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Regulation of Stem Elongation in Chinese Cabbage by Inflorescence Removal and Application of Growth Regulators

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Abstract. The effect of inflorescence removal on stem elongation in Chinese cabbage cv. Spring A was studied. Removal of the inflorescence before its visibility, or upon its appearance but before the beginning of bolting (stages 1–3), markedly reduced the stem length. Removal after the beginning of bolting (stage 5) had no effect on stem length.

Application of GA_3 to the treated plants partially or fully restored the elongation of the flowering stem, whereas paclobutrazol inhibited the elongation of the treated, as well as the control stems. Indole-3-acetic acid (IAA) or kinetin was ineffective in restoring stem elongation of the plants from which the inflorescence had been removed. Inflorescences at stages 1–2 were found to secrete about 10 times more gibberellic acid (GA)-like activity compared with control apices or inflorescences at stage 5.

It is suggested that the developing inflorescence is the major source of GAs which control stem elongation. However, shortly after the appearance of the inflorescence at the onset of bolting, stem elongation is no longer dependent on GAs derived from the apical inflorescence but require GAs from other sources.

Chinese cabbage (*Brassica campestris* subsp. pekinensis) is a biennial plant which requires low temperature for the induction of bolting and flowering (Lorenz 1946; Pressman and Negbi 1981). In cold-requiring plants, the assumption that the shoot apex is the site which senses and responds to low temperature is widely accepted (Lang 1965; Vince-Prue 1975). It is generally agreed that endogenous gibberellins (GAs) play an important role in mediating the response to low temperature (Zeevaart 1984). However, Metzger (1985) could not find any changes in GA-like substances related to the thermoinduced stem elongation in a causal manner. As to the source of GAs, Zeevaart (1978) postulated that flower buds are the source of the increase in the level of GA-like substances following vernalization. This generalization was in agreement with data of Kaufman et al. (1976) showing that the inflorescence and nodes of *Avena* are the major source of native GAs. Op den Kelder et al. (1971) found that the removal of tulip flower buds inhibited the elongation of the last internode. Indole-3-acetic acid (IAA) or NAA could replace the flower, whereas similar applications of GA and kinetin were ineffective.

In the 'Spring A' cultivar of Chinese cabbage, the inflorescence appears before visible bolting begins and it is possible to remove it at different stages. The effect of this treatment on stem elongation was studied in the present work. The hypothesis that the inflorescence as a source of endogenous GAs plays a major role in stem elongation is discussed.

Materials and Methods

Plant Growth and Apices Removal

Seeds of Chinese cabbage cv. 'Spring A' were sown in polystyrene trays in a greenhouse with day and night temperatures of 25° and 17°C, respectively. One-month-old plants (five to six leaves) were vernalized for 1 month in a growth chamber at a constant temperature of 6 ± 1°C with 8 h of light (60 $\mu E\ cm^{-2}\ S^{-1}$ at plant level) provided by 40 W fluorescent (Tadiran, Israel) tubes. After vernalization the plants were placed in 15-cm plastic pots containing a mixture of volcanic ash (tuff), peat, and vermiculite (1:1:1 vol/vol) and then placed in a growth chamber with a constant temperature of 20 ± 2°C and 16 h of light at the above intensity.

Under these conditions the inflorescences became visible

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about 3 weeks after the end of vernalization. The young leaves were carefully separated and the inflorescences were removed at different stages of development, using fine forceps.

The five stages examined were (1) 5–6 days before the expected appearance of the inflorescence; (2) 2–3 days later; (3) at inflorescence appearance; (4) 2–3 days after its appearance but before the beginning of bolting; and (5) 2–3 days later, when bolting started (stem length of 2–3 mm).

The growth regulators GA_3, kinetin, IAA (Sigma, USA), or paclobutrazol [2RS, 3RS-1-(4-chlorophenyl)-4, 4-dimethy-2 (1H-1,2,4 triazol-1-yl) penta-3-ol] (I.C.I., England) were applied with a micropipette directly to the center of the rosette.

Every experiment was repeated at least two times with 12 plants for each treatment.

GA Collection and Bioassay

A combination of the agar diffusion (Jones and Phillip 1964) and the immersion assay (Murakami 1973) techniques was used to analyze changes in GA-like substances. Apices and inflorescences at the following stages of development were sampled: (1) apices of control nonvernalized plants, (2) inflorescences of stages 1 and 2, and (3) inflorescences at stage 5. After the removal of the young visible leaves, 10 excised apices at each stage were placed (slightly inserted) on 3 ml of 0.8% agar blocks held in small plastic cups. The cups were placed in plastic boxes on wet filter paper and covered with a glass cover. The boxes were placed in a growth chamber at 22°C continuously illuminated with fluorescent tubes providing 120 $\mu E\ m^{-2}\ s^{-1}$.

After 3 days the apices were replaced by 10 germinating seeds of dwarf rice (cv. 'Tan ginbozu') and the temperature was raised to 28°C. The length of the second leaf sheath was measured 6 days after planting. To establish a standard curve, a known amount of GA_3 was mixed with agar and germinating dwarf rice seeds were placed in it.

Results

In a preliminary experiment it was found that removing the inflorescence before visible bolting started caused a marked reduction in the flower stem length. Application of GA_3 to the treated plants partially restored growth, while paclobutrazol prevented the elongation of stems of both treated and control plants.

Therefore, an experiment was initiated to examine the effect of the removal of the inflorescence, at different stages of development, on stem elongation (Fig. 1). Removing the inflorescence at stages earlier than or just at its visible appearance (stages 1–3) greatly reduced stem growth and the stems were not significantly different in length. However, plants treated 2–3 days after the appearance of the inflorescences (stage 4) or after bolting had already started (stage 5), produced stems as long as or slightly longer than those of the control plants. In general the stems of plants decapitated at stages 4 and 5 and those of the control plants were significantly longer than those treated at stages 1–3. The removal of the inflorescences at any stage of development had no effect on plant weight (Fig. 1).

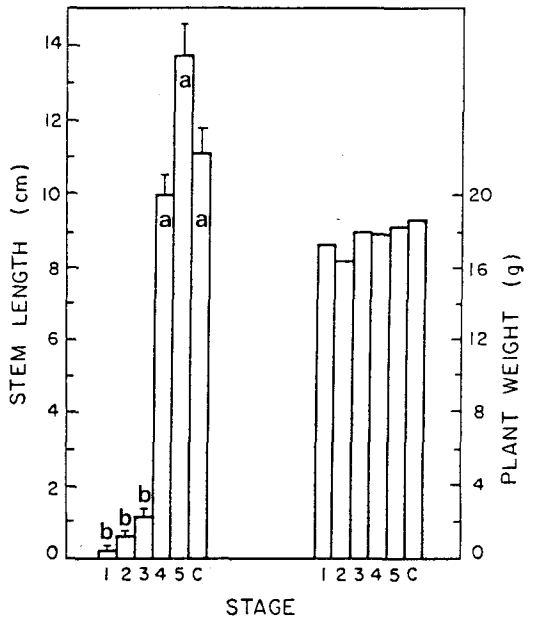

Fig. 1. The effect of inflorescence removal at five stages on stem length (left) and plant weight (right) of Chinese cabbage cv. Spring A compared with intact, control plants (c). Values of the stem length followed by the same letter are not statistically different at $p = 0.05$ by the multiple range test. Vertical bars represent the standard error.

Plants with flower buds removed at stage 3 (young visible inflorescence) developed stems significantly shorter (13%) than the control, at stage 4 (visible inflorescence) the stem length was 60% of the control (not significantly different), and at stage 5 stem length was not reduced. Application of GA_3 to the debudded plants at stage 3 caused a fivefold increase in stem length, similar to that of the control plants. At stage 4 it caused a 3.5-fold increase in stem length, double that of the control stems and even longer, although not significantly, than the GA_3-treated control stems. At stage 5 the effect of GA_3 was similar to its effect on the control plants (Fig. 2).

Daminozide, chloromequat chloride, and paclobutrazol were found to inhibit stem elongation in intact plants with a dose–response effect. GA_3 was able to restore the elongation of stems inhibited by the growth regulators, and the restoration was dependent on the concentration of the applied GA_3 (data not shown). Data in Fig. 2 show that paclobutrazol at 50 ppm markedly inhibited elongation of control, as well as debudded plants, and that GA_3 at 250 ppm reversed the inhibitory effects of debudding.

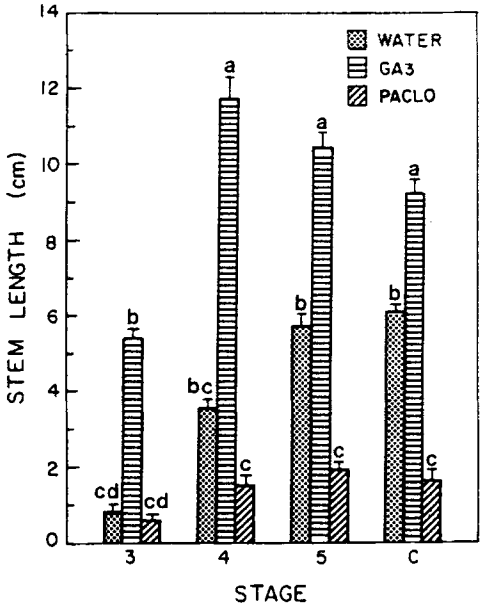

Fig. 2. The effect of GA_3 (250 ppm) and paclobutrazol (Paclo, 50 ppm) treatment on inflorescence-removed (three stages) and intact (control, c) Chinese cabbage cv. Spring A plants. Values followed by the same letter are not statistically different at p = 0.05 by the multiple range test. Vertical bars represent the standard error.

The removal of the inflorescence at any stage did not affect plant weight or leaf length (Table 1), while GA_3 tended to increase and paclobutrazol significantly decreased these two parameters. Average plant weight and leaf length were increased by 13 and 24%, respectively, by GA_3 and decreased by 35 and 40%, respectively, by paclobutrazol. The number of leaves was not affected by the growth regulators (data not presented).

IAA or kinetin, whether applied individually or together, could not restore the inhibitory effect of inflorescence removal. Moreover, they slightly, although not significantly, decreased the promoting effect of GA_3. Plant weight (approximately 10 g) was not affected by the growth regulators nor by inflorescence removal (data not presented).

Data in Fig. 3 show that both control nonvernalized apices and stage 5 inflorescences secreted GA-like activity equivalent to 0.1 ng/ml of GA_3. However, the very young inflorescences (at stages 1 and 2) secreated nearly 10 times higher GA-like activity.

Discussion

Removal of the floral buds was found to reduce stem elongation in tulip (Op der Kelder et al. 1971) and the penultimate internode in *Avena* (Koning et al. 1977). The removal of floral buds 2 days before

Table 1. The effect of inflorescence removal and application of GA_3 (250 ppm) and paclobutrazol (Paclo, 50 ppm) on leaf length (cm) and plant weight (g) in Chinese cabbage cv. Spring A.

Removal stage	Plant weight			Leaf weight		
	GA_3	Paclo	Water	GA_3	Paclo	Water
3	34.4 a	16.1 d	27.3 ab	11.2 a	5.3 c	8.7 b
4	34.1 a	18.1 cd	30.0 ab	10.9 a	5.5 c	9.1 ab
5	27.1 ab	17.2 d	26.4 bc	11.0 a	5.1 c	8.6 b
Control	28.3 ab	17.4 d	24.0 bc	10.7 a	5.4 c	8.8 b

Values, for each parameter, followed by the same letter are not statistically different at p = 0.05 by the multiple range test.

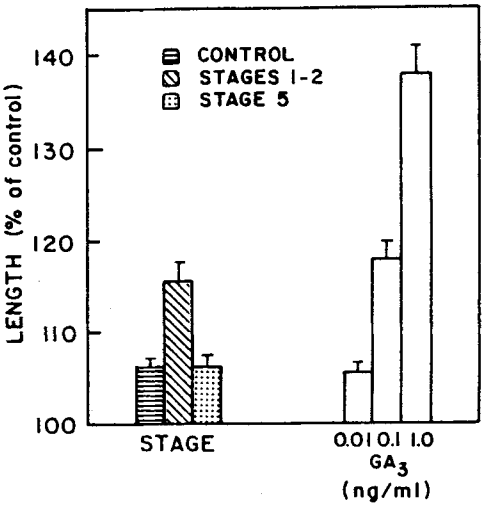

Fig. 3. GA-like activity of the substances secreted by Chinese cabbage apices, removed at different stages, to agar blocks. Activity was detected by the elongation of dwarf rice second leaf sheath.

anthesis markedly decreased the height of petunia plants, and removal at other stages had a lesser effect (Pressman et al. 1985).

In the present work a marked reduction in stem length of Chinese cabbage cv. Spring A was obtained by removing the inflorescence at different stages before the beginning of visible bolting (Figs. 1 and 2). Differentiation of the inflorescence in Chinese cabbage was found to start 10 days after the end of vernalization (Po-jen 1969). In the present work the first treatment took place 24 days after the end of vernalization, which meant that differentiated inflorescences, although not visible, were removed. However, upon the beginning of bolting inflorescence removal did not affect stem length (Figs. 1 and 2).

In *Avena*, inflorescence has been postulated to be a major source of GAs which control stem elongation (Kaufman et al. 1976; Koning et al. 1977). Like-

wise, in view of the present results indicating that Chinese cabbage inflorescences also secrete GA-like substances (Fig. 3), it is suggested that the endogenous GAs which regulate stem elongation are also derived from this organ. The fact that in Chinese cabbage, as in *Avena* (Koning et al. 1977) and petunia (Pressman et al. 1985), but not in tulips (Op der Kelder et al. 1971), the inhibitory effect of the inflorescence removal could be partially or fully reversed with exogenous GA_3 (Fig. 2), but not with IAA and/or kinetin, supports this suggestion. Kigel (1981) found that GA promoted internode elongation in decapitated bean plants and that the addition of IAA enhanced elongation. In the present work IAA did not show a synergistic effect with GA_3 (data not presented).

The data in Figs. 1 and 3 suggest that the basipetal transport of GAs to the stem takes place only during the early stages of development of the flower buds (stages 1–3). Upon full appearance of the inflorescence (stage 4) or at the beginning of bolting (stage 5), stem elongation was no longer dependent on this source. On the other hand, paclobutrazol inhibited the elongation of stage 5-treated stems to the same extent as the control plants, which were treated immediately after the end of vernalization (Fig. 2). This indicates that organs other than the apical inflorescence may be sources of GAs. One source might be inactive or root-activated GAs moving in the vascular system (Metzger and Zeevaart 1980). Other sources could be young leaves or flowering side shoots.

The size of the plant affects its bolting intensity in such a way that small plants bolt more slowly than big plants (E. Pressman, unpublished data). Inflorescence removal at any stage did not affect the plant size (Fig. 1), indicating that the treatment had a direct effect on stem elongation. GA_3 increased the plant weight by increasing the leaf size (Table 1), while paclobutrazol had the opposite effect. However, the effect of the two applied regulators on stem elongation was much greater than on plant size, indicating a direct effect on the former.

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