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Influence of ABA, Gibberellin and Kinetin on IAA Induced Adventitious Root Development on Hypocotyl Cuttings of Mungbean

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Abstract: GA_3 dissolved in 0.2% ethanol inhibited the adventitious root formation in mungbean cuttings. Conversely, when it is dissolved in water, GA_3 promoted the adventitious rooting response at 10^{-7} and 10^{-8} M concentrations. Ethanol suppressed the promoting effects of GA_3. Generally, it has been observed that low concentration of GA_3, favour rooting and high concentration inhibit it. Furthermore no evidence of synergism between supplied GA_3 and IAA was observed. Kinetin, at low concentration and in the presence of IAA promoted rooting response. ABA at 10^{-5} M and in the presence of IAA promoted adventitious root formation. The stimulatory effects of ABA was more pronounced at 5×10^{-4} concentration of ABA.

Key words: Dventitious roots, gibberellins, kinetin, indole acetic acid, hypocotyl cutting

Introduction

Plant growth regulators are capable of either inhibition or promoting adventitious root regeneration although the reason of this paradoxical situation is obscure. With respect to ethylene, ABA, gibberellins and cytokinins both promotory and inhibitory effects on adventitious root formation have been reported. This is occasionally so even where the same species has been used by different investigators. For example Mullins (1972) reported that ethylene inhibited root formation in cuttings of mungbean, whereas Roy and Basu (1972) described stimulatory effects of ethylene. Likewise in pea cuttings, gibberellic acid has been reported to inhibit (Brian ***et al.***, 1960) and stimulate (Adhikari and Bajracharya, 1978) adventitious root formation. Such contradictory results may reflect the different experimental conditions and the concentration of the supplied chemicals used by various research workers (Yasmin ***et al.***, 1993, Soomro ***et al.***, 1994). The present investigation was undertaken to examine the effects of various concentrations of GA_3, ABA and Kinetin in the presence of IAA on rooting response of stem cuttings of ***Vigna radiata***. Some of the experiments described here have investigated the effect of GA_3, ABA, kinetin in the presence of supplied auxin IAA.

Materials and Methods

Seed germination and preparation of stem cuttings has been described elsewhere, (Yasmin ***et al.***, 1997). Seeds of mungbean were surface sterilized by submergence in a filtered solution of 4% (W/V) calcium hypochlorite for 15 minutes. They were rinsed thoroughly, then soaked overnight in running tap water. Seeds were sown onto vermiculite for 10 days in distilled and deionized water. Seedlings were rinsed and grown throughout at 25^{0}C under continuous light from warm white fluorescent tubes giving the intensity of light 2000 Lux. Cuttings of mungbean consisted of an apical bud 3 cm hypocotyl, entire epicotyl, a pair of cotyledons and a pair of primary leaves.

Preparation of test solutions

Solutions of IAA, GA_3, ABA and kinetin were prepared by first dissolving them in absolute ethanol, then adding distilled and deionized water to the required volume. Appropriate concentrations of auxins and ethanol are stated in the legends to tables.

Results

Effect of GA_3

Since ethanol is often used, for convenience, to dissolve GA_3 its influence on GA_3-mediated rooting was determined prior to further experimentation of the GA_3-auxin interaction in rooting. When GA_3 was dissolved in ethanol it proved slightly inhibitory to rooting at 10^{-6} M and more so at 10^{-5} M (Table 1). At 10^{-7} and 10^{-8} M, GA_3 was without effect on the mean number of roots developing per cutting. In the absence of ethanol, 10^{-5} GA_3 was similarly inhibitory to the rooting response. However, the two lowest concentrations of GA_3 tested, namely 10^{-7} and 10^{-8} M, significantly stimulated root regeneration (Table 1). Clearly 2 ml L^{-1} ethanol which is without effect on auxin induced rooting (Middleton ***et al.***, 1978a), masks a stimulatory influence of GA_3 which is evident in the absence of ethanol (Table 1). When dissolved in ethanol GA_3, between 10^{-5} and 10^{-8} M, was without significant effect on mean root length (Table 1). However, in the absence of ethanol, GA_3, inhibited root growth at all concentrations tested, with the most inhibitory effect evident at the lowest concentration employed, 10^{-8} M.

Interaction between GA_3 and IAA

The influence of GA_3, in the presence of IAA on rooting is shown (Table 2). Approximately 10 roots/cutting developed in cuttings treated with 3×10^{-4} M IAA. The presence of GA_3 during the auxin treatment stimulate the number of roots developing per cutting at all concentrations of GA_3 employed. Maximum rooting occurred when GA_3 was supplied at 10^{-4} M, approximately 19 roots per cutting being recorded. In the presence of IAA, GA_3 was without influence on root growth (Table 2).

Table 1: The influence of GA_3 on adventitious root development

Treatments	Mean number of roots cutting^{-1}	Mean root length (mm)
2% ethanol	13.2±1.4	8.8±0.8
10^{-5} M GA_3	5.1±0.9	8.2±0.8
10^{-6} M GA_3	7.0±1.4	7.7±0.5
10^{-7} M GA_3	11.5±2.2	7.5±0.7
10^{-8} M GA_3	13.0±1.7	7.9±0.8
Water	10.1±1.2	9.7±0.6
10^{-5} M GA_3	7.9±11.7	7.8±10.8
10^{-6} M GA_3	11.4±1.9	7.6±0.4
10^{-7} M GA_3	17.8±2.4	7.5±0.7
10^{-8} M GA_3	16.2±1.6	6.8±0.6

Table 2: The influence of GA_3 and IAA in adventitious root development

Treatments	Mean number of roots cutting^{-1}	Mean root length (mm)
3×10^{-4} M IAA	9.7±1.0	8.7±1.1
3×10^{-4} M IAA+10^{-5} M GA_3	17.9±2.8	8.5±0.8
3×10^{-4} M IAA+10^{-6} M GA_3	19.1±3.8	8.5±1.0
3×10^{-4} M IAA+10^{-7} M GA_3	17.4±3.5	9.5±1.1
3×10^{-4} M IAA+10^{-8} M GA_3	14.6±3.3	8.0±1.3

Table 3: The interaction between ABA and IAA in adventitious root development

Treatments	Mean number of roots/cutting	Mean root length (mm)
3×10^{-4} M IAA .	9.7±1.0	8.7±1.1
3×10^{-4} M IAA+5 $\times$ 10^{-4} M ABA.	30.1±2.7	2.9±0.7
3×10^{-4} M IAA+10^{-4} M ABA	28.6±5.4	6.3±0.6
3×10^{-4} M IAA+10^{-5} M ABA	18.3±3.1	8.6±0.8
3×10^{-4} M IAA+10^{-6} M ABA	12.0±2.5	10.2±0.5

Data are presented ±95% confidence limits

Interaction between ABA and IAA

A marked promotion of rooting was evident when ABA was supplied at higher concentrations in the presence of 3×10^{-4} M IAA. (Table 3). The greatest stimulation of the root formation was evident with the highest concentration of ABA employed, 5×10^{-4} M and rooting response diminished with decreasing concentration of ABA, although all concentrations employed except 10^{-6} M, were stimulatory. The increased number of roots developed in response to high level of ABA supplied with IAA, was much greater than those resulting from treatment with ABA alone (Yasmin ***et al.***, 1993).

ABA supplied at 5×10^{-4} M and 10^{-4} M in the presence of IAA proved inhibitory to root growth as evident by root growth (Table 3). The inhibitory effects of ABA decreased with decreasing

Table 4: The interaction between Kinetin and IAA in adventitious root development

Treatments	Mean number of roots cutting^{-1}	Mean root length (mm)
3×10^{-4} M IAA	9.7±1.0	8.7±1.1
3×10^{-4} M IAA+10^{-5} M Kinetin	14.0±2.8	8.7±0.8
3×10^{-4} M IAA+10^{-6} M Kinetin	16.7±3.8	8.2±0.5
3×10^{-4} M IAA+10^{-7} M Kinetin	12.5±2.0	7.9±0.6
3×10^{-4} M IAA+10^{-8} M Kinetin	11.4±2.4	7.7±0.8

Data are presented ±95% confidence limits

concentration and in one instance where 10^{-6} M ABA was supplied with 3×10^{-4}M IAA, a prominent stimulation in the root length was observed. In the presence of IAA, ABA was the most inhibitory to root growth but it was most promotory to the number of root developed per cutting.

The interaction between kinetin and IAA

Cuttings treated with 3×10^{-4} M IAA prior to transfer to boric acid eventually produced approximately 10 roots/cutting (Table 4). Kinetin supplied with IAA enhanced the number of roots developed when used at 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M concentrations. The largest number of roots per cutting resulted from treatment with IAA in the presence of 10^{-6}M kinetin. Here approximately 17 roots per cutting developed. The only concentration of kinetin without significant effect on rooting response was 10^{-8}M.

There was no significant effect of kinetin on root growth at any of the concentrations employed. This is in contrast with the inhibitory influence of kinetin on root growth when supplied in the absence of supplied IAA or IBA (Yasmin ***et al.***, 1993).

Discussion

Gibberellins (GA_3) dissolved in ethanol was either inhibitory or without any effect on adventitious root development. In the absence of ethanol, however, GA_3 promoted rooting significantly at 10^{-7}M and 10^{-8}M. Clearly ethanol suppressed the potential promotory effects of GA_3 (Table 1). The promotory effects of GA_3 could be explained in the light of previous reports which suggests increased auxin production in axillary meristem (Eriksen, 1971; Anand ***et al.***, 1972). Alternatively, GA_3, could increase mobilization of reserve food material as reported by Nanda ***et al.*** (1968), Mertz (1966) and Anand ***et al.*** (1972). Adhikari and Bajracharya (1978) suggested that an appropriate combination of GA_3 and auxin is necessary for root initiation. Data presented here clearly shown that at 10^{-5} and 0^{-6} M concentrations of GA_3 employed, comparatively few roots developed compared to number induced by IAA. (Tables 1 and 2). Furthermore no evidence of synergism between supplied GA_3 and IAA was observed. The number of roots induced by 10^{-7} M GA_3, alone was essentially the same as that induced in the presence

of supplied IAA (Table 2). However, a marked synergism between GA_3 at 10^{-7} and 10^{-8} M was observed in the presence of 5×10^{-6} M IBA (Jarvis and Yasmin, 1987). Clearly the rooting response was controlled by both the concentrations of supplied GA_3 and auxin. The results described here concerning the effect of supplied GA_3 on rooting do relatively little to unreveal the apparent complexities inferred by previous contradictory reports (Batten and Goodwin, 1978). Nevertheless several points need to be stressed. First the use of ethanol readily facilitate the dissolving of GA_3 can mask the relatively small promotory effect of GA_3 encountered where GA_3 is supplied at 10^{-7} or 10^{-8} M. Secondly, GA_3 supplied with IBA is most effective in stimulating rooting when IBA concentration is well below its own optimum concentration for initiating root formation (Jarvis and Yasmin, 1987). Thirdly, high concentration of GA_3 (10^{-5} M) is inhibitory to rooting whether supplied alone or with IAA. Fourthly, the concentrations of GA_3 which is themselves, are without influence on root number or even inhibitory, may enhance the rooting response to supplied auxin.

In general low concentrations of GA_3 favour rooting whereas high concentrations are inhibitory. Broadly similar result were obtained with supplied kinetin with the exception that no stimulatory effect of kinetin was recorded when it was supplied alone (Yasmin ***et al.***, 1993). Kinetin in low concentrations, in the presence of auxin promoted adventitious root development. These results are consistent with the findings of Mission (1988) Jarvis and Yasmin, (1987) and Rashida ***et al.*** (2000). There could be two alternate explanations for the later observation. Either the failure to demonstrate a stimulatory effect simply reflect the presence of ethanol, or the supplied concentrations of kinetin has disbalanced the auxin/kinetin ratio favourable for root formation. It has been suggested that root formation required both favourable concentration of auxin/cytokinin ratio.

The effect of GA_3 and kinetin are consistent with idea that low concentrations are necessary for root initiation. Such low concentrations may naturally prevail in cuttings. Since removal of the root system would remove a potential source of both those type of compounds.

Indeed there is a limited evidence for production of an inhibitory factor within the root which could be gibberellin or cytokinin (Batten and Goodwin, 1978). Another point need to be emphasized, Gibberellic acid at least may enhance rooting via an indirect effect on leaves. In cuttings, for example GA_3 stimulation of rooting is dependent upon prevailing irradiance condition, during growth of the stalk plant from which cuttings are made (Hansen, 1976).

Further more application of TIBA (2,3,5-Triiodobenzoic acid) below the pulvinus of leaf cuttings (Varga and Humpharies, 1974) and morphactin below the apical bud of mungbean hypocotyl inhibits the stimulatory effect of GA_3 and other growth regulators, (Jarvis and Yasmin, 1987). Such observations coupled with the interactions between supplied regulators and exogenous auxin, emphasize the need for further experiment to identify the possible indirect effect of these regulators. In particular their possible influence on auxin transports to there site

of regeneration needs to be investigated. In the absence of such indirect effects, these plant growth regulators could be investigated in term of their effect on IAA oxidase activity, since all have been shown to enhance and diminish IAA oxidase or peroxidase activity at different concentrations (Schneider and Wightman, 1974).

ABA at 5×10^{-5} M promoted adventitious root development, the stimulation of rooting caused by ABA could be explained in the light of previous reports that ABA can promote cell division (Altman and Goren, 1971; Minocha, 1979) or stimulate in rooting response may be due to increased photosynthesis at the base of the cuttings (Hartung ***et al.***, 1980).

The stimulatory influence of ABA on root formation was evident when a concentration of 5×10^{-4} M was employed, much larger number of roots result from ABA supplied in the presence of IAA. The possibilities arises therefore that ABA, may interact with auxin or have a preparatory action similar to that suggested for other non auxin compounds (Gorter, 1969). However, given the concentration required for stimulated rooting, it seems unlikely that ABA has any major effect in natural regeneration. Indeed the effect of supplied ABA could well be an indirect one since lateral bud break may be influenced during the rooting period (Hartung ***et al.***, 1980; Rasmussen, 1980). On the basis of the present investigation we infer that ABA is transported to the apex and its action may depend on the illuminated leaves. This possibility needs to be investigated by the use of disbudded and leafless cuttings and using TIBA application.

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