	10	40
α -NAA (4000 p.p.m.) + DMSO + Triton X-100	13	13	45
IBA (2000 p.p.m.) + DMSO + Triton X-100	11	14	40
IBA (4000 p.p.m.) + DMSO + Triton X-100	32	75	80

Each treatment consisted of five plants.

was included in this experiment. The results are given in Table 6.

Generally, treatments in which IBA was included, gave a markedly higher percentage of rooting of stumps compared with all other treatments. Treatment with CIRP alone increased the number of stumps which rooted.

Application of 2000 p.p.m. IBA to the tap roots of budded stumps gave significantly higher dry weight of roots compared with the control; CIRP itself did not induce much rooting. Application of IBA at 2000 p.p.m. with 7 g or 14 g CIRP gave higher dry weights of roots compared with the treatment in which IBA was applied at 2000 p.p.m. and Mag-X at 10 g per plant.

A marked variation of response to rooting between stumps was observed (Figure 2).

Statistical Confirmation of the Effect of Indolebutyric Acid on Root Production, Extension Growth and Girdling

A large-scale trial was carried out to confirm observations of the effects of IBA on extension growth, lateral root production and girdling; each treatment consisted of 310 stumps. Benzyladenine (BA) was sprayed on to the budpatches of each stump to enhance bud-break. About 0.5 ml of the hormone solution was sprayed on to each budpatch.

Observations on bud-break were made weekly over a period of two months. The

TABLE 5. EFFECT OF TREATMENT WITH INDOLEBUTYRIC ACID AND MAG-X ON ROOT AND SHOOT PRODUCTION

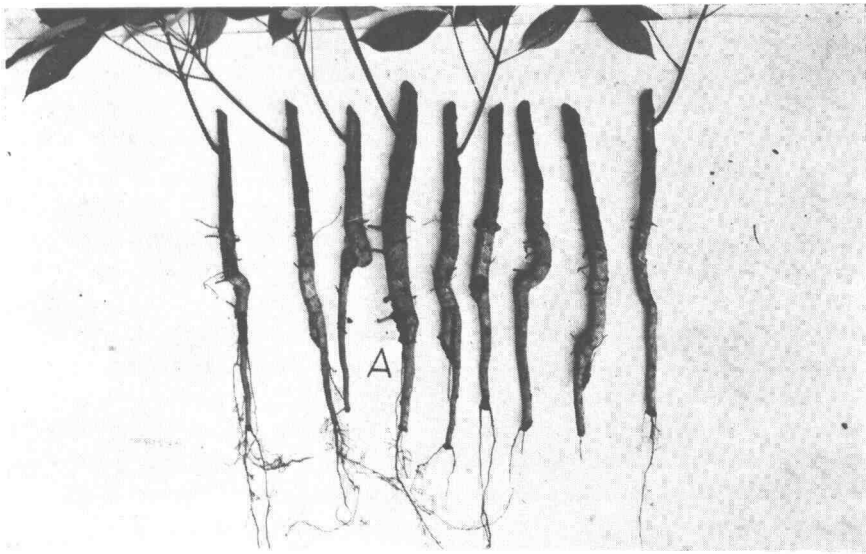
Treatment	Total no. of stumps	Stumps rooted No.	%	Extension growth cm	%
Control	14	2	14	8.9	100
Mag-X (10 g/bag)	14	3	21	17.2	193
IBA (2000 p.p.m.)	14	10	72	23.7	266
IBA (2000 p.p.m.) + Mag-X	15	12	80	31.3	352

Measurements were made two-and-a-half months after treatment. Rootstocks used in this experiment were twelve months old.

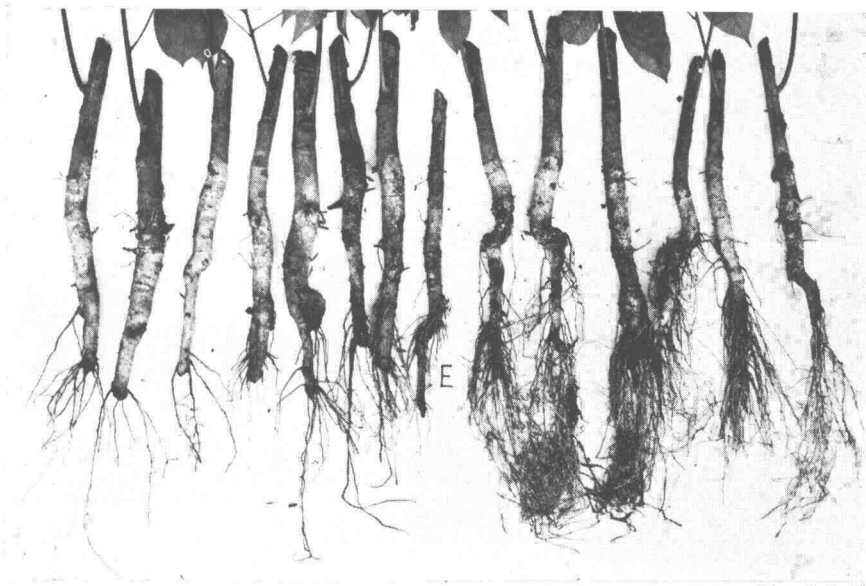
TABLE 6. EFFECT OF CHRISTMAS ISLAND ROCK PHOSPHATE ON RESPONSE TO ROOTING

Treatment	Stumps rooted (%)	Dry weight of roots (g)	Response (%)
Control	47	0.33	100
CIRP (7 g/plant)	53	0.35	106
CIRP (7 g/plant) + IBA (2000 p.p.m.)	93	0.74	224
CIRP (14 g/plant)	73	0.32	97
CIRP (14 g/plant) + IBA (2000 p.p.m.)	93	0.86	261
IBA (2000 p.p.m.)	92	0.71	215
Mag-X (10 g/plant) + IBA (2000 p.p.m.)	80	0.63	191

Sampling was made two months after treatment. Each treatment consisted of fifteen budded stumps. The rootstocks used in this experiment were six months old and budded with RRIM 703.



Control



Treated with 2000 p.p.m. IBA and 14 g per plant CIRP

Figure 2. Marked variation in rooting response to 2000 p.p.m. IBA. No variation in the control stumps.

results are summarised in *Table 7*. It can be seen that the treated stumps gave a higher percentage of bud-break after two weeks compared with the control. The increase in bud-break was found to be between 13% (second week) and 7% (two months).

Examination of the stumps after six months from initial application showed that treatment of the tap root of stumps with IBA, gave significantly higher dry weight of new laterals and extension growth compared with the control. No significant differences were observed in the girth of stems. These results are summarised in *Table 8*.

DISCUSSION AND CONCLUSION

A number of compounds were screened for root-promoting activity in budded stumps. Among these, it was found that IBA, α -NAA and Furadan were promising.

Torrey⁵ showed that treatment of seedling roots with auxins initiated a crop of laterals.

It was also shown that application of auxins to the tap root of budded stumps initiated lateral roots. The present study indicated that IBA and α -NAA were most active.

The spraying, dipping and painting methods of application of growth regulators to the tap roots of stumps were tested. Of these the dipping and painting methods were found to be simple and effective. This method involved mixing of the powdered formulation in water to a slurry of suitable consistency. The tap roots of the stumps were then either dipped into this slurry or applied with a paint brush and planted directly in the soil.

A suitable formulation was developed, which consisted of IBA, Captan and potassium nitrate. Captan was included in this formulation because several investigators¹⁰⁻¹⁴ have noted increased rooting with this fungicide. Hansen and Hartmann¹⁵ investigated the effects of IBA and Captan singly and in combination on rooting and survival of hardwood cuttings. They found

TABLE 7. OBSERVATIONS OF THE EFFECT OF BENZYLADENINE ON BUD-BREAK

Treatment	No. of bud-break				
	1st wk	2nd wk	3rd wk	4th wk	2 mth
Control	51 (17%)	193 (62%)	223 (72%)	259 (84%)	274 (88%)
BA (2000 p.p.m.)	50 (16%)	232 (75%)	254 (82%)	289 (93%)	296 (95%)

Each treatment consisted of 310 stumps.

TABLE 8. EFFECT OF INDOLEBUTYRIC ACID ON ROOT PRODUCTION, EXTENSION GROWTH AND GIRTHING

Type of measurements	Control	2000 p.p.m. IBA
Dry weight of roots (g)	9.17 \pm 4.7	11.74 \pm 5.1***
Extension growth (cm)	82.58 \pm 33.4	91.55 \pm 33.8*
Girth (cm)	1.23 \pm 0.3	1.28 \pm 0.3

*** Significant at 0.1%

* Significant at 5%

Each treatment consisted of 310 stumps. Sampling was made six months after treatment.

that mixtures of IBA with Captan resulted in a higher survival rate. However, they concluded that the increased survival rate which resulted from Captan treatment (25% wettable powder) was probably due to the protection it provided against soil-borne organisms during the rooting period rather than direct stimulation of root formation or development. However, in the present studies, Captan did not show evidence of root promoting activity. It was included in the formulation purely as a fungicide for wider use of rooting in *Hevea* and other hardwood cuttings.

Potassium nitrate was added to the formulation because it has been reported that in some cases nitrogen nutrition, as nitrate¹⁶, ammonium or amino acids, might also promote rooting^{16,17,18}. Potassium nitrate added to the formulation in some experiments acted as an adjuvant while in others, it did not enhance the effects of IBA. Earlier studies have shown that application of potassium nitrate alone did not initiate much rooting in budded stumps but only enhance further production of roots after its initiation by IBA. Part of the variability of the response to root production by potassium nitrate in the presence of IBA may be due to other factors such as age of stumps and clonal differences in rootstocks as all rootstocks in these experiments were derived from illegitimate seedlings. Another reason for this variability may be attributed to small sample size due to dieback.

Studies on the effect of rooting response with various concentrations of IBA on six- to seven-month-old rootstocks and twenty- to twenty-two month-old rootstocks showed that the optimum concentration of IBA for rooting response varied between 2000 p.p.m. and 3000 p.p.m. for both classes of stumps. Both girth classes gave higher rooting response when the adjuvants

potassium nitrate and Captan-50 were included in the formulation with 2000 p.p.m. and 3000 p.p.m. IBA. The hormone treatment induced earlier rooting and gave better rooting systems compared to control.

Preliminary experiments to test the effect of a mixed fertiliser Mag-X and rock phosphate (CIRP) singly and in combination with IBA on root number and growth, showed that CIRP increased survival of budded stumps while Mag-X did not show this effect. However, addition of Mag-X increased extension growth of scion shoots. Application of CIRP with IBA at 2000 p.p.m. increased dry weight of roots compared with IBA alone applied at the same concentration. Christmas Island Rock Phosphate did not give much increase in dry weight of lateral roots during the initial two months from application.

Cytokinins, such as BA and kinetin (KIN) have been shown to induce lateral buds to break in young *Hevea* seedlings¹⁹. Spraying 2000 p.p.m. of BA to bud-patches of budded stumps enhanced budbreak by about 13% after two weeks. This increase was half maintained over a period of two months.

The early initiation of roots could be valuable aid to earlier establishment of stumps in the field which could lead to uniform budbreak and vigorous scions. This formulation is currently being tested on a large scale.

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