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POLYAMINES AND ROOT FORMATION IN MUNG BEAN HYPOCOTYL CUTTINGS

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Abstract

The role of polyamines in the control of root formation in mung bean hypocotyl cuttings was investigated. Polyamine seems to be involved in the regulation of root formation in mung bean cuttings. This conclusion was based on the observations that (a) exogenous application of polyamines promoted root formation, (b) L-arginine and L-ornithine, precursors of polyamine biosynthesis, promoted root formation, (c) analogs of precursors of polyamine biosynthesis inhibited root formation, (d) methylglyoxal-bis (guanyl hydrazone) (MGBG), which blocks polyamine biosynthesis by inhibiting S-adenosylmethionine decarboxylase, inhibited root formation, (e) β-hydroxyethylhydrazine, an inhibitor of polyamine oxidase, promoted root formation.

The polyamine effect in enhancing root formation is not dependent on exogenous supply of boron, but dependent on its presence in leaves.

Key words: Auxin; *Phaseolus aureus*; polyamines; rooting; spermidine; spermine.

Introduction

It has long been recognized that polyamines are synthesized in higher plants (Smith, 1971). Recent studies, including our own, indicated that polyamines may play significant role in regulating plant senescence (Altman, 1982; Cheng and Kao, 1983; Cheng *et al.*, 1984; Cohen *et al.*, 1979; Kaur-Sawhney and Galston, 1979). Auxin-induced root formation requires cell division and appears to involve delaying or reversal of senescence process (Middleton *et al.*, 1980). Furthermore, exogenous application of arginine, a precursor of polyamine synthesis, was found to be effective in promoting root formation of some cuttings (van Overbeek *et al.*, 1946). It, therefore, seems that polyamines may be involved in the control of root formation in cuttings. Recent work of Friedman *et al.* (1982) and Jarvis *et al.* (1983) suggested that polyamines were involved in root formation in cuttings.

The present work was undertaken to study the effect of polyamines on root formation in hypocotyl cuttings taken from light grown seedlings of mung bean (*Phaseolus aureus* Roxb.).

Materials and Methods

Seeds of mung bean (*Phaseolus aureus* Roxb. cv. Tainan Select 3), generously provided by AVRDC, were sterilized by sodium hypochlorite for 20 min prior to sowing in vermiculite moistened with water. Seedlings were raised at 27°C under continuous light by white fluorescent tubes at an irradiance of 16.7 Wm^{-2}. Unless otherwise stated, hypocotyl cuttings were made from 10-day-old seedlings and consisted of an apical bud, a pair of primary leaves, epicotyl and 3 cm hypocotyl. Where was necessary primary leaves were excised by severing the petiole as close to the epicotyl as possible by means of a razor blade. Eight cuttings, two per vial, were used per treatment. Freshly prepared hypocotyl cuttings were introduced into glass vials containing test solution which covered the entire hypocotyl for 24 h and were then transferred to deionized water or boric acid (10 μg/ml) for subsequent six days. Subjected to the period of seven days, cuttings were maintained in the same condition used to raise seedlings. Rooting ability was expressed by the number of roots per cutting.

Results and Discussion

The effect of polyamines on root formation in mung bean hypocotyl cuttings is shown in Table 1. Polyamines, spermindine and spermine, effectively increased the number of roots per cutting. Friedman *et al.* (1982) failed to promote the root formation in mung bean cuttings by exogenous application of polyamines. Jarvis *et al.* (1983) reported that spermine stimulated the number of roots developed per cutting only when the mung bean cuttings were subsequently transferred to boric acid. Middleton *et al.* (1978) also showed that no roots developed in cuttings from

Table 1. *The effect of polyamines in root formation in mung bean cuttings*

Treatment		Number of roots/cutting
Initial 24 h	Subsequent 6 days	
Water	Water	6.7±0.4
Water	Boric acid (10 μg/ml)	7.2±0.5
Spermidine, 10^{-4} M	Water	19.0±2.4
Spermidine, 10^{-4} M	Boric acid (10 μg/ml)	24.7±3.3
Spermine, 10^{-4} M	Water	15.5±1.0
Spermine, 10^{-4} M	Boric acid (10 μg/ml)	14.8±1.8

light-grown mung bean seedlings without exogenous boric acid, irrespective of auxin pretreatment. It seems to suggest that boron is prerequisite for the polyamine- or auxin-induced root formation in mung bean cuttings. However, our results showed that root formation promoted by polyamines or indole-3-butyric acid (IBA) treatment was not dependent on exogenous supply of boron (Table 1 and Fig. 1), suggesting that the cuttings used in our work contained sufficient endogenous level of boron. For the subsequent experiments, only the results of those cuttings treated with test solution for 24 h and then transferred to water for subsequent 6 days were reported.

Since polyamines are synthesized in plants from L-arginine and L-ornithine, the effect of these precursors and their analogs (D-arginine and α-methyl ornithine) on root formation was studied. L-arginine and L-ornithine effectively promoted root formation in cuttings, although the latter was far more effective than the former (Fig. 2). Analogs of polyamine biosynthesis precursors significantly inhibited root formation in mung bean cuttings (Table 2). Similarly, methylglyoxal-bis (guanyl hydrazone) (MGBG), which blocks spermidine and spermine biosynthesis by inhibiting S-adenosylmethionine decarboxylase (Raina *et al.*, 1981), also inhibited root formation in mung bean cuttings in the presence or absence of IBA (Table 3). All these results seem to suggest that spermidine and spermine are involved in root formation in cuttings. The involvement of polyamines in regulating root formation was further supported by the observation that β-hydroxylethylhydrazine (HEH) effectivelly promoted root formation (Table 4). HEH is known to inhibit polyamine oxidase (Kaur-Sawhney *et al.*, 1981; Shih *et al.*, 1982), which catalyzes the conversion

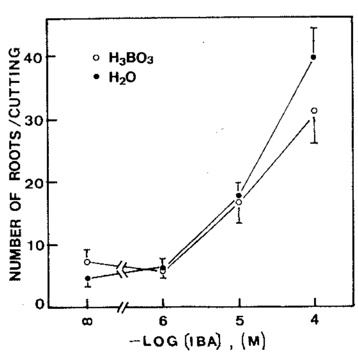

Fig. 1. The effect of IBA on root formation in mung bean cuttings. Cuttings were treated with IBA for 24 h and were then transferred to deionized water (●) and boric acid (10 μg/ml) (○), respectively, for subsequent 6 days.

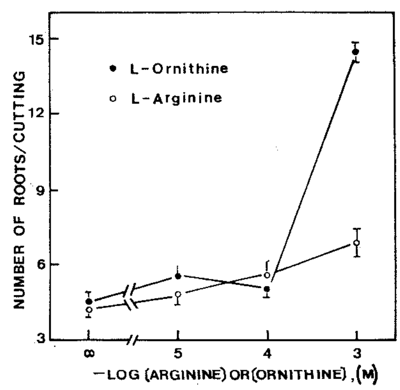

Fig. 2. The effect of L-arginine and L-orthinine on root formation in mung bean cuttings. Cuttings were treated with L-arginine and L-orthinine for 24 h and were then transferred to deionized water for subsequent 6 days.

Table 2. *The effect of D-arginine and α-methyl ornithine on root formation in mung bean cuttings*

Cuttings were treated with D-arginine and α-methylornithine for 24 h and were then transferred to deionized water for subsequent 6 days.

Treatment	Number of roots/cutting
Control	10.8±1.8
D-arginine, 10^{-5} M	7.3±1.3
10^{-4} M	6.9±0.9
α-methylorthinine, 10^{-5} M	7.6±1.9
10^{-4} M	7.3±1.1

Table 3. *The effect of MGBG on root formation in mung bean cuttings treated with or without IBA*

Cuttings were treated with MGBG, IBA, MGBG+IBA for 24 h and were then transferred to deionized water for subsequent 6 days.

Treat	ment Number of roots/cutting
Control	10.8±1.8
MGBG, 10^{-4} M	6.1±1.2
IBA, 10^{-4} M	39.3±3.6
IBA, 10^{-4} M + MGBG, 10^{-4} M	32.0±3.3

Table 4. *The effect of DAP and HEH on root formation in mung bean cuttings*

Cuttings were treated with DAP and HEH for 24 h were then transferred to deionized water for subsequent 6 days.

Treatment	Number of roots/cutting
Experiment I	
Control	5.3±0.3
DAP, 10^{-6} M	6.6±0.6
10^{-5} M	4.8±0.3
10^{-4} M	6.3±0.7
Experiment II	
Control	10.8±1.0
HEH, 10^{-5} M	10.0±4.2
10^{-4} M	14.3±0.9

Table 5. *The effect of leaf removal on root formation in mung bean cuttings*

Cuttings were treated with IBA, spermidine and spermine for 24 h and were transferred to deionized water for subsequent 6 days. The concentration of IBA, spermidine and spermine is 10^{-4}M.

Treatment	Part excised	Number of roots/cutting
Water	None (control)	4.8±0.5
Water	One leaf	3.6±0.1
Water	Both leaves	0.3±0.1
IBA	None (control)	34.0±2.9
IBA	One leaf	25.4±1.6
IBA	Both leaves	2.3±0.1
Spermidine	None (control)	10.3±0.4
Spermidine	One leaf	7.4±0.9
Spermidine	Both leaves	2.3±0.3
Spermine	None (control)	11.4±1.6
Spermine	One leaf	7.5±0.4
Spermine	Both leaves	1.8±0.8

of spermidine or spermine into 1,3-diaminopropane (DAP). Since DAP did not affect root formation (Table 4), the promotion effect of HEH in inducing root formation may possibly be caused by the accumulation of endogenous polyamines in hypocotyl.

When primary leaves of mung bean cutting were excised, the effect of polyamine- or IBA-induced root formation decreased significantly (Table 5). It is apparent that the effect of polyamines and auxin is dependent on the presence of the leaves, suggesting that a supply of rooting factors from leaves to hypocotyl is required for root formation. Middleton *et al.* (1980) also found that the promotion of rooting by IBA occurred only when leaves are present. Current work in this laboratory is aimed at elucidating the roles of leaves in polyamine-dependent rooting of mung bean cuttings.

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Polyamines 與綠豆子葉下軸根形成關係之研究

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本試驗主要探討 palyamines 對綠豆子葉下軸發根之影響。試驗結果顯示 polyamines 具有控制發根之能力。主要之證據爲(一)外加 polyamines 如 spermidine 與 spermine 促進發根，(二)合成 polyamines 之前驅物，L-arginine 與 L-ormithine 均可促進發，(三) L-arginine 與 L-ornithine 之類似物如 D-arginine 與 α-methylornithine 抑制發根，(四) polyamines 合成之抑制劑 MGBG 抑制發根，(五) polyamine oxidase 之抑制劑如 β-hydroxyethylhydrazine 促進發根。結果亦顯示 polyamine 促進發根之效應，不需外加硼，但需葉片之存在。