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Comparative Effects of Chlormequat Chloride (CCC) and Gibberellic Acid (GA_3) on the Flowering and Seed Production of Lettuce

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ABSTRACT

The objective of the present study was to determine if the application of chlormequat chloride (CCC) at the rosette stage would have a positive effect on seed stalk growth, flowering, seed yield and seed quality of lettuce in comparison with gibberellin (GA_3) or water (control) applied at the same stage. Seeds of lettuce were sown on 16 October (cvs. 'Great Lakes' and 'Parris Island') and 20 January ('Parris Island') and the plants of both sowings were cultivated in an unheated greenhouse until July and August, respectively. At the rosette stage (8 leaves), the plants were sprayed with 30 ppm GA_3, 500 ppm CCC or water (control). Although GA_3 induced rapid bolting, its effect on the time of flowering and harvest were less pronounced. GA_3 increased seed yield and in some cases the mean 1000-seed weight and percent germination. However, the seed stalks of GA_3-treated plants were longer and thinner than those of the control. Treatment with CCC at the 8-leaf stage produced plants with better seed stalk characteristics (shorter and thicker), but yield and seed quality (mean 1000 seed weight, germination) were similar to the control and significantly less than that of the GA_3 treated plants. In 'Parris Island', the yield and quality of seed were better in the October sown crop than in the January sown crop. Whereas the application of GA_3 is indicated for lettuce seed production, especially in tightly-heading cultivars such as 'Great Lakes', CCC is beneficial only in terms of seed stalk characteristics.

Keywords: bolting, lodging, flower stalk, germination, seed size

INTRODUCTION

Certain lettuce (*Lactuca sativa* L.) cultivars, particularly those of the crisphead (iceberg) type, form tightly wrapped heads that inhibit the elongation of the flower stalk during seed production and may therefore require mechanical injury (such as slashing or deheading) to assist the emergence of the flower stalk (George 1985). Alternatively, the application of gibberellin to lettuce at the rosette stage of plant growth can induce bolting without the intermediate formation of a vegetative head (Harrington 1960), leading to greater uniformity of flowering and increased seed yield (Gray *et al*. 1986). However, gibberellin also promotes elongation of the flower stalk, leading to a reduction in stalk strength.

The application of the anti-gibberellin chlormequat chloride (CCC) to field crops of cereals at the 3-5 leaf growth stage reduced the height of wheat (*Triticum aestivum* L.) and to a lesser extent barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.), thus delaying lodging. In all three crops, height reduction was cultivar related (Clark and Fedak 1977). In common bent (*Agrostis capillaris* L.), CCC application was recommended to reduce flower stalk height, and therefore lodging (Aamlid *et al*. 2007). Lettuce flower stalks are not woody and their relative susceptibility to lodging is increased by the use of gibberellin. Although the flower stalk height of gibberellin-treated plants was reduced by the subsequent application of 1500 ppm CCC at the bolting stage, this treatment caused a decrease in yield (Koutri *et al*. 2007; Passam *et al.* 2008).

Therefore, we investigated the effects of CCC and gibberellin application at the rosette stage on the subsequent pattern of flowering and seed production in a lettuce cultivar that normally forms a tightly wrapped head ('Great Lakes', crisphead type) and one that forms a looser head ('Parris Island', Cos type). The objective of the study was to see if CCC would positively affect flower stalk growth (reduced height, increased diameter), seed yield and quality (seed size, germination) in comparison with gibberellin.

MATERIALS AND METHODS

Two lettuce (*Lactuca sativa* L.) crops were grown at the Agricultural University of Athens (37° 58′ 55″ latitude and 23° 32′ 14″ longitude, 30 m above sea level) during the period October 2003–August 2004. The average temperature throughout the growth period was 14.77°C and 17.83°C in the autumn and winter sowings respectively, while average solar radiation was 151.09 W m^{-2} and 212.01 W m^{-2} in the autumn and winter sowings respectively. In the autumn-sown crop, seeds of cv. 'Parris Island' (Cos type, *L. sativa* var. *longifolia* Lam.) and cv. 'Great Lakes' (crisphead type, *L. sativa* var. *capitata* L.) were sown in compartments (8.5 x 8.5 x 4 cm) within polystyrene trays (40 compartments per tray) containing peat compost (KTS1, Klasmann-Deilmann Gmbh, Geeste, Germany) on 16 October followed by transplantation to the soil of an unheated, plastic-covered greenhouse on 30 November. Three or four seeds were sown per compartment, but after emergence only one plant (the most robust) was retained. In the winter-sown crop, seeds of cv. 'Parris Island' were sown on 20 January, followed by transplantation on 23 February. Although seed crops of lettuce are more commonly grown outdoors in the spring, the use of greenhouses, especially for basic seed production, not only increases yield per m^2 but enables earlier sowing and improves isolation against unwanted pests, diseases and possible cross-pollination (George 1985; Saplaouras *et al*. 2001). The plants (45 per crop) were placed in double rows with distances of 40 cm between rows, 25 cm between plants within the rows and with 1.0 m paths between each double row. At the rosette stage (8 leaves of mini-

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Short Communication

mum lamina length 5 cm), the plants were subjected to the following treatments: (1) 30 ppm gibberellic acid (GA_3, British Drug Houses Ltd., London), (2) 500 ppm chlormequat chloride (CCC, Cycocel 40AS, American Cyanamid Co. Parsippany, N.J.), (3) deionized water. The concentration of GA_3 was based on that used by Gray *et al*. (1986), whereas the concentration of CCC was selected empirically on the basis of previous work (not published). Treatments were applied by hand spraying until run off. Each treatment consisted of three completely randomized replicates of 5 plants each. The cultivation procedures were typical of greenhouse crops grown for seed under cover (Saplaouras *et al*. 2001). Seeds were harvested sequentially by hand every 8-10 days from the appearance of the first mature seeds, i.e. dry achenes with a fully developed pappus. In total, 4-5 harvests were carried out per plant. The seeds were cleaned by hand rubbing, sieving and winnowing.

On completion of the harvest period, the height (measured from the soil surface to the top of the flower stalk) and diameter (20 cm above the soil and 10 cm below the first inflorescence branch) of the flower stalk, and the number of inflorescence branches per plant were recorded. The inflorescence branches of lettuce develop sequentially from the flower stalk, the first (or oldest) forming at the lowest site of branching and the younger branches arising from progressively higher points. The seed weight per plant and the 1000-seed weight were recorded. Seed germination was measured on seed batches (4 Petri dishes containing 50 seeds each) in the dark at 20°C according to the guidelines of the International Seed Testing Association (Anonymous 1985).

Experimental data were subjected to analysis of variance and means compared by the least significance difference test ($p = 0.05$) using the statistical packages Statgraphics Plus 5.1 and JMP 4.02. (Statistical Graphics Corp., Herndon VA, USA).

RESULTS

Plants of cv. 'Parris Island' that were sprayed with GA_3 at the rosette (8-leaf) stage bolted 53 days (autumn sowing) or 41 days (winter sowing) earlier than the control (sprayed with water), whereas those sprayed with CCC at the same stage bolted 15 or 10 days (autumn and winter sowings respectively) later than the control. Despite the promotion of bolting by GA_3, both the start of flowering and the first harvest were promoted by only 8 days (autumn sowing) or 6 days and 4 days respectively (winter sowing) relative to the control, whereas CCC delayed flowering and first harvest by 21 and 3 days (autumn sowing) or 17 and 7 days (winter sowing) respectively. In 'Great Lakes', GA_3 promoted bolting by 50 days and in this cultivar the promotive effect of GA_3 on flowering (23 days) and first harvest (15 days) was more pronounced than in 'Parris Island'. In 'Great Lakes', CCC delayed bolting and the start of flowering by 18 and 24 days respectively, but the first harvest occurred only 7 days later than the control (**Table 1**).

The flower stalks of plants that were treated with GA_3 were 25% and 36% (autumn and winter sowings of 'Parris Island', respectively) or 40% ('Great Lakes') taller than the control, whereas plants that were treated with CCC were 7 and 11% (autumn and winter sowings of 'Parris Island', respectively) or 26% ('Great Lakes') shorter than the control (**Table 2**). In all cases the diameter of the flower stalk, measured 20 cm above the soil, was significantly lower (GA_3) or significantly higher (CCC) than the control. The diameter of the flower stalk towards the apex (10 cm below the first inflorescence branch) was also significantly less in GA_3 treated plants, but the diameter of the flower stalk of CCC treated plants 10 cm below the first inflorescence branch was significantly higher than the control only in 'Great Lakes' (**Table 3**).

Table 1 The effect of spraying lettuce plants with water, GA_3 or CCC at the 8-leaf (rosette) stage on the subsequent flowering pattern of the plant.

Cultivar/crop	Treatment	Days from sowing until:		
		Bolting	Flowering	First harvest
Parris Island 1*	Water	146	174	212
	GA_3	93	166	204
	CCC	161	195	215
Parris Island 2**	Water	91	111	143
	GA_3	50	105	139
	CCC	101	128	150
Great Lakes	Water	159	193	235
	GA_3	109	170	220
	CCC	177	217	242

*1 = autumn sowing; **2 = winter sowing.

Table 2 The effect of water, GA_3 and CCC application at the 8-leaf stage on the mean height at flowering of lettuce cvs. 'Parris Island' and 'Great Lakes'.

Treatment	Mean plant height at flowering (m)		
	Parris Island 1*	Parris Island 2**	Great Lakes
Water	1.51 ± 0.08 b	1.25 ± 0.05 b	1.67 ± 0.15 b
GA_3	1.89 ± 0.10 a	1.70 ± 0.25 a	2.33 ± 0.40 a
CCC	1.41 ± 0.06 c	1.12 ± 0.10 c	1.23 ± 0.12 c

Means in columns followed by the same letter are not significantly different according to the L.S.D. test (p=0.05).
*1 = autumn sowing; **2 = winter sowing.

Although GA_3 increased the number of inflorescence branches per plant in all crops, CCC had either no effect (autumn sowing of 'Parris Island', 'Great Lakes') or reduced the number of inflorescence branches (winter sowing of 'Parris Island') in comparison with the control (**Table 4**). This result was further reflected in the yield of seed per plant where GA_3 increased the seed yield significantly in comparison with the control, but CCC had no effect (**Table 5**). GA_3 increased the mean 1000-seed weight of 'Parris Island' sown in the autumn, but not in the other crops, whereas CCC had no effect on seed size in any case (**Table 5**). In both crops of 'Parris Island', CCC caused a reduction in the percent germination of seeds compared to the control, but not in 'Great Lakes'. GA_3 increased the germination of 'Great Lakes' but not 'Parris Island' (**Table 5**).

DISCUSSION

The treatment of lettuce plants with GA_3 at the rosette (8-leaf) stage not only assists flower stalk elongation in cultivars that form tightly wrapped heads (Harrington 1960), but also significantly increases seed yield per plant (**Table 5**). Despite the promotion of bolting by GA_3, the time of flowering and first harvest of the autumn and winter sowings of 'Parris Island' is advanced by only 7 ± 1 and 6 ± 2 days, respectively (**Table 1**). This presumably relates to the effect of photoperiod, which means that in the autumn sown crop GA_3 treated plants rapidly bolt, but the flower buds remain closed until the photoperiod is favourable, whereas in

Table 3 The effect of water, GA_3 and CCC application at the 8-leaf stage on the diameter of the flower stalk 20 cm above the soil and 10 cm below the first inflorescence branch of lettuce cvs. 'Parris Island' and 'Great Lakes'.

Treatment	Mean flower stalk diameter (cm)					
	20 cm above soil			10 cm below inflorescence		
	Parris Island 1*	Parris Island 2**	Great Lakes	Parris Island 1*	Parris Island 2**	Great Lakes
Water	1.67 ± 0.07 b	1.68 ± 0.05 b	1.49 ± 0.01 b	1.33 ± 0.07 a	1.33 ± 0.02 a	1.20 ± 0.10 b
GA_3	1.55 ± 0.03 c	1.57 ± 0.04 c	1.26 ± 0.03 c	1.24 ± 0.04 b	1.24 ± 0.07 b	1.03 ± 0.04 c
CCC	1.73 ± 0.04 a	1.75 ± 0.02 a	1.56 ± 0.03 a	1.34 ± 0.03 a	1.35 ± 0.03 a	1.35 ± 0.02 a

Means in columns followed by the same letter are not significantly different according to the L.S.D. test (p=0.05).
*1 = autumn sowing; **2 = winter sowing.

Table 4 The effect of water, GA_3 and CCC application at the 8-leaf stage on the number of inflorescence branches formed per plant of lettuce cvs. 'Parris Island' and 'Great Lakes'.

Treatment	Number of inflorescence branches plant^{-1}		
	Parris Island 1*	Parris Island 2**	Great Lakes
Water	18.7 ± 4.2 b	15.3 ± 3.3 b	14.0 ± 1.4 b
GA_3	24.2 ± 0.7 a	20.3 ± 1.6 a	18.3 ± 1,2 a
CCC	16.0 ± 1.2 b	12.0 ± 2.7 c	15.3 ± 2.0 b

Means in columns followed by the same letter are not significantly different according to the L.S.D. test (p=0.05).
*1 = autumn sowing; **2 = winter sowing

Table 5 The effect of water, GA_3 and CCC application at the 8-leaf stage on the mean seed yield per plant, mean 1000-seed weight and percent seed germination of lettuce cvs. 'Parris Island' and 'Great Lakes'.

Treatment	Parris Island 1*	Parris Island 2**	Great Lakes
Mean seed yield plant^{-1}			
Water	4.23 ± 0.25 b	2.44 ± 0.25 b	2.30 ± 1.82 b
GA_3	6.56 ± 0.60 a	4.10 ± 1.72 a	8.03 ± 2.63 a
CCC	3.58 ± 0.96 b	1.53 ± 0.85 b	1.93 ± 0.29 b
Mean 1000-seed weight (g)			
Water	1.20 ± 0.12 b	0.77 ± 0.05 a	0.85 ± 0.21 a
GA_3	1.65 ± 0.07 a	0.85 ± 0.11 a	0.90 ± 0.24 a
CCC	1.27 ± 0.10 b	0.84 ± 0.32 a	0.70 ± 0.00 a
Germination (%)			
Water	98.5 ± 0.0 a	74.0 ± 2.0 a	85.8 ± 5.0 b
GA_3	99.0 ± 1.0 a	72.0 ± 1.5 a	94.5 ± 1.1 a
CCC	93.5 ± 1.0 b	66.0 ± 1.5 b	82.0 ± 1.8 b

The means of each characteristic presented in columns and followed by the same letter are not significantly different according to the L.S.D. test (p=0.05).
*1 = autumn sowing; **2 = winter sowing.

the winter sown crop the required photoperiod is more quickly attained, so the GA_3 effect is less (Saplaouras *et al.* 2001). Similarly, the application of GA_{4+7} has been shown to improve the uniformity of flowering and seed yield (Gray *et al.* 1986). However, a significant problem that arises from GA_3 application is that the flower stalks are longer and thinner than those of the control (plants treated with water at the same growth stage) and although the flower stalk height of gibberellin-treated plants may be reduced by the application of 1500 ppm CCC at the bolting stage this is achieved only at the expense of seed yield (Koutri *et al.* 2007). Tall, thin stalks are susceptible to lodging, which makes harvesting more difficult and may lead to a loss of seeds (George 1985).

In lettuce, the application of CCC at the 8-leaf stage delays bolting and the onset of flowering (**Table 1**), but the time until the first harvest is less affected, indicating that seed matures more rapidly in CCC treated plants. CCC also significantly reduces the height and increases the diameter of the seed stalk, thus increasing seed stalk strength and thereby reducing the tendency to lodge. In cereals (Clark and Fedak 1977; Knapp *et al.* 1987) and common bent (Aamlid *et al.* 2007), CCC has been shown to reduce flower stalk height, but the anti-lodging effects of CCC in cereals result only from stalk shortening and not from strengthening (Peltonen-Sainio *et al.* 2003). Unlike GA_3, CCC does not increase the number of inflorescence branches per plant and therefore seed yield is not increased. Moreover, CCC in 'Parris Island' appears to lower the percent seed germination, thus indicating a reduction in seed quality.

Seed yield and quality of lettuce also relate to cultivar and crop season (Passam *et al.* 2008). Thus, the seed yield, mean 1000-seed weight and percent germination were higher in the long-term crop of 'Parris Island' (autumn sown) than in the short-term crop (winter sown), as noted too by Saplaouras *et al.* (2001). Large 1000-seed weight in lettuce is desirable since it correlates with high vigour (Smith *et al.* 1973). GA_3 treated plants of both cultivars did not form heads, but CCC did not affect head formation. The low yield of both the control and CCC treated plants of 'Great Lakes' relates to the much denser head formation in these two treatments than in the correspondingly treated plants of 'Parris Island'.

It is concluded that for satisfactory production of lettuce seed under cover under warm, Mediterranean conditions, autumn sowing is preferable to spring sowing, both in terms of seed yield and quality. In addition, gibberellic acid (GA_3) application at the 8-leaf stage may be used to promote bolting and increase seed yield, particularly in tightly-heading cultivars such as 'Great Lakes'. Although the application of CCC at the same stage improves seed stalk characteristics (shorter, thicker stalks) it does not improve seed yield or quality.

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