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The effect of 6-benzyladenine derivatives on the rooting of *Phaseolus vulgaris* L. primary leaf cuttings

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Abstract. The effects of 6-benzyladenine (BA), its 3-glucoside (BA-3-G) and 9-glucoside (BA-9-G), and its riboside (BA-R) on the rooting of primary leaf explants of *Phaseolus vulgaris* L. were compared, alone or in combination with indole-3-butyric acid (IBA). At all except the lowest concentration used (4.4×10^{-7} *M*), BA and BA-3-G reduced the number of roots and their averge length relative to a distilled water control. Addition of IBA (4.4×10^{-5} *M*) increased rooting beyond the control, but not to the level of IBA alone. This contrasted with BA-9-G where a response very similar to that of the control was recorded. The riboside of BA was more inhibitory than the free base, its effect extending to the lowest concentration used (4.4×10^{-7} *M*).

1. Introduction

Cytokinin ribosides usually show biological activity in bioassays, but appreciably less so than their corresponding free bases [19, 21, 25]. The 7- and 9-glucosides of cytokinins are postulated to be storage forms [6, 18]. They show little biological activity relative to their corresponding free bases [14, 15, 24]. Formation of 3-glucosides from free bases has been reported to have little effect on cytokinin activity and these derivatives are more active than the 7- or 9-glucosides [14, 15]. Although the physiological response of plants to cytokinin derivatives has often been assessed in standard bioassays, knowledge of their effects in other systems is lacking. We are not aware of any studies which have focused on cytokinin derivatives in relation to the rooting of cuttings. This is probably due to their previous non-availability for use in such investigations. Most reports in the literature indicate that cytokinins inhibit rooting [1, 12] while auxins promote this process [3, 11], although these two hormones may well interact [2]. To date, the predominant cytokinins tested with respect to rooting have been the synthetic free

base forms of BA and kinetin. In this study, the rooting response of *Phaseolus vulgaris* leaf explants to the application of BA, BA-3-G, BA-9-G and BA-R has been tested.

2. Materials and methods

Plants of *Phaseolus vulgaris* L. (cv. Contender) were grown in trays of vermiculite in a greenhouse under natural summer day length of about 14 h light. After 12 days, the trays were transferred to a controlled environment chamber and maintained at 25 ± 1 °C in continuous low light of 50 $\mu E\,m^{2\,-1}$. After 2 days, explants consisting of the expanded primary leaves were taken and the petioles trimmed to a uniform length of 25 mm. The cuttings were transferred randomly to 18 ml vials filled with various concentrations of BA (Sigma), BA-R (Calbiochem), BA-3-G (Apex Organics), BA-9-G (Apex Organics) or distilled water, alone or in combination with IBA (Sigma). Preliminary studies had indicated that 4.4×10^{-5} *M* was a suitable IBA concentration to use for root induction. The vials were inserted into holes in wooden blocks, thereby keeping the petiole bases in the dark, a situation more natural for rooting. The eight replicates per treatment were then returned to the aforementioned controlled environment chamber.

After 24 h, the test solutions were replaced by quarter-strength Hoagland's nutrient solution (pH 6.5). The number of roots per petiole and the average root length were determined after 10 days. The results were subjected to a one-way analysis of variance and the least significant differences determined.

3. Results

3.1 BA and BA + IBA

After 10 days, the control cuttings, which had not been exposed to a hormone treatment, had developed a considerable root system. An average of 18 roots was produced per petiole (Figure 1) and their mean length was 19.8 mm (Figure 2). With BA alone, rooting was severely inhibited by all but the lowest concentration (4.4×10^{-7} *M*). No roots were produced in the treatments with 4.4×10^{-5} *M* and 2.2×10^{-5} *M* BA (Figure 1), although in some cases, small amounts of callus were observed at the petiole base. Similarly, BA reduced root length, relative to the control (Figure 2). When petioles were pulsed with solutions of BA plus IBA, the level of rooting was

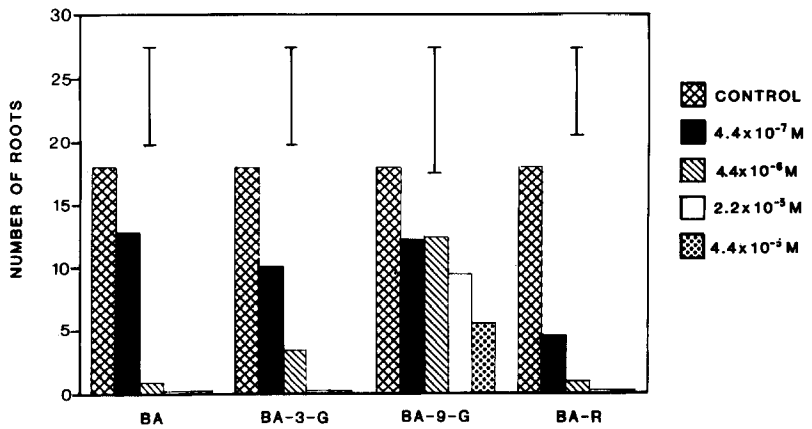

Fig. 1. Number of roots per primary bean leaf explant treated with BA, BA-3-G, BA-9-G or BA-R, or left untreated. Bar indicates the LSD (P = 0.05).

greater than that of the distilled water control (Figure 3), but not significantly so. This was significantly lower than the IBA control, which promoted root initiation, but not elongation (Figures 3 and 4). A combination of 2.2×10^{-5} *M* BA with IBA led to a significantly reduced average root length relative to either the water or IBA control (Figure 4), but none of the other concentrations had this effect.

3.2 Glucosyl-BA and glucosyl-BA + IBA

A reduced number of roots developed on the cuttings when 4.4×10^{-5} *M* and 2.2×10^{-5} *M* BA-3-G were applied. The average length of the roots

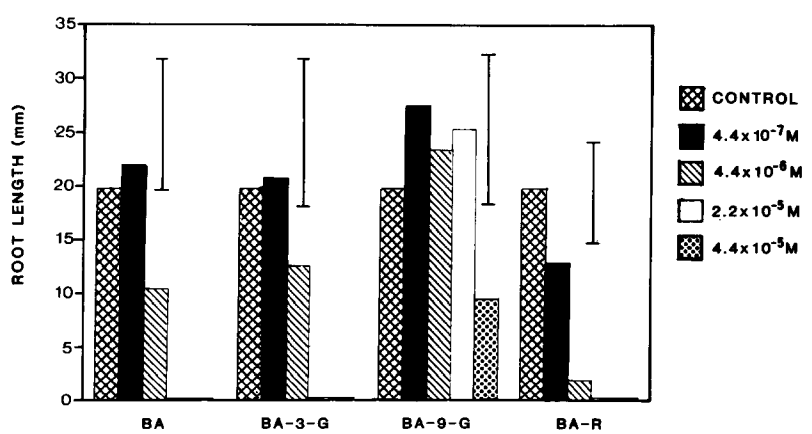

Fig. 2. Average root length per primary bean leaf explant treated with BA, BA-3-G, BA-9-G or BA-R, or left untreated. Bar indicates the LSD (P = 0.05).

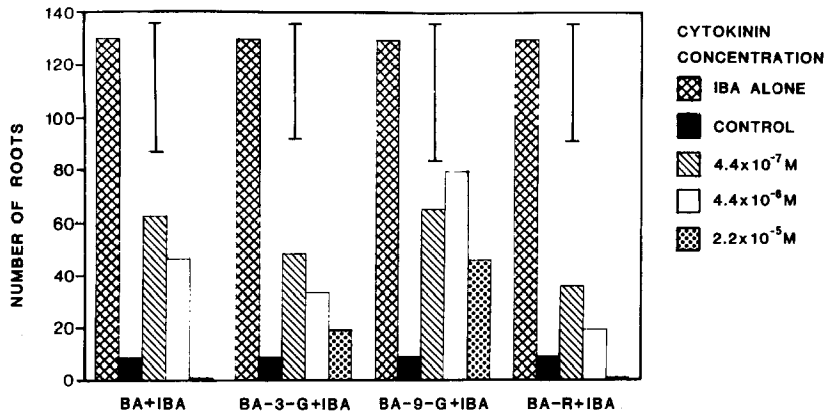

Fig. 3. Number of roots per primary bean leaf explant treated with IBA (4.4×10^{-5} *M*) plus BA, BA-3-G, BA-9-G or BA-R, IBA alone (4.4×10^{-5} *M*), or left untreated. Bar indicates the LSD (P = 0.05).

produced was less than the water control (Figures 1 and 2). As was found for the free base, a concentration of 4.4×10^{-6} *M* produced significantly fewer roots than the control, but the extension of these roots was not impaired (Figure 2). The inhibitory efects of BA and BA-3-G were essentially the same. Inhibition was not as marked when IBA was added (Figures 3 and 4).

Application of BA-9-G alone (Figures 1 and 2) resulted in a slight reduction in root number. This was only statistically significant at the highest concentration (4.4×10^{-5} *M*), where the average number of roots

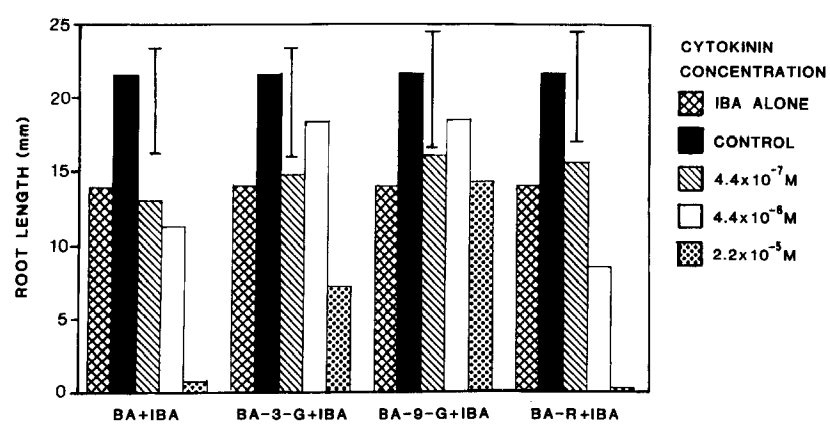

Fig. 4. Average root length per primary bean leaf explant treated with IBA (4.4×10^{-5} *M*) plus BA, BA-3-G, BA-9-G or BA-R, IBA alone (4.4×10^{-5} *M*), or left untreated. Bar indicates the LSD (P = 0.05).

produced per petiole was half that of the control. However, the length of these roots was not significantly reduced (Figure 2). Treatment with the lower concentrations resulted in a slight but non-significant increase in root length relative to the control. When BA-9-G was applied together with IBA (Figure 3), the number of roots produced per petiole was not significantly greater than in the distilled water control. Nor, was the number of roots produced significantly less than the IBA control when the two lowest BA-9-G concentrations and IBA were applied simultaneously. When 2.2×10^{-5} *M* BA-9-G was used in combination with IBA, significantly fewer roots were produced than with IBA alone, which suggests that this cytokinin concentration was able to exert an effect. Root length (Figure 4) was not affected by any of the BA-9-G concentrations.

3.3 BA-R and BA-R + IBA

The results indicate that BA-R is a more potent inhibitor of rooting than its corresponding free base. Even the lowest concentration of the riboside (4.4×10^{-7} *M*) resulted in significantly fewer roots being formed than in the control (Figure 1), although root length was not significantly reduced (Figure 2). The greater inhibitory activity of BA-R, relative to BA and BA-3-G, was largely overcome by the simultaneous application of IBA (Figures 3 and 4), since with this combination, neither the number of roots produced per petiole, nor the average root length, was significantly different from the distilled water control.

4. Discussion

The presence of both O- and N-cytokinin glucosides in plants is well-established [22, 25]. The metabolism of applied cytokinin free bases or ribosides to glucosides has also been observed [9, 10, 15, 16, 24]. Such metabolism resulted in the accumulation of cytokinin glucosides in mature leaves [4, 9]. It has therefore been suggested that cytokinin glucosides in leaves could have a significant effect on the rooting ability of cuttings [4].

There are few reports concerning the biological activity of 3-glucosides. Where it has been tested, for example in the radish cotyledon [15], oat leaf senescence and tobacco pith bioassays [14], these compounds were relatively active. The high biological activity may be due to the cleavage of the 3-glucoside moiety, to release BA [14]. If this occurs in *Phaseolus vulgaris* cuttings, it would have to be rapid and virtually quantitative, since BA and its 3-glucoside gave similar inhibitory effects on root number and root

length. The possibility that BA-3-G itself has inhibitory activity equivalent to that of BA cannot be ruled out.

In contrast to BA-3-G, BA-9-G had little inhibitory effect in the rooting of cuttings. A comparison between the rooting pattern when the cytokinin derivatives were applied alone and in combination with IBA, confirmed the low level of inhibition of BA-9-G, relative to BA, BA-3-G and BA-R. Following a thorough study involving the radish cotyledon, *Amaranthus* betacynin, oat leaf senescence and tobacco pith bioassays, it was concluded that BA-9-G has low biological activity [14]. With respect to rooting, it certainly was less inhibitory than the other BA metabolites.

Cytokinin ribosides were reported to be slightly less inhibitory to lateral root formation in isolated pea roots, than the free bases [23]. Similarly, when the effects of BA-R, ribosylkinetin and ribosylzeatin on root growth of wheat and flax seedlings were compared, it was found that the free bases were more inhibitory than the ribosides [20].

In the carrot callus assay and a chlorophyll retention test, cytokinin ribosides displayed biological activity equal to that of the free base. Ribosylzeatin was found to be at least eight times less active than zeatin in the tobacco callus bioassay [19]. Similarly, BA, at a concentration of 10^{-6} to 10^{-9} M was reported to be more active in the soybean callus bioassay than BA-R [21]. The activity of cytokinin ribosides therefore appears to differ between systems, although, they always exhibit some level of activity, either promotive, as in callus, or inhibitory, as in rooting. Cytokinin riboside activity could be due to a rapid conversion to the free base form. However, BA-R, in the absence of IBA, was more inhibitory than BA in bean explants, which suggests that this may not be the case. Alternatively, free base cytokinins are known to be readily ribosylated in plant systems [13]. It has been indicated [13] that there are no experiments which have precluded the 9-riboside as a principal active form of cytokinin.

The well-documented inhibitory effect of cytokinin free bases on rooting was confirmed in this study. The inhibition does not, however, appear to be a direct effect on root initiation. Rather, the cytokinins seem to interact with auxin and thus affect the rooting response of the cuttings to auxin. It was reported earlier [8] that a high auxin to cytokinin ratio leads to reduced inhibition of rooting in *Begonia* cuttings [8]. An auxin to cytokinin ratio of 1:10 is promotive in the rooting of softwood peach cuttings [7]. The hormones may therefore interact by mutual effects on transport and metabolism. Information about the interactions between these hormones is, however, very contradictory.

The pronounced inhibitory effect that application of BA-R or BA-3-G has on rooting may be due to a direct mode of action, or the conversion of these

derivatives to BA. In the presence of these derivatives and IBA, rooting increased beyond that of the control, but remained lower than with IBA alone. This could be due to IBA affecting cytokinin metabolism. Degradative metabolism of ribosylzeatin has been reported to increase at progressively higher levels of NAA in tobacco pith callus [17]. The presence of auxin also accelerates degradative metabolism of BA in excised carnation ovaries [5]. The IBA could therefore be decreasing the amount of biologically active BA in the bean leaf explants, thus reducing inhibition of rooting.

Acknowledgements

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