INDUCTION OF FLOWERING IN CABBAGE PLANTS BY *IN VITRO* VERNALIZATION, GIBBERELLIC ACID TREATMENT AND RATOONING

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ABSTRACT

A study was carried out to determine whether applying cold to plants in vitro, or gibberellic acid treatment later to green plants in soil, alone or in combination, can induce flowering in cabbage plants raised from seeds at 20°C. Sterilized seeds of four lines of cabbage were cultured in glass jars and kept in the growth room 25°C for 56 d after which they were cold treated (6°-13°C) for another 56 d. The seedlings generated were later transplanted into 15 cm-plastic pots and 250 mg l^{-1} gibberellic acid (GA_3) was later applied to the leaves of plants, followed by six further applications at 1-week intervals. Results showed that one line, 'HRI 003202', produced more leaves than the other three lines but the stem girth of the lines was not affected after 8 weeks cold treatment. At 21 d after transplanting, 'HRI 003202' had the smallest stem girth, shortest leaves and stem and highest leaf number, however all lines varied significantly from each other in terms of stem height. For line 'HRI 006556', GA_3 did not have any effect ($P > 0.05$) on cold treated plants but reduced both the leaf number and number of days at flowering of the control plants. One of the three replicates of line 'HRT 007617' flowered when a combination of cold and GA_3 treatments was applied to plants.

INTRODUCTION

Cabbage is one of the popular 'exotic' vegetables in many tropical countries, especially in West Africa. However, its large scale production and utilization are hampered partly by the high cost of seed. The crop is known to have a diploid chromosome number (2n) of 18 with the genome and the cytoplasm denoted as cc and C respectively (Tindal, 1983; Dickson and Wallace, 1986). Brassicas of such genetic make up are known to flower only when green plants complete a juvenile period and exposed to cold treatment (2°-10°C) for a critical duration of time (Inouye and Kuo, 1981). It is therefore difficult to produce the cabbage seed in most tropical countries because of the absence of the low temperature necessary for flower induction (vernalization).

However, previous work (Nyarko *et al.*, 2007) showed that, GA_3 treatment could induce flowering in at least one line of cabbage provided the plants are raised from cold-treated seeds. This has raised a strong suspicion of gibberellic acid (GA_3) involvement in cabbage flowering. Some researchers have also used *in vitro* techniques to achieve flowering in plants (Tang *et al.*, 1983; Yoshikawa and Furuya, 1983; Naik and Latha, 1996; Virupakshi *et al.*, 2002). It has been further reported that in some species, excised apices grown *in vitro* are sensitive to some flower promoting factor like photoperiodic treatments (Francis, 1987), and it is likely that cabbage can be sensitive to cold *in vitro*.

The aim of this experiment is to determine whether cabbage plants raised from seeds and cold-treated *in vitro* can flower with or without GA_3 application at relatively high temperatures (20°C) as an alternative to the normal green plant vernalization.

MATERIALS AND METHODS

Seeds of four cabbage lines ('HRI 009617', 'HRI 006556', 'HRI 003202', and 'K- K Cross') were separately dipped in 2 ml of 5 % Parozone bleach for 5 minutes. The seeds were then washed five times in 4 ml of purified water and cultured in 100-ml screw-capped glass jar (Beatson Clark and Co Ltd, Rotherham, UK) containing 15 ml of heat sterilized agar medium (Murashige and Skoog, 1962) with 3 % sucrose (weight / volume). The sucrose was added to the agar before autoclaving. The cultured plants were kept at 25°C for 56 d after which they were cold treated (6-13 °C) for another 56 d while in culture. The control plants were kept at 25°C for 66 days. Plants generated were transplanted into 4 cm-square plastic modular trays (filled with 46 g Levington F2S compost). When seedlings were 21 days old, they were again planted into plastic pots of 15 cm top diameter containing 0.58 kg Levington M2 soil mix. Then, 4 g ammonium nitrate per pot was applied as a top dressing on two occasions to all plants, i.e. one month after transfer of plants to Levington M2 compost in 15 cm pots and one month later (a total of 8 g ammonium nitrate).

Plants were arranged in a randomised complete block design with three replications. In each block, a plant which had been cold-treated *in vitro* and a control treatment of each line was sprayed until run off with a GA_3 solution, and another with distilled water, using a hand sprayer. Two drops of Tween 80 (wetting agent) were added l^{-1} to each spray. The GA_3 solutions were prepared at 250 mg l^{-1} by dissolving 250 mg of GA_3 in 10 ml ethanol and making up to 1 l with distilled water. The GA_3 application started 14 d after planting out from the glass jars and there were seven applications at one-week intervals.

Parameters measured included stem girth and leaf number just after 56 d cold treatment. Leaf number, length of the longest leaf, stem girth and height were also measured at 21 d (before GA_3 application), 60 d (i.e. 30 d after first GA_3 was applied) after transplanting (DAT) and at flowering. Also measured were days to flowering after transplanting (DFAT) of all plants that flowered.

RESULTS

Data taken immediately after the 56 d cold treatment showed that line and cold treatments significantly affected leaf number independently. Line 'HRI 003202' produced more leaves ($P = < 0.001$) than the other three lines (Table 1), which did not differ in the number of leaves produced within the period. Plants which were given cold treatment also produced more leaves ($P = 0.027$) than the control plants (Table 1). The four lines did not show any differences in stem girth ($P = 0.05$) after the cold treatment

At 21 DAT, 'HRI 003202' had the smallest stem girth, shortest leaves and stem and highest leaf number (Table 2). The length of the longest leaf and stem girth were not different for the other three lines, however all lines varied significantly from each other in terms of stem height; line 'HRT 006556' was the tallest. Plants exposed to the eight weeks cold treatment had significantly taller stems ($P = 0.010$) as compared the controls (data not shown)

Similar to what happened at 21 DAT, plants which were exposed to eight weeks cold treatment grew taller than the untreated plants at 60 DAT. GA_3 generally increased the stem height, leaf number and length of the longest leaf of all cabbage lines at 20°C (Table 3)

Among the four cabbage lines tested, only one ('HRT 006556') was able to flower in the growth room without prior vernalization. The combination of GA_3 and cold treatment affected leaf number at flowering and number of days to flowering of line 'HRT 006556' (Figure 1). GA_3 did not have any effect ($P = 0.05$) on cold treated plants but reduced both the leaf number at flowering and DFAT of plants that did not receive the cold treatment (control). The stem height at flowering of 'HRT 006556' was not affected by any of the treatments (Table 4). Cold treated plants had narrower stem girth at flowering as compared to the controls.

For the other three cabbage lines, neither GA_3 nor cold treatments applied without the other was able

Table 1: Cabbage line and cold treatment main effects on leaf number after 58 days exposure to cold (6-13°C)

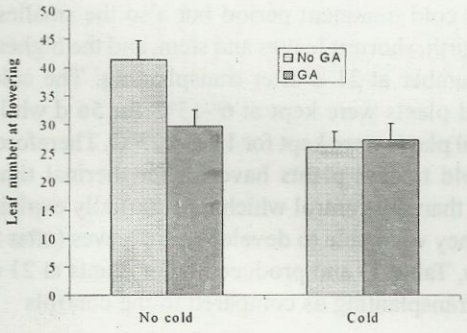
Line	Leaf number	Treatment	Leaf number
'HRI 006556'	2.67	No cold treatment	2.70
'HRI 009617'	2.71	Cold treatment	2.84
'HRI 003202'	3.03		
'K-K cross'	2.67		
SED#	0.089	SED#	0.063
df	24	df	24
Probability	<0.001	Probability	0.027

Data were square root transformed and transformed data are presented.
SED = standard errors of the differences of means.

Table 2: Cabbage line main effects on leaf and stem parameters at 21 d after transplanting

Line	Leaf number	Length of longest leaf	Stem girth	Stem height
HRI 006556	3.17	2.08	1.28	2.90
HRI 009617	3.07	2.24	1.34	2.35
HRI 003202	3.41	1.85	1.01	1.95
K K cross	3.28	2.25	1.30	2.44
SED#	0.080	0.157	0.10	0.159
Df	14	14	14	14
Probability (*p*)	0.004	0.074	0.021	<0.001

Data were square root transformed and the transformed data are presented.
SED = standard errors of the differences of means.



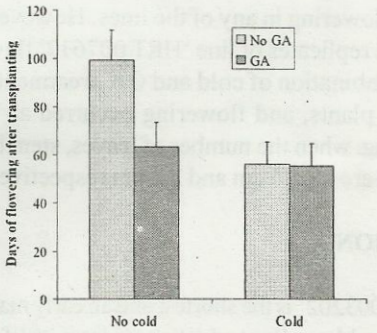


FIGURE 1: The combined effects of GA_3 and cold treatment on the leaf number at flowering and number of days to flowering after transplanting of 'HRT 006556' cabbage.
Error bars = one standard error of the mean predicted from regression mode

Table 3: The effects cold treatment and GA_3 treatments on stem girth, stem height, leaf number and length of longest leaf of four lines of cabbage at 60 d after transplanting

Line	Stem girth ($cm^{1/2}$)				Stem height ($cm^{1/2}$)				Leaf number				Length of longest leaf ($cm^{1/2}$)			
	No cold		Cold		No cold		Cold		No cold		Cold		No cold		Cold	
	GA	NGA	GA	NGA	GA	NGA	GA	NGA	GA	NGA	GA	NGA	GA	NGA	GA	NGA
HRT 003202	2.0	2.0	2.0	1.8	4.8	2.5	4.9	2.6	5.0	4.2	5.0	4.6	5.2	5.3	5.1	4.9
HRT 006556	2.1	2.0	1.9	19	5.3	3.7	5.3	4.9	4.8	4.6	5.5	4.7	5.8	5.7	6.0	5.6
HRT 009617	2.0	2.0	2.1	1.9	5.0	2.6	5.6	3.3	4.6	4.2	4.6	3.8	5.4	5.3	5.6	5.4
K K cross	2.2	2.0	2.0	2.0	4.1	2.9	4.7	3.2	5.3	5.1	4.8	4.8	5.5	5.2	5.5	5.3
Df		30				30				30				30		
Replication		3				3				3				3		

Data were square root transformed before analysis and the transformed data are presented

Table 4: GA_3 and cold treatments main effects on cabbage length of longest leaf, stem girth and stem height (stem ht) of 'HRT 006556' taken at the day of flowering

Treatment	Longest leaf (cm)	Stem ht (cm)	Treatment	Stem girth (cm)	Stem ht (cm)
No GA_3	29.83	31.0	No cold treatment	5.17	3.68
GA_3 treatment	34.17	40.5	Cold treatment	3.92	3.47
SED#	1.351	6.02	SED#	0.180	6.02
Df	6	6	df	6	6
Probability	0.018	0.166	Probability	0.003	0.731

Data were square root transformed and the transformed data are presented.
SED = standard errors of the differences of means.

to induce flowering in any of the lines. However, one of the three replicates of line 'HRT 007617' flowered when a combination of cold and GA_3 treatments was applied to plants, and flowering occurred at 148 d after sowing, when the number of leaves, stem height and girth were 48, 96 cm and 2.6 cm respectively.

DISCUSSION

Line 'HRI 003202' is the shortest and an early maturing of the four cabbage lines which completes its lifecycle earliest as compared to the others. This fact may not only have accounted for the ability of that line to produce the highest number of leaves within the 8 weeks cold treatment period but also the smallest stem girth, shortest leaves and stem, and the highest leaf number at 21 d after transplanting. The cold treated plants were kept at 6°-13°C for 56 d while control plants were kept for 11 d at 25°C. Therefore, the cold treated plants have higher thermal time (°Cd) than the control which may partially explain why they were able to develop more leaves (after 8 weeks, Table 1) and produced taller plants at 21 d after transplanting as compared to the controls

It was not surprising that GA_3 increased the stem height of all the cabbage lines at 60 d after transplanting. Gibberellins (e.g. GA_3) are known to

increase stem height of many crops (Suge and Rappaport, 1968; Nyarko *et al*, 2007). The biophysical mechanism triggered by GA_3 to cause stem elongation in plant is still a contentious issue. For example, Clerand *et al* (1968) proposed that GA_3 increases the elongation rate in plants by exerting greater osmotic pressure on the growing cells which generate greater turgor pressure and subsequently drive cell wall extension at greater rate. However, this conclusion was contradicted (Bahringer *et al*, 1990) and it was rather suggested that that GA enhances stem elongation by both decreasing the wall yield threshold and increasing the wall yield co-efficient.

Both the cold treated and control plants of 'HRT 006556' were able to flower, which confirmed the earlier assertion (Nyarko *et al*, 2007) that, it undergoes another flowering pathway different from the three lines. It was also revealed (Figure 1) that GA_3 was able to reduce the leaf number and DFAT only of plants that have not received the cold treatments and the combined effect was similar to when the factors are applied without the other. The implication is that GA_3 can be substituted for cold in that line to promote early flowering and that it is not necessary to use both factors to achieve the same result. The stem girth at flowering of the cold treated plants was smaller than control (Table 3) probably because the former flowered earlier. The control plants therefore had more time to grow and increased in stem girth before flowering. The treatments did not have any effect on the stem height at flowering because both the treated and control plants elongated their stems (bolted) to reach similar height before flowering.

A previous study showed that when eight weeks old potted plants (with more than 7 leaves and stem girth greater than 2 cm) of two of the cabbage lines ('HRI 009617'and 'HRI 003202',) were exposed to cold (0°-4°C) for 8 weeks, they were subsequently able to flower (Nyarko *et al*., 2006). That result affirmed the earlier observations that some cabbage lines need about seven-to-nine leaves, or a stem diameter of at least 6 mm (stem girth = 1.9 cm) before they become sensitive to low temperatures (i.e., plant-vernalisation responsive type, Friend, 1985; Lin *et al*., 2005). In this present work, the minimum leaf number (> 7 leaves) required for the plants to sense cold was attained after growing the plant *in vitro* for 8 weeks. However, the plants could only attain a stem girth between 0.4-0.5 cm instead of the required 1.9 cm, due to growth restriction in the glass jar. It is therefore likely that, most of the plants of the three lines could not sense the cold to allow for the subsequent induction of flowering, because they had not reached the critical stem girth. Attainment of threshold size was reported to be particularly important in biennials such as cabbage since it is related to the amount resources accumulated (Bernier and Perilleux, 2005). It may therefore be useful, in future experiments, to ensure that the critical girth of 1.9 cm be attained before cold treatment for vernalization is imposed.

It was expected that the combined effect of cold and GA_3 would cause flower induction in 'HRI 009617' as happened in a previous work (Nyarko *et al*., 2007) when the cold (0°-4°C) was applied on primed seeds. One out of the three replicates of that line was able to flower in this case when the combined treatments was applied, giving an indication that the others might have sensed the cold but not enough to cause flower induction. Unlike the previous work where a cold temperature range of 0-4°C was applied, in this present study a temperature of 6°-13°C was applied because that was the minimum temperature range that could be obtained in the growth room during the experimental period (hot summer). The temperature range for vernalization in many brassicas does not usually exceed 10°C (Nieuwhof, 1969; Yamaguchi, 1983). Hence, the combined effect of the factors may not have induced all the plants to flower probably because the cold temperatures applied were not optimal for vernalization. The fact that one plant of the line ('HRI 009617') flowered in response to the combined effects of cold and GA_3 may be due to chance but it may also be a confirmation of the earlier report (Nyarko *et al*., 2007) that the same line flowered after vernalization as seed and that not all cabbage lines need to reach certain developmental stage and

size before they are sensitive to flower induction factors. Further work is needed however, perhaps with lower temperatures and longer vernalization durations to confirm this.

CONCLUSION

For cabbage line 'HRI 006556' which flowers even without cold treatment, GA_3 did not have any effect ($P > 0.05$) on cold treated plants but reduced both the leaf number and number of days at flowering of plants that did not receive the cold treatment (control). The combined effect of *in vitro* cold treatment and GA_3 induced flowering in one of the three replicates of line 'HRI 009617'.

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