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Early development of cotton as affected by seed treatment with cyclanilide combined with mepiquat chloride

Leonardo Cirilo da Silva Soares[1], Juan Piero Antonio Raphael[2], Henrique da Ros Carvalho[3], Ederaldo José Chiavegato[1], Durval Dourado Neto[1]

[1] Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Departamento de Produção Vegetal, Av. Pádua Dias, 11, Agronomia, CEP 13418-900, Piracicaba-SP, Brasil. Caixa Postal 9. E-mail: leonardo.cs.soares@gmail.com; ejchiave.esalq@usp.br; ddourado@usp.br
[2] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agronômicas, Departamento de Produção e Melhoramento Vegetal, Rua José Barbosa de Barros, 1780, Jardim Paraíso, CEP 18610-307, Botucatu-SP, Brasil. E-mail: juanpiero1@gmail.com
[3] Texas A&M University., 370 Olsen Blvd, 343 Heep Center, TAMU MS 2474, ZIP 778432474, College Station-TX, United States of America. E-mail: henrique.carvalho05@neo.tamu.edu

ABSTRACT

Greenhouse and laboratory experiments were carried out with the purpose of studying effects of the plant growth regulators (PGR) mepiquat chloride (MC) and cyclanilide (CY) as seed treatments on the early growth of cotton. Seeds of cultivars NuOpal, Fibermax-993, Fibermax-910, FMT-701 and FMT-523 were treated with MC (4.50 g kg^{-1} seed), MC+CY (1.60+0.40 g kg^{-1} seed) and MC+CY (4.50+1.12 g kg^{-1} seed). In laboratory, seed storage periods of 4 and 50 days were also tested. MC+CY treatments decreased plant height by approximately 50%, whereas MC decreased plant height about 30 to 40%. The greatest and lowest rates of MC+CY decreased the height of cotyledon node by 33 and 12% compared to MC treatment, respectively. MC and control treatments did not differ from each other on radicle length (RaL), taproot length (RL) and secondary root initiation depth (SRI). The greatest MC+CY rate resulted in the greatest SRI values, increased RL by 7% and R_aL by 29% compared to the control. Seed storage period did not affect consistently the performance of treated seeds. Short-cycle cultivars have a greater sensitivity to seed treatment with PGRs, whereas CY synergizes with MC to reduce shoot growth and inhibits emission of secondary roots.

Key words: auxin, gibberellin, *Gossypium hirsutum*, vegetative growth

Desenvolvimento inicial do algodoeiro após tratamento de sementes com ciclanilida associada ao cloreto de mepiquat

RESUMO

Experimentos em casa de vegetação e laboratório foram conduzidos para estudo de efeitos dos reguladores vegetais (RV) cloreto de mepiquat (CM) e ciclanilida (CY) em tratamento de sementes sobre o crescimento do algodoeiro. Sementes das cultivares NuOpal, Fibermax-993, Fibermax-910, FMT-701 e FMT-523 foram tratadas com CM (4,50 g kg^{-1} semente), CM+CY (1,60+0,40 g kg^{-1} semente) e CM+CY (4,50+1,12 g kg^{-1} semente). Em laboratório, testaram-se períodos de armazenamento de sementes de 4 e 50 dias. Tratamentos CM+CY reduziram a altura de plantas em aproximadamente 50%, enquanto foi diminuída entre 30 e 40% pelo CM. A maior e a menor dose de CM+CY diminuíram, respectivamente, a altura de inserção cotiledonar em 33 e 12% comparativamente ao CM. CM e controle não diferiram entre si em comprimento de radícula (CRa), comprimento de raiz (CR) e profundidade de início de raízes secundárias (IRS). A maior dose de CM+CY resultou em maiores valores de IRS, aumentou CR em 7% e CRa em 29% em comparação ao controle. O tempo de armazenamento não afetou consistentemente as sementes tratadas. Cultivares precoces são mais responsivas ao tratamento de sementes com RVs, enquanto CY atua sinergisticamente com CM na redução de crescimento de parte aérea e inibe a emissão de raízes secundárias.

Palavras-chave: auxina, giberelina, *Gossypium hirsutum*, crescimento vegetativo

Introduction

Over the past decade cotton has been increasingly grown as a second crop in Brazil, with reduced row spacing, mainly after harvesting early-cycle soybeans (Belot & Campelo Junior, 2010). There is a difficulty to manage the crop to meet the recommended ratio of final plant height to row spacing being equal to 1.5 m, especially for narrow row cotton, so the use of plant growth regulator (PGR) has become essential (Nagashima et al., 2005). In Brazil, the two commercial plant growth regulators (PGRs) recommended to control the vegetative growth of cotton are mepiquat chloride (MC) and chlormequat chloride (CC), which inhibit the biosynthesis of gibberellic acid (Rademacher, 2000). MC is also applied in combination with cyclanilide (CY), although commercial products with both active ingredients are not available in Brazil. Cyclanilide is a PGR used in combination with other molecules such as ethephon, in order to accelerate defoliation and opening of bolls, and with mepiquat chloride to control vegetative growth. In both cases, cyclanilide appears to enhance the effects of the compound it is mixed with (Burton et al., 2008). Although there are still speculations about its mechanism, there are indications that cyclanilide can act as an auxin transport inhibitor (Burton et al., 2008; Pedersen et al., 2006).

At locations where narrow-row cotton is grown in Brazil, the early growth commonly occurs under high rainfall and temperature. A more efficient control of vegetative growth may be obtained with the anticipation of the first PGR application. In this context, PGRs applied by seed treatment may be an alternative management strategy (Nagashima et al., 2005) as long as the reduction in growth rate is not severe or limiting. This procedure may be helpful by providing more time for defining the moment of first foliar application.

According to recent research data, seed treatment with MC affects cotton growth by decreasing plant height, leaf area, height of the cotyledon node and shoot dry mass, but without influencing root length (Nagashima et al., 2005; Nagashima et al., 2007; Oliveira et al., 2011; Almeida & Rosolem, 2012). However, there are no studies about effects on cotton growth by seed treatment with MC+CY. It is also possible that a longer seed storage period reduces PGR activity and, thus, influences its effects on early growth of cotton seedlings. Therefore, the objective of this study was to evaluate the effects of MC in combination with CY on early development of cotton cultivars by seed treatment.

Material and Methods

Two experiments were carried out in 2009, in Piracicaba, Brazil. Experiment I and Experiment II were conducted in greenhouse and seed laboratory, respectively.

In experiment I, the plots consisted of plants grown in polyethylene bags (diameter of 12 cm and height of 40 cm) filled with clay soil of moderate fertility, in a completely randomized design with factorial arrangement (4 x 5) with five replications. The treatments consisted of four PGR treatments combined with five cotton cultivars. The PGR treatments were: mepiquat chloride (MC) at the rate of 4.50 g kg^{-1} seed, mepiquat chloride + cyclanilide (MC+CY) at the rates of 1.60+0.40 and 4.50+1.12 g kg^{-1} seed, and a control treatment. The cultivars used were NuOpal, Fibermax-993, Fibermax-910, FMT-701 and FMT-523. PGR treatments were applied in 100 grams of seeds.

The PGR solutions were added by using a syringe into the bags containing the seeds. Water was added in the control treatment. The seeds were homogenized, transferred into "Kraft-paper" bags, and stored for 4 days under no light, temperature of 20ºC and air relative humidity ranging from 50 to 60% until planting. The sowing was performed in 21 May 2009. No fertilizer was used as well as other chemicals such as insecticides and fungicides on the seeds. Each plot received a total of 3 seeds. Thinning was done after emergence, and only one plant was left per polyethylene bag. Soil moisture was maintained by replacing the same volume of water in the plots daily. The greenhouse temperature was maintained below 30ºC. The plants were grown up to stage B_1, which corresponds to first flower buds visible in at least 50% of the plants (Marur & Ruano, 2001). This occurred 75 days after emergence (DAE) due to low temperatures during the study. Plant height (PH) was measured at 6, 10, 14, 21, 31, 38, 48 and 75 DAE, determined as the height of the youngest leaf in the first 3 evaluations and as the height of apical bud in the remaining evaluations. Taproot length (RL), secondary root initiation depth (SRI), height of the cotyledon node (HC) and leaf area (LA) were evaluated at B_1 stage (75 DAE). RL was determined as the distance between its upper and lower ends with a graduated ruler. SRI was determined by measuring the taproot length from the soil surface to the first secondary root with a ruler. HC was determined by measuring the length from cotyledon node to soil surface. LA determinations were done using an area meter of the LI-COR 3100 (LI-COR, Lincoln, Nebraska, USA).

Experiment II was conducted in June 2009 in a seed laboratory, with controlled temperature at 25±1ºC, following the method of Brasil (2009) for germination tests. PGR treatments and cultivars were the same used in Experiment I. In addition to these two factors, in this experiment the seeds were stored for 4 or 50 days before starting the experiment, under the same conditions described in Experiment I, in order to evaluate the effect of storage period on the treated seeds. The experimental design was completely randomized with factorial arrangement (4 x 5 x 2) and 3 replications. Experimental units comprised of rolled paper towels (Germitest®) moistened to 2.5 times the weight of dry paper, with 10 seeds spaced equidistantly, placed in the upper third of the towel paper. The rolled paper towels with treated seeds were placed in germination chamber for 4 days and then were removed for radicle length (R_aL) measurements.

Results were subjected to analysis of variance (F-test) and the averages were compared by the Tukey test at the 5% probability level.

Results and Discussion

In experiment I there was interaction between cultivars and PGR only for secondary root initiation depth (SRI). Except for Fibermax-910, which showed the greatest values of height and height of cotyledon node, all cultivars reached similar height during the initial stage of the study. Subsequently, Fibermax-910 and Fibermax-993 had greater mean height compared to the other cultivars (Table 1). MC+CY treatments reduced plant height in about 50% compared with the control, whereas the reduction by MC treatment was between 30 and 40% across measurement dates (Table 1).

Compared to the control, the PGR treatments decreased HC by 40 to 60% (Table 2). The highest rate of MC+CY resulted in the greatest reduction in HC and increased the taproot length compared with the control. The decrease in leaf area promoted by the PGR treatments varied from 8-10% compared to the control treatment (Table 2). No significance was found between PGR treatments. Cultivar FMT-523 showed leaf area about 50% smaller than the other cultivars.

The interaction between cultivars and PGRs found for SRI revealed differences between cultivars only within the greatest MC+CY rate (Table 3). MC+CY treatments resulted in greater SRI values among all cultivars tested (Table 3), especially when the greatest rate was used (Figure 1).

Table 1. Plant height (PH) of cotton plants at 6, 10, 14, 21, 31, 38, 48 e 75 days after emergence (DAE) as affected by cultivars and plant growth regulators (PGRs) in Experiment I. Piracicaba, Brazil, 2009

Cultivar	Plant height							
	Days after emergence (DAE)							
	6	10	14	21	31	38	48	75
	(cm)							
FMT-523	4.9 b	5.7 b	6.6 b	6.6 b	6.7 b	7.6 bc	8.9 b	11.0 c
NuOpal	5.3 ab	6.0 b	6.7 b	6.7 b	6.8 b	7.3 c	8.8 b	12.2 bc
FMT-701	5.3 ab	6.3 b	7.0 b	6.8 ab	7.2 b	8.3 ab	9.6 ab	12.3 b
Fibermax-993	5.2 b	6.1 b	7.2 b	6.6 b	7.2 b	8.3 ab	10.2 a	13.2 ab
Fibermax-910	6.0 a	7.1 a	7.9 a	7.4 a	8.4 a	9.1 a	10.7 a	13.6 a
PGR (g a.i. kg^{-1})								
Control	7.7 A	9.2 A	10.6 A	10.7 A	11.5 A	12.5 A	14.0 A	16.3 A
MC (4.50)	5.4 B	6.2 B	7.0 B	7.2 B	7.3 B	8.2 B	10.1 B	13.1 B
MC+CY (1.60+0.40)	4.6 C	5.5 C	6.0 C	6.1 C	6.1 C	6.8 C	8.2 C	11.3 C
MC+CY (4.50+1.12)	3.8 D	4.2 D	4.4 D	4.6 D	4.7 D	5.1 D	6.4 D	9.2 D
CV (%)	14.22	13.74	14.26	14.02	12.61	12.96	12.3	10.74

Means followed by different letters, lower-case letters within cultivars and upper-case letters within plant growth regulators (PGR) differ from each other by the Tukey test ($p<0.05$). MC: mepiquat chloride; CV: coefficient of variation; CY: cyclanilide; g a.i.: grams of active ingredient.

Table 2. Taproot length (RL), height of cotyledon node (HC) and leaf area (LA) of cotton plants at B$_1$ stage as affected by cultivars and plant growth regulators (PGRs) in Experiment I. Piracicaba, Brazil, 2009

Cultivar	RL	HC	LA
	(cm)		(cm^2 plant^{-1})
FMT-523	29.9 a	6.3 b	82.2 b
NuOpal	30.4 a	6.7 b	158.8 a
FMT-701	30.8 a	6.9 b	160.1 a
Fibermax-993	30.1 a	6.9 b	154.0 a
Fibermax-910	30.4 a	7.9 a	148.9 a
PGR (g a.i. kg^{-1})			
Control	29.3 B	10.8 A	151.9 A
MC (4.50)	29.9 AB	6.6 B	135.5 B
MC+CY (1.60+0.40)	30.7 AB	5.8 C	136.3 B
MC+CY (4.50+1.12)	31.4 A	4.4 D	139.6 B
CV (%)	7.81	11.00	11.06

Means followed by different letters, lower-case letters within cultivars and upper-case letters within plant growth regulators (PGR) differ from each other by the Tukey test ($p<0.05$). MC: mepiquat chloride; CV: coefficient of variation; CY: cyclanilide; g a.i.: grams of active ingredient.

Table 3. Secondary root initiation depth (SRI) of cotton plants at B$_1$ stage as affected by cultivars and plant growth regulators in Experiment I. Piracicaba, Brazil, 2009

Cultivar	Secondary root initiation depth			
	Plant growth regulator (g a.i. kg^{1} seed)			
	(cm)			
	Control	MC (4.50)	MC + CY (1.60 + 0.40)	MC + CY (4.50 + 1.12)
FMT-523	1.4 Ab	1.4 Ab	3.5 Aa	3.3 Ba
NuOpal	0.7 Ac	1.2 Ac	4.2 Ab	5.9 Aa
FMT-701	1.0 Ab	1.1 Ab	2.9 Aa	3.7 Ba
Fibermax-993	0.2 Ac	1.7 Abc	2.9 Ab	4.5 ABa
Fibermax-910	1.0 Ac	1.3 Ac	3.1 Ab	4.8 ABa
CV (%)	38.06			

Means followed by different letters, upper-case letters within a column and lower-case letters within a row differ from each other by the Tukey test ($p<0.05$). MC: mepiquat chloride; CV: coefficient of variation; CY: cyclanilide; g a.i.: grams of active ingredient.

Figure 1. Taproot length and secondary root initiation depth (cm) at B$_1$ stage by seed treatment with plant growth regulators (PGR) on cotton cultivars in Experiment I. MC (4.5): mepiquat chloride at a rate of 4.5 grams of active ingredient per kg of seeds (g a. i. kg^{-1}); MC+CY (1.6+0.4): mepiquat chloride + cyclanilide at a rate of 1.6+1.4 g a.i. kg^{-1}; MC+CY (4.5+1.12): mepiquat chloride + cyclanilide at a rate of 4.5+1.12 g a.i. kg^{-1}. Piracicaba, Brazil, 2009

In experiment II, Fibermax-910 had the greatest radicle length (Table 4). FMT-701 and NuOpal showed intermediate values, but did not differ from FMT-523, which had the shortest radicle (Table 4). An interaction between seed storage period and PGR treatment was found. Seeds treated with MC+CY that were stored for 4 days showed greater R_aL compared with the MC and control treatments (Table 4). Compared with the control treatment, the greatest and the lowest MC+CY rates increased R_aL by 29% and 25%, respectively (Table 4).

The performance of cotton cultivars suggests that root and shoot growth are affected by the maturity group. Fibermax-993 and FMT-701 are classified as late-maturing cultivars; Fibermax-910 as intermediate; and NuOpal and FMT-523 as early-maturing. In general, the early cultivars had a slower height growth. These results suggest that short-cycle cultivars are the most affected by seed treatment with plant growth regulators. This

Table 4. Radicle length (R_aL) emitted from seeds of cotton cultivars treated with plant growth regulators (PGR) and stored for 4 and 50 days after four days in germination chamber (Experiment II). Piracicaba, Brazil, 2009

	Seed storage period		Mean
	4 days	50 days	
	radicle length (cm)		
PGR (g a.i. kg^{-1})			
Control	7.9 Ba	8.9 Ba	8.4
MC (4.5)	7.5 Bb	9.1 Ba	8.3
MC+CY(1.60+0.40)	9.9 Aa	10.6 Aa	10.3
MC+CY(4.50+1.12)	10.2 Aa	9.9 ABa	10.0
Mean	8.9	9.6	
Cultivar			
FMT 523	8.6	8.7	8.7 C
NuOpal	8.0	9.5	8.8 BC
FMT 701	8.7	9.3	9.0 BC
Fibermax-993	9.2	10.0	9.6 AB
Fibermax-910	9.7	10.5	10.1 A
Mean	8.9	9.6	

Means followed by different letters, upper-case letters within a column and lower-case letters within a row differ from each other by the Tukey test ($p<0.05$). MC: mepiquat chloride; CV: coefficient of variation; CY: cyclanilide; g a.i.: grams of active ingredient.

is in agreement with the work of Bogiani & Rosolem (2009), who found that short-cycle cultivars are the most responsive to foliar MC applications. The R_aL values in experiment II indicate that Fibermax-910 has a rapid initial growth, which may be related to the greatest values of plant height and height of cotyledon node found in experiment I. This pattern, even less pronounced, is also observed from late-cycle cultivars (Fibermax-993 and FMT-701). No differences regarding taproot length at B_1 stage (Table 2) indicate that the influence of cultivars on root development was gradually offset over the time.

In comparing the results of seed treatment, plant height and height of cotyledon node were decreased by plant growth regulators (Tables 1 and 2), according to results of previous experiments (Iqbal et al., 2005; Nagashima et al., 2005; Nagashima et al., 2010a; Nagashima et al., 2010b). The height-growth rates calculated from original height data revealed that the treatment of seeds with plant growth regulators retarded shoot growth until 31 DAE (MC and the lowest MC+CY rate) and 38 DAE (the highest MC+CY rate) compared with the control (data not shown). The combination of mepiquat chloride with cyclanilide intensified the reduction of plant height (Table 1) and height of cotyledon node (Table 2), especially with the highest MC+CY rate.

The differences between plant growth regulators observed in experiments I and II indicate that the presence of cyclanilide was related to a greater radicle length (Table 4) and taproot length at B_1 stage (Table 2), and that mepiquat chloride alone was not able to induce significant effects. Since the total amount of active ingredients in the lowest concentration of MC+CY is lower than that found in MC treatment, the presence of cyclanilide alters the root growth and enhance the effects of mepiquat chloride on plant height and height of cotyledon node. In general, the more pronounced effects of MC+CY on shoot growth are according to the synergism recorded with both of active ingredients (Pedersen et al., 2006; Burton et al., 2008). The inhibition of gibberellin biosynthesis by mepiquat chloride is mediated by auxin, which induces enzymes to metabolize the inactive form GA_{20} to the active GA_1 (Rademacher, 2000; Tanimoto, 2012). Thus, the action of a probable inhibitor of auxin transport, such as cyclanilide, may increase the efficiency of a gibberellin-biosynthesis inhibitor such as mepiquat chloride (Burton et al., 2008). The increase of radicle length and secondary root initiation depth only in the presence of cyclanilide highlights that MC+CY-plants prioritized root elongation during the first few days of seedling development over the emission of secondary roots. This effect is in agreement with references mentioning that auxin inhibitors suppress the formation of lateral roots (Muday & Haworth, 1994), causing the taproot to elongate for a longer time until the first secondary roots are emitted. The lack of effects by MC alone on root evaluations is in accordance with results from Nagashima et al. (2010a) and Almeida & Rosolem (2012) after testing different rates of mepiquat chloride by treatment of seeds. In experiment II, the increase of radicle length by the longest storage period when the MC treatment was used does not necessarily indicate an effect on the treatment of seeds since there was no difference in comparison with the control (Table 4). In addition, the absence of differences between seed storage periods for MC+CY, regardless of its rate, indicates that the activity of the products tested is not reduced as the storage period increases.

The combination of mepiquat chloride and cyclanilide enhanced the effects on shoot growth of cotton plants even when its respective concentration of mepiquat chloride was lower than that used for MC-alone treatment, indicating that cyclanilide synergized with mepiquat chloride. However, this synergism was observed in comparison with a single MC-rate of 4.5 g kg^{-1} seeds, which is low compared with MC-rates tested in previous studies (Nagashima, 2010b; Oliveira et al., 2011). It is possible that the synergism between mepiquat chloride and cyclanilide occurs only with low MC-rates. However, to confirm this possibility, new experiments testing increased rates of both MC and MC+CY active ingredients should be conducted.

Conclusions

MC and MC+CY as seed treatment reduce growth in cotton. The plant growth regulator MC+CY enhances reductions in shoot-growth compared with MC alone and inhibits the development secondary roots.

Short-cycle cultivars are the most affected by the plant growth regulators MC and MC+CY.

Seed storage period does not affect consistently the radicle length determined from seeds treated with the plant growth regulators MC and MC+CY.

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