Plant Growth Regulation **14**: 15–19, 1994.

Synergistic effects of plant growth retardants and IBA on the formation of adventitious roots in hypocotyl cuttings of mung bean

Ruichi Pan & Zhijia Zhao
Department of Biology, South China Normal University, Guangzhou, P. R. China

Received 1 August 1992; accepted 11 May 1993

Key words: adventitious root formation, daminozide, indole-3-butyric acid, mung bean cuttings, paclobutrazol, triadimefon

Abstract

The synergistic effect of plant growth retardants, such as daminozide, paclobutrazol and triadimefon, and of indole-3-butyric acid (IBA) on the formation of adventitious roots in hypocotyl cuttings of mung bean was studied. The three retardants and IBA all stimulated adventitious root growth, but IBA was the most effective. However, mixtures of the retardants with IBA have proven generally more effective than IBA alone in promoting adventitious root formation. When IBA was applied to the hypocotyls one day after cutting preparation followed by the growth retardant on the second day, there were even more adventitious roots produced than if applied in the reverse order. The effectiveness of the treatments were in the order, IBA followed by growth retardant, IBA + growth retardant together, and IBA alone.

Abbreviations: IBA = indole-3-butyric acid; GA = gibberellin

1. Introduction

Indole butyric acid (IBA) is one of the most widely used rooting promoters [4], but plant growth retardants may also promote the formation of adventitious roots. For example, triadimefon affected mung bean rooting [8], paclobutrazol affected mung bean [15] and bean [3], and daminozide stimulated *Protea* rooting [2], but the magnitude of effect of the growth retardants was less than that of IBA. A proposed scheme of event associated with various phases of adventitious root formation has been suggested by Jarvis [13]: the induction phase of root primordia is promoted by IAA accumulation and the early initiation phase of root primordia is inhibited by GA. Since growth retardants inhibit the biosynthesis of GA, it was thought that IBA and growth retardants could have a synergistic effect on rooting when applied simultaneously or sequentially. The present study attempted to clarify the relationship between IBA and growth retardants on the development of adventitious roots, and to develop methods of enhancing the adventitious rooting achieved with IBA alone.

2. Materials and methods

Mung bean (*Phaseolus aureus* L.) seedlings were grown from seeds at $27 \pm 2\,°C$ in the dark for 48 h, and then transfered to an incubator for 3 days. The experimental condition were: 12 h light each day; light intensity $30\,W/m^2$; temperature $27 \pm 2\,°C$; relative humidity 80%. For maximal uniformity the cuttings were selected according to height, hypocotyl diameter, size of leaf and cotyledon. Material was prepared by cutting the seedlings 4 cm below the cotyledons. The cuttings were dipped in 3.5 cm deep solutions of daminozide, paclobutrazol or triadimefon alone or in combination with IBA respectively, at various concentrations. The bases of the cuttings were then washed with distilled water in order to remove excess chemicals from the surface. The cuttings then were

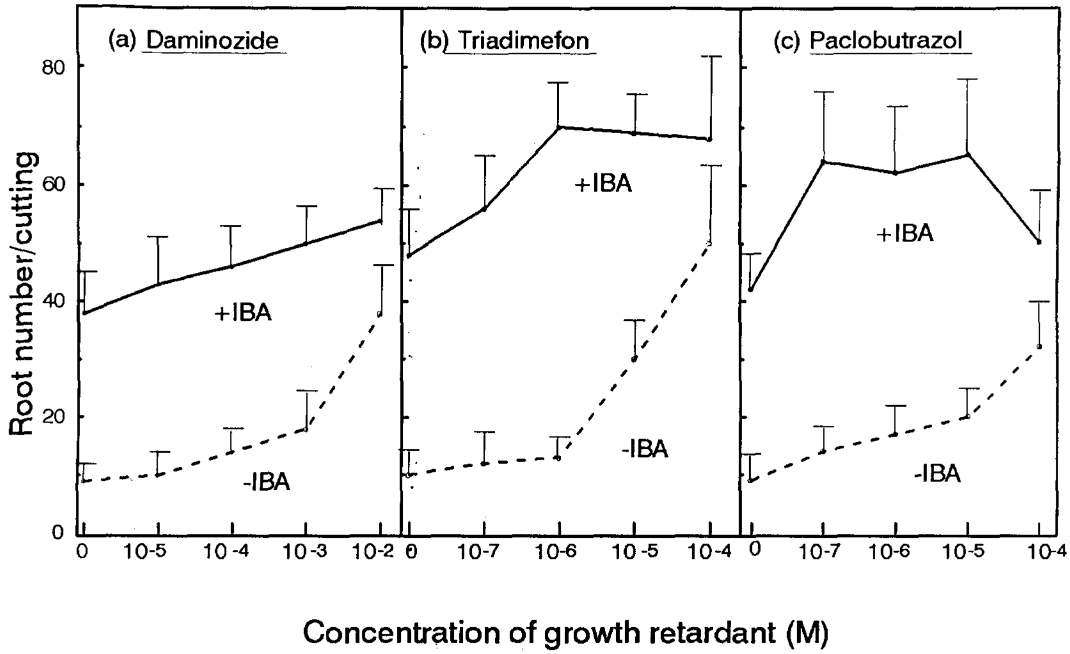

Fig. 1. Effects of different concentration of growth retardants alone or in combination with $10^{-4}M$ IBA on the rooting of hypocotyl cuttings of mung bean seedlings.

maintained in wet sand and watered every day. The number of adventitious roots were counted 6 days later. The data presented are the means of three or four experiments with 15–30 hypocotyls per treatment.

3. Results

3.1 Application of plant growth retardant and IBA combinations

Daminozide at concentrations ranging from $10^{-5}M$ to $10^{-3}M$ slightly increased adventitious root development, but $10^{-2}M$ was much more effective. As compared with daminozide and IBA applied alone, a mixture of daminozide and $10^{-4}M$ IBA strongly increased the formation of adventitious roots (Fig. 1a).

Concentrations of paclobutrazol from $10^{-7}M$ to $10^{-4}M$ slightly increased the number of roots, with increasing concentration (Fig. 1c). Mixtures of paclobutrazol with $10^{-4}M$ IBA treatment significantly increased adventitious root number, particularly at concentration between $10^{-7}M$ to $10^{-5}M$. This data confirms previously reported information [15].

The structure of triadimefon is similar to that of paclobutrazol and it exhibits comparable growth regulatory properties. Treatment with triadimefon at $10^{-5}M$ and $10^{-4}M$ for 24 h significantly promoted the growth of adventitious roots (Fig. 1b). Mixtures of $10^{-4}M$ IBA with different concentration of triadimefon were much more stimulatory

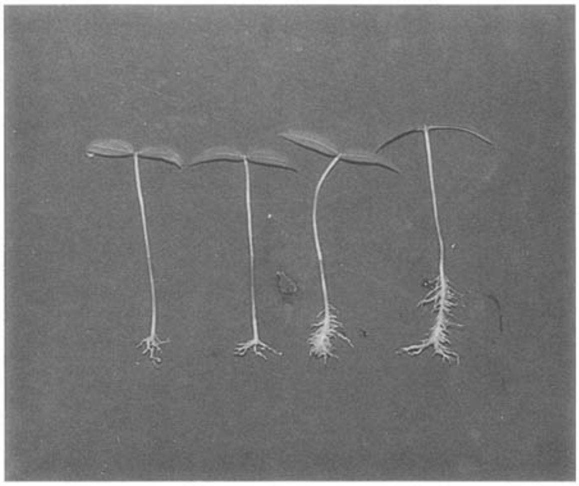

Fig 2. Effects of triadimefon alone or in combination with IBA on the rooting of hypocotyl cuttings of mung bean seedlings. From left to right: CK; $10^{-6}M$ triadimefon: $10^{-4}M$ IBA; $10^{-6}M$ triadimefon + $10^{-4}M$ IBA.

Table 1. Effects of daminozide, pacobutrazol and triadimefon in combination with IBA respectively on the rooting of mung bean hypocotyl cuttings

Treatment	Root number/cutting	%
IBA ($10^{-4}M$)	50.5 ± 7.4 d	100
IBA ($10^{-4}M$) + daminozide ($10^{-2}M$)	61.7 ± 9.1 c	122.2
IBA ($10^{-4}M$) + paclobutrazol ($10^{-7}M$)	71.1 ± 7.8 b	140.8
IBA ($10^{-4}M$) + triadimefon ($10^{-6}M$)	75.5 ± 7.4 a	149.1

Values followed by the same letter are not significantly different at 5% level (Duncan's Multiple Range Test).

than treatment with either chemical alone (Fig. 2). In the treatments concerned, not only more adventitious root were produced, but they were formed more extensively along the hypocotyl.

These data shown in Figure 1 were obtained from separate experiments. In order to have a more exact comparison of promotory effects of the chemical combinations, the most effective concentration of each growth retardant was chosen, i.e. daminozide at $10^{-2}M$, paclobutrazol at $10^{-7}M$ and triadimefon at $10^{-6}M$, and a further experiment was set up. Table 1 shows that each growth retardant combined with $10^{-4}M$ IBA significantly produced more adventitious roots than IBA alone. Among these combinations, the treatments of IBA with paclobutrazol or triadimefon were superior to the IBA plus daminozide treatment, in respect to rooting.

Thus, it is clear that treatments of all three growth retardants mixed with IBA were more effective in promoting adventitious root number than any of the single chemical treatments, including IBA.

3.2 Sequential application of IBA and growth retardants

Chemicals were applied sequentially as indicated in Table 2. Treatment 1 was the control treated with distilled water only and this produced few adventitious roots. Solutions were applied on two occasions, firstly between 0 and 24 h (first day) after cutting preparation, and then between 24 and 48 h (second day). Treatment with IBA plus daminozide produced the same effect irrespective of the time of application, causing more adventitious root development as compared to controls (treatments 2 and 3). Similar effects were observed from IBA with paclobutrazol (treatments 4 and 5), and with triadimefon (treatments 6 and 7). Thus there was no appreciable difference in root number whether IBA/growth retardant combinations were applied on the first or second day.

When IBA and the growth retardants were applied on different days, significant differences in the production of adventitious roots were observed. In general, IBA applied on the first day fol-

Table 2. Effect of mixed or sequential applications of daminozide ($10^{-2}M$), paclobutrazol ($10^{-7}M$), triadimefon ($10^{-6}M$) and IBA ($10^{-4}M$) applied on different days on the rooting of hypocotyl cuttings from mung bean seedlings

Treatment	First day	Second day	Root number/cutting
1	H_2O	H_2O	7.2 ± 2.2 f
2	IBA + dam	H_2O	61.6 ± 11.1 d
3	H_2O	IBA + dam	58.4 ± 10.4 d
4	IBA + pac	H_2O	66.1 ± 10.4 b
5	H_2O	IBA + pac	63.1 ± 6.6 c
6	IBA + tri	H_2O	69.8 ± 5.6 b
7	H_2O	IBA + tri	70.0 ± 4.2 b
8	IBA	dam	69.0 ± 9.2 b
9	dam	IBA	54.0 ± 8.7 e
10	IBA	tri	81.0 ± 4.8 a
11	tri	IBA	63.2 ± 5.2 c
12	IBA	pac	80.6 ± 7.1 a
13	pac	IBA	54.2 ± 6.0 e

Values followed by the same letter are not significantly different at 5% level (Duncan's Multiple Range Test).

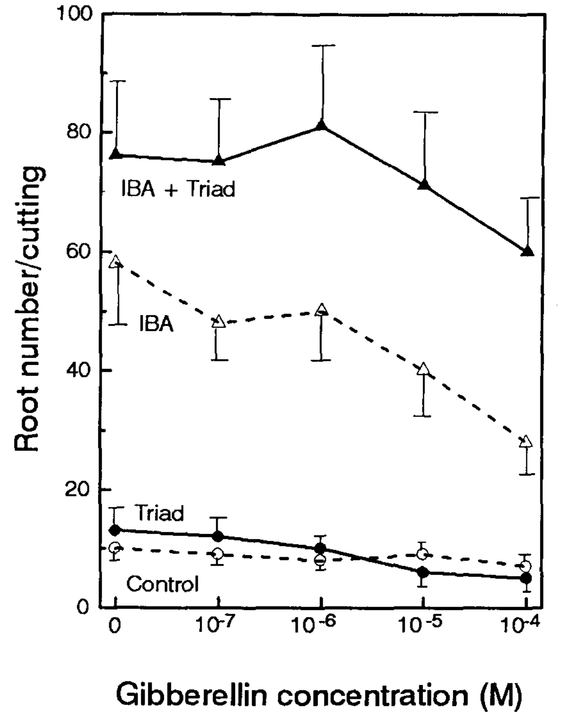

Fig. 3. Effects of different concentration of GA on the rooting of hypocotyl cuttings of mung bean seedlings treated with $10^{-4}M$ IBA, $10^{-6}M$ triadimefon, or $10^{-4}M$ IBA + $10^{-6}M$ triadimefon.

lowed by growth retardant on the second day (treatments 8, 10 and 12) induced more rooting than applications in the reverse order, namely, growth retardant applied on the first day and IBA on the second day (treatments 9, 11 and 13). The fact that IBA and growth retardant applied on different days caused a large effect on rooting has not been reported previously.

4. Discussion

It is well known that the production of adventitious roots is controlled by growth substances [6, 7]. Auxins are the main hormones for promoting rooting, and play a direct role in this process [9]. Application of GA has been widely reported to inhibit adventitious root formation in cuttings of a variety of species [1, 14]. In the mung bean hypocotyl, adventitious root development is also inhibited by GA alone, GA mixed respectively with IBA or triadimefon, or by both (Fig. 3). This indicates that GA reduces the rooting induced by IBA or growth retardants. Since plant growth retardants inhibit GA biosynthesis, so decreasing the endogenous GA levels it seemed likely that such compounds could enhance adventitious root formation. Indeed this has been demonstrated in several species [4, 5].

The synergistic effects of indole, phenylacetic acid and phenylbutyric acid on IAA-inducing rooting have also been reported [10, 11, 12]. In media enriched with IAA and paclobutrazol, rooting percentage of cherry *in vitro* increased in one species but was unaffected in another two species [16]. We found that treatment with IBA plus paclobutrazol induced a synergistic influence on the rooting of mung bean cutting [15]. The present study further demonstrated that hypocotyl rooting was also hastened by the addition of daminozide, triadmefon or paclobutrazol in the presence of IBA. Hence, mixtures of IBA with growth retardant may induce more rooting than IBA or growth retardant alone.

According to Jarvis' hypothesis [13], the first phase (inductive or preparatory phase) of the regeneration process is essentially characterized by a lack of cell division. Auxins are accumulated during this phase. The second phase (early initiation phase) is characterised by cell division and GA inhibits this process. In the present study, IBA and growth retardants applied sequentially were more effective in enhancing the number of adventitious roots than when mixed together and applied simultaneously. Whether this is related to the fact that auxin is sufficient for the first development phase and growth retardants are more effective during the second phase is not clear. Whatever the reason, it is clear that the sequential application of IBA and growth retardants was more effective in terms of the extent of rooting and the number of roots produced, than when they were applied as mixture or alone.

References

1. Brian PW, Hemming HG and Lowe D (1960) Inhibition of rooting of cuttings by gibberellic acid. Ann Bot 24: 407–419
2. Criley RA and Parvin PE (1979) Promotive effects of auxins, ethephon and daminozide on the rooting of *Protea mellifolia* cuttings. J Am Soc Hort Sci 104: 592–596
3. Davis TD, Sankla N, Walsee R and Upadhyaya A (1985) Promotion of adventitious root formation on cuttings by paclobutrazol. Hort Sci 20: 883–884
4. Davis TD (1986) Influence of triazole growth retardants on

adventitious root formation in bean hypocotyl cuttings. Proc Plant Growth Reg Soc Am 13: 217–223
5. Davis TD and Sankhla N (1988) Effect of shoot growth retardants and inhibitors on adventitious rooting. In: Davis TD, Haissig BE and Sankhla N (eds) Adventitious Root Formation in Cuttings, Advances in Plant Sciences Series, Vol 2, pp 174–184. Dioscorides Press, Portland, OR
6. Davis TD and Hassig BE (1990) Chemical control of adventitious root formation in cuttings. Bull Plant Growth Reg Soc Am 18: 1–17
7. Fibijian D, Taylor JS and Reid DM (1981) Adventitious rooting in hypocotyls of sunflower (*Helianthus annus*) seedlings. II. Action of gibberellins, cytokinins, auxins and ethylene. Physiol Plant 53: 589–597
8. Fletcher RE, Asare-Boamah NK, Kreig LC, Hofstra G and Dumbroff EB (1988) Triadimefon stimulates rooting in bean hypocotyl. Physiol Plant 73: 401–405
9. Gaspar T and Hofinger M (1988) Auxin metabolism during adventitious rooting. In Davis TD, Haissig BE and Sankhla N (eds) Adventitious Root Formation in cuttings. Advances in Plant Sciences Series, Vol 2, pp 117–131. Dioscorides Press, Portland, OR
10. Gorter CJ (1958) Synergism of indole and indole-3-acetic acid in root production of *Phaseolus* cuttings. Physiol Plant 11: 1–9
11. Hansen J (1988) Influence of gibberellins on adventitious root formation. In Davis TD, Haissig BE and Sankhla N (eds) Adventitious Root Formation in Cuttings, Advances in Plant Sciences Series, Vol 2, pp 162–173. Dioscorides Press, Portland, OR
12. Haissig BE (1974) Influences of auxins and auxin synergists on adventitious root primordium initiation and development. NZ J For Sci 4: 311–323
13. Jarvis BC (1986) Endogenous control of adventitious rooting in non-woody cuttings. In Jackson MB (ed) New Root Formation in Plants and Cuttings, pp 191–222 Martinus Nijhoff Publishers, The Netherlands
14. Kato J (1958) Studies on the physiological effect of gibberellin. II. On the interaction of gibberellin with auxin and growth inhibitors. Physiol Plant 11: 10–15
15. Mai YY and Pan RC (1988) Effect of indole butyric acid and PP333 on adventitious root formation in hypocotyl cuttings of mung bean seedlings. J South China Normal Univ (Natural Science Edition), Biol issue: 12–15
16. Marino G (1988) The effect of paclobutrazol on *in vitro* rooting, transplant establishment and growth of fruit plants. Plant Growth Reg 7: 237–247