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Effect of methyl jasmonate on the metabolism of phenolic compounds in cotton (*Gossypium Hirsutum* L., CV. W471B)

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Abstract

The aim of this study was to understand the stimulation of plants' natural defenses in general and by methyl jasmonate. It is thus right that we followed the influence of the latter on the metabolism of phenolic compounds of cotton leaves (*Gossypium hirsutum L.* Cv. W471B). It is clear that the phenol content, which is 48.16 mg/g of dry matter at 2.5 mM of MeJA spray, increases to reach its maximum at 5 mM, i.e., 64.33 mg/g of dry matter. PAL is the enzyme that participates the most in the biosynthesis of total phenols following the treatment of cotton leaves with 5 mM MeJA. On the other hand, the activity of polyphenoloxidase (PPO), which was initially 2.83 ΔDO /g DM in the untreated control leaves, fell to 1.81 ΔDO /g DM in the leaves treated with 5 mM MeJA, i.e., a 36.04% decrease in phenol degradation activity. Peroxidase (POD) activity also decreased from 2.5 ΔDO /g DM in control leaves to 0.25 ΔDO /g DM in the leaves sprayed with 5 mM MeJA, i.e., a decrease in phenol degradation activity of 90%. TAL is, therefore, the enzyme that participates more in the phenol degradation activity following the application of 5mM MeJA on cotton leaves.

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Introduction

Cotton is a multipurpose textile and protein-oil plant **(**Shahrajabian *et al.*, 2020**).** It is grown in many tropical and subtropical countries for its fibers and seeds, which contain 18-20% lipids and 35-40% protein. The useful exploitation of these proteins would allow covering 5 to 6% of the world's protein needs (Rachuonyo *et al.*, 2002). Cotton cultivation occupies nearly 2.5% of the world's arable land. World production is about 12.7 million tons of cotton and represents 60% of the raw material for the textile industry (FAO, 1991). The United States, China, Uzbekistan, India and Pakistan are the largest producers. Africa accounts for only 6% of world production. In Côte d'Ivoire, cotton is the third-largest export product after coffee and cocoa and contributes 1.7% of the gross domestic product. It provides substantial income to the peasantry in the center and north of the country, which are the main cotton-producing regions. With approximately 340,000 tons of cotton expected this year compared to 260,000 last year, Côte d'Ivoire is the 3rd largest cotton producer in West Africa, after Burkina Faso and Mali (Anonyme, 2013). However, cotton is threatened by various pests and diseases that reduce the production and quality of the fibers and seeds (Pettigrew. 2008). Of all the diseases of cryptogamic origin, fusariosis caused by *Fusarium oxysporum* f. *vasinfectum* (FOV) seems to be the most formidable. Indeed, conventional fungicides are unable to control this disease, which causes enormous damage in cotton farms (Stoddard *et al.*, 2010). Some authors propose farm abandonment or crop rotation as a solution to these infections. However, studies have shown that when faced with pathogen aggression, plants produce secondary metabolites, notably phenolic compounds, to defend themselves (Rehman *et al.*, 2012). Thus, the cotton plant produces a high number of phenolic compounds that are determinant in disease resistance (Makoi and Ndakidemi, 2007). One of the characteristics of plant resistance to biotic agents is indeed the capacity to induce specific defense reactions at the site of infection (Elad *et al.*, 2011) and in areas far from the point of infection, within tissues that are a priori not infected, characterized by systemic resistance (Vallad and Goodman, 2004). In this last case, the notion of systematicity implies the existence of a signal that will command the activation or the amplification of defense reactions over long distances (Heil and Ton 2008). A review of the literature reveals an abundance of references concerning three hormones with important functions in the installation of resistance. These are salicylic acid, ethylene and jasmonic acid. The last one mentioned is a hormone that has long been associated with plant responses induced by injury (Bajguz and Hayat 2009). According to Kazan and Manner (2008), it is, in fact, jasmonic acid, its methylated form (MeAJ) and its derivatives (called methyl jasmonate) that have potential bioactive properties in the regulation of defense to biotic stresses. In order to understand the stimulation of plants' natural defenses in general and by methyl jasmonate in particular, we followed the influence of the latter on the metabolism of phenolic compounds of cotton leaves (*Gossypium hirsutum* L. Cv. W471B).

Materials and methods

Plant material

The plant material consists of cotton seeds (*Gossypium hirsutum* L.cv. W471B). These seeds come from the Korhogo region (north of Côte d'Ivoire) and were supplied by the CIDT (Compagnie Ivoirienne pour le Développement du Textile).

Growing medium and conditions

The culture medium for our study is soil put in pots and arranged under shelters. This soil was previously autoclaved for 30 min at a temperature of 121 °C under a pressure of 1 bar.

Surface sterilisation and in vivo germination of seeds

The seeds are first disintegrated with concentrated sulphuric acid. After several rinses, the seeds are immersed in water. Floating seeds are classified as non-viable and are removed. Viable seeds are air-dried. These seeds are then sterilised under a laminar flow hood by a quick soak (1 min) in 70% ethanol,

followed by a 20 min immersion in sodium hypochlorite (3.6% active chlorine). After three (3) successive rinses with sterile distilled water for 3 to 5 min, the seeds were soaked in a jar containing sterile distilled water and placed in the dark in the culture room (Figure 1). After 48 hours, the seeds that have developed a pointed radicle are pricked out in the prepared pots. The whole set is then grown under a shelter covered with a transparent plastic film where the light and temperature conditions are close to natural conditions. (Figure 2).

Effect of methyl jasmonate on phenolic compounds

MeJA-treated plants

Twenty-five (25) seedlings were sprayed with 2.5 mM, 5 mM, 10 mM, 15 mM, and 20 mM methyl jasmonate (MeJA) previously dissolved in 1% ethanol added to an aqueous solution containing triton (0.1%). The sprays were applied for 24 h, 48 h and 72 h. Each plant received 50 ml of these elicitors at each application. The plants were watered according to the humidity of the substrate. Cotton plants that had not received any treatment were used as controls.

Content of phenolic compounds

The extraction of total phenols is done according to the method of SINGLETON *et al.* (1999). Half a gram (0.5g) of leaves taken from each batch were freeze-dried and cold-ground in 80% ethanol. The grindings were centrifuged at 5000 rpm for 5 min. The supernatant obtained constitutes the crude extract on which the determination of total phenols will be performed.

The determination of total phenols was done according to the method of Singh *et al.* (2002), modified and adapted to our plant material. The reaction mixture is mainly composed of phosphotungstic acid and phosphomolibdic acid, which will be reduced in an alkaline medium in parallel with the oxidation of phenols. The presence of the phenols is revealed by the addition of 0.5 ml of Folin Ciocalteu 1 N reagent, 1.5 ml of 17 % sodium carbonate and 0.5 ml of crude extract. The intensity of the coloration produced by this reaction, in proportion to the concentration of phenolic compounds in the extract, is monitored by a spectrometer at 765 nm. During the assay, a control is carried out where the phenolic extract is replaced by distilled water. The level of total phenols is determined using a standard curve with different concentrations of a gallic acid stock solution (200 µg /ml) and is expressed in milligrams per gram of dry matter (mg /g DM).

Activity of phenolic metabolism enzymes

Enzymes extraction

The buffer required for the extraction of the enzyme substances will depend on the enzymes studied and will therefore be specified later for each of them. The extraction of enzymes is carried out at a cold temperature of 4°C (to prevent the inhibitory actions of phenolic compounds and proteolytic enzymes) by grinding the plant material in an extraction buffer. During grinding, 1.2 ml of an extraction medium solution composed of 0.5 ml polyethylene glycol 6000 (PEG 6000) is used to fix the phenolic compounds, 0.25% sodium thiosulphate to protect the enzyme sites, 15% glycerol for the stability of the enzyme systems, 1 mM EDTA as complexing agent and 15 mM mercaptoethanol is added as reducing compounds to prevent enzymatic oxidations. After centrifugation, at 5000 rpm for 20 min, the resulting supernatant represents the crude extract of cytoplasm-soluble enzymes. The residue is depleted 3 times as before. Then 1 ml of 1% TRITON X 100 is added (to solubilise the enzymes bound to the walls), and 1 ml of the extraction medium. After centrifugation (under the same conditions), the supernatant is removed, the residue is taken up in the same buffer for a new extraction conducted in the presence of polyvinylpyrrolidone (PVP), followed by one hour of incubation. After centrifugation, the supernatant obtained represents the crude enzymatic extract bound to the wall.

Purification of crude extracts

A crude extract contains a large number of inhibitors. Most of the inhibitors are ionic in nature. To fix them, we used a basic anion exchange resin: DOWEX 2. It is

dissolved in our crude extract and then incubated for 30 min. under agitation. Centrifugation is carried out in order to eliminate the DOWEX 2 (which has fixed the inhibitor ions). The supernatant obtained constitutes the purified enzyme fraction ready for analysis.

Essay of phenolic biosynthesis enzymes

Ammonia-lyases (PAL and TAL)

The determination of these two enzymes was carried out using the method described by REGNIER (1994), modified and adapted to our plant material.

The basic buffer used is 0.2 M sodium borate at pH 8.8.

After 10 min of incubation at room temperature, the activity of PAL and TAL, which is proportional to the amount of cinnamic acid and p-coumaric acid, respectively, is monitored by spectrophotometer at a wavelength of 290 nm. In the assay, a control test is performed for each extract in which phenylalanine or tyrosine is replaced by 0.2 M sodium borate buffer at pH 8.8. The activity of PAL and TAL is respectively expressed in millimoles of cinnamic acid or p-coumaric acid formed per minute per gram of dry matter, considering that the molar extinction coefficient of cinnamic acid is equal to 19600 cm-1 mol-1 and that of p-coumaric acid is equal to 17600 cm-1 mol-1.

Phenolic oxidation enzyme activity

Peroxidases (POD)

The activity of peroxidases was determined according to the technique described by SANTIMONE (1973). The base buffer used was 0.1 M sodium phosphate at pH 7.5.

After shaking, the mixture is incubated for 10 min. in the dark to prevent partial destruction (by light) of the reddish-brown oxidation product formed from guaiacol in the presence of hydrogen peroxide. A delay of one minute is allowed between tubes when adding the enzyme extract to the substrate. The oxidation of guaiacol is monitored with a spectrophotometer at a wavelength of 470 nm, always respecting the one-minute delay between tubes. Control is made where the substrate is replaced by 0.1 M sodium phosphate buffer at pH 7.5.

The molar extinction coefficient of the product formed at a wavelength of 470 nm is equal to 26.6 cm-1 mol-1 (Santimone, 1973).

Polyphenoloxidases (PPO)

The determination of PPO activity was done according to the method of Cano *et al.* (2011), modified and adapted to our plant material. The base buffer used was 0.1 M phosphate citrate at pH 6.5. Before the addition of DOWEX 2, two volumes (2xVml) of 100% acetone (to precipitate the enzymes contained in the supernatant) are added to volume (v) of the supernatant. Final centrifugation under the same conditions as above yields a supernatant that represents the crude PPO extract.

The oxidation of pyrocatechol is monitored by a spectrophotometer at a wavelength of 500 nm against a control assay in which pyrocatechol is replaced by 0.1 M phosphate citrate buffer at pH 6.5. PPO activity is expressed as the change in optical density (ΔDO) per minute per gram of fresh material (NGALANI *et al.*, 1993).

Statistical analysis

For the analysis of the results, a statistical test was carried out. This is the analysis of variance with 1 classification criterion (ANOVA1), which was carried out in order to compare the different biochemical parameters. The smallest significant difference test (SSDT) was used to classify the parameters when there was a significant difference between them. The statistical analysis was performed using SAS software.

Results

Effect of different MeJA concentrations and treatment time on total phenol content of cotton leaves

The analysis of Fig. 3 shows that the phenol content, which is 48.16 mg/g of dry matter at 2.5 mM of MeJA

sprayed, increases to reach its maximum at 5 mM, i.e., 64.33 mg/g of dry matter. This content remains more or less the same at 10 mM or 64.25 mg/g dry matter. However, 5 mM is slightly higher than 10 mM. From this concentration onwards, there was a decrease in the total phenol content in the leaves, from 15 to 20 mM MeJA sprayed respectively 46.25 and 32.77 mg/g dry matter. Fig. 4 shows that there is not a big difference between the total phenol content in the cotton leaves obtained after spraying with 5 mM MeJA for 48 h and 72 h. However, 72 h of treatment gave the highest total phenol content with 51.77 mg /g DM. Fig. 5 shows the combined effect of MeJA concentration and application time on total phenol content in leaves. The analysis of this table confirms that 5 mM gives the maximum phenolic compounds after 72 h of MeJA application with 75.75 mg /g DM.

Table 1. Total phenol content and activity of phenolic metabolism enzymes as a function of MeJA concentration

	Concentration (mM)		
Elements	0 mM	5mM	Rate of variation (%)
Phenols (mg /g MS)	35,08 ± 8,57b	4,33 ± 13,39a	(+) 83,38 %)
PAL (µM /g DL)	160,22±4,56b	210,51 ± 7,41a	(+) 31,38 %)
TAL (µM /g MS)	95,02 ± 2,03b	145,07± 14,9a	(+) 52,67%)
PPO (ΔDO /g MS)	2,83 ± 8,73a	1,81 ± 0,57b	(-) 36,04 %)
POD (Mol /g MS)	2,5 ± 7,83 a	0,25 ± 0,08b	(-90 %)

Standard error; DM: dry matter on the same line, means followed by the same letter are not significantly different (PPDS test at 5%); values represent the average of three replicates; (+) growth; (-) decrease; MeJA : methyl jasmonate.

Effect of 5 mM MeJA on the metabolism of phenolic compounds in cotton leaves

The results obtained have been grouped in Table 1. The analysis of this table shows that the initial phenol content of 35.08 mg /g DM in the untreated leaves (control) increases to 64.33 mg /g DM in the leaves sprayed with MeJA 5mM, i.e., an increase in phenol content of 83.38%.

Also, the activity of phenylalamine ammonia-lyase (PAL), which is 160.22 µM /g DM in the untreated leaves (control), increases to 210.51 µM /g DM in the treated leaves, i.e., an increase in the biosynthesis activity of this enzyme of about 31.38%.

Similarly, the activity of tyrosine ammonia-lyase (TAL), which is initially 95.02 µM / g DM in the untreated control, increases to 145.07 µM /g DM in the sprayed leaves, i.e., an increase in activity of about 52.67%. PAL is the enzyme most involved in the biosynthesis of total phenols following treatment of cotton leaves with 5 mM MeJA. On the other hand, the activity of polyphenoloxidase (PPO), which was initially 2.83 ΔDO /g DM in the untreated control leaves, fell to 1.81 ΔDO /g DM in the leaves treated with 5 mM MeJA, i.e., a 36.04% decrease in phenol degradation activity. Peroxidase (POD) activity also decreased from 2.5 ΔDO /g DM in control leaves to 0.25 ΔDO /g DM in the leaves sprayed with 5 mM MeJA, i.e., a decrease in phenol degradation activity of 90%. TAL is, therefore the enzyme that participates more in the phenol degradation activity following the application of 5mM MeJA on cotton leaves.

Discussion

The results obtained show that the phenol content of *Gossypium hirsutum* L. cv. W471B cotton leaves varied significantly according to the concentration of MeJA and the treatment time. Thus, the concentration of 5mM MeJA sprayed for 72h gave the highest levels with 75.75 mg /g DM. These results are in agreement with those of El- Habbak (20013), who showed that the treatment of 5mM MeJA for 72h gave the highest phenol content in grape leaves.

Thus, 5mM MeJA applied for 72 h seems to give the maximum phenols regardless of the plant material used.

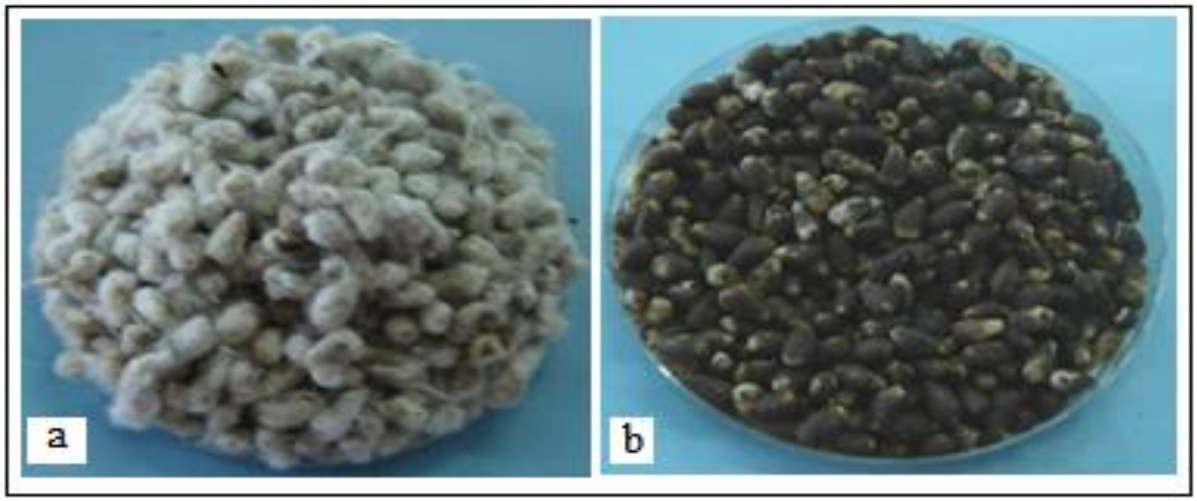

Fig. 1. Cotton seeds a: fibre-bearing seeds, b: seeds with fibres removed (delinted).

The results obtained in the study of the effect of MeJA on the metabolism of phenolic compounds show a significant increase in the phenol content of the order of 83.38% in the treated leaves compared to the controls (untreated). Several research studies have shown the inducing power of MeJA in the synthesis of numerous secondary metabolites *in vitro* or *in vivo* (Al Balkhi, 2008).

Fig. 2. Cotton seedlings were grown under shelter.

However, it should be noted that the phenol contents measured at a given stage are the results of biosynthesis and transformation, in particular by oxidative degradation (Kouakou, 2003). Indeed, some authors have reported a close correlation between an intense activity of biosynthesis enzymes and a high content of total phenols. Similarly, a direct link was established between an increase in the activity of degradation enzymes and a decrease in the level of total phenols in several plants. Thus, our results showed an intense activity of PAL and TAL in treated leaves compared to controls (untreated). These results are in agreement with those of Bouarab *et al.* (2004), who showed that MeJA stimulates PAL and TAL activity in both the gametophyte and sporophyte of the alga C. *Cripus*.

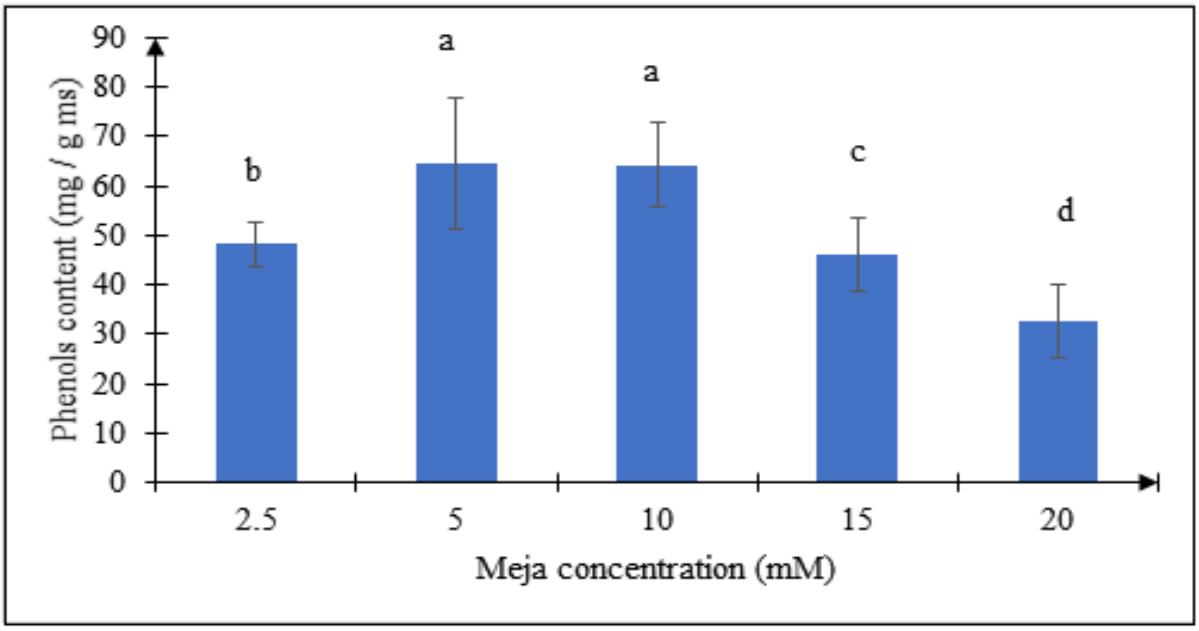

Fig. 3. Total phenol levels in cotton leaves as a function of different concentrations of spray-applied MeJA.

For some authors, the inducing effect of MeJA is often preceded by an activation of the synthesis of enzymes involved in the biosynthesis of various metabolites such as PAL (Choi *et al.*,2005). This is in agreement with our results. Furthermore, our results showed an increase in PAL and TAL activity of about 31.38% and 52.67%, respectively, in MeJA-treated leaves compared to the control (untreated).

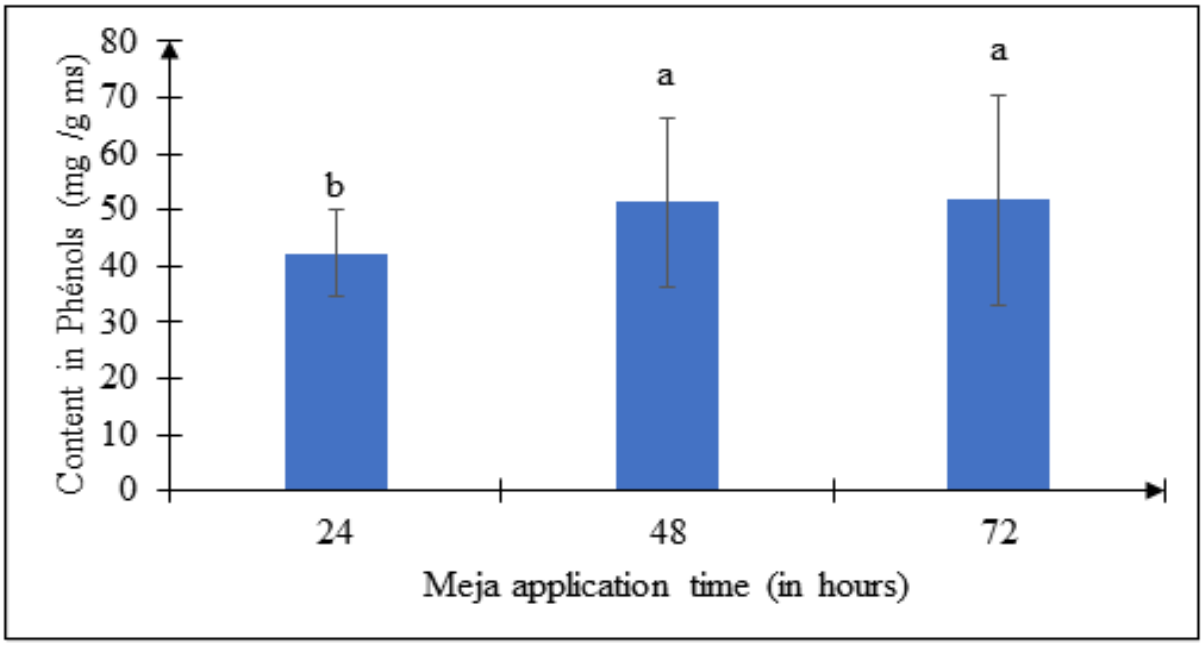

Fig. 4. Total phenol content of cotton leaves as a function of MeJA treatment time applied.

These results suggest that the Tyrosine pathway is more involved than the Phenylalanine pathway in the accumulation of total phenols in cotton leaves following the exogenous (spray) application of MeJA. This preponderance of the TAL pathway has already been demonstrated *in vitro* plants and in FOV-inoculated cotton callus (Konan, 2006). In addition, analysis of phenol degrading enzyme activities shows a decrease in POD and PPO activity in sprayed leaves compared to controls. This decrease in activity is thus

opposite to the increase in total phenol content in the treated leaves. This correlation had already been reported by Al Shamsi *et al.* (2021), who had suggested the increase in the activities of Peroxidases (POD) and Polyphenoloxidases (PPO) coincided with the decrease of phenol contents in date palm roots and inversely. In this activity of degradation of total phenols, our results showed a decrease in the activity of the Peroxidases of the order of 90 % compared to that of the PPOs, which is 36.04 %. This would mean that PPOs would have a higher degradation activity than peroxidases in MeJA sprayed cotton leaves.

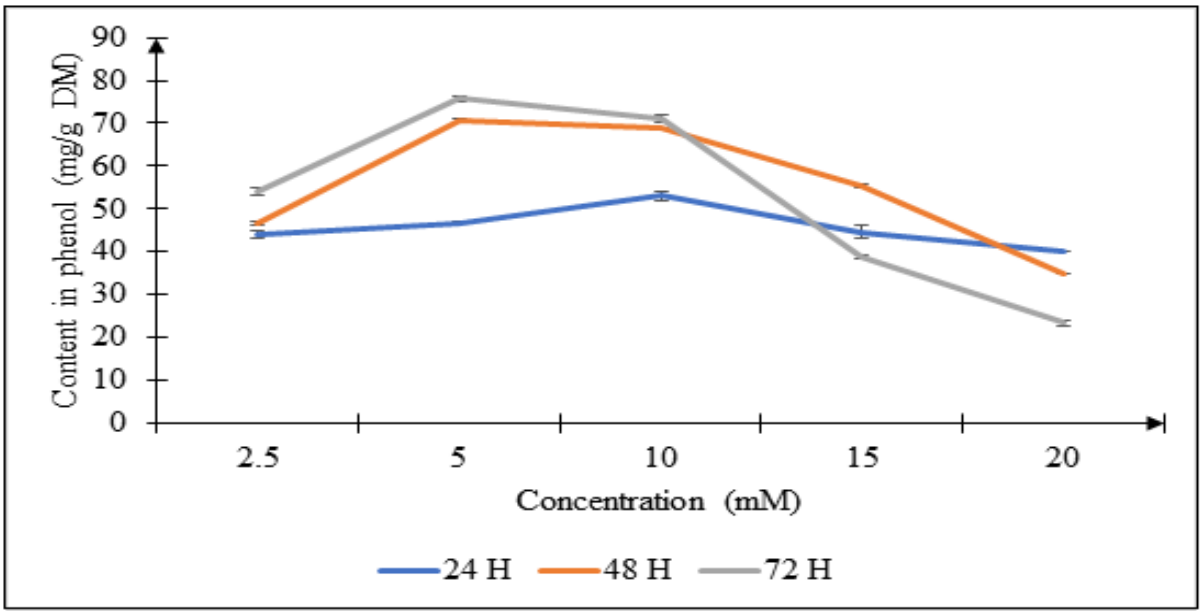

Fig. 5. Total phenol levels in cotton leaves as a function of concentration and MeJA spray time.

All these results could constitute a contribution to the study of the stimulation of natural plant defenses.

Conclusion

The objective of our work is to study the effect of MeJA on the metabolism of phenolic compounds in the leaves of cotton *Gossypium hirsutum* L.Cv. W471 B. At the end of this study, we note that 5 mM and 72 h are respectively the concentration of MeJA and the time of spray treatment for which the content of total phenols is the highest. On the other hand, the analysis of the metabolism of phenolic compounds revealed an increase in phenol content compared to the control. This would be the result of the activities of biosynthetic enzymes (PAL and TAL increased in treated leaves compared to the control) and the activities of degradation enzymes (PPO and POD) decreased in treated leaves compared to the control). This study also indicated that the tyrosine pathway is more involved than the phenylalanine pathway in the accumulation of total phenols.

References

Al Balkhi M. 2008. Exploration phytochimique de la voie de biosynthèse des alcaloides tropaniques chez les racines transgéniques de Datura innoxia Mill. Thèse de Doctorat, Université de Picardie, Jules Verne.

Al Shamsi SR, Rabert GA, Kurup SS, Alyafei MAM, Jaleel A. 2021. Biochemical Changes and Antioxidant Variations in Date Palm (*Phoenix dactylifera* L.) Varieties during Flower Induction and Development. Plants **10(11),** 2550.

Anonyme. 2013. Association des Industrie de la Filière Oléagineuse (AIFO). www.aifo-uemoa.org

Bajguz A, Hayat S. 2009. Effects of brassinosteroids on the plant responses to environmental stresses. Plant physiology and biochemistry **47(1),** 1-8.

Bouarab K, Adas F, Gaquerel E, Kloareg B, Salaun JP, Potin P. 2004. The innate immunity of a marine red alga involves oxylipins from both the eicosanoid and octadecanoid pathways. Plant Physiology **135(3),** 1838-1848.

Cano W. 2011. Novas determinações sobre as questões regionale urbana após 1980. Revista Brasileira de Estudos Urbanos e Regionais (RBEUR), **13(2),** 27-53. https://doi.org/10.22296/2317-1529.2011v13n2p27

Choi DW, Jung J, Ha YI, Park HW, In DS, Chung HJ, Liu JR. 2005. Analysis of transcripts in methyl jasmonate-treated ginseng hairy roots to identify genes involved in the biosynthesis of ginsenosides and other secondary metabolites. Plant Cell Reports **23(8),** 557-566.

Constabel CP, Bergey DR, Ryan CA. 1995. Systemic activates synthesis of wound inducible tomato leaf polyphénols oxidase via octadecanoid defence signalling pathway. Proceedings of the National Academy of Sciences **92,** 407-411, 275-283.

Elad Y, Cytryn E, Harel YM, Lew B, Graber ER. 2011. The biochar effect: plant resistance to biotic stresses. Phytopathologia Mediterranea **50(3),** 335-349.

El-Habbak M. 2013. Overexpression/silencing of selected soybean genes alters resistance to pathogens. University of Kentucky.

FAO. Food and Agriculture Organisation. 1991. Production Year Book **44,** 184.

Heil M, Ton J. 2008. Distance Signalling en plant defense trends. Plant Science **13,** 264-72.

Kanzan K, Manners J. 2008. Jasmenale Signaling, toward an integrated view. Plant physiology **146,** 1459-68.

Konan YK. 2006. Métabolisme des composés phénoliques au cours de l'infection des vitroplants et des cals de cotonnier (Gossypium hirsutum L. cv. R405-2000) par Fusarium oxysporium f. sp. Vasinfectum, agent causal de la fusariose. Mémoire de DEA, Université de Cocody, Abidjan, Côte d'Ivoire. 47 p.

Kouakou TH. 2003. Contribution à l'étude de l'embryogenese somatique chez le cotonnier (*Gossypium hirsutum* L.): Evolution de quelques parametres biochimiques au cours de la callogen,èse et de cultures de suspensions cellulaires. Thèse doctorat troisième cycle. Physiologie végétale. UFR Biosciences .Université de Cocody (Abidjan Côte d'Ivoire). p 147.

Makoi JH, Ndakidemi PA. 2007. Biological, ecological and agronomic significance of plant phenolic compounds in rhizosphere of the symbiotic legumes. African Journal of Biotechnology **6(12).**

Munro JM. 1987. Cotton Longman and Technical, England, p 436.

Ngalani JA, Signoret A, Crouzet J. 1993. Partial purification and properties of plantain polyphenol oxidase. Food Chemistry **48(4),** 341-347.

Pettigrew WT. 2008. Potassium influences on yield and quality production for maize, wheat, soybean and cotton. Physiologia plantarum **133(4),** 670-681.

Pontier D, Balagué C, Roby D. 1998. The hypersensitive response. A programmed cell death associated with plant resistance. Comptes Rendus de l'Académie des Sciences-Series III-Sciences de la Vie, **321(9),** 721-734.

Rachuonyo HA, Pond WG, McGlone JJ. 2002. Effects of stocking rate and crude protein intake during gestation on ground cover, soil-nitrate concentration, and sow and litter performance in an outdoor swine production system. Journal of animal science **80(6),** 1451-1461.

Regnier T. 1994. Composés phénoliques du blé dur (Triticum turgidum L. var durum); Variation au cours du développement et de la maturation du grain relation avec l'apparition de la moucheture. Thèse de sciences et technique de langue doc, base de la production végétale, Univ. Montpellier II, Fr., p 177.

Rehman F, Khan FA, Badruddin SMA. 2012. Role of phenolics in plant defense against insect herbivory. In Chemistry of phytopotentials: health, energy and environmental perspectives (p 309-313). Springer, Berlin, Heidelberg.

Santimone M. 1973. Mécanismes des réactions d'oxydation péroxydasique. Thèse d'Etat, Université d'AIX Marseille II. N° AO. 8321.

Shahrajabian MH, Sun W, Cheng Q. 2020. Considering white gold, cotton, for its fiber, seed oil, traditional and modern health benefits.

Singh N, Singh RK, Bhunia AK, Stroshine RL. 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing Escherichia coli O157: H7 on lettuce and baby carrots. LWT-Food Science and Technology **35(8),** 720-729.

Singleton VL, Orthofer R, Lamuela-Raventós RM. 1999. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology **299,** 152-178.

Stoddard FL, Nicholas AH, Rubiales D, Thomas J, Villegas-Fernández AM. 2010. Integrated pest management in faba bean. Field crops research **115(3),** 308-318.

Vaissayre M. 1994. Dix années d'expérimentation pour la protection du cotonnier en Côte d'Ivoire (1981-1990). Doc. CIRAD/CA **3(93),** 1-57.

Vallad GE, Goodman RM. 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. Crop science **44(6),** 1920-1934.

Stoddard FL, Nicholas AH, Rubiales D, Thomas J, Villegas-Fernández AM. 2010. Integrated pest management in faba bean. Field crops research **115(3),** 308-318.