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Original Research Article

Combined Effects of Mepiquat Chloride and Trinexapac-ethyl on Oil Content, Lignan, Seed Yield and Endogenous Gibberellins in Flax (*Linum usitatissimum* L.)

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Abstract - Flax (*Linum usitatissimum* L.) has been used for the only edible oil in Korea. We carried out the field experiment in order to investigate the possibly combined effects of mepiquat chloride (MC) and trinexapac-ethyl (TE) on oil composition, lignan content, seed yield and endogenous gibberellins content of flax cultivar. Plant growth retardants mepiquat chloride (300 and 600 ppm) and trinexapac-ethyl (100, 200 and 300 ppm) were foliar-sprayed to flax plant at 50days after seeding. The plant height was decreased in the combination of mepiquat chloride 600 ppm with trinexapac-ethyl 100, 200 and 300 ppm. Mepiquat chloride treatment combined with trinexapac-ethyl observed the highest response on seed yield, followed by mepiquat chloride 300 ppm with trinexapac-ethyl 100 ppm, mepiquat chloride 300 ppm with trinexapac-ethyl 200 ppm and mepiquat chloride 300 ppm with trinexapac-ethyl 300 ppm. Lignan content was increased in all of the combination treatments. It concludes that the combination of mepiquat chloride 300 ppm with trinexapac-ethyl 300 ppm will be useful to increasing oil and lignan content in flax plants.

Key words - *Linum usitatissimum*, Ripened seed rate, Linoleic acid, Linolenic acid

Introduction

Flaxseeds, an annual crop belonging to the family Linaceae which are mainly used for oils (Simmonda 1976). It is grown worldwide either for the oil extracted from the seed or for fiber from the stem. Flax is grown mainly in Manitoba, Saskatchewan and Alberta in Canada. The majority of flax utilized in North America is still consumed as feed; while China and India along with Japan and Korea is mostly consumed as food (SCAAFC, 2012). Flax seed production in the world is estimated approximately 177MMT and cultural area is also assumed at 426,684ha (SCAAFC, 2012). It was estimated that Canadian flaxseed exports to China would be up to about 106,000 MT from 2011 to 2012.

The remnants after oil extraction is fed to animals as a protein supplement (Lennerts, 1983). Flaxseed is composed of 35 to 40% protein and together with cottonseed and sunflower supplies about 23% of the world's oilcake and meal (Hatje, 1989). High α-linolenic acid flaxseed is one of the richest dietary sources for α- linolenic acid and is also a good source of soluble fiber mucilage (Cunnane *et al*., 1993).

Plant growth regulators, particularly growth retardants can enhance crop productivity by modifying internal hormonal balance and improving sink-source relationships (Singh *et al*., 1987). Mepiquat chloride, one of the 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 structures such as 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 (Jung *et al*., 1975; Willard *et al*., 1976; Stuart *et al*., 1984; Kerby 1985; Hodges *et al*., 1991; Reddy *et al*., 1992). TE is one of the newest growth regulators in agriculture and horticulture. It acts by inhibiting gibberellins biosynthesis resulting in shorter internode length and it inhibits gibberellin production

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much later in the biosynthetic pathway than MC, chloromequat, and triazole compounds (Rademacher, 2000; Hafner, 2001). In a past study, it revealed that foliar application of mepiquat chloride increases seed oil content and promotes unsaturated fatty acids in flax (Kim *et al.,* 2011). Considering the above mentioned information, the field experiment was conducted to study the response of combined plant growth retardants both mepiquat chloride and trinexapac-ethyl on the change of seed yield, oil and lignan content, and endogenous gibberellin levels in flax plant.

Materials and Methods

Field experiment was conducted at the experimental field of Gyeongsangbuk-do Provincial Agricultural Research & Extension Services, Daegu, Korea. Flax seeds were planted in 4.8 m (16 rows) with 6.0 m (row length) plots covered with black polyethylene vinyl on March 2010. 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 90 kg/ha, incorporating as basal and top dressing (7:3, w/w) to the soil, respectively. The climate was temperate with mean annual maximum and minimum daily air temperatures of 17.5℃ and 8.3℃, respectively, and precipitation of 1,410 mm.

Main soil physical and chemical properties were loamy soil consisting of 40.2% in silt, 21.2% in clay, and 38.6% in sand. The details of the treatments comprised of T_1: control, T_2: mepiquat chloride (MC) 1.346 kg a.i./ha (300 ppm) + trinexapac-ethyl (TE) 0.756 kg a.i./ha (100 ppm), T_3: MC 1.346 kg a.i./ha (300 ppm) + TE 1.512 kg a.i./ha (200 ppm), T_4: MC 1.346 kg a.i./ha (300 ppm) + TE 2.668 kg a.i./ha (300 ppm), T_5: MC 2.691 kg a.i./ha (600 ppm) + TE 0.756 kg a.i./ha (100 ppm), T_6: MC 1.346 kg a.i./ha (600 ppm) + TE 1.512 kg a.i./ha (200 ppm), T_7: MC 1.346 kg a.i./ha (600 ppm) + TE 2.668 kg a.i./ha (300 ppm).

Each combined MC and TE was foliar-sprayed at 50 days after seeding. The each solution volume was applied at the rate of 30 L/ha. 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. Flaxseeds were harvested on 10 August 2010 depending on seed maturity rate. Oil content and fatty acid composition were determined from matured flaxseed. Crushed flaxseed (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 200 ml of a 1% (w/v) solution of sodium methoxide in methanol as described (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.

Extraction and analysis of lignans including SDG (secoisolariciresinoldiglucoside), SECO (secoisolariciresinol), and ANHSEC (anhydrosecoisolariciresinol) followed the reference (Meagher *et al*., 1999). Defatted flax powder was extracted with 50 ml of 80% methanol for 5hours 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 1h 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 114 M 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 55min, then back to 70A:30B, for equilibration of the system over 5 min.

Extraction and analysis of gibberellin metabolites were followed as described by Lee *et al*. (1998). The seeds harvested were immediately stored at -80℃. When all of the required materials for GA analysis had been collected, the samples were lyophilized for 48 hours. The extraction of endogenous gibberellins was followed as described by Lee *et al*. (1998).

The GAs were chromatographed on a 3.9 × 300 mm μ BondaPak C_{18} column (Waters) and eluted at 1.5 ml /min 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 μl) 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. Three or five prominent ions were analyzed by GC-MS-SIM (Finnigan Mat GCQ) with dwell times of 100 ms. The 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 flaxseed yield were analyzed by using SAS package for Duncan's multiple range tests.

Results and Discussion

Growth attributes

Different treatments significantly influenced the plant height as well as number of capsule/plant (Table 1). The lowest plant height (76.4 cm) and the highest number of capsule/plant (22.6) were observed in treatment T_7 involving combination of MC 600 ppm + TE 300 ppm. MC regulating plant growth has been extensively researched and well documented in cotton (York, 1983a; 1983b). It was reported that MC tends to be shorter and more compact than untreated plants (Jung *et al*., 1975; Willard *et al*., 1976; Stuart *et al*., 1984; Kerby, 1985; Hodges *et al*., 1991; Reddy *et al*., 1992).

The reduction in plant height was more effective in treatment T_5, T_6 and T_7 compared to the treatment T_1, T_2, T_3 and T_4. The plant height in these treatments T_5, T_6 and T_7 was reduced by 7.4, 8.8, and 10.2% compared to the treatment T_1, and reductions in plant height were closely correlated with the final height of untreated flax. Most plant growth retardants inhibit the formation of growth-active gibberellins and thus can be used to reduce unwanted shoot elongation (Hedden and Kamiya, 1997). It implies that mepiquat chloride (MC) acts strongly to decrease stem growth compared to the trinexapac-ethyl (TE). It is known that mepiquat chloride is a plant growth regulator which suppresses vegetative growth in cotton (York, 1983b).

Qian (1998) reported that TE reduces stem elongation and mowing requirements stimulating a favorable vertical shoot growth in turfgrass. The combination treatment of lower MC and elevated TE (treatment T_2, T_3, T_4) was found to have a significant effect over control compared to the higher MC and elevated TE (treatment T_5, T_6, T_7). In a previous study, we

Table 1. Combined effects of MC and TE on growth characteristics and seed yield in flax

Treatment	Plant height (cm)	No. of branch/ plant	No. of capsule/ plant	Ripened seed rate (%)	1,000-seed weight (g)	Seed yield (kg/ha)
T_1	85.1a	6.2cd	16.8d	85.2d	5.75c	1,701c
T_2	82.0b	6.7c	18.6c	88.7b	6.45a	1,822b
T_3	82.2b	7.6b	20.2ab	92.4a	6.62a	1,874b
T_4	81.3b	7.4b	19.8b	92.6a	6.61a	1,946a
T_5	78.8c	6.9bc	19.9b	86.1bc	5.98b	1,808b
T_6	77.6c	8.8a	21.8a	86.8bc	5.96b	1,813b
T_7	76.4c	9.2a	22.6a	86.7bc	5.98b	1,820b

The same letters in each column were not significantly different at 5% by DMRT.
T_1: control, T_2: mepiquat chloride (MC) 1.346 kg a.i. /ha (300 ppm) + trinexapac-ethyl (TE) 0.756 kg a.i./ha (100 ppm), T_3: MC 1.346 kg a.i./ha (300 ppm) + TE 1.512 kg a.i./ha (200 ppm), T_4: MC 1.346 kg a.i./ha (300 ppm) + TE 2.668 kg a.i./ha (300 ppm), T_5: MC 2.691 kg a.i./ha (600 ppm) + TE 0.756 kg a.i./ha (100 ppm), T_6: MC 1.346 kg a.i. /ha (600 ppm) + TE 1.512 kg a.i. /ha (200 ppm), and T_7: MC 1.346 kg a.i. /ha (600 ppm) + TE 2.668 kg a.i./ha (300 ppm).

reported that single MC treatment had a higher seed ripening than TE treatment (Kim *et al*., 2011). In this study, however, combined treatment of MC with TE gave a lower seed ripening rate than that of single MC treatment. The highest seed yield recoded with treatment T_4 compared to control and other combinations. This result would be due to the increased seed ripening rate accompanying the elevated 1,000-seed weight. The treatment T_2 and T_3 caused an increased seed yield compared to the treatment T_5, T_6 and T_7. However, the differences between these treatments were statistically insignificant.

Content of oil and lignan, and fatty acid composition

Combined application of MC and TE showed significant response on the increased oil and lignan content (Table 2). All combined treatments of MC and TE applied to flax plants produced high amounts of oil. The oil content in the treatment T_2, T_3 and T_4 was increased by 5.9 to 11.8% compared to the control. Meanwhile, the oil content in the treatment T_5, T_6 and T_7 was increased by 3.0 to 6.7% compared to the control. It suggests that the increased oil content might be depended on an elevated MC concentration with or without TE treatment. Farooqi and Sharma (1988) reported that plant growth retardant such as chlormequat chloride increased significantly oil contents and inhibited growth in Japanese mint.

Kim *et al.* (2011) reported that the foliar spray of single treatment with MC caused higher oil content than that of TE. Change of lignan associated with plant growth retardants has not been reported yet in flax plants. Lignan content was increased in all the treatments. The highest lignan content was observed in the treatment T_4 followed by the treatment T_3 compared to the control.

In the combination of MC 300 ppm, when TE concentration was increased oil content was significantly increased in treatment T_2, T_3, T_4. Like an above tendency, in the combination of MC 600 ppm, oil content was significantly increased in treatment T_5, T_6, T_7. Palmitic acid in composition of fatty acids was ranged 4.0 to 4.8% in all of the treatment except for control.

In unsaturated fatty acids, the highest oleic acid was only observed in the treatment T_4. Linolenic acid content was reduced in the treatment T_2, T_3, T_4, T_6 and T_7 except for the treatment T_5 compared to the control. These results suggest that the equal combination of two plant growth retardants with same ratio affects partially changes of some unsaturated fatty acid and the combination of relatively high concentration of mepiquat chloride with low trinexapac-ethyl. In earlier study, it revealed that single treatment of MC increased oil content when MC concentration increased (Kim *et al*., 2011). The present studies implied that combined treatment of MC and TE may be associated reversely with contrary effect between plant hormones.

Change of endogenous gibberellins

The bioactive gibberellins (GA_1 and GA_4) and total endogenous gibberellin content were measured from flax seed applied

Table 2. Combined effects of MC and TE on oil, SDG content and fatty acid composition in flax

Treatment	Oil content (g/kg FW)	Lignan (mg/g)	Fatty acid composition (%)				
			Palmitic	Stearic	Oleic	Linoleic	Linolenic
T1	350e	11.0c	5.4	3.2	16.9	17.1	57.4
T2	372c	11.2c	4.8	3.0	17.6	17.6	57.0
T3	384b	13.5b	4.3	3.0	18.2	17.5	57.0
T4	397a	14.6a	4.4	2.6	22.1	18.3	52.6
T5	361d	14.5a	5.3	3.1	16.1	17.5	58.0
T6	370c	13.8b	4.4	2.7	17.8	19.2	55.9
T7	375c	13.7b	4.0	2.7	18.4	19.6	55.3

The same letters in each column were not significantly different at 5% by DMRT.

T_1: control, T_2: mepiquat chloride (MC) 1.346 kg a.i. /ha (300 ppm) + trinexapac-ethyl (TE) 0.756 kg a.i./ha (100 ppm), T_3: MC 1.346 kg a.i./ha (300 ppm) + TE 1.512 kg a.i./ha (200 ppm), T_4: MC 1.346 kg a.i./ha (300 ppm) + TE 2.668 kg a.i./ha (300 ppm), T_5: MC 2.691 kg a.i./ha (600 ppm) + TE 0.756 kg a.i./ha (100 ppm), T_6: MC 1.346 kg a.i. /ha (600 ppm) + TE 1.512 kg a.i. /ha (200 ppm), and T_7: MC 1.346 kg a.i. /ha (600 ppm) + TE 2.668 kg a.i./ha (300 ppm).

Table 3. Combined effects of MC and TE on GA_1, GA_4 and total gibberellin content in flax

Treatment	GA_1 content (ng/g DW)	GA_4 content (ng/g DW)	ECH (ng/g DW)	NCH (ng/g DW)	Total GAs content (ng/g DW)
T_1	0.41a	1.63a	7.91	21.30	29.21a
T_2	0.37b	1.58a	7.58	18.73	26.31b
T_3	0.38b	1.51a	7.44	16.22	23.66c
T_4	0.31c	1.43b	6.78	15.84	22.62cd
T_5	0.25d	1.36b	6.23	17.31	23.54c
T_6	0.26d	1.29b	5.95	16.02	21.97d
T_7	0.24d	1.13c	5.48	15.85	21.33d

ECH, early-13-hydroxylation pathway, NCH, non-13-hydroxylation pathway.
The same letters in each column were not significantly different at 5% by DMRT.
T_1: control, T_2: mepiquat chloride (MC) 1.346 kg a.i./ha (300 ppm) + trinexapac-ethyl (TE) 0.756 kg a.i./ha (100 ppm), T_3: MC 1.346 kg a.i./ha (300 ppm) + TE 1.512 kg a.i./ha (200 ppm), T_4: MC 1.346 kg a.i./ha (300 ppm) + TE 2.668 kg a.i./ha (300 ppm), T_5: MC 2.691 kg a.i./ha (600 ppm) + TE 0.756 kg a.i./ha (100 ppm), T_6: MC 1.346 kg a.i. /ha (600 ppm) + TE 1.512 kg a.i./ha (200 ppm), and T_7: MC 1.346 kg a.i./ha (600 ppm) + TE 2.668 kg a.i./ha (300 ppm).

with different concentration of two plant growth retardants, MC and TE (Table 3). GA_1 content was gradually decreased in all treatments. GA_1 content was more decreased in the treatment T_2, T_3 and T_4 than that of T_5, T_6 and T_7. GA_1 level in the combination of MC and TE was decreased by higher concentration, which is attributed to the significant increase in plant height. Otherwise, GA_4 content was not significantly different in the treatment T_1 and T_2 compared to the control although it was reduced significantly in the treatment T_4, T_5, T_6 and T_7. Gibberellin content of early C-13 hydroxylation groups including bioactive GA_1 was gradually decreased with the increased MC and TE concentrations. Gibberellin content in non C-13 hydroxylation groups including bioactive GA_4 was also reduced with increasing MC and TE. In spite of increased MC and TE concentrations, major gibberellin pathway was always the non C-13 hydroxylation route leading GA_4. In a previous report, we found that eight endogenous gibberellins were identified and the non-C13 hydroxylation pathway was also dominantly operated during seed development in flax plant (Kim *et al*., 2009). Total gibberellin content including early and non C-13 hydroxylation routes was decreased with elevating MC and TE treatments. Among these treatments, the lowest total gibberellin level was observed in the combination of MC 600 ppm and TE 300 ppm (T_7). Present study revealed that the combination of MC and TE had positive effects to increase the seed ripening rate, oil and lignan content and seed yield in flax plant.

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