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PREMATURE ABSCISSION OF PHASEOLUS BEAN PODS: THE ROLE OF PLANT GROWTH SUBSTANCES

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ABSTRACT

A 2x5 factorial experiment was conducted to study the effect of concentrations of 6-benzyladenine (BA) applied at five post anthesis stages on pod development of *Phaseolus vulgaris* L., especially in relation to abscission. The terminal raceme of a five-node cultivar (cv 344) was used for the study. There were no significant differences between treated plants and controls with respect to numbers of flowers that opened and those that developed into pods. BA not only delayed the onset of pod abscission but also slowed it down, giving it a protracted duration. The cytokinin was effective in reducing pod abscission when application commenced between anthesis and six days after anthesis. BA effected pod retention on the distal triad of the terminal raceme where normally all pods drop off. All treated racemes had significantly ($P<0.01$) more seeds at the time of harvest than controls, the overall increase in seed number being due to increased seed number in pods borne on the medial and distal triads of the raceme. BA increased pod retention and seed yield without any significant effect on the number of pods on other nodes, and dry matter of the stem and leaves. When BA was applied beginning at the time of anthesis and using the higher dosage (400 ppm), normally abscising fruits on the distal triad were retained with full seed development.

Key Words: **Abscission, cytokinin, *Phaseolus vulgaris*, post-anthesis, seed yield**

RÉSUMÉ

Une étude factorielle de 2 x 5 a été menée pour évaluer les effets des concentrations de benzyladdenine-6 (BA) appliquées au cours de cinq étapes post-anthèse du développement du gousse du *Phaseolus vulgaris* L., en relation particulière avec l'abscission. Le recème terminal d'un cultivar à cinq noeuds (cv 344) a été utilisé pour l'étude. Il n'y avait pas de différences significatives entre les plantes traitées et les témoins en ce qui concerne les nombres de fleurs qui se sont ouvertes et celles qui se sont développées en gousses. Le BA a non seulement retardé le début de l'abscission des gousses, mais l'a ralentie, lui donnant une duré prolongée. La cytokinine était effective dans la réduction de l'abscission des gousses quant l'application débutait entre l'anthèse et six jours après l'anthèse. Le BA a affecté la retention des gousses sur les triades distales du recème terminal, où normalement toutes les gousses chutent. Tous les recèmes traités avaient significativement ($P<0.01$) plus de graines au moment de la récolte que les témoins, l'augmentation générale du nombre de graines étant due à l'augmentation du nombre de graines dans les gousses produitent sur les triades médiales et distales du recème. Le BA a augmenter la retention de gousses et le rendement de graines sans effets significatifs sur le nombres de gousses des autres noeuds et sur les matières sèches de la tige et des feuilles. Quand le BA était appliqué débutant au moment de l'anthèse et utilisant le dosage plus élevé (400ppm); les fruits normalement abscissants sur les triades distales on été conservés avec des graines complètement développées

Mots Clés: **Abscission, cytokinine, post-anthèse, *Phaseolus vulgaris,* rendement de graines**

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the most widely cultivated of the Phaseolus species (Laing *et al.*, 1984), although individual plant yields of this species are considerably lower than those of the other Phaseolus species. Whereas insect pests (Van Schoonhoven and Cardona 1980) and diseases (Sanders and Schwartz, 1980) are reported to be major constraints to bean yields, the application of pesticides has still not made significant improvement to grain yields (Ojejowun, 1986). One of the possible causes of low yield in this crop is abscission of flower buds, flowers and immature pods. Work by Binnie and Clifford (1980) suggests that available assimilate supply limits harvestable yields in this crop. However, the fact that plants abort flowers and fruits even under good management conditions (Ojehomon, 1968; Arach, 1988) indicates that factors other than assimilate availability may be involved in abscission. Studies in soybeans have shown that pods at various stages of development require adequate plant growth substances for proper development up to maturity (Crosby *et al.*, 1981). Legume pods have particularly been reported to contain cytokinins or cytokinin-like substances (Davey and Van Staden, 1978; Lindoo and Nooden, 1978), required for early embryo growth (Bennici and Cionni, 1979) and successful embryo development (Nesling and Morris, 1979). Subsequent work by Okelana and Adedipe (1982) has shown that cytokinins are some of the growth substances that likely become limiting, leading to abscission.

Although the efficacy of cytokinins in reducing abscission in *P. vulgaris* has been demonstrated, the emphasis has been on flower bud abscission (e.g. Saad, 1972; Bentley, 1974; Bentley *et al.*, 1975; Lynas, 1981; Morgan and Morgan, 1984) and there is a dearth of information regarding their potential in reducing pod abscission. Since bean grain yield is dependent on pod and seed numbers, which are in turn dependent upon successful pod formation, it may be assumed that in the absence of other constraints, the yield of dry beans might be increased if abscission of young pods could be reduced. This experiment sought to show the effect of an exogenously applied cytokinin on pod set and development on the terminal receme. 6-benzyladenine (BA) was chosen because earlier work (e.g. Crosby *et al.*, 1981) showed that plants are particularly responsive to this growth substance.

MATERIALS AND METHODS

The study was conducted at the field station of the Department of Applied Biology, University of Cambridge, in the summer of 1985.

Plant material. Bean seeds of a determinate five-node bean cultivar, No. 344, were sown in 15 cm diameter pots containg a John Innes II soil mixture. The pots were placed on 60x120 cm sand trays which were watered every other day so that the plants received water from the sand by capillary action. The plants were grown in a heated glass-house with an automatic ventilation system. Maximum and minimum temperatures averaged 21.6 and 19.8°C, respectively. Twenty eight days after sowing, when the flower buds had attained macroscopic size, the plants were selected to ten treatment combinations involving two concentrations of BA applied at five post-anthesis stages of development. A randomized complete block design was used, each treatment being replicated five times.

BA preparation and application. Four mg of BA (Sigma Chemicals) was weighed out in a glass vial and 0.352 ml of 0.1 N hydrochloric acid added to it using a 1.0 ml pipette. The mixture was gently warmed at 60°C on a hot plate until the BA powder dissolved completely. The solution was made to 10 ml by adding 9.648 ml of 0.1 % Tween 20, which served as solvent and surfactant. This solution contained 4.0mg BA per 0.01ml equivalent to 400 ppm BA. One ml of the solution was pipetted into another glass vial and diluted with 9ml of 0.1% Tween 20, to give a solution containing 0.4mg BA per 0.01ml, equivalent to 40ppm BA.

An 'Agla" micrometer syringe (Wellcome Ltd., U.K) was used to deliver the cytokinin solutions to the target organs. Application of cytokinin started at (i) anthesis, (ii) three days after anthesis, (iii) six days after anthesis, (iv) nine days after anthesis, and (v) twelve days after anthesis. At each of these stages of development, each individual flower bud, flower or pod at the terminal raceme received one drop (equivalent to 0.01ml) of BA daily for five consecutive days, the cytokinin being applied at the base of the reproductive organ into the "calyx cup". The control plants received a solvent without BA, for five consecutive days starting at the time of anthesis.

Daily counts were made of the flowers that opened and pods formed, flowers and pods that were shed, final pod number on each of the triads on the terminal raceme, and final pod number on each of the remaining untreated nodes of the plant. At physiological maturity, the pods were harvested separately for each of the three triads on the terminal raceme, oven-dried at 75°C for 96 hours and weighed. The pods were separated into seeds and pod wall, the average seed number per pod recorded for each triad, and seed and pod wall weighed. The total seed number for the whole terminal raceme was also recorded. The leaves and stems were separated, oven-dried at 75°C for 96 hours, and weighed.

RESULTS

Taking the terminal raceme as a whole, the treatments did not show significant differences with respect to the total number of flowers produced and those flowers that developed into pods. Figure 1 shows the daily pattern of pod abscission as influenced by BA applied at different times. When applied at anthesis, BA delayed the onset of pod abscission by nearly a week. Furthermore, the process of pod senescence and abscission was slowed down, prolonging the abscission period in plants where BA treatment started at anthesis and three days after anthesis. In contrast, pod abscission was concentrated in a shorter period in the controls and those plants where treatment with BA was delayed up to six days from anthesis.

The control plants shed significantly more pods on the terminal raceme than treated plants ($P<0.05$). The time of BA treatment significantly ($P<0.05$) influenced the number of pods that were shed and this effect varied between the different triads (Table 1). Pod abscission on the proximal triad was reduced to zero even when application was delayed to six days after anthesis. Later applications starting nine and twelve days after anthesis also reduced the number of pods shed from this triad. Although the picture is less clear with fruits on the medial triad, it appears that BA applied between anthesis and six days after anthesis reduced pod abscission in this position. Pod abscission on the distal triad was markedly reduced by treatments starting at anthesis and three days after anthesis, the effect being greater with the higher concentration of BA. Later treatments starting six days after anthesis did not reduce the number of pods shed from the distal triad. Considering the terminal raceme as a whole, BA reduced pod abscission when applied between anthesis and six days thereafter.

The average number of pods retained to maturity and the same values expressed as percentage of flowers that opened are shown in Table 1. Treated plants retained more pods on the terminal raceme than control plants, the effect being more marked

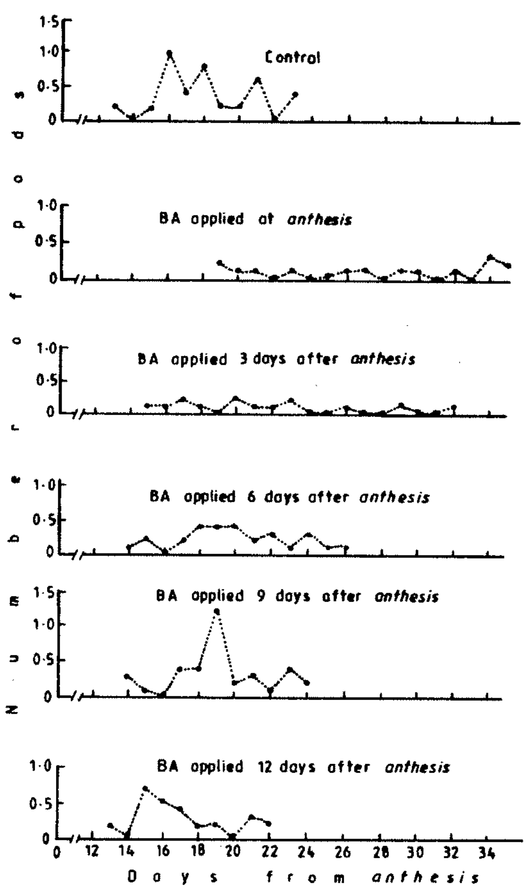

Figure 1. The daily pattern of pod abscission on the terminal raceme as influenced by time of BA application

on the medial and distal triads. There were no pods retained on the distal triad of the terminal raceme of control plants and those plants where BA treatment started later than three days after anthesis. Furthermore, the lower dosage of BA (40 ppm) was not effective in retaining any pods on the distal triad to maturity when application was delayed after start of anthesis.

Figure 2 shows the total seed number for the whole terminal raceme. All treated racemes had significantly more seeds at the time of harvest ($P<0.01$) than controls. The time of application also had a significant effect on seed number ($P<0.01$), earlier applications starting at anthesis and three days later having a greater effect than later applications. The differences in seed number were due to increased seed numbers over the control, and retention of pods containing seeds on the distal triad. The controls and treated plants did not differ significantly in the number of seeds pod^{-1} on the proximal triad. With respect to the distal triad, there were no pods from control plants for comparison since they all abscised. Figure 3 shows x-ray radiographs of pods from some of the racemes that were treated with BA. Although BA reduced pod abscission on the distal triad, the pods retained were mostly non-harvestable. Only the treatment where the growth substance was applied from the time of anthesis and at the higher dose (400 ppm) retained some otherwise normally abscising pods that contained fully developed seeds.

Pods from the proximal triad of treated plants did not differ significantly in weight from their counterparts of control plants (Table 2). In contrast, pods from the medial triad on treated plants were significantly heavier than those of control plants ($P<0.01$) regardless of concentration and time of BA application. There were no pods from the distal triad on control plants for comparison, but in treated plants, the higher dosage of BA produced more dry matter in pods from this triad than the lower dosage.

Table 2 also shows the mean dry weights of seeds from the treated triads. While there was no response from seeds on the proximal triad, seeds

TABLE 1. The average number of pods shed and retained on the three triads of the terminal raceme after BA application at rates of 40 and 400 ppm (means of 5 plants)

Triad		Time (days from anthesis) of BA application (ppm)									
		0		3		6		9		12	
	Control	40	400	40	400	40	400	40	400	40	400
Number of pods shed											
Proximal	0.6	0	0	0	0	0	0	0.2	0.4	0.2	0.4
Medial	1.2	0.6	0.8	0.6	0.2	0.8	0.6	1.1	0.8	0.6	0.8
Distal	1.7	1.1	0.4	1.5	0.4	1.3	1.9	1.9	2.2	1.7	1.3
Number of pods retained at maturity											
Proximal	1.7	2.3	2.1	1.9	2.1	1.9	1.9	1.9	1.9	1.9	1.3
Medial	0.8	1.5	2.1	2.1	2.8	1.3	1.7	1.9	1.1	1.9	1.3
Distal	0	0.6	1.5	0	1.7	0	0	0	0	0	0
Pod retention as a percentage of flowers that opened											
Proximal	70	100	100	100	99	99	100	99	98	99	98
Medial	28	76	72	80	100	56	60	64	52	68	52
Distal	0	32	76	0	80	0	0	0	0	0	0
Pod retention as a percentage of pods that were set											
Proximal	80	100	100	100	100	100	100	99	98	99	98
Medial	40	72	76	80	100	64	76	68	60	80	64
Distal	0	40	80	0	84	0	0	0	0	0	0

from the medial triad of treated plants were heavier than their counterparts on control plants. In treated plants, weight seed^{-1} was lowest for seeds harvested from the distal triad. The higher level of BA applied at anthesis produced heavier seeds on the distal triad than the lower level because the latter concentration retained pods on this triad that did not contain fully developed seeds. The final pod number on the untreated nodes of the plant and the leaf and stem dry matter were not affected by the cytokinin treatments.

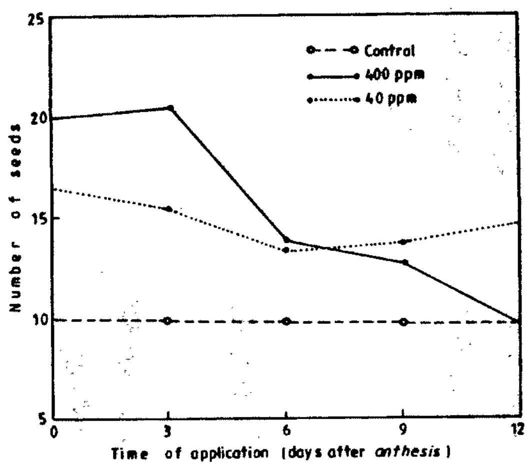

Figure 2. Variation in number of seeds in terminal raceme following BA application (ppm) at two levels 0-12 days after anthesis

DISCUSSION

Results from this study have shown that 6-benzyladenine applied between anthesis and three days after anthesis increases the vigour of normally abscising bean pods on the apical triad of the terminal raceme. As a result, pod abscission is both delayed and reduced. These results seem to confirm histological studies which showed that the processes of cell separation are initiated within about four days of flowering in normally abscising pods on the distal triad of the terminal raceme (Wajja-Musukwe, 1985). BA application commencing within the first three days of flowering therefore appears to prevent the onset of cell separation processes. Once the latter have started, it is not possible to halt them with exogenously supplied cytokinin. The fact that BA was most effective during the early days of flowering agrees with similar observation in *Vicia faba* (Morgan, 1985) and corroborates Bennici and Cionni's (1979) findings which suggested that cytokinins are required for early embryo growth. The observation that BA application starting after three days, and as late as six days, from anthesis reduced pod abscission on the medial and basal triads, respectively, suggests that the onset of cell separation process in pods destined to abscise is different for the three triads on the raceme.

The response of bean pods to exogenous cytokinin as noted in the present study underlines

TABLE 2. The average dry weight (g) of pods and seeds from the three triads of the terminal raceme following BA application at 40 and 400ppm

Triad		Time (days from anthesis) of BA application (ppm)									
		0		3		6		9		12	
	Control	40	400	40	400	40	400	40	400	40	400
Dry weight of pods											
Proximal	0.80	0.84	0.84	0.80	0.87	0.74	0.80	0.84	0.78	0.78	0.8
Medial	0.43	0.74	0.93	0.78	0.74	0.56	0.71	0.59	0.62	0.53	0.56
Distal	0	0.08	0.29	0	0.15	.0	0	0	0	0	0
Dry weight of seeds											
Proximal	0.56	0.53	0.49	0.53	0.56	0.45	0.51	0.49	0.51	0.49	0.51
Medial	0.21	0.53	0.54	0.49	0.44	0.39	0.45	0.38	0.42	0.39	0.38
Distal	0	0.07	0.19	0	0.9	0	0	0	0	0	0

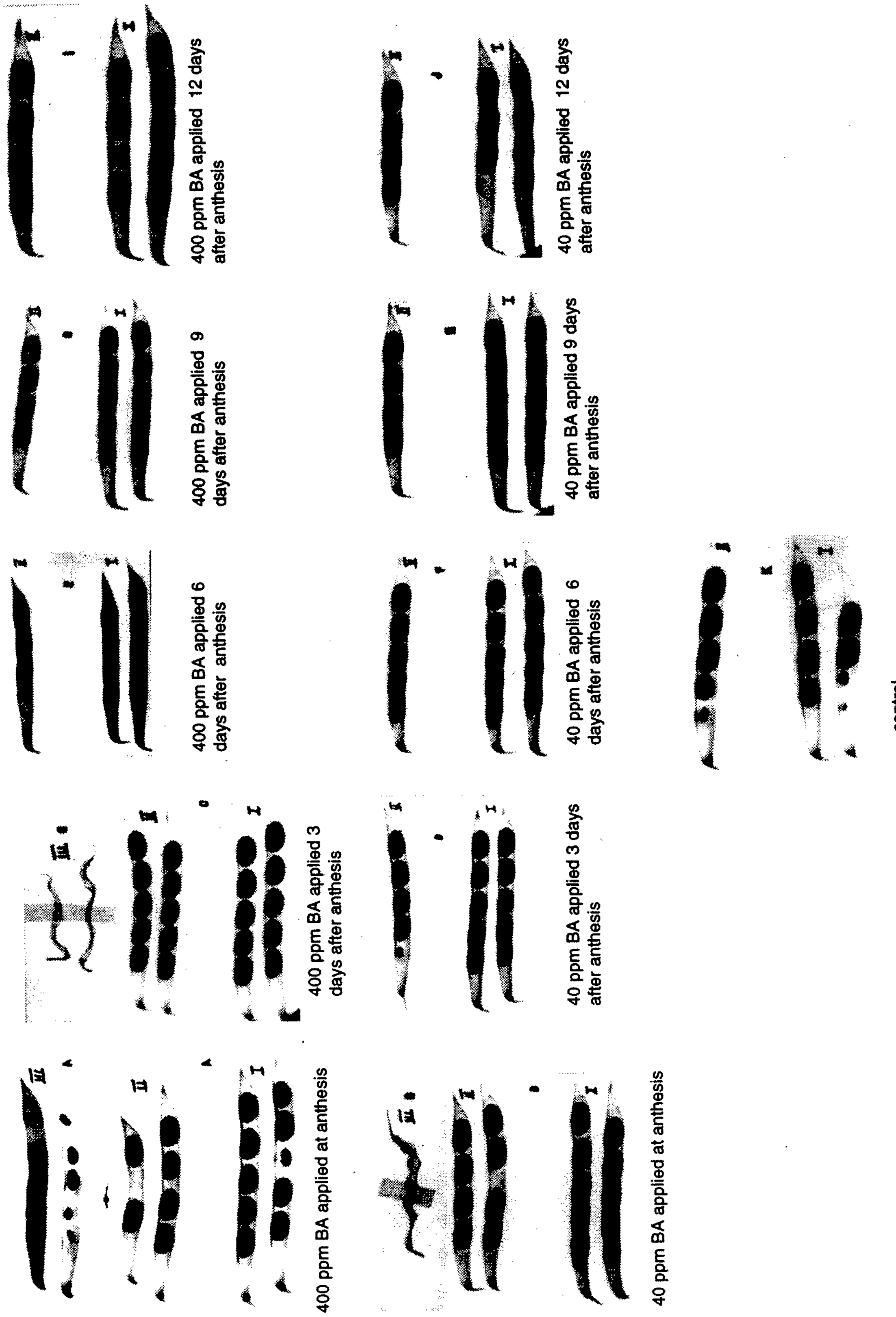

FIGURE 3. X-ray radiographs of pods treated with 6-benzyladenine

the importance of cytokinins for bean fruit development and suggests that fruit abscission may be caused by a lack of vital endogenous growth promoting substances. Other published reports (e.g. Adedipe *et al.*, 1976; Clifford, 1981; Crosby *et al.*, 1981, Okelana and Adedipe, 1982) have shown that cytokinins are some of the growth substances that might become limiting, leading to abscission.

That pods and seeds from the medial triad were more responsive to BA in terms of dry matter accumulation than those on the proximal triad is similar to results obtained in cowpea by Adedipe *et al.* (1976) who found that the distal young pods on the inflorescence sequestered more 14C following BA treatment than the older pods lower down the inflorescence. This failure of the lowermost pods to respond to BA may suggest that they initially contain adequate amounts of cytokinins. Thus, it is reasonable to assume that there is a concentration gradient of growth promoting substances along the inflorescence, which favours the lower most pods. Since cytokinins are synthesised in the roots (Torrey, 1976) it could be that earlier formed proximal pods intercept cytokinins and other growth substances before they reach distal pods. Future work aimed at clarifying the role played by plant growth substances in bean pod development may involve systematic monitoring of the distribution of these substances in different sections of the inflorescence at specific intervals of the reproductive phase. The effect of BA in increasing bean seed yield corroborates earlier observations in cowpea (Okelana and Adedipe, 1982) and soybean (Crosby *et al.*, 1981). In view of earlier reports that cytokinin levels increase in seed tissue during periods of active seed development (Burrows and Carr, 1970; Davey and van Staden, 1978) it seems clear that seed requirements for cytokinins are fairly high. Therefore, the number of fertilized ovules that develop into mature seed would be expected to increase pod retention and seed yield without any significant effect on the number of pods on other nodes and dry matter of the stem and leaves. Since it is known that fertilization is not a limiting factor in *Phaseolus vulgaris* (Gabelman and Williams, 1962), the seed yield increment could be attributed to the growth substance causing redistribution of dry matter within the treated reproductive organs in favour of seed development.

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