Korean J. Plant Res. 27(3):229-235(2014)
http://dx.doi.org/10.7732/kjpr.2014.27.3.229

Print ISSN 1226-3591
Online ISSN 2287-8203

Original Research Article

Effects of Gibberellin Biosynthetic Inhibitors on Oil, Secoisolaresonolodiglucoside, Seed Yield and Endogenous Gibberellin Content in Flax

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Abstract - Flax (*Linum usitatissimum* L.) has been used for the edible oil in Korea. The evaluate the effect of plant growth retardants on flaxseed yield, oil content, and gibberellin level, chloromequat chloride (CMC), paclobutrazol (PBZ), and prohexadione-Ca (PHC) were used as plant growth retardants in this study. These plant growth retardants were foliar-sprayed to flax plant at 55 days after seeding. The concentrations of each plant growth retardant were as followed: CMC 250, 500, 1000 ppm, PBZ 40, 80, 160 ppm and PHC 500, 1000, 2000 ppm. PHC treatment to forming bolls was more stimulated than CMC and PBZ. The highest ripened seed rate was observed in PHC treatment at 2000ppm. The high see yields were obtained in PHC treatment following PBZ and CMC, in turn. Seed yield that significantly increased in PBZ and PHC was found to be increased 12.4 ~ 23.9% as compared to the control. The PHC showed higher flaxseed production and oil yield than that of CMC and PBZ. The results obtained in the present study suggest that higher concentration of plant growth retardant (PHC) increased flaxseed yield and oil content. The optimal concentration of PHC treatment was observed in 2000 ppm. It concludes that the foliar application of PHC 2000 ppm may be useful for the increasing oil and higher seed production in flax plants.

Key words - Chloromequat-Cl, Paclobutrazol, Prohexadione-Ca, Lignans, Gibberellin

Introduction

Flax (*Linum usitatissimum* L.) is used for fiber and oil is an annual crop belonging to the family Linaceae (Simmonda, 1976). Flaxseed meal is 35 to 40% proteins, and together with cottonseed and sunflower supplies about 23% of the world's oilcake and meal (Hatje, 1989). In flax culture, it has been tried to raise seed and oil productivity. Application of plant growth regulators (PGR), particularly growth retardants, may maintain internal hormonal balance, i.e., efficient sink–source relationship, thus enhancing crop productivity (Singh *et al*., 1987). Mepiquat chloride and chloromequat chloride were found by Dippenaar *et al*. (1990) and Pípolo *et al*. (1993) to restrict vegetative growth, thus enhancing reproductive organs (Wang *et al*., 1995). Mepiquat chloride as one of gibberellins biosynthetic inhibitors has been found to restrict the vegetative growth in the cost of enhanced reproductive organs (Wang *et al*., 1995). Fan *et al*. (1999) reported that mepiquat chloride improved photosynthetic efficiency. In addition, the good population type and canopy structure for dwarf plants, smaller leaves and bigger bolls could be achieved by mepiquat chloride application. Mepiquat chloride and trinexapac-ethyl tend to be shorter and more compact than untreated plants (Hodges *et al*., 1991; Jung *et al*., 1975; Kerby, 1985; Reddy *et al*., 1992; Stuart *et al*., 1984; Willard *et al*., 1976).

Mepiquat chloride, chloromequat chloride, and daminozide also have been associated with increased photosynthesis through increased total chlorophyll concentration in plant leaves (Nepomuceno *et al*., 1997; Wu *et al*., 1985).

Sawan *et al*. (1991) indicated that application of mepiquat chloride to cotton plants increased protein and oil yields and also caused a general decrease in the oil saturated fatty acids, associated with an increase in unsaturated fatty acids. Al-Gharbi and Yousif (1989) observed that application of chloromequat chloride increased sunflower oil seed content.

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Osman and Abu-Lila (1985) showed that spraying flax plants with chloromequat chloride increased the levels of unsaturated fatty acids. Osman and Ahmed (1985) found that spraying sesame plants with daminozide caused an increase in the oil unsaturated fatty acids. Zafirova *et al*. (1987) reported that Alar increased seed and oil yields of sunflower. We have focused on changes of endogenous gibberellins to get a better understanding together with better agronomic practices. In a previous study, it was revealed that foliar application of mepiquat chloride increases seed oil content and promotes unsaturated fatty acids in flax (Kim *et al*., 2011). The present study aimed to evaluate the optimal concentration of plant growth retardants in order to increase seed yield and oil content in flax plants.

Materials and Methods

Field experiment was conducted at the experimental field of Gyeongsangbuk-do Provincial Agricultural Research & Extension Services, Daegu, Republic of Korea. Flax (*Linum usitatissimum* L. cv. Hwabaek) seeds were planted in 4.8 m (16 rows) with 6.0 m (row length) plots covered with black polyethylene vinyl on March 12 2011 and March 11 2012, respectively. Row spacing (wide) was 0.3 m and plant density was 11,111/ha. Prior to seeding, fertilizer was supplied with nitrogen, phosphorus, and potassium at the 100, 25, and 90kg/ha, incorporating as basal and top dressing (7:3, w/w) to the soil, respectively.

Three plant growth retardants, chloromequat chloride (CMC), paclobutrazol (PBZ), and prohexadione-Ca (PHC), were treated to flax plants. The concentrations of each plant growth retardant were as followed: CMC 250, 500, and 1000 ppm, PBZ 40, 80, 160 ppm and PHC 500, 1000, 2000 ppm. Each plant growth retardant was foliar-sprayed at 55 days after seeding. The each solution volume applied was also 930 L ha^{-1}. The untreated check was sprayed with distilled water. All foliar spray was done early hours of the day to reduce evaporation in the morning. A randomized complete block design with four replications was used.

Flax seeds were harvested on 10 August in 2011 and 9 August in 2012, depending on seed ripening degree. Oil content and fatty acid composition were determined from matured flax seed. Crushed flax seed (5 g fresh weight) was extracted by percolating with diethyl ether 100 ml using soxhlet apparatus. For analysis of fatty acid, the extracted solvent derived from soxhlet was evaporated and concentrated. Fatty acid methyl esters were prepared from in 200 ml of a 1% (w/v) solution of sodium methoxide in methanol as described previously (Hitz et al., 1994). After 20 min of incubation at room temperature, fatty acid methyl esters were recovered by the addition of 250 ml of 1 M sodium chloride and extraction with 250 ml of heptane and analyzed using a gas chromatogram (Model HP 5890, USA). Fatty acid methyl esters were resolved using an Omegawax 320 column (Supelco, PA, USA), and the oven temperature was programmed from 185℃ to 215℃ at a rate of 2.5℃ min^{-1}.

Extraction and analysis of SDG (secoisolariciresinol diglucoside) followed the reference (Meagher *et al*., 1999). Defatted flax powder was extracted with 50 ml of 80% methanol for 5h at 55℃ in a shaking water bath. The methanolic extract was filtered and concentrated by rotary evaporation. The resulting aqueous extract was hydrolyzed with 0.8 ml of 1M hydrochloric acid for 1 h at 100℃. The acid hydrolysate was diluted with water and extracted twice ethyl acetate/hexanes (1:1). The dry samples were redissolved in methanol, filtered and applied to the HPLC. HPLC semipreparative separations were carried out on a Beckman 114M HPLC system with a Microsorb semipreparative C_{18} column. The HPLC system was equipped with an HP 1100 DAD, with detection set at 280 nm and 400 nm for identification. Elution was carried out with a flow rate of 0.6 ml/min using following solvent systems: solvent A=water/glacial acetic acid (99.8:0.2 v/v) and solvent B=acetonitrile. An initial ratio of 70A:30B was followed by a linear gradient to 50A:50B, over 55 min, then back to 70A:30B, for equilibration of the system over 5 min.

Extraction and analysis of gibberellin metabolites followed the reference (Lee *et al*., 1998). The seeds harvested were immediately frozen in liquid nitrogen and stored at -80ºC. When all the required materials for GA analysis had been collected, the samples were lyophilized for 48 h. The extraction of endogenous gibberellins was followed as described by Lee *et al*. (1998). The GAs were chromatographed on a 3.9×300 mm μBonda-Pak C_{18} column (Waters) and eluted at 1.5 ml

min^{-1} with following gradient: 0 to 5 min, isocratic 28% MeOH in 1% aqueous acetic acid; 5 to 35 min, linear gradient from 28 to 86% MeOH; 35 to 36 min, 86 to 100% MeOH; 36 to 40 min, isocratic 100% MeOH. Up to 50 fractions of 1.5 ml each were collected. Small aliquots (15㎕) from each fraction were taken, and radioactivity was measured with liquid scintillation spectrometry (Beckman, LS 1801) to determine accurate retention times of each GA based upon the elution of 3H-GA standards. The fractions were dried on a Savant Speedvac and combined according to the retention times of 3H-GA standards and previously determined retention times of the labeled (deuterated) GA standards. GAs were quantified using [17, 17-2H_2]-GAs (20 ng each) as internal standards (purchased from Prof. L. N. Mander, Australian National University, Canberra, Australia). The three or five prominent ions were analyzed by GC-MS-SIM (Finnigan Mat GCQ) with dwell times of 100 ms. Endogenous GA contents were calculated from the peak area ratios respectively and retention time was determined by the hydrocarbon standards to calculate the KRI value.

The collected data for endogenous gibberellins and tuber yield were analyzed by using SAS package for Duncan's multiple range tests.

Results and Discussion

The study was performed to test the effects of three gibberellin biosynthetic inhibitors (chloromequat chloride: CMC, paclobutrazol: PBZ, and prohexadione-calcium: PHC) on changes of oil, lignan, seed yield and gibberellins in flax plant. Table 1 shows influences of three plant growth retardants on growth characteristics and seed yield of flax plant. Three plant growth retardants significantly reduced plant height. Plant height reduction was related to shortening of the internodes. The CMC, PBZ, and PHC are used widely in agriculture and horticulture. It acts by inhibiting gibberellins biosynthesis resulting in shorter internode length and it inhibits gibberellin production much later in the biosynthetic pathway than MC, chloromequat, and triazole compounds (Hafner, 2001; Rademacher, 2000).

Qian (1998) reported that TE reduces stem elongation and mowing requirements stimulating a favorable vertical shoot growth in turfgrass.

Kim *et al*. (2011) reported that single MC treatment had a higher seed ripening than TE treatment. CMC and PBZ had a similar effect in reducing plant height, otherwise PHC showed more significant reduction than CMC and PBZ in plant height. There was significant difference in number of branch per plant treated with CMC, PBZ and PHC. As compared with the control, the incidence of branch occurrence in flax plant was significantly increased by treatment with PHZ and PHC, and it was same tendency by treatment with CMC. PHZ and PHC also proved to be more effective than PHC treatment. In comparison with control, number of boll per branch was also increased by plant growth retardants. However, two plant growth retardants both CMC and PBZ

Table 1. Influences of plant growth retardants on growth characteristics and seed yield in flax

Treatment	Plant height (cm)	No. of branch/plant	No. of boll/branch	Ripened seed rate (%)	1,000-seed weight (g)	Seed yield (kg/ha)
Control	84.3c	6.2c	16.8c	85.2f	5.75f	1,587d
CMC 250	83.9c	6.3c	16.6cd	86.0e	5.76f	1,590d
CMC 500	82.2b	6.2c	17.2c	85.7f	5.74f	1,601d
CMC 1000	82.0b	6.2c	17.4c	87.5e	5.88e	1,625d
PBZ 40	83.1bc	6.6c	17.1c	88.4de	6.21d	1,784c
PBZ 80	82.5b	7.2b	17.4c	88.9d	6.45c	1,900b
PBZ 160	81.3b	7.8b	17.3c	89.2d	6.52b	1,953a
PHC 500	82.2b	7.5b	18.2b	92.6c	6.50b	1,949a
PHC 1000	79.4a	8.7a	19.3ab	94.1b	6.63a	1,967a
PHC 2000	78.1a	8.8a	21.2a	96.4a	6.62a	1,960a

Note: CMC = chloromequat chloride; PBZ = paclobutrazol; PHC = prohexadione-ca. Different letters in the column indicate significant difference at $\alpha = 0.05$.

did not alter the number of boll per branch as compared with control. PHC-treated plant showed higher capsule than that of CMC and PBZ treatment. PHC treatment to ability forming bolls was more stimulated than that of CMC and PBZ. The highest capsule productions were observed in PHC at 2000 ppm. The ripened seed rate was significantly increased with PBZ and PHC except for CMC. The highest ripened seed rate was observed in PHC treatment at 2000 ppm. The high see yields were obtained in PHC treatment following PBZ and CMC, in turn. Seed yield was significantly increased in PBZ and PHC. These treatments were found to be increased 12.4 ~ 23.9% as compared to the control.

Change of oil and fatty acid contents in flax seeds treated with three plant growth retardants are presented in Table 2. The treatment of flax plant with foliar growth retardants in oil content resulted in an increase in the proportion of concentrations. Among these plant growth retardants, highest oil content was observed in plants treated with PHC, whereas lowest oil content was observed in plants treated with CMC. Kim *et al*. (2011) reported that the foliar application with mepiquat chloride causes higher oil content than trinexapac-ethyl. Furthermore, it was also revealed that the treatment of mepiquat chloride (MC) causes increased oil content when the higher concentration of mepiquat chloride was applied (Kim *et al*., 2011).

However, in our study, CMC that has same inhibiting point of gibberellin biosynthesis like a MC showed low oil content. This was probably due to the cultivars of flax and applied concentration. Two saturated fatty acids both palmitic acid and stearic acid were not changed by three plant growth

Table 2. Oil and fatty acid contents of flax seeds as affected by plant growth retardants

Treatment	Oil content (g/kg DW)	Fatty acid composition (%)				
		palmitic	stearic	oleic	linoleic	linolenic
Control	350e	5.4	3.2	16.9	17.1	57.4c
CMC 250	353e	5.1	3.1	16.6	17.2	58.0b
CMC 500	352e	4.5	3.3	17.4	16.6	58.2b
CMC 1000	361d	4.7	3.1	17.2	16.6	58.4b
PBZ 40	376c	5.2	3.3	16.9	16.7	57.9b
PBZ 80	384b	4.8	3.1	16.7	16.8	58.6b
PBZ 160	385b	4.4	3.2	16.2	16.9	59.3b
PHC 500	382b	4.4	3.0	15.8	16.7	60.1ab
PHC 1000	391a	4.3	3.3	15.6	15.4	61.4a
PHC 2000	395a	4.2	3.2	14.8	14.9	62.9a

Note: CMC = chloromequat chloride; PBZ = paclobutrazol; PHC = prohexadione-ca. Different letters in the column indicate significant difference at $\alpha = 0.01$.

Table 3. Endogenous gibberellin content of flax seed as affected by plant growth retardants

Treatment	GA_1 content (ng/g DW)	GA_4 content (ng/g DW)	ECH (ng/g DW)	NCH (ng/g DW)	Total GA content (ng/g DW)
Control	0.40a	1.76a	7.91a	21.30a	29.21a
CMC 250	0.37a	1.73a	7.44a	19.47b	26.91b
CMC 500	0.35a	1.68ab	7.47a	19.03b	26.50b
CMC 1000	0.33a	1.55c	7.43a	18.22c	25.65b
PBZ 40	0.36a	1.72a	7.75a	20.47a	28.22a
PBZ 80	0.34a	1.74a	7.63a	18.76bc	26.39b
PBZ 160	0.32a	1.65b	7.58a	18.55c	26.13b
PHC 500	0.35a	1.63b	7.80a	18.32c	26.12b
PHC 1000	0.34a	1.55c	7.57a	16.39d	23.96c
PHC 2000	0.34a	1.38d	7.43a	15.01e	22.44d

Note: CMC = chloromequat chloride; PBZ = paclobutrazol; PHC = prohexadione-ca. ECH = Early C-13 hydroxylation group including GA_{53}, GA_{44}, GA_{19}, GA_{20} and GA^1; NCH = Non C-13 hydroxylation group including GA_{12}, GA_{15}, GA_{24}, GA_9 and GA_4. Total GA indicates sum of ECH and NCH. Different letters in the column indicate significant difference at $\alpha = 0.01$.

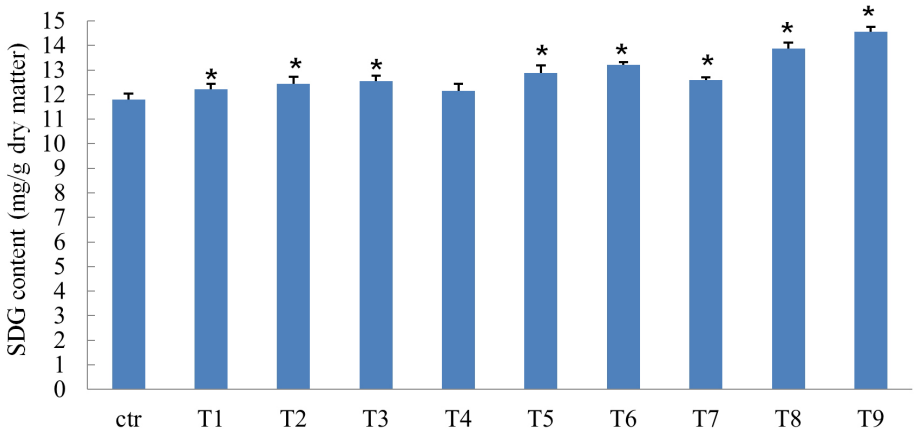

Fig. 1. SDG contents of whole flaxseed. Note: ctr = distilled water applied; T1 = CMC 250ppm; T2 = CMC 500ppm; T3 = CMC 1000ppm; T4 = PBZ 40ppm; T5 = PBZ 80ppm; T6 = PBZ160ppm; T7 = PHC 500ppm; T8 = PHC 1000ppm; T9 = PHC 2000ppm; CMC = chloromequat chloride; PBZ = paclobutrazol; PHC = prohexadione-ca. Vertical bars means SE (n=3). Asterisk on a vertical bar means significant difference at $P = 0.01$.

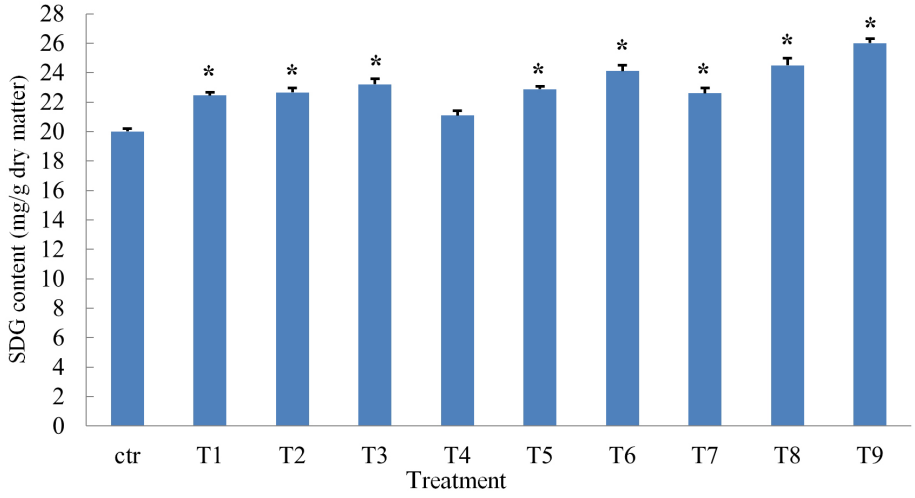

Fig. 2. SDG contents of defatted flaxseed. Note: ctr = distilled water applied; T1 = CMC 250ppm; T2 = CMC 500ppm; T3 = CMC 1000ppm; T4 = PBZ 40ppm; T5 = PBZ 80ppm; T6 = PBZ160ppm; T7 = PHC 500ppm; T8 = PHC 1000ppm; T9 = PHC 2000ppm; CMC = chloromequat chloride; PBZ = paclobutrazol; PHC = prohexadione-ca. Vertical bars means SE (n=3). Asterisk on a vertical bar means significant difference at $P = 0.01$.

retardants. In change of linolenic acid, the highest content was found in plants treated with PHC. In particular, PHC treatment gave the higher unsaturated fatty acid content than that of CMC and PBZ. Meanwhile, the oleic and linoleic acid contents were slightly decreased to increased concentration of PBZ and PHC. Low content of saturated fatty acids is desirable for edible uses. The bioactive and total gibberellin content was determined by GC-MS from flax seeds treated with CMC, PBZ and PHC (Table 3).

In our previous study, it was first reported that eight gibberellins were identified and quantified operating two different gibberellin biosynthetic pathways, early C-13 hydroxylation and non C-13 hydroxylation routes (Kim *et al*., 2009). Two endogenous bioactive gibberellins (GA_1 and GA_4) in flax seeds were decreased by CMC, PBZ and PHC. Although higher concentration of these plant growth retardants in bioactive gibberellin (GA_1) showed lower content as compared to the control, there was no statistically significant difference

between concentration and plant growth retardants.

Bioactive gibberellin (GA_1) content was always 3.8 ~ 4.7 times higher than that of GA_4 in all treatments. Bioactive gibberellin (GA_1) was less suppressed than GA_4 by plant growth retardants. It showed that gibberellin content of the early C-13 hydroxylation pathway including GA_{53}, GA_{44}, GA_{19}, GA_{20} and GA_1 was not significantly changed by plant growth retardants. However, it was tended to decrease with increased concentration of plant growth retardants.

We also revealed that gibberellin content of the non C-13 hydroxylation pathway including GA_{12}, GA_{15}, GA_{24}, GA_9 and GA_4 was significantly decreased by plant growth retardants. In particular, it was observed that PHC treatment with 2000 ppm caused dramatic decrease in gibberellin content of the group of non C-13 hydroxylation pathway. It is known that PHC (prohexiadione-Ca) as acylcyclohexanediones like a trinexapac-ethyl, block particularly 3ß-hydroxylation, thereby strongly inhibiting the formation of highly active GAs from inactive precursors. Lignans, secondary plant metabolites, are widely distributed in the plant kingdom. Flaxseed is known to be rich source of lignans such as secoisolariciresinol (SECO) and matairesinol (MATAI).

In the present study, this is the first report of the lignans being determined in flaxseed treated with plant growth retardants. Fig. 1 shows SDG content of whole flaxseed as affected by different concentrations of three plant growth retardants. The results showed that the flax plants applied with CMC, PBZ and PHC except for PBZ 400ppm produced much higher SDG content than that of control. The SDG contents in plant growth retardant treated flaxseeds were ranged from 12.5 to 14.8 mg. Among these treatments, the flaxseed treated with highest concentration of PBZ recorded a highest SDG content. The SDG content from defatted flaxseed was also determined (Fig. 2). The SDG content of defatted flaxseeds treated with three plant growth retardants were 21.5 to 26.1 mg. The higher concentration applied to flax plants, the more SDG content increased. The more significant increase of SDG content was observed in much higher concentration of PHC treatment. Our results obtained in the present study suggest that plant growth retardants (CMC, PBZ, and PHC) enhanced flaxseed yield, oil and SDG content.

Acknowledgment

This work (12-1-70-72) was supported by Researcher Program through grant funded by the DEGEC.

References

Al-Gharbi, A.S. and I.R. Yousif. 1989. Effect of nitrogen and growth regulators on seed yield and quality of sunflower. Zanco 2:51.

Dippenaar, M.C., C.R. Nolte and C. Barnard. 1990. Controlling excessive growth in cotton by multiple applications of low concentrations of mepiquat chloride. South Afr. J. Plant Soil 7:50-54.

Fan, S., X. Yuzhang and Z. Chaojun. 1999. Effects of nitrogen, phosphorus and potassium on the development of cotton bolls in summer. Acta Gossypii Sinica 11:24-30.

Hafner, V. 2001. Moddus-universal product for lodging prevention in cereals. 5th Slovenian Conference on Plant Protection. Catez ob Savi, Slovenia. 6-8 March. pp. 167-172.

Hatje, G. 1989. World importance of oil crops and their products. *In* Robbellen, G., R.K. Downey and A. Ashri (eds.), Oil Crops of the World; Their Breeding and Utilization, McGraw-Hill Pub. Co., New York, USA. pp. 1-21.

Hedden, P. and Y. Kamiya. 1997. Gibberellin biosynthesis: enzymes, genes and their regulation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:431-460.

Hitz, W.D., T.J. Carlson, J.J.R. Booth, A.J. Kinney, K.L. Stecca and N.S. Yadav. 1994. Cloning of a higher-plant plastid [omega]-6 fatty acid desaturase cDNA and its expression in a *Cyanobacterium*. Plant Physiol. 105(2):635-641.

Hodges, H.F., V.R. Reddy and K.R. Reddy. 1991. Mepiquat chloride and temperature effects on photosynthesis and respiration of fruiting cotton. Crop Sci. 31:1302-1308.

Jung, J. B. and W.V. Amsberg. 1975. Biological activity of new onium compounds in cotton and other crops. *In* Abstracts. Meeting of the Plant Growth Regulator Working Group, Sullivan E.F. (ed.), Longmont, Co., Chicago, IL (USA). p. 13.

Kerby, T.A. 1985. Cotton response to mepiquat chloride. Agron. J. 77:515-518.

Kim, S.K., E.Y. Sohn and I.J. Lee. 2009. Quantification of endogenous gibberellins in two flax (*Linum usitatissimum* L.) cultivars during seed development. J. Crop Sci. Biotech. 12(1):43-46.

Kim, S.K., H.D. Lee and H.J. Choi. 2011. Effect of mepiquat chloride and trinexapac-ethyl on oil composition, seed yield and endogenous gibberellins in flax. Korean J. Plant Res. 24(6):696-701.

Lee, I.J., K.R. Foster and P.W. Morgan. 1998. Photoperiod control of gibberellin levels and flowering in sorghum. Plant Physiol. 116:1003-1010.

Meagher, L.P., G.R. Beecher, V.P. Flanagan and B.W. Li. 1999. Isolation and characterization of the lignans isolariciresinol and pinoresinol in flaxseed meal. J. Agric. Food Chem. 47:3173-3180.

Nepomuceno, A.L., D.M. Oosterhuis and A. Steger. 1997. Duration of activity of the plant growth regulators PGR-IV and mepiquat chloride, special report—Agricultural Experiment Station, Division of Agriculture, University of Arkansas. 183:136-139.

Osman, R.O. and B.H. Abu-Lila. 1985. Studies on the effect of gibberellic acid and cycocel on flax plants (*Linum usitatissimum* L.), seed oil content and oil composition. J. Agron. Crop Sci. 155: 82-88.

Osman, R.O. and F.A. Ahmed. 1982. Biochemical studies on the effect of B9 on sesame plants. Ann. Fac. Agric., Ain Shams Univ. 28:517.

Pípolo, A.E., M.L.F. Athayde, V.C. Pípolo and S. Parducci, 1993. Comparison of different rates of chlorocholine chloride applied to Herbaceous Cotton. Pesquisa Agropecuária Brasileira 28:915-923.

Qian, Y.L. 1998. Trinexapac-ethyl restricts shoot growth and improves quality of 'Diamond' zoysiagrass under shade. Hort. Sci. 33(6):1019-1022.

Rademacher, W. 2000. Growth retardants: effects on gibberellins biosynthesis and other metabolic pathways. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51:501-531.

Reddy, V. R., A. Trent and B. Acock, 1992. Mepiquat chloride and irrigation versus cotton growth and development. Agron. J. 84:930-933.

Sawan, Z.M., R.A. Sakr, F.A. Ahmed and A.M. Abd-Al-Samad. 1991. Effect of 1,1-dimethyl piperidinium chloride (Pix) on the seed, protein, oil and fatty acids of Egyptian cotton. J. Agron. Crop Sci. 166:157-161.

Simmonda, N.W. 1976. Evolution of Crop Plants. Longman Inc., New York, USA. p. 64.

Singh, H., S.C. Chandra and R.S. Jolly, 1987. Effect of growth regulators in relation to time of sowing and yield of soybean cultivars. Ann. Biol. 3:36-43.

Stuart, B.L., V.R. Isbell, C.W. Wendt and J.R. Abernathy, 1984. Modification of cotton water relations and growth with mepiquat chloride. Agron. J. 76:651-655.

Wang, Z. L., Y.P. Yin and X.Z. Sun, 1995. The effect of DPC (*N*,*N*-dimethyl piperidinium chloride) on the $^{14}CO_2$-assimilation and partitioning of ^{14}C assimilates within the cotton plants interplanted in a wheat stand. Photosynthetica 31:197-202.

Willard, J.M., M. Schroeder, J. Thompson, J. Daniel and C. Carter, 1976. Effects of 1,1-dimethyl piperidinium chloride (BAS 083 00 E) of cotton yield and development. *In* Abstracts. Meeting of the Plant Growth Regulator Working Group. Sullivan E.F. (ed.), Longmont, Co., LA. (USA). p. 8.

Wu, L.Y., Z.H. Chen, Y.N. Yuan and H.Y. Hu, 1985. A comparison of physiological effects of DPC and CCC on cotton plants. Plant Physiol. Commun. 5:17-19.

Zafirova, T., C. Christov and V. Iliev, 1987. The influence of some growth regulators on the sunflower production. *In* Proceedings of the International Symposium of Plant Growth Regulators, Lilov, D., G. Vassilev, C. Christov, A. T. Sofia, Bulgaria and M. Popov (eds.), Institute of Plant Physiology. pp. 797-800.

(Received 14 April 2014 ; Revised 14 May 2014 ; Accepted 21 May 2014)