

CORRELATIVE INFLUENCE OF SEEDLING AGE, COTYLEDONS AND TERMINAL BUDS ON ADVENTITIOUS ROOT FORMATION IN STEM CUTTINGS OF MUNG BEAN

Abdullah Ibrahim Shaheed

Department of Biol., Coll. of Sci., Univ. of Babylon, P. O. Box 4, Hilla, Iraq

ABSTRACT

Rooting response in stem cuttings of mung bean increased considerably with increasing seedling age, due to endogenous IAA or supplied IBA. However, after the day 7- or 8-old of seedling age. The cotyledons shrivel and drop-off spontaneously at day-8 of seedling age. So that cotyledons excision after cuttings were made during the period between seedling emergence (the day 4) and cotyledons dropping off (which starts at day 8 and its completion at day 10) causes decrease in rooting at any time during cutting treatment, in particular, at zero time. In addition, results of this study revealed that terminal buds do not influence significantly adventitious root formation whether IBA supplied or not. Whereas in leafless cuttings, excision of terminal buds at any time enhance rooting of cuttings specially at zero time, compared with its presence. The correlative role of cotyledons and terminal buds as a source of endogenous IAA and rooting co-factors and their influence on seedling development and subsequently on rooting response of cuttings derived from them. In addition to role of leaves on uptake and subsequent transport of supplied IBA have been discussed.

Key Words: Adventitious roots, Auxin, Stem cuttings, Seedling age, Cotyledons, Terminal buds, Correlative phenomena.

INTRODUCTION

The promotory effect of leaves and buds on adventitious root formation (ARF) has been published earlier by Van der Lek (1925). In contrast, decapitation and disbudding of terminal bud in Pea cuttings have inhibitory effect on root formation (Eriksen, 1973). These organs are considered as a source of IAA biosynthesis (Moore, 1969). Furthermore, Eriksen and Mohammed (1974) showed that auxin treatment of cuttings is capable of substituting for the effect of leaves and buds together partially or completely. On the other hand cotyledons also influence rooting and its excision from young seedlings of sunflower inhibit root formation (Fabijan *et al.*, 1981). However, Varga and Lenart (1974) have been suggested that auxin may be synthesized by cotyledons. It appears that leaves, cotyledons and terminal buds influence ARF in terms of supply of both auxin and nutritional factors (Katsumi *et al.*, 1969; White and Lovell, 1984). Consequently the work presented here assesses the importance of the correlative influence of these vegetative organs on ARF of mung bean cuttings as a response to endogenous IAA or exogenously supplied IBA.

MATERIALS AND METHODS

Growth of Stock Plants:

Seeds of mung bean (*Phaseolus aureus* Roxb. Var. Local) were soaked overnight in running tap water. Germination was done in fine granular sowedust moistened with tap water in small plastic trays (37x27x5 cm) perforated with small holes in the bottom. Each small tray contained over 70 seeds in 6 rows each of 12 seeds which were covered with a layer of

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sowdust 1 cm deep. Each tray was placed in a large plastic tray (43x31x8 cm) containing 3 liters of tap water. The level of water in the large tray was maintained by daily addition after emergence of seedlings.

Seedlings were grown for 10 days in growth cabinet at 25±1°C under continuous irradiance supplied by warm white fluorescent tubes (64-66 Lux) and a relative humidity of 65-70%.

Preparation of Cuttings:

Stem cuttings were prepared according to Hess (1961) from 10-day-old light-grown seedlings having one pair of expanded primary leaves, a small apical bud, the entire epicotyl and 3 cm of the hypocotyl after removal of the root system. Cuttings were employed in one experiment with different physiological age (4-day-old seedling) with the same characteristics mentioned above. In all experiments cuttings were held under the conditions employed to raise the stock seedling.

Basal treatment of cuttings:

Indole butyric acid (IBA) was initially dissolved in absolute ethanol to which d/H₂O was added to prepare the required stock solution. Ethanol was present at a final concentration of 2 ml/L. At these concentrations ethanol has no significant effect on rooting of mung bean cuttings (Middleton *et al.*, 1978a).

For rooting tests twelve cuttings were used per treatment by placing 4 per glass vial (7.5x1.3 or 3.7x1.3 cm) containing 3 cm depth (=15 ml) of the appropriate solution for 24 hr, after which they were transferred to vials containing 15 ml of boric acid (10 µg/ml). This level was maintained by daily addition of d/H₂O. A supply of borate is essential for formation of root primordia, as well as subsequent root growth (Middleton *et al.*, 1978b). Root numbers were determined 6 days after transfer of cutting to borate on a rooting period.

Excision of cotyledons and terminal buds:

Cotyledons and terminal buds were excised from their bases with a fine forceps during different time intervals as mentioned in each experiment.

Determination of root number and statistical analysis:

At the end of rooting period which was 6 days after transfer of cuttings to boric acid, root numbers were calculated. The mean root number was presented in terms of standard error (S. E.) of the mean root number per cutting for all treatment according to (Spiegel, 1975).

RESULTS AND DISCUSSION

Data presented in figure 1 shows the rooting response of cutting to different concentrations of IBA. Few roots developed (14.9±2.2) when cuttings are not treated with IBA. Presumably those few roots which were formed in absence of exogenous auxins attributed to endogenous IAA and other hormones in their initiation. However, when cuttings are treated with IBA then transferred to boric acid, substantially increased the number of roots per cutting. The lowest concentrations of IBA employed, 10⁻⁸ to 10⁻⁶ M, had no effect on the number of roots per cutting compared with the control treatment, but the rooting response increased markedly with increasing conc. of IBA up to 10⁻⁴ M. At this concentration the no. of roots per cutting was approximately 78, this represent 5 folds that of the control. With further increase in conc. of IBA to 10⁻³ M, rooting response was totally inhibited, probably because of wilting and decaying of cuttings during the initial 24 h of auxin treatment as observed. Studies in this field (e.g. Haissig, 1974) have been emphasized the importance of auxins and their control on root formation in cuttings compared with all chemicals tested for their bioassay. However, Middleton *et al.* (1980) suggested that the major effect of IBA is in the leaves such that synthesis of endogenous IAA is promoted. Shaheed (1987) confirmed such results by supplying IBA to the base of cuttings, which enhanced basipetal transport of C¹⁴-IAA from primary leaves to the root initiation zone (Hypocotyl). The latter worker showed that root

formation on the hypocotyl inhibited by local application of polar transport inhibitors such as Triiodobenzoic acid (TIBA) and Morphactin when applied 3 mm above the base of epicotyl (coteledonary nodes). Such treatments inhibit the accumulation of C^{14} -IAA in hypocotyl when C^{14} -IAA applied to primary leaves as described by Morris *et al.* (1969) for movement of auxin from young leaves and apex.

Figure 2a shows the rooting response of cuttings in relation to seedling age in absence of supplied IBA. Root no. increase with increasing seedling age from which cutting were taken between 4- and 8-day old of seedling age, which may be attributed to the presence of cotyledons as a source of nutritional factors (Vargan and Lenart, 1974). Or coincides with expansion of primary leaves and may be related to high conc. of auxin which occurs during leaf enlargement (e.g. Wetmore and Jacobs, 1953). The decrease in rooting response of cuttings taken from older seedlings particularly after the day eight, could be resulted from a limited supply of auxin from the aging primary leaves (Middleton *et al.*, 1980). Or may be associated with cotyledons when shrivels and drop-off spontaneously during the period between 8- and 10-day old of seedling age. It represents the senescence phase of cotyledons characterized by depletion of nutritional storage (Mohammed and Al-Mashhadani, 1976). The storage carbohydrate and protein of cotyledons is considered as essential prerequisite during initial growth of primary leaves and roots (Lovell and Moore, 1970). Alternatively, the development of 1st trifoliated true leaf in older seedlings/cuttings may create a metabolic sink for competition on nutritional factors with developing roots (Shaheed, 1995).

In the presence of supplied IBA as shown in figure 2b, the rooting response and its relation to seedling age does not differ from the general trend that observed in absence of IBA (See figure 2a) except the marked increase in root number in all treatments as a response to supplied IBA. Generally, these foregoing findings confirm that obtained by Jarvis and Booth (1981) on stem cuttings of *Phaseolus aureus* Roxb, cv. Berkin.

The influence of cotyledons excision on root formation is shown in figure 3. Cuttings were taken from 4-day-old stock plants to study this phenomenon as a special case for two reasons. First, because emergence of seedlings needs 4 days after seed germination (Sowing time) , according to the environmental factors mentioned in metrology. Second, cotyledons shrivels and drop-off spontaneously needs at least 8 day after sowing. On these bases, this phenomenon were studied during the time interval between the day 4 and 8 after sowing time. So, in absence of IBA , data presented in figure 3a shows that cotyledons excision reduce rooting response at any time during the 1st 72 h after cuttings were made, such reductions increases when cotyledons were excised earlier . Excision of cotyledons at zero time after cutting were made reduce root no., of approximately 55% compared with its excision 72 h later (the root no. reduce from 10.1 ± 0.8 to 4.5 ± 0.2). When cotyledons lasting 72 h on cutting , the latter gives better rooting compared with any previous time for excision. This time associates with visible root emergence (72 h after cuttings were made) and emphasizes the necessity of cotyledons as a source of nutritional factors for root primordial development . Furthermore , the decrease of rooting response at 96 h and there after coincide with the day 8 (4-day- old seedlings + 96 h after cuttings were made = 8 days), the time of cotyledons shrivels and drop-off spontaneously whether on cuttings or on seedlings before cuttings were taken. The correlative influence of cotyledons during this time on seedling growth and subsequently on ARF on cuttings has been discussed above in figure 2a,b.

In presence of IBA (figure 3b), the influence of cotyledons excision on rooting response of cuttings supplied with IBA has the same general trend as that dependent on endogenous IAA except that the response in terms of root number is higher due to IBA application for all treatments. The response was continued approximately, in the same average, after 72 h, up to the end of experimental period. These results indicate that exogenous auxin is capable of substituting for cotyledons that already dropping off, which considered as a source of auxin biosynthesis (Cotyledons are dropping off cuttings at 96 h and beyond). Such results are in

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agreement with Fabijan *et al.* (1981) who mentioned that cotyledons affect root formation and its removal from young seedlings of sunflower inhibit rooting although they used different plants. However, Vargan and Lenart (1974) have been suggested that auxin considered as one of the endogenous cofactors, which synthesized by cotyledons, and the latter could be substituted by auxin treatment.

Table.1: The influence of terminal buds on rooting of mung bean cuttings in presence or absence of supplied IBA.

Time of terminal bud excision	Mean root No./cuttings± S. E.		Coefficient of variation %	
	-IBA	+IBA	-IBA	+IBA
No excision (control=144 h)	17.8±3.4	27.0±4.7	19.1	17.4
Zero-time	17.8±2.5	28.6±4.0	14.0	13.9
24 h later	19.9±3.5	24.7±3.7	17.5	14.7
48 h later	16.4±2.7	25.8±4.8	16.3	18.6
72 h later	18.3±3.3	28.6±6.7	18.0	23.4
96 h later	22.7±4.6	31.0±2.8	20.2	9.0

Table.1 shows the relation between terminal buds excision and rooting of stem cuttings. In absence of exogenous IBA, excision of terminal buds at any time after cuttings has no significant effect on number of roots developed. The mean root number between (16.5±2.7) and (22.7±4.6) per cutting for all treatment.

In presence of supplied IBA, the rooting response was increased in all excision times, compared with its absence (Table 1). Such response had the same trend and has no significant differences. The foregoing findings indicate that the terminal buds had no influence on ARF in stem cuttings of mung bean in presence or absence of supplied IBA particularly in presence of primary leaves. The importance of leaves in root formation does not only attributed to produce leaf-cofactors which are important in rooting as suggested by Audus (1963), but correlatively, leaves may control not only the uptake of auxin supplied to the base of cuttings through transpiration stream, but also its reloading into an appropriate transport system to the hypocotyl after its accumulation in leaves as proposed by Jarvis and Shaheed (1986).

It is noteworthy that in case of leafless cuttings taken from 10-day-old seedlings, after dropping off cotyledons at day-8, is as shown in figure 4. The presence of terminal buds alone on stem cuttings until the end of rooting period (control) have inhibitory effect on rooting. Whereas the root number increased when the terminal buds excised from cuttings at any previous time. However, no. of roots increased when terminal buds were excised earlier. For instance, the root no. when terminal buds were excised at zero time is (3.4±0.3) compared with presence of terminal bud to the end of experiment, 144 h (control, 1.9±0.3). These results are in agreement with Biran & Halvey (1973). They shows that excision of growing terminal buds (vegetative or reproductive) from cuttings of *Dahlia* increase their rooting response. The latter workers suggested that growing buds may effect root formation in two opposed direction. The first, inhibit rooting through their competition with developing roots on metabolites. The second, promote rooting by enhancing of cambial activity.

Data presented above suggested that although the presence of terminal buds on cuttings are suitable for rooting as demonstrated by (Bachelard and Stow, 1963), but terminal buds have no influence on rooting at least in mung bean cuttings in presence of primary leaves, since cotyledons have been shrivels and drop-off from 10-day-old seedlings before cuttings were taken. Alternatively, terminal buds may retain some of IAA synthesized in leaves, hence

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reduce the amount transported basipetally to hypocotyl (regeneration zone) (Shaheed unpublished data). Such retention should create metabolic sink in the terminal meristem distinguished by a high rate of growth by competing with the root for metabolites. Consequently more critical approaches to the precise roles of cotyledons and terminal buds in ARF with specific experimental system will be the subsequent study.

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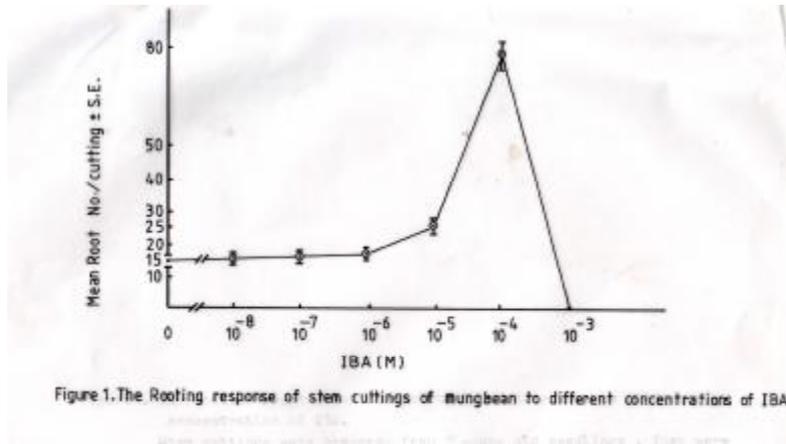


Figure 1. The Rooting response of stem cuttings of mung bean to different concentrations of IBA.

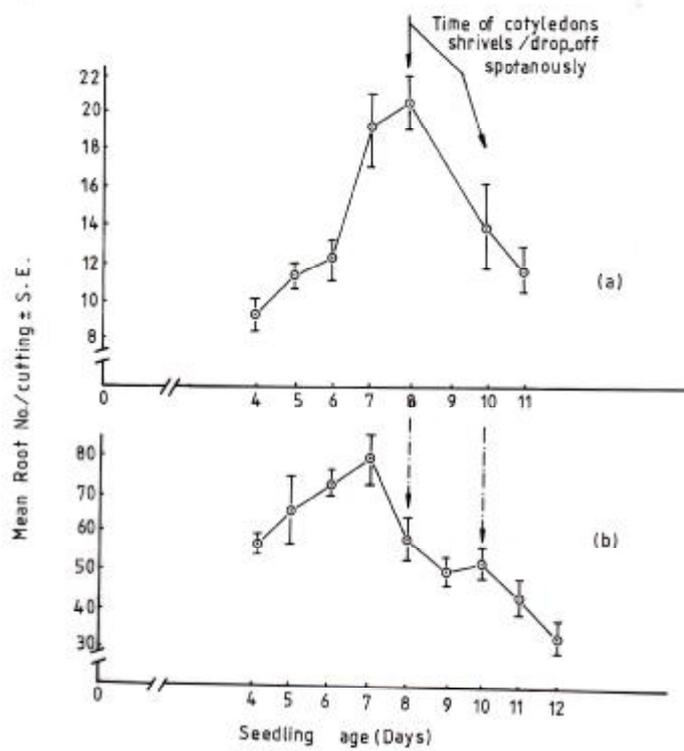


Figure 2. The influence of seeding age on rooting response of stem cutting of mung bean, (a) IBA (b) +IBA.

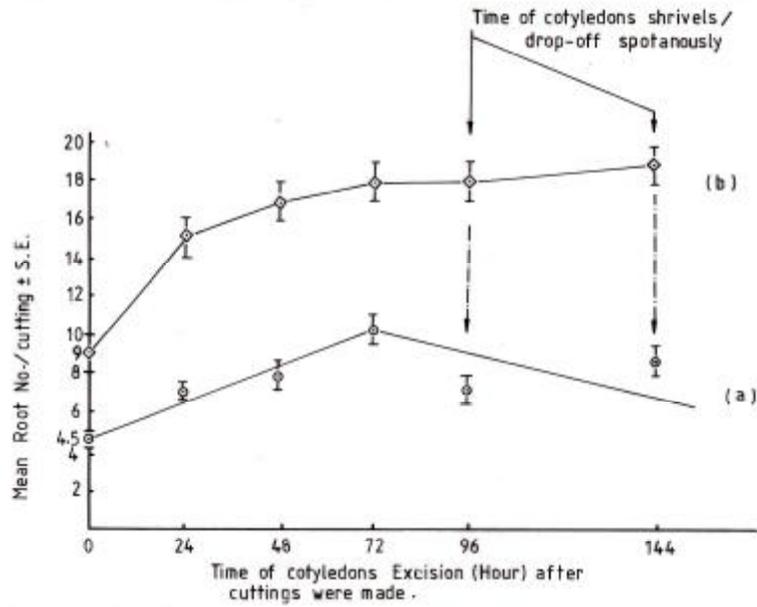


Figure 3. The influence of cotyledons on rooting response of mung bean cuttings in absence (a) or presence (b) of IBA.

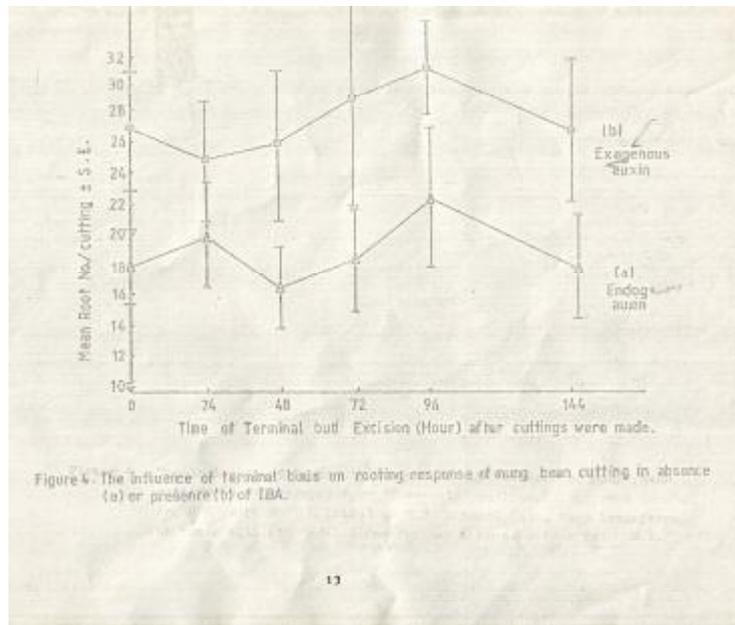


Figure 4. The influence of terminal buds on rooting response of mung bean cutting in absence (a) or presence (b) of IBA.

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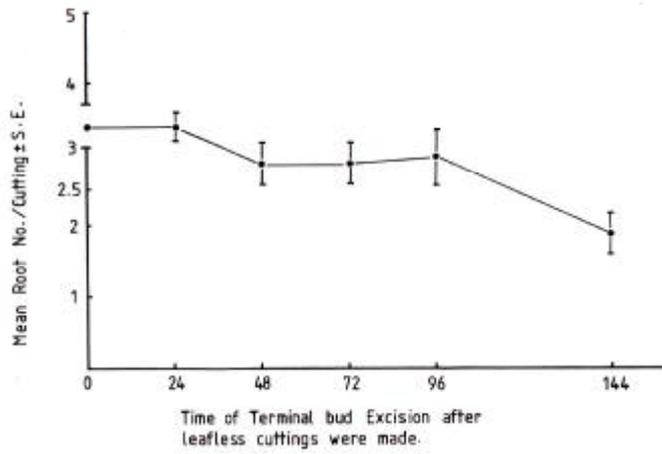


Figure 5. The influence of terminal buds on rooting response of mung bean leafless cuttings due to endogenous IAA.