CHANGES IN ROOTING FACTORS DURING THE REGENERATION OF ROOTS ON CUTTINGS OF EASY - AND DIFFICULT-TO-ROOT CULTIVARS OF *BOUGAINVILLEA* AND *HIBISCUS*

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Summary

A significant decrease in auxin level was noted during the course of root formation in the easy-to-root cuttings of *Bougainvillea glabra* cv Partha and *Hibiscus rosa-sinensis* cv My Beauty. No significant auxin activity could be detected in the cuttings of the difficult-to-root materials, *B. glabra* cv Formosa and *H. rosa-sinensis* cv Sweet Heart, either initially or during root formation in the presence or absence of indolebutyric acid (IBA). The activity of the mung bean rooting factors which was higher in the easy-rooting materials, decreased during the regeneration of roots and the reduction was more in the cuttings treated with IBA. In the easy-to-root materials, the three phenolics, *p*-hydroxybenzoic acid, ferulic acid and *p*-coumaric acid ocurred in appreciable concentrations but in the difficult-to-root ones only *p*-hydroxybenzoic acid was present in high concentrations; the other two phenolics were not present in detectable amounts. It has been shown that the above-noted phenolic acids were responsible for the coleoptile straight growth inhibition as well as the mung bean rooting activity between Rfs. 0.35-0.50 (in isopropanol : water, 8 : 2 v/v) of the chromatograms of the easy-to-root materials.

Introduction

The rooting of cuttings from a number of herbaceous plants has been found to be related to the presence or accumulation of free auxins in the bases of cuttings (Odom and Carpenter, 1965). Although the rapidity and vigour in root formation paralleled the presence or accumulation of acidic auxins, no consistent correlation could be made between the inhibitors and the rooting response of the cuttings. Taylor and Odom (1970), however, suggest that the presence of inhibitory substances appears to play a significant role in the rooting complex of pecan stem cuttings. According to Turetskaya *et al.* (1970), the process of rooting depends on the auxin-inhibitor ratio. During the rooting of black poplar cuttings the amount of auxins increased at first, reaching the maximum value after the emergence of roots but the level decreased thereafter. Although the level of gibberellin-like substances rose during root growth, the level of the growth inhibitors decreased markedly during the process of root formation (Michniewicz and Krissel, 1970).

The differences in the natural rooting potentiality of cuttings have been suggested to be due, at least in part, to the occurrence of endogenous root-promoting substances (Hess, 1968). A comparison of the changes in endogenous growth promoters, inhibitors and root-promoting substances during the regeneration of roots on cuttings from easy-and difficult-to-root plants is, therefore, expected to give a better understanding of the role of the different substances in adventitious root formation. Attempts have been made in the present study to correlate actual root formation with the changes in the coleoptile straight growth promoters and inhibitors and mung bean root-promoting substances during the course of root formation of *Hibiscus* and *Bougainvillea*, employing an easy-to-root and a difficult-to-root cultivar of each species.

MATERIALS AND METHODS

The materials used were *Bougainvillea glabra* cv Partha (easy-to-root) and cv Formosa (difficult-to-root) and *Hibiscus rosa-sinensis* cv. My Beauty (easy-to-root) and cv. Sweet Heart (difficult-to-root).

In the case of *Bougainvillea*, 25 cm long leafless cuttings from one year old shoots were used. The cuttings of *Hibiscus* were made from the upper portion of the shoot and 4 leaves (including the tip) were retained on each cutting. Half of the total number of cuttings of each cultivar were treated with IBA (6000 mg/l in talc) and the other half with talc only (control) before planting in sterile sand beds under polythene cover in a propagation house. The root-forming region of the cuttings (4 cm of the basal portion of each cutting) was sampled initially (before planting), and at different stages of regeneration of roots after planting for the assay of the endogenous auxins and other rooting factors. The stages were: S_1 – initial on the day of planting; S_2 – 4 days after planting; S_3 – 6 days after planting and S_4 – 10 days after planting. S_2 corresponds to early and S_3 late root initiation and S_4 to root emergence. Data on root formation was collected 45 days after planting. Fifty cuttings were employed for each treatment at each stage of sampling. The root-forming regions were cut into small pieces and 30 g material in each case was extracted with absolute ethanol at 0°C (Kawase, 1965). The extracts were fractionated with peroxide-free ether into four fractions, viz. (i) free neutral (ii) free acidic (iii) bound neutral and (iv) bound acidic, following in principle the methods of Kefford (1959). Ascending chromatography was done on Whatman No. 3 filter paper strips (25× 2.5 cm) in cylindrical glass jars (Nitsch, 1956). Usually, isopropanol : water (8 : 2 v/v) was employed as the solvent. Chromatography was done in the dark at a temperature of 22 ± 1°C and a total run of 20 cm was allowed after which the paper was air-dried and used for bioassay.

The wheat coleoptile straight growth test (Mer *et al.*, 1962) was adopted for the growth promoters and inhibitors and the mung bean rooting test (Hess, 1964) was used for the rooting factors. The chromatogram sections were eluted with sucrose-phosphate buffer (0.01 M KH_2 PO_4 with 2% sucrose) for the wheat coleoptile test and with phosphate-phosphate buffer (0.01 M, pH 5.0) for the mung bean rooting test. The tests were repeated three times and the data were analysed statistically.

The identification of indole and phenolic compounds on the chromatograms, was done by cochromatography with synthetic chemicals, UV-fluorescence and absorbence and by the use of different chromogenic reagents (viz., Ehrlich's reagent, diazotized *p*-nitroaniline, diazotized sulphanilic acid and $FeCl_3$).

Results

Rooting response.—The easy-and difficult-to-root cultivars of *Bougainvillea* and *Hibiscus* showed marked variation in their response to

Table 1. Rooting of Bougainvillea *and* Hibiscus *cuttings (data taken 45 days after planting)*

	Percentage of cuttings rooted		Mean number of roots per cutting		Mean dry wt of roots per cutting (mg)	
	Control	IBA	Control	IBA	Control	IBA
Bougainvillea						
Partha	70	100	14.5	52.5	62	190
Formosa	0	20	—	6.1	—	45
Hibiscus						
My Beauty	65	100	8.0	19.6	108	285
Sweet Heart	0	20	—	3.5	—	66

IBA (Table I). IBA greatly promoted the rooting of easy-to-root varieties. The beneficial effect of IBA was evident even in the difficult-to-root cultivars as 20% of these cuttings also rooted.

Coleoptile straight growth.—The biological activities of different fractions, as measured by the growth of wheat coleoptile sections, are presented in Figs. 1 and 2.

Bougainvillea : The bio-histograms of the easy-rooting *Bougainvillea* cv Partha showed the presence of a growth-promoting substance between Rf 0.3–0.4. This has been found to be identical with β-indoleacetic acid as was evident from the chromatographic resolution in different solvent systems and colour reactions with Ehrlich's reagent. In the control Partha cuttings, the level of the auxin was not altered much till S_3 stage, but the level dropped significantly at the time of root emergence. The growth-promoting zone was also noted in IBA treatment but the level dropped sharply at S_3 stage and was absent altogether at S_4 stage. In the IBA treatment, another promoting zone between Rf 0.5–0.6 was noted at S_2 and S_3 stages, the identity of which could not be established. The difficult-to-root *Bougainvillea* cv Formosa did not show any promoting zone on the chromatogram of the free acid fraction of control, but in IBA-treated cuttings a promoting zone between Rf 0.5–0.6 was noted in this cultivar. The chromatograms of the free acid as well as the bound acid fractions showed extensive inhibiting zones. In the easy-rooting Partha, the inhibiting zones in the free acid fraction of the control cuttings, were more extensive than in the difficult-to-root Formosa. It was of interest to note that in the latter cultivar IBA tended to increase the level of the free acidic inhibitors. The chromatograms of the bound acid fractions of both the cultivars revealed the presence of extensive growth inhibitory zones and in either case the level of the inhibitors decreased during the period from S_1 to S_4 stage in control as well as in IBA-treated cuttings.

Hibiscus : The sequence of changes in the levels of growth-promoting substances in the two *Hibiscus* cultivars was similar to that noted in *Bougainvillea.* In *Hibiscus* also, the level of the acid auxins in the easy-rooting My beauty decreased during regeneration whereas in the difficult-to-root Sweet Heart no significant growth promoting zone could be noted. IBA treatment resulted in an increase in the level of the free acidic inhibitors in Sweet Heart. The bound acidic inhibitors decreased in concentration during the regeneration of both the *Hibiscus* cultivars.

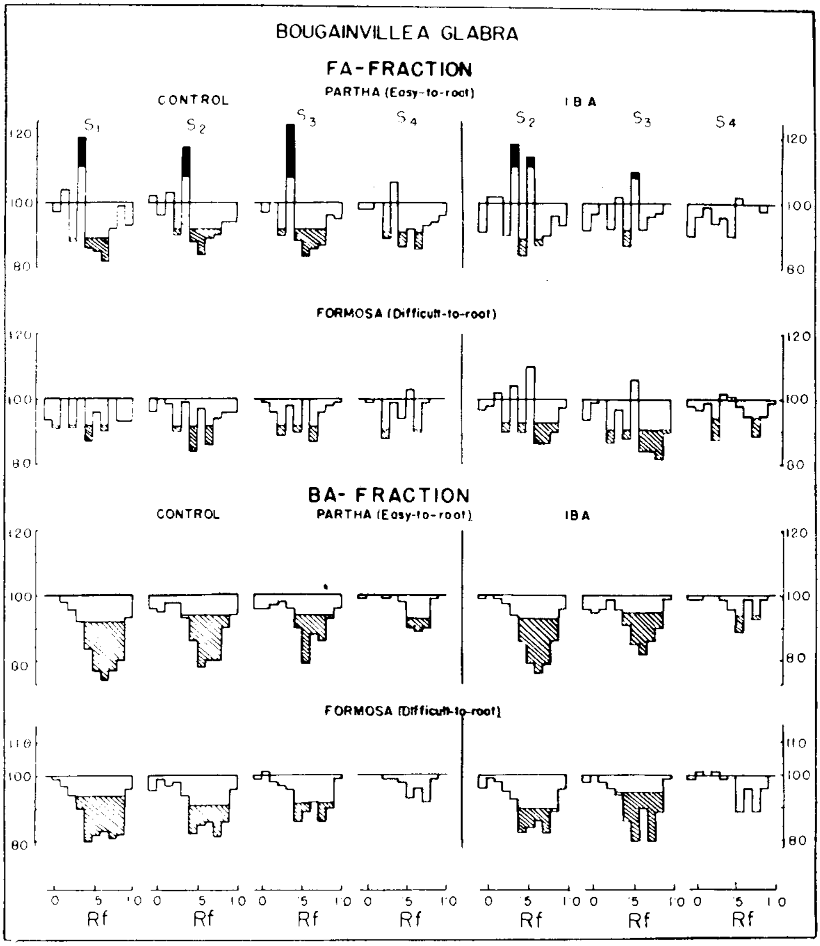

FIG. 1. Histograms showing the bioactivity at different Rf values of chromatograms, as determined by the wheat coleoptile straight growth test, in free acid (FA) and bound acid (BA) fractions of the extracts of root-forming region of easy-and difficult-to-root, *Bougainvillea* cuttings.

Values transformed to percentages of respective controls (blanks).

Shaded areas denote significance at 0.05 p.

Stages of sampling: S_1, S_2, S_3 and S_4 corresponding to 0 (initial), 4, 6 and 10 days respectively after starting the treatments.

Solvent used for ascending chromatography, isopropanol : water (8 : 2 v/v) ; loading 2.5 g fresh wt equivalent.

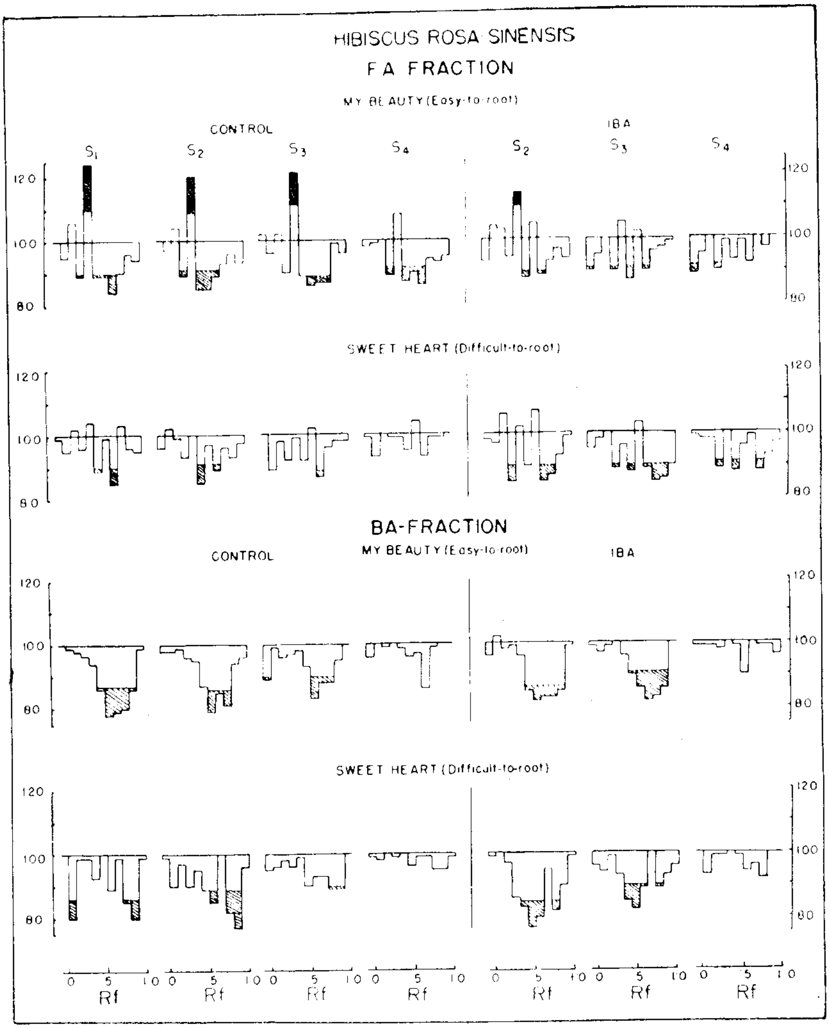

FIG. 2. Histograms showing the bioactivity at different Rf values of chromatograms, as determined by wheat coleoptile straight growth test, in free acid (FA) and bound acid (BA) fractions of the extracts of root-forming region of easy-and difficult-to-root *Hibiscus* cuttings.

Other details same as in Fig. 1.

Rooting factors.—The activity of the free acid and bound acid fractions of the *Bougainvillea* and *Hibiscus* cultivars in mung bean bioasay is shown in the biohistograms in Figs. 3, 4, 5 and 6. There was no significant activity of rooting factors in the neutral fractions (both free and bound) of the extracts.

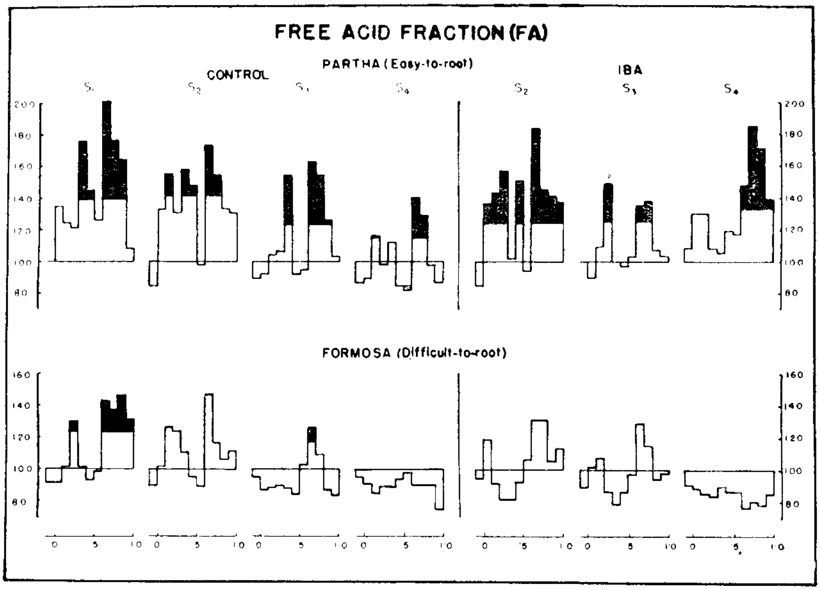

FIG. 3. Histograms showing the bioactivity at different Rf values of chromatograms, as determined by the mung bean rooting test, in free acid (FA) fraction of the extracts of the root-forming region of easy-and difficult-to-root *Bougainvillea* cuttings.

Other details same as in Fig. 1.

Bougainvillea : Three peaks of the activity of rooting factors were noted in the chromatograms of the free acid fractions of easy-to-root Partha, between Rfs. 0–0.2, 0.3–0.5 and 0.6–1.0 (Fig. 3). The third zone was usually more prominent of the three. In the control cuttings, there was a decrease in the activity of rooting factors through the stages of regeneration, but with IBA treatment (although there was a significant decrease in rooting activity at S_3), the third rooting zone significantly increased at the S_4 stage. In the difficult-to-root Formosa, the level of the rooting factors was low but here too there was a decrease in the level of rooting factors through all the stages in the control cuttings (Fig. 3). With the application of IBA, although there was no significant variation of the rooting factors, a tendency towards a decrease in the activity was noted. The bound acid fraction showed a more or less similar pattern as in the case of free acid fraction (Fig. 4). However, the decrease in the

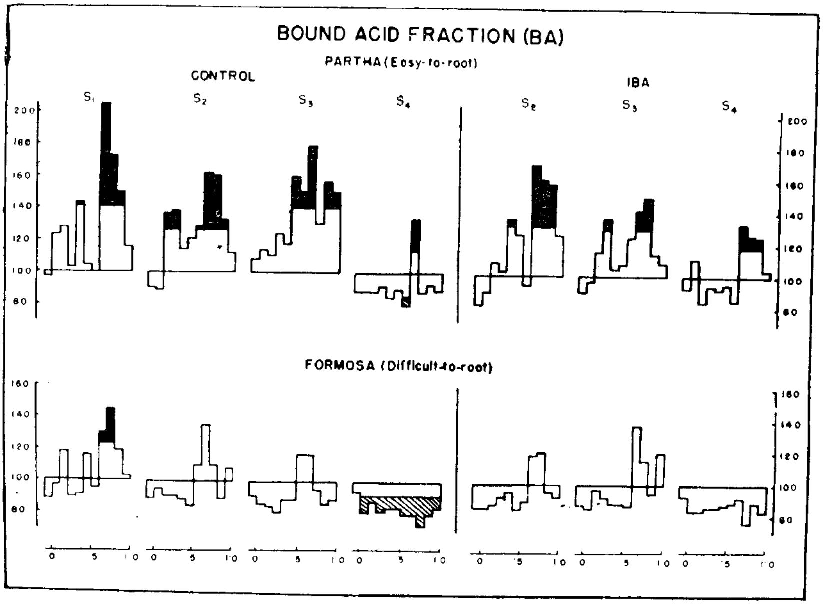

FIG. 4. Histograms showing the bioactivity at different Rf values of chromatograms, as determined by the mung been rooting test, in bound acid (BA) fraction of the extracts of the root-forming region of easy-and difficult-to-root *Bougainvillea* cuttings.

Other details same as in Fig. 1.

activity of rooting factors in the IBA-treated Partha cuttings was gradual, there being no increase in the level of the rooting factors at S_4 as was noted in the free acid fraction. In the difficult-to-root Formosa, the level of bound rooting factors was much lower compared to Partha. Nevertheless, the rooting factors tended to decrease in concentration through S_1 to S_4 stages.

Hibiscus : The rooting factors in the free acid fraction of easy-to-root My Beauty decreased in concentration in both the control and IBA-treated cuttings during the course of regeneration of roots (Fig.5). In the final stage (S_4), the chromatograms were charaterized by the presence of a number of root inhibitory zones in both the treatments. In the difficult-to-root Sweet Heart also, there was a decrease in the level of rooting factors in both control and IBA-treated cuttings. This cultivar, however, initially showed a lower activity of rooting factors than the easy-rooting My Beauty.

The bound acid fraction of the two *Hibiscus* cultivars showed a sequential decrease in the levels of the rooting factors similar to those

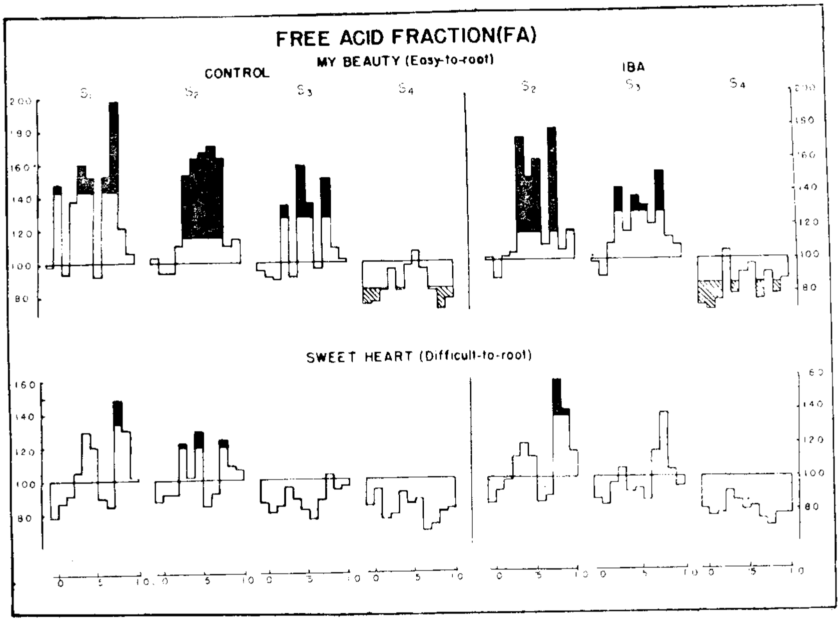

FIG. 5. Histograms showing the bioactivity at different Rf values of chromatograms, as determined by the mung bean rooting test, in free acid (FA) fraction of the extracts of root-forming region of easy-and difficult-to-root *Hibiscus* cuttings.

Other details same as in Fig. 1.

noted with the free acid fraction (Fig. 6). But in this fraction, the second zone of activity of the rooting factors (Rf 0.4) was more prominent than the third zone in the case of My Beauty. In Sweet Heart, the third zone was more prominent than the second but the overall activity was much lower compared to My Beauty.

As pointed out earlier, the coleoptile straight growth-promoting regions of the chromatograms (Rf 0.3–0.4) in the free acid fractions of the easy-to-root cultivars of *Bougainvillea* and *Hibiscus* corresponded to that of synthetic IAA. Chromatography in different solvent systems coupled with studies on the fluorescence and absorbence patterns in UV-light and colour reactions with diazotized *p*-nitroaniline and diazotized sulphanilic acid have shown the presence of ferulic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid in the extracts of the easy-rooting cultivars of *Bougainvillea* and *Hibiscus*. In the difficult-to-root cultivars of the two species, only *p*-hydroxybenzoic acid was detected in sufficient concentrations.

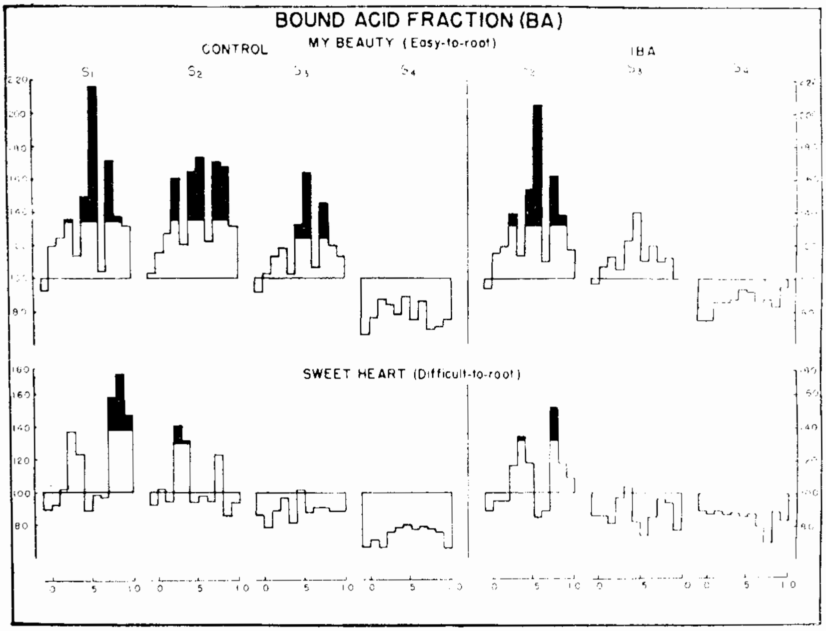

FIG. 6. Histograms showing the bioactivity at different Rf values of chromatograms, as determined by the mung bean rooting test, in bound acid (BA) fraction of the extracts of root-forming region of easy-and difficult-to-root *Hibiscus* cuttings.

Others details same as in Fig. 1.

DISCUSSION

The results presented in this paper show the accumulation of free auxins in the root-forming regions of cuttings during the regeneration of roots. On the other hand, there was a significant decrease in the auxin level during the rooting of the easy-to-root cuttings. These results suggest that during the process of adventitious root formation the auxin is metabolized and converted to a form which is no longer bioactive. A decrease in auxin level during the regeneration of roots has also been noted by Kawase (1965). Stoltz and Hess (1965) in their studies on the effect of girdling of the shoot on the rooting of cuttings of easy-and difficult-to-root *Hibiscus* cultivars, noted that the auxin concentration decreased below the original level 10 days after girdling and suggested that the reduction could be due to decrease in synthesis, destruction or metabolism. As the girdling of shoot is highly beneficial for the rooting of cuttings, these results further imply that the accumulation of auxin may not be a crucial factor in regeneration of roots.

The higher concentrations of free acidic growth inhibitors in the easy-to-root cultivars of *Bougainvillea* and *Hibiscus* in comparison to the difficult-to-root cultivars of either species, and the marked fall in the levels of the inhibitors during the course of root formation, are considered highly significant from the point of view of root regeneration. The fall in the levels of the bound inhibitors was, however, noted in both the easy-to-root as well as difficult-to-root cultivars, implying that the change may not be related to actual root formation. While such a supposition can not be ignored, the close coincidence of the activity of rooting factors and the activity of growth inhibitor activity, both of which decreased during rooting, indicates that the inhibitory substances may be involved, at least in part, in the process of adventitious root formation. Moreover, the level of the free acidic inhibitors showed a greater decrease in IBA-treated easy-to-root materials, suggesting their greater utilization in the root-forming processes. The three phenolic compounds *p*-hydroxybenzoic acid, *p*-coumaric acid and ferulic acid, which were identified in the easy-rooting cuttings, have been found to act as auxin-synergists in a number of materials (Basu, 1969 ; Basu, 1970 ; Bose *et al.*, 1971). The monophenolic compounds, however, act as inhibitors of straight growth and antagonize the auxin-induced extension growth of coleoptile sections. An examination of the biohistograms of coleoptile growth-promoting and inhibiting activity and the activity of rooting factors shows that the phenolic compounds, singly or in combination, were most likely responsible for the observed coleoptile straight growth inhibition as well as mung bean root promotion between Rf. 0.35–0.50 (in isopropanol : water, 8 : 2 v/v) of the chromatograms of the free acid fractions of the extracts. The absence (or presence in very small concentration) of ferulic acid and *p*-coumaric acid would explain the relatively low level of growth inhibitor activity and low rooting factor activity in the extracts of the difficult-to-root materials. The rooting factor activity of the second zone was due to the above-noted phenolic acids, but the first (Rf. 0.0–0.2) and third zone (Rf 0.6–1.0) could not be chemically characterized. The third zone might reveal the presence of abscisic acid which acts as a strong mung bean root-promoting chemical (Basu *et al*, 1968 ; Basu *et al* , 1970 and Chin *et al*, 1969). No attempt has, however, been made in the present investigation to characterise abscisic acid in the extracts.

The mode of action of the rooting factors in regeneration is yet to be elucidated. How they are metabolised during the course of regeneration, resulting in a decrease in their concentration, is also not known ? It needs to be mentioned that while the polyphenolic synergists may be oxidized to quinones, through the action of the polyphenol oxidases, the above-noted monophenols are highly resistant to oxidation. Additional hydroxylation of the molecules through the action of hydroxylating enzymes may, however, make them more susceptible to biological oxidation (Poapst and Durkee, 1967). We have practically no information on these lines and further experimentation might elucidate the metabolic pathways of the monophenols during the process of adventitious root formation.

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