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Effect of GA_3 and nitrogen on yield and marketability of lettuce (*Lactuca sativa* L.)

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Abstract

In the present study the effect of both nitrogen application rate and gibberellic acid (GA_3) on yield and earliness of production and marketability (plant height and leaf color) of lettuce was examined. Three commercial cultivars [cv. 'Kismy' and 'Marady' (loose-leaf types) and cv. 'Adranita' (romaine type)] were cultivated in an unheated plastic greenhouse for three consecutive growing periods. During cultivation, four nitrogen rates (0, 150, 300 and 450 mg L^{-1} of N) and two foliar sprayings with two concentrations of GA_3 (0 and 50 mg L^{-1} for the first and second sowing and 0 and 25 mg L^{-1} for the third sowing) were applied. Total fresh and dry weight significantly decreased and increased, respectively, by GA_3 application, especially in the second sowing date, whereas high nitrogen rates (300 and 450 mg L^{-1}) resulted in higher fresh weight (by 11.2%) and lower dry weight (by 7.5%) respectively. Plant height was significantly increased by GA_3 application, except for cv. 'Adranita' in the third sowing date, whereas nitrogen application did not affect plant height for all the cultivars studied. The application of GA_3 and high nitrogen rates resulted in an increase of the total number of leaves per plant and a decrease of chlorophyll content of leaves during the first sowing date and third sowing date. In conclusion, nitrogen application could be beneficial for total yield and the total number of leaves, whereas medium GA_3 concentration (25 mg L^{-1}) should be applied during spring (third sowing date) as it results in plants with higher number of leaves, total fresh weight (cv 'Kismy') and marketable height.

Keywords: Chlorophyll content; GA_3; gibberellin; *Lactuca sativa* L.; lettuce; nitrogen; yield.
Abbreviations: CCC_Chlormequat Cloride; DAT_Days After Transplantation; GA_3_Gibberellic acid.

Introduction

Yield and quality of lettuce can be affected by various factors such as environmental condition (La Malfa and Ruggeri, 1988), nutritional management (Acar et al., 2008; Khah and Arvanitoyannis, 2003) and growing season (Saplaouras et al., 2001; Khah et al., 2012). A serious problem in lettuce crops is the emergence of seed stalks during vegetative growth (bolting) that affects severely the quality of the final product by rendering it unmarketable. Nitrogen application rate in relation to cultivar significantly affects the yield of romaine lettuce, as being reported by Maryam et al. (2007) and Boroujerdnia et al. (2007) who studied the effect of four nitrogen levels on two cultivars of lettuce and suggested that the application of mid to high level of nitrogen (120 Kg ha^{-1}) resulted to the highest yield. Konstantopoulou et al. (2010) reported that nitrogen application not only results in higher yield but also affects quality features such as nitrate, vitamin C and chlorophyll content in leaves. In addition, nitrogen application rate has been reported to affect various growth parameters such as photosynthesis and transpiration rate and stomatal conductance (Konstantopoulou et al., 2012). In contrast, Acar et al. (2008) reported that nitrogen application rate did not affect significantly total fresh weight and marketable weight per plant, as well as the number of leaves.
Gibberellins play an important role in inducing anthesis during the vegetative growth stage in many species, including leafy vegetables such as lettuce. This phenomenon known as bolting, affects severely the yield and quality of lettuce due to stem elongation and is stimulated by both high temperatures and endogenous metabolism of gibberellic acid (GA) (Fukuda et al., 2009; Fukuda et al., 2012). Harrington et al. (1960) reported that spraying lettuce plants at the stage of 4 and 8 leaves with 3 to 10 mg L^{-1} resulted in earliness for harvesting date for 14 days, whereas plant growth and development were extremely uniform. In a similar study, Lovato et al. (2000) reported that foliage spraying with 20 ppm GA_3 before bolting induced a slight earliness in seed maturity and increased seed yield. Apart from its role in inducing anthesis, GA has been suggested to increase growth rate of plants and total seed yield by Passam et al. (2008), especially when GA application is combined with Chlormequat Chloride (CCC). Gray et al. (1986) reported that GA_{4+7} application at the rosette stage, resulted in higher survival rate of plants till flowering stage, in higher seed yield and more uniform development of plants. Reghin et al. (2000) applied 4 different doses of gibberellic acid on three bolting-resistant varieties of lettuce and reported increased flowering percentage, seed yield, earliness of the anthesis and seed maturation. They also reported that the application of 23 ppm of GA_3 resulted in the highest seed yield in lettuce cv. 'Veronica' and 'Marisa' and concluded that the effect of GA_3 application was higher on cultivars which were ready for harvest later or were more resistant to bolting. The aim of the present study was to evaluate the effect of nitrogen application rate and GA_3 on the production of two types of lettuce, as well as to evaluate the effect of GA_3 on the

Table 1. Nitrogen doses (total amount of applied nitrogen in Kg ha^{-1}) in relation to sowing date.

Nitrogen application rate	Sowing date		
	1st	2nd	3rd
Control (30 mg L^{-1})	0	0	0
150 mg L^{-1}	67.5	105.0	82.5
300 mg L^{-1}	135.0	210.0	165.0
450 mg L^{-1}	202.5	315.0	247.5

Table 2. Analysis of variance of experimental data.

Source	Number of leaves ($p>F$)	Fresh weight ($p>F$)	Dry weight ($p>F$)	Final height ($p>F$)	Chlorophyll content ($p>F$)
A: Cultivar	*	*	*	*	*
B: Gibberellic acid	*	*	*	*	*
C: Sowing date	*	*	*	*	*
D: N-fertlization	*	*	*	*	*
AB	*	ns	ns	*	*
AC	*	*	*	*	*
AD	ns	ns	ns	ns	*
BC	ns	*	*	*	*
BD	ns	ns	ns	ns	ns
CD	ns	ns	ns	*	ns
ABC	*	*	ns	*	*
ABD	ns	ns	ns	ns	ns
ACD	ns	ns	ns	ns	ns
BCD	ns	ns	ns	ns	*
ABCD	ns	ns	ns	ns	ns

*: significant effect ($p \leq 0.05$), ns: not significant ($p \leq 0.05$)

earliness and marketability (plant height and leaf color) of the final produce.

Results

The factorial analysis of the experimental data showed no significant interaction among the four factors, except for third and second order interaction in some cases (Table 2).

Number of leaves

The application of gibberellic acid (GA_3) on the foliage, twice during cultivation, resulted in a significant increase of the number of leaves for all the cultivars in the first sowing date (Table 3), whereas in the case of cv. 'Kismy' the effect of GA_3 was beneficial for all the sowing dates, regardless of the dose. In contrast, Abbas (2013) reports that the application of GA_3 at concentrations of 250 and 500 mg L^{-1} had no effect on both the number of leaves and branches of dill plants. Number of leaves was also significantly affected by sowing date, where plants formed higher number of leaves during only the second or the second and the third sowing date, according to the application of GA_3 and the cultivar (Table 3). Regarding nitrogen rate application, increasing nitrogen level (from 300 to 450 mg L^{-1}) resulted in higher number of leaves comparing to control (0 mg L^{-1}), whereas no significant differences were observed among the various levels of nitrogen (Table 4).

Fresh and dry weight

The application of GA_3 had no effect on plant fresh weight during the first sowing date, whereas in the second sowing date had a negative effect and resulted in a significant decrease of fresh weight (by 38, 53.1 ad 39.3% for cv 'Kismy', 'Marady' and 'Adranita', respectively) comparing to plants where no GA_3 was applied (Table 5 and Table 6). The cultivar and the head type have a significant effect, especially when climate conditions are favorable to plant development (third sowing date), with a statistically significant increase of plant fresh weight for cv. 'Kismy' (loose-leaf type) and a statistically significant decrease for cv. 'Adranita' (romaine type) when the lower dose of GA_3 is applied (Table 5). Nitrogen application had a beneficial effect on plant fresh weight, only when high nitrogen rates (300 and 450 mg L^{-1}) were applied, whereas no significant differences were observed among the various nitrogen rates (Table 4). Regarding plant dry weight, significant differences were observed between the three cultivars, depending on the sowing date, whereas GA_3 application resulted in higher plant dry weight (increased by 55%) comparing to plants where no GA_3 was applied, regardless of cultivar (Table 7). In addition, increased nitrogen application rate resulted in decreased plant dry weight, regardless of the nitrogen rate (Table 4). Considering that GA_3 application resulted in a decrease in fresh weight during the second sowing date, that increases even more the dry weight of plants.

Plant height

The application of GA_3 resulted in an increase of plant height for all the cultivars and sowing dates of the present study (Table 8), whereas plants had the lowest height in the third sowing date when foliage was sprayed with the lower dose of GA_3, especially for cv. 'Kismy' (30.1 cm), without loss quality due to lack of head formation (e.g. cv. 'Adranita'). In the first sowing, the application of GA_3 resulted in stem elongation and lack of head formation for cv. 'Adranita' rendering the final product unmarketable. In contrast, when no GA_3 was applied, plant height was the highest in the third sowing date. Regarding nitrogen application, plant height was not significantly affected by nitrogen at any rate for all the sowing dates (data not shown).

Chlorophyll content

Chlorophyll content of all cultivars, as expressed by SPAD index, was affected by GA_3 application especially during the

Table 3. The effect of gibberellic acid (GA_3) application on the number of leaves in relation to cultivar and sowing date.

		Sowing date		
Cultivar	GA_3 treatment	1st	2nd	3rd
Kismy	no GA_3	31.2 b(b)	44.4 b(a)	45.3 b(a)
	GA_3	41.2 a(c)	66.3 a(a)	56.9 a(b)
Marady	no GA_3	37.7 b(c)	49.2 a(b)	64.7 b(a)
	GA_3	49.0 a(b)	48.2 a(b)	71.2 a(a)
Adranita	no GA_3	37.7 b(b)	42.0 a(b)	50.5 a(a)
	GA_3	46.7 a(b)	42.3 a(b)	53.3 a(a)
	GA_3 treatment x Cultivar	1st	2nd	3rd
LSD	no GA_3 x Cultivar	5.1	4.1	5.0
LSD	GA_3 x Cultivar	3.1	7.0	5.2

Mean separation in columns and rows by t test and LSD test, respectively ($p \leq 0.05$). Differences between means of the same row are indicated by Latin letters with parenthesis, whereas the differences between the means of the same column and cultivar are indicated by Latin letters without parenthesis.

Table 4. The effect of nitrogen application rate on the number of leaves, fresh and dry weight (g).

Nitrogen level	Number of leaves	Fresh weight (g)	Dry weight (g)
Control (30 mg L^{-1})	47.7 b	596.4 b	4.3 a
150 mg L^{-1}	50.1 ab	625.9 ab	4.0 b
300 mg L^{-1}	52.1 a	663.1 a	4.0 b
450 mg L^{-1}	53.4 a	663.4 a	4.0 b

Mean separation in columns by DMRT at $p \leq 0.05$. Differences between means of the same column are indicated by different Latin letters.

first and third sowing date where a significant decrease was observed (Table 9). However, during the first sowing date cv. 'Kismy' and 'Marady' (loose-leaf type cultivars) retained their marketability despite the paler green color of leaves, whereas for cv. 'Adranita' (romaine type) the leaves were very pale and lost their marketability. In the third sowing date, the decrease in chlorophyll content was also significant without however affecting the marketability for any cultivar tested in our study due to the lower dose of GA_3 applied. Nitrogen application resulted in a significant increase in chlorophyll content during the first sowing date and only when no GA_3 was applied, whereas for plants treated with GA_3 no significant effect from nitrogen application was observed (Table 10).

Discussion

Lettuce yield and quality is severely affected by the early emergence of seed stalks during vegetative growth, a phenomenon known as bolting. Climate conditions, especially high temperatures and long days, and GA_3 metabolism play an important role on the occurrence of this phenomenon, with great variation among cultivars (Jenni and Yan, 2009; Fukuda et al., 2012). GA_3 is usually applied to promote seed stalk emergence, when plants are cultivated for seed production, whereas apart from its role in inducing anthesis, its application has been suggested to increase growth rate of plants and total seed yield of various species (Passam et al., 2008; Singh et al., 2011; Sharma et al., 2013). Javid et al. (2011) have suggested that GA application would be implemented as a means for alleviating salt stress by NaCl, mostly by increasing plant growth. Maggio et al. (2010) also reported that GA_3 exogenous application on tomato plants grown under salinity stress was associated with reduced stomatal resistance and higher water use efficiency, thus alleviating the negative effects of high salinity and allowing for better plant growth at medium levels of salinity.

In our study, GA_3 application showed the best results when applied at the first sowing date, resulting in higher number of leaves and higher plants comparing to the other two sowing dates, whereas plant fresh weight did not differ significantly from plants where no GA_3 was applied. In addition, differences were observed among cultivars, where cv. 'Kismy' was beneficially affected by GA_3 application, regardless of sowing date and especially during the third sowing date where plant fresh weight was significantly higher for plants treated with the lower dose of GA_3. The fact that GA_3 increased the number of leaves without increasing plant fresh weight could be due to the smaller size of leaves (longer and narrower leaves). However, in the case of cv. 'Kismy' in the third growing period, the significantly higher number of leaves also resulted in an increase of fresh weight. Therefore, the growing conditions during the third period resulted in higher fresh weight and higher plant height due to the larger number of leaves. Chlorophyll content decreased significantly by GA_3 application during the first sowing date, results that come in agreement with those of Wittwer and Bucovac (1958) who reported as a general effect of GA_3 the formation of paler and chlorotic new growth. In addition, Sekimoto et al. (1998) reported that the application of a gibberellin-biosynthesis inhibitor (uniconazole-P) enhanced greening and chlorophyll content of *Cucumis sativus* leaves, whereas the chlorophyll content reduction is correlated with quality loss for leafy vegetables. However, in our study the marketability of the product was severely affected only for cv. 'Adranita', whereas for cv. 'Kismy' the decrease of chlorophyll content during the third sowing date was not as high as in the first one, a result that could be attributed to the lower dose of GA_3. The fact that plants can achieve the marketable size by forming more and longer leaves and having a more upright growth could be a useful means to increase plant density and therefore increase total yield. Nitrogen application has been previously reported to affect yield of lettuce, especially when mid to high nitrogen rates (120 Kg ha^{-1}) were applied (Maryam et al., 2007; Boroujerdnia et al., 2007), whereas the nitrogen concentration of nutrient solution (5.0 mol m^{-3} of NO_3^-) has affected biomass production and leaf number of hydroponically grown basil. In our study, even lower doses (67.5 to 105 Kg ha^{-1}) resulted in higher yield comparing to control, whereas larger doses had no significant effect on total yield. In addition, considering that high nitrogen rates could result in high nitrate content within lettuce plant tissues increasing health risks, the fact that high yields could achieved by implementing middle doses of nitrogen should be taken into consideration when crop nutritional regime is planned.

Table 5. The effect of gibberellic acid (GA_3) application on plant fresh weight (g) in relation to cultivar and sowing date.

		Sowing date		
Cultivar	GA treatment	1st	2nd	3rd
Kismy	no GA_3	253.1 a(c)	559.2 a(b)	880.2 b(a)
	GA_3	272.1 a(b)	345.3 b(b)	1023.6 a(a)
Marady	no GA_3	277.8 a(c)	454.1 a(b)	909.8 a(a)
	GA_3	264.1 a(b)	212.7 b(b)	963.8 a(a)
Adranita	no GA_3	387.7 a(b)	469.8 a(b)	953.5 a(a)
	GA_3	432.4 a(b)	285.3 b(c)	808.5 b(a)
	GA_3 treatment x Cultivar	1st	2nd	3rd
LSD	no GA_3 x Cultivar	54.6	83.4	91.4
LSD	GA_3 x Cultivar	63.8	40.7	93.1

Mean separation in rows and columns by LSD and t tests, respectively ($p \leq 0.05$). Differences between means of the same row are indicated by Latin letters with parenthesis, whereas the differences between the means of the same column and cultivar are indicated by Latin letters without parenthesis.

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Table 6. The effect of gibberellic acid (GA_3) application on plant fresh weight (g) in relation to sowing date.

	Sowing date		
Treatment	1st	2nd	3rd
no GA	306.2 a(c)	494.4 a(b)	914.5 a(a)
GA_3	322.8 a(b)	281.1 b(b)	932.0 a(a)
LSD	46.2	43.4	56.7

Mean separation in rows and columns by LSD test at $p \leq 0.05$. Differences between means of the same row and column are indicated by Latin letters with and without parenthesis, respectively.

Table 7. The effect of giberellinic acid (GA_3) application on plant dry weight (% of fresh weight) in relation to sowing date and cultivar.

	Sowing date		
GA treatment	1st	2nd	3rd
no GA_3	5.2 a(a)	4.4 b(b)	2.8 a(c)
GA_3	5.0 a(b)	6.8 a(a)	3.1 a(c)
	Sowing date		
Cultivar	1st	2nd	3rd
Kismy	4.8 b(a)	5.3 b(a)	3.9 a(b)
Marady	4.7 b(a)	5.0 b(a)	2.3 b(b)
Adranita	5.8 a(b)	6.6 a(a)	2.6 b(c)

Mean separation in rows and columns by LSD and t test, respectively ($p \leq 0.05$). Differences between means of the same row and column are indicated by Latin letters with and without parenthesis, respectively.

The adverse effect of GA_3 on plant fresh weight in the second sowing date could be attributed to the lower temperatures comparing to the first and third sowing date that resulted in a prolonged cultivation period (102 DAT), comparing to the first (65 DAT) and third sowing date (81 DAT) and a higher total nitrogen dose (67.5 to 202.5, 105 to 313 and 82.5 to 247.5 Kg ha^{-1} for the first, second and third sowing date, respectively, whereas the cultivar as well the type of lettuce (romaine or loose-leaf lettuce) seems to be of major importance regarding the response to GA_3.

Materials and Methods

Plant material

The experiment was carried out in an unheated greenhouse, at the region of Servota, municipality of Karditsa, Greece (latitude 39° 50′ 86″, longitude 21° 93′ 41″), for three consecutive growing periods. Young seedlings of three wide-spread in Greek market commercial lettuce cultivars [cv. 'Kismy' and 'Marady' (loose-leaf types) and cv. 'Adranita') (romaine type)] were obtained in disk trays 35-40 days after sowing (stage of three true leaves), from commercial nurseries of Spirou House of Agriculture (Athens, Greece) and Plantas S.A. (Thiva, Greece) and transplanted directly to the soil. Plants were arranged in double rows with 20 cm distance between rows and within each row and 40 cm between each pair of rows (115.000 plants per ha). During cultivation, foliar spraying with Decis 2.5 EC (Deltamethrin 2.5% w/v) and Altacor 35WG (Chlorantraniliprole 35% w/w) for pest control, and Rovral Aquaflo 50SC (Iprodione 50% w/v) and Aliette 80WG (Fosetyl-Al 80% w/w) for disease control were applied.

Experimental conditions

Plants were grown for three consecutive growing periods and transplanted to the soil on 07/10/2011, 01/11/2011 and 27/01/2012 for the first, second and third growing period respectively. Harvest took place on 11/12/2011 [65 days after transplantation (DAT)], 11/02/2012 (102 DAT) and 17/04/2012 (81 DAT) for the first, second and third growing period respectively. Treatments consisted of four nitrogen application rates [30 (Control), 150, 300 and 450 mg L^{-1} of nitrogen] and two levels of GA_3 [0 (Control), 50 mg L^{-1} (for the first and second sowing date) and 25 mg L^{-1} (for the third sowing date)]. In order to obtain the desired nitrogen rates, proper amounts of Folur fertilizer (Geoponiki, S.A., Greece) (20% of nitrogen in urea form) dissolved in bore water (Control). Nitrogen application was carried out with manual application of 0.5 L per plant once a week throughout each growing period (Table 1). GA_3 (Gibberellic acid 10% in tablets of 100 g) (Greenfarm Chemicals S.A., Greece) was applied with foliar spraying to run off (15 ml per plant approximately), twice during each growing period, with the first application taking place at the stage of rosette (on 07/11/2011, 21/11/2011 and 17/03/2012 for the first, second and third sowing date respectively) and the second application

Table 8. The effect of gibberellic acid (GA_3) application on plant height (cm) in relation to sowing date and cultivar.

		Sowing date		
Cultivar	GA treatment	1st	2nd	3rd
Kismy	no GA	15.9 a(c)	20.8 a(b)	22.9 a(a)
	GA_3	32.8 b(b)	39.7 b(a)	30.1 b(c)
Marady	no GA_3	16.2 a(c)	19.5 a(b)	24.7 a(a)
	GA_3	39.6 b(b)	43.7 b(a)	32.0 b(c)
Adranita	no GA_3	33.6 a(b)	35.2 a(b)	37.7 a(a)
	GA_3	44.2 b(a)	47.3 b(a)	38.7 a(c)
	GA_3 treatment x Cultivar	1st	2nd	3rd
LSD	no GA_3 x Cultivar	1.8	1.7	1.1
LSD	GA_3 x Cultivar	2.2	2.3	1.5

Mean separation in rows and columns by LSD and t test, respectively ($p \leq 0.05$). Differences between means of the same row are indicated by Latin letters with parenthesis, whereas the differences between the means of the same column and cultivar are indicated by Latin letters without parenthesis.

Table 9. The effect of giberellic acid (GA_3) application on chlorophyll content (SPAD index) in relation to sowing date and cultivar.

		Sowing date		
Cultivar	GA_3 treatment	1st	2nd	3rd
Kismy	no GA_3	13.4 a(b)	20.5 a(a)	17.9 a(a)
	GA_3	5.5 b(b)	18.0 a(a)	15.3 b(a)
Marady	no GA_3	7.6 a(c)	12.2 a(a)	9.8 a(b)
	GA_3	3.2 b(c)	10.4 a(a)	5.9 b(b)
Adranita	no GA_3	35.7 a(b)	42.0 a(a)	25.7 a(c)
	GA_3	14.6 b(b)	30.5 b(a)	18.7 b(b)
	GA_3 treatment x Cultivar	1st	2nd	3rd
LSD	no GA x Cultivar	5.1	4.2	2.5
LSD	GA_3 x Cultivar	2.0	4.5	2.4

Mean separation in rows and columns by LSD and t test, respectively ($p \leq 0.05$). Differences between means of the same row are indicated by Latin letters with parenthesis, whereas the differences between the means of the same column and cultivar are indicated by Latin letters without parenthesis.

Table 10. The effect of giberellic acid (GA_3) application on chlorophyll content (SPAD index) in relation to sowing date and nitrogen application rate.

		Sowing date		
Nitrogen rate	GA Treatment	1st	2nd	3rd
Control (30 mg L^{-1})	no GA_3	14.0 a(b)	22.7 a(a)	16.6 a(b)
	GA_3	6.7 b(b)	13.9 b(a)	12.1 b(a)
150 mg L^{-1}	no GA_3	18.2 a(b)	26.5 a(a)	18.2 a(a)
	GA_3	8.0 b(b)	20.8 b(a)	12.6 b(b)
300 mg L^{-1}	no GA_3	21.0 a(a)	24.3 a(a)	19.4 a(a)
	GA_3	7.8 b(c)	21.5 a(a)	14.7 b(b)
450 mg L^{-1}	no GA_3	22.4 a(b)	26.0 a(a)	17.1 a(c)
	GA_3	8.6 b(b)	22.4 a(a)	13.7 a(b)
	GA_3 treatment x Cultivar	1st	2nd	3rd
LSD	no GA_3 x Cultivar	5.2	4.1	3.3
LSD	GA_3 x Cultivar	5.5	5.3	2.7

Mean separation in rows and columns by LSD test, respectively ($p \leq 0.05$). Differences between means of the same row are indicated by Latin letters with parenthesis, whereas the differences between the means of the same column and cultivar are indicated by Latin letters without parenthesis.

two weeks later (on 21/11/2011, 05/12/2011 and 31/03/2011 for the first, second and third sowing date respectively). Throughout the experiment the air temperature was recorded (min and max temperature for day and night). For the first growing period air temperature during day ranged from 16-20 °C, whereas night temperatures ranged from 6-13 °C. For the second growing period, temperatures ranged from 10-18 and (-3)-9 °C for day and night respectively. For the third growing period, the temperatures ranged from 10-25 and 0-15 °C for day and night, respectively. Regarding soil properties, soil texture was CL (30% sand, 31% clay and 39% silt), pH=7.90, EC=2.47 dS/m, $CaCO_3$=8.20% (total), organic matter=9.20% and P Olsen=122 mg $Kg^{-1.}$

Measurements

During cultivation, plant height, number of leaves and chlorophyll content of leaves were recorded every week. For chlorophyll content, measurements were made with the aid of SPAD 502 Plus (Spectrum Technologies Inc., USA) at three points on the same leaf (the third leaf from outside to the inner part of the head or rosette). At the time of harvest, fresh weight of each plant was recorded, whereas samples of leaves were dried at 85 °C to a constant weight for 72 hours.

Statistical analysis

The experiment was laid out according the Randomized Complete Blocks design, with three replications (n=3) and 8 plants per replication (24 plants per treatment for each cultivar and 192 plants in total for each cultivar). Statistical analysis was carried out with the aid of Statgraphics Plus Version 5.1 program (Statistical Graphics Corporation). Experimental data were subjected to analysis of variance and means compared by the least significance difference test (LSD) and Duncan Multiple Range (DMRT) or t Test at $p \leq 0.05$.

Conclusions

In conclusion, GA_3 application did not significantly affect plant fresh weight, however it increased leaf number and plant height and could allow for higher plant density and therefore higher total yield. Climate conditions as well as the implemented dose are of major importance for GA_3 application in order to have the desirable effect and low temperatures (second sowing date) and high doses (50 mg L^{-1}) of GA_3 should be avoided as they result in significant lower yield and quality. In addition, lettuce types and cultivars did not have a similar response, with cv. 'Kismy' being positively affected by GA_3 application which resulted in higher fresh weight in the third sowing, whereas chlorophyll content was not severely affected and leaves retained their marketability. Regarding nitrogen application rate, low to middle doses (67.5 to 105 Kg ha^{-1}) are suggested for higher yield without compromising product quality. Therefore, in any case, high doses of GA_3 should be avoided regardless of growing conditions and nitrogen application rate. GA_3 should be applied in low doses (up to 25 mg L^{-1}) in combination with low to middle doses of nitrogen and when climate conditions are not impeding plant growth (medium day temperatures and low to medium night temperatures), whereas the cultivar and head type of lettuce (e.g. loose-leaf type cv. 'Kismy') is of major importance in order to obtain the desired result and have higher yields.

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