EFFECT OF THE OLIGOSACCHARID FRACTION OF *FUSARIUM OXYSPORUM* f. sp. *VASINFECTUM* ON THE ACTIVITY OF PHENOLIC METABOLISM ENZYMES IN COTTON LEAVES

ABSTRACT

The synthesis of secondary metabolites, notably phenolic compounds, is part of the plant's defense reactions. The enzymes of phenolic metabolism can constitute a parameter of measurement of the defense of the plants. The aim of this work is to evaluate the activity of phenolic metabolism enzymes and an antioxidant enzyme after treatment of cotton plants with a fungal elicitor. Cotton plants obtained from the cultivar Y764AG3 were treated with the oligosaccharide fraction (FOS) of *Fusarium oxysporum* f. sp. *Vasinfectum* (FOV). The extraction of the enzymes is carried out cold by grinding the leaves in extraction buffer in the presence of PVP (0.5%) and 0.1 M sodium phosphate buffer. The supernatant obtained after centrifugation of the filtrate was purified with Dowex-2 in order to obtain the purified enzymatic fraction. This fraction was used for the determination of enzyme activity by reading the absorbances. The results showed that the activity of ammonia-lyase activity was higher in the treated plants than in the control. This reflects an increase in the biosynthesis of phenolic compounds in the treated plants. On the other hand, oxidoreductase activity was higher in control plants than in treated plants. This indicates a decrease in the degradation of phenolic compounds in the treated plants. This study revealed the existence of an active phenolic metabolism in the FOS-treated plants. Thus defence mechanisms are activated in plants treated with the oligosaccharide fraction of *Fusarium oxysporum* f. sp. *Vasinfectum*.

Key words: Cotton plant, enzymes,*Fusarium oxysporium* f. sp. *Vasinfectum*, oligosaccharide fraction,phenolic metabolisms.

1. INTRODUCTION

The cotton plant is grown because of its fibres and its seeds. Its fibres are mainly used in the textile industry and its seeds are used in human and animal nutrition [1]. However, the production and quality of cotton fibres and seeds are affected by various diseases [2] In West Africa, and particularly in Côte d'Ivoire, cotton diseases are one of the main causes of production losses, estimated at between 15 and 25%. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *Vasinfectum* is the most damaging disease in cotton crops. The incidence of parasitism on the cotton crop in tropical climates is such that chemical control is the dominant control strategy. Unfortunately, pesticides are increasingly being singled out for toxicity, environmental pollution, health and even biodiversity destruction [3].

Many studies have shown that the application of elicitor to a plant activates defence reactions and also leads to an increase in its resistance to pathogens [4]. Elicitors are generally molecules secreted by microorganisms, derived from the cell wall of the fungus, bacterium and/or host plant [5]. Other molecules classified as elicitors are also capable of initiating a defence response in the host plant. These molecules are

mostly oligosaccharides released by the pathogen or plant cells [6]. Studies have shown the action of oligosaccharides in the case of plant/fungus interaction. They are derived from the degradation of polysaccharides in the fungal wall or are secreted by the fungus and recognised on the surface by receptors that activate the plant's defence systems [7]. Furthermore, some authors have shown that the oligosaccharide fraction of fungal origin rich in reducing sugars was able to induce defence reactions [8]. Studies have shown that among the defence reactions of plants is the synthesis of secondary metabolites, especially phenolic compounds [4]. Thus Konan *et al.*[9] and N'goran *et al.*[10] have shown the production of a large number of phenolic compounds that are crucial for disease resistance in cotton. Indeed, during the cotton-fusarium interaction, the level of phenolic compounds increases in cotton [9]. Furthermore, Vessere [11] noted that the degradation or oxidation product of phenols has a bactericidal action. While Nicholson and Hammerschidt [12] showed their antifungal action. Phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL), the main precursors of many phenolic compounds, would possess fungal activity [13]. Similarly, several authors have reported activation of phenolic biosynthetic enzymes under the effect of MeJA and summerphon in cotton [14, 15]. The work of EL Bellaj and EL Hadrami [16] also showed that peroxidase activity in resistant plants increases during pathogen attack, in order to fight against their invasion. Furthermore, the activity of polyphenoloxidases increases during fungal attacks in plants [17]. Therefore, these enzymes may constitute a parameter for measuring plant defence. Hence the aim of this work which is to evaluate the activity of phenolic metabolism enzymes and an antioxidant enzyme after treatment of cotton plants with a fungal elicitor.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant material

The plant material consisted of cotton plants obtained from the seeds of cotton (*Gossypium hirsutum* L.), cultivar Y764AG3. The seeds, originating from Côte d'Ivoire, were supplied by the Centre National de Recherche Agronomique (CNRA). This variety is very susceptible to fusarium.

2.1.2. Fungal material

The fungal material is *Fusarium oxysporum* f. sp. *Vasinfectum* (FOV) strain 14. The FOV strain was provided by the Phytopathology Laboratory of the Institut National Polytechnique Houphouët-Boigny (INPHB) in Yamoussoukro (Côte d'Ivoire). The strain was characterised and collected at the Centraal-bureau voor Schimmelcultures (CBS) in the Netherlands. Strain 14, which is less virulent, is collected under the number 116623.

2.2. Methods

2.2.1. Cotton plant production

Pure sulfuric acidis used to delint cotton seeds. Mature and healthy seeds were sterilised by quick soak (1 min) in 70 % ethanol followed by 20 min immersion in 3.6%sodium hypochlorite. The seeds were rinsed three times successively with sterile distilled water for 5 min. The seeds were sown in pots containing soil autoclaved at 121°C for 30 min under a pressure of 1 bar. They are germinated under a shelter covered with transparent plastic film for two months.

2.2.2. Preparation of the potentially eliciting fungal fraction

After cultivation of FOV on PDA medium, the spores are suspended in sterile distilled water containing tween 20. Aliquots of this spore suspension are added to the modified Czapek-Dox liquid medium which has been autoclaved at 121 °C for 30 min under a pressure of 1 bar. The final concentration is approximately $2{,}5.10^4$spores/ml. The cultures are placed at 21 °C, in the dark and without agitation for 28 days [18]. The spore suspensions were shaken and then filtered on sterile Wattman paper. The filtrate obtained constituted the exocellular oligosaccharide fraction (FOS). This fraction was sterilised in an autoclave at 121°C for 30 min. After sterilisation, it constituted the fungal filtrate or FOV oligosaccharide fraction.

2.2.3. Treatment of cotton plants

Two-month-old cotton plants were treated with 10 ml of the 10% oligosaccharide fraction containing 0.1 ml of triton X-100 (0.1%). According to N'goran et al. (2014) [8], the 10% oligosaccharide fraction is the one that strongly stimulates the production of phenolic compounds involved in the defence of plants against pathogens. Plants are treated at the root (PTR), on the leaves (PTF) and simultaneously at the root and on the leaves (PTRF). A batch of plants untreated (PNT) with the oligosaccharide fraction is made. For each treatment, 12 cotton plants were used and then exposed under a shelter covered with transparent plastic film in a completely randomised manner. The experiment was repeated three times. The plants were watered according to the humidity of the substrate.

2.2.4. Enzyme extraction

Enzyme extractions were performed according to the method described by Kouakou *et al.*[19]. Enzymes were extracted cold (4 °C) by grinding 0.5 g of leaves in 1.2 ml of extraction buffer in the presence of PVP (0.5%) and 0.1 M sodium phosphate buffer. The extraction buffer consists of 0.5 ml polyethylene glycol 6000 (PEG 6000), 0.25% sodium thiosulphate, 15% glycerol, 1 mM EDTA and 15 Mm mercaptoethanol. The filtrate was centrifuged at 5000 rpm for 20 min. The resulting supernatant constituting the crude enzyme extract is stored at -25 °C. In order to get rid of inhibiting ions, Dowex-2 was added to the crude enzyme extract and the mixture was incubated for 30 min at 4°C. The supernatant was collected and centrifuged under the same conditions as above. The resulting supernatant constituted the purified enzyme fraction and was used to determine enzyme activities.

2.2.5. Determination of enzymatic activities

For this study, the absorbances were read using a Mitton Roy spectrophotometer (model Spectronic 601). The activity of the phenolic compounds biosynthesis enzymes (phenylalanine ammonia-lyase and tyrosine ammonia-lyase) on the one hand and the phenolic compounds degradation enzymes (peroxidases, polyphenoloxidases) on the other hand are measured.

2.2.5.1. Phenylalanine ammonia-lyase

The determination of phenylalanine ammonia-lyase (PAL) is carried out using the method described by Régnier [20] modified and adapted to our plant material.

The reaction medium is composed of 1.9 ml of 0.2 M sodium borate buffer at Ph 8.8; 1 ml of 0.1 M phenylalanine and 0.1 ml of enzyme extract. After 10 min of incubation at room temperature, the activity of PAL, which is proportional to the amount of cinnamic acid formed, is monitored by spectrophotometer at a

wavelength of 290 nm. PAL activity is expressed as enzyme activity per gram of fresh material (AE/g of MF), considering that the molar extinction coefficient of the cinnamic acid formed is equal to 19600 M^{-1} cm^{-1}.

2.2.5.2. Tyrosine ammonia-lyase

The determination of tyrosine ammonia-lyase (TAL) is carried out using the method described by Régnier [20] modified and adapted to our plant material.

The reaction medium is composed of 1.9 ml of 0.2 M sodium borate buffer at pH 8.8; 1 ml of 0.1 M tyrosine and 0.1 ml of enzyme extract. After 10 min of incubation at room temperature, the activity of TAL, which is proportional to the amount of pcoumaric acid formed, is monitored by spectrophotometer at a wavelength of 333 nm. The activity of TAL is expressed as AE/g of MF, considering that the molar extinction coefficient of the formed p-coumaric acid is equal to 17100 M^{-1} cm^{-1}.

2.2.5.3. Peroxidases

Peroxidase (POD) activity is determined according to the technique described by Blancas et al. [21] The base buffer used is 0.1 M sodium phosphate at pH 6. The reaction mixture consisted of 1.8 ml of buffer, 1 ml of 25 mM guaiacol and 0.1 ml of enzyme extract. The reaction was started with the addition of 0.1 ml of hydrogen peroxide (H_2O_2). The mixture was incubated for 3 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. The oxidation of guaiacol is monitored by spectrophotometer at a wavelength of 470 nm. POD activity is expressed as AE/g of MF. The molar extinction coefficient of the product formed at the wavelength of 470 nm is equal to 26.6.106 $M^{-1}cm^{-1}$.

2.2.5.4. Polyphenoloxidases

The determination of Polyphenoloxidase (PPO) activity is performed according to the method described by Coseteng and Lee [22], modified and adapted to our plant material. The reaction medium, incubated for 10 min at room temperature, consists of 2.95 ml of 10 mM catechol in 0.1 M sodium phosphate buffer pH 6.5 and 50 µl of enzyme solution. The formation of quinones is monitored by spectrophotometer at 420 nm. The PPO activity is expressed as AE/g of MF, assuming that the molar extinction coefficient of the product formed is equal to 1400 $M^{-1}cm^{-1}$.

2.2.6. Evaluation of enzymatic activities

The rate of participation of each enzyme in the metabolism of phenolic compounds is evaluated and defined as follows:

$$RP = \frac{EA}{EAg} \times 100$$

RP = rate of participation of enzymes in the metabolism of phenolic compounds

EA = enzyme activity

EAg = sum of group enzymatic activities

2.2.7. Evaluation of the activity of an antioxidant enzyme : catalase

The activity of catalases is determined according to the method of Zhou et al [23] modified and adapted to our plant material. The determination of catalase activity is performed with 3 ml of reaction volume. It is composed of 0.1 ml of enzyme extract, 1 ml of hydrogen peroxide and 1.9 ml of Tris-HCl buffer. A control test was carried out in which the hydrogen peroxide was replaced by the Tris-HCl buffer. Catalase activity is monitored by spectrophotometer at 240 nm and expressed as AE/g of MF. The molar extinction coefficient of the product formed at the wavelength of 240 nm is equal to 36.10^{-6} $M^{-1}.cm^{-1}$.

2.2.8. Statistical analyses

The experimental results obtained are analyzed using Statistica software version 6.0. An analysis of variance (ANOVA) is performed to determine differences significant. The 5% Duncan test is used for comparison of means. For the percentages, the Kruskal-Wallis test at the 5% threshold is used to determine the significant differences between treatments.

3. RESULTS

3.1. Activities of ammonia-lyases

The results showed that the ammonia-lyases have the same evolution (fig. 1), that is to say an increase in their activity compared to the control whatever the treatment. However, the activity of phenylalanine ammonia-lyase (PAL) is significantly greater than that of tyrosine ammonia-lyase (TAL) whatever the treatment mode applied to the cotton plants.

PAL activity of 167.77 AE/g of MF in the control changed slightly after application of the oligosaccharide fraction (FOS) on cotton roots (PTR) to 189.28 AE/g of MF. However, there was no significant difference between PAL activity in the control and the RTPs. This activity increases strongly in the plants treated at leaf level (PTF) (272.07 AE/g of MF) and in the plants treated simultaneously at root and leaf level (PTRF) (284.01 AE/g of MF). There was no significant difference between these two types of treatment (PTF and PTRF) in PAL activity.

Concerning tyrosine ammonia-lyase (TAL) activity, the application of FOS strongly increases its activity. Thus, from 102.57 AE/g of MF in the control, TAL activity increased to 154.45 AE/g of MF in PTR, 206.64 AE/g of MF in PTF and 218.90 AE/g of MF in PTRF. From the control to the TFPs, there is a significant difference between the TAL activities. On the other hand, the TAL activities of PTF and PTRF are not significantly different. The increase in ammonia-lyase activity reflects an increase in the biosynthesis of phenolic compounds in the treated plants.

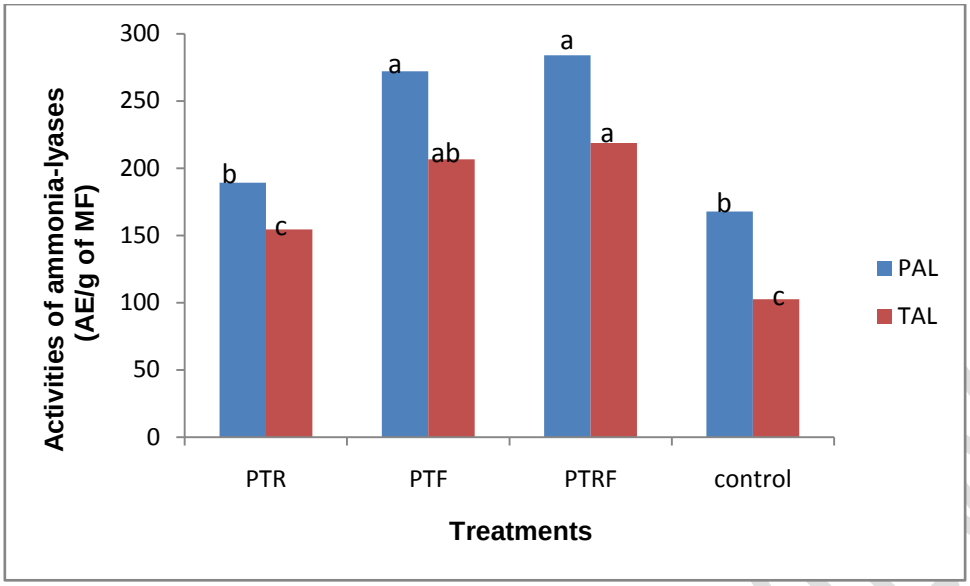

Fig. 1: Evolution of ammonia-lyase activity in leaves of cotton plants treated or not with FOS from FOV FOS, oligosaccharide fraction; FOV, *Fusarium oxysporum* f. sp. *Vasinfectum*; PTR, root-treated plant; PTF, leaf-treated plant; PTRF, root and leaf-treated plant; PAL, phenylalanine ammonia-lyase; TAL, tyrosine ammonia-lyase; means followed by the same letter are not significantly different (Duncan's test at 5%); values represent the mean of three replicates

3.2. Oxidoreductase activities

The activity of oxidoreductases evolves in the same direction with a significantly higher activity of polyphenoloxidases than of peroxidases (fig. 2). On the other hand, the activity of oxidoreductases decreases in plants treated or not by FOS. The activity of polyphenoloxidases (PPO) in control cotton leaves of 3.04 AE/g of MF decreased strongly in cotton plants after FOS application.

Thus, PPO activity increased to 2.39 AE/g of MF in root-treated plants (PTR), 2.26 AE/g of MF in leaf-treated plants (PTF) and 1.71 AE/g of MF in root and leaf-treated plants (PTRF). There is a significant difference between the PPO activities of each treatment and the control.

The activity of peroxidases (POD) shows a similar pattern to that of PPO (figure 2). Thus, it is high in control plants (1.61 AE/g of MF) followed by PTR plants (1.34 AE/g of MF), PTF plants (0.65 AE/g of MF) and PTRF plants (0.39 AE/g of MF). There was also a significant difference between the POD activities of each treatment and the control. This decrease in oxidoreductase activity reflects a decrease in the degradation of phenolic compounds in the treated plants.

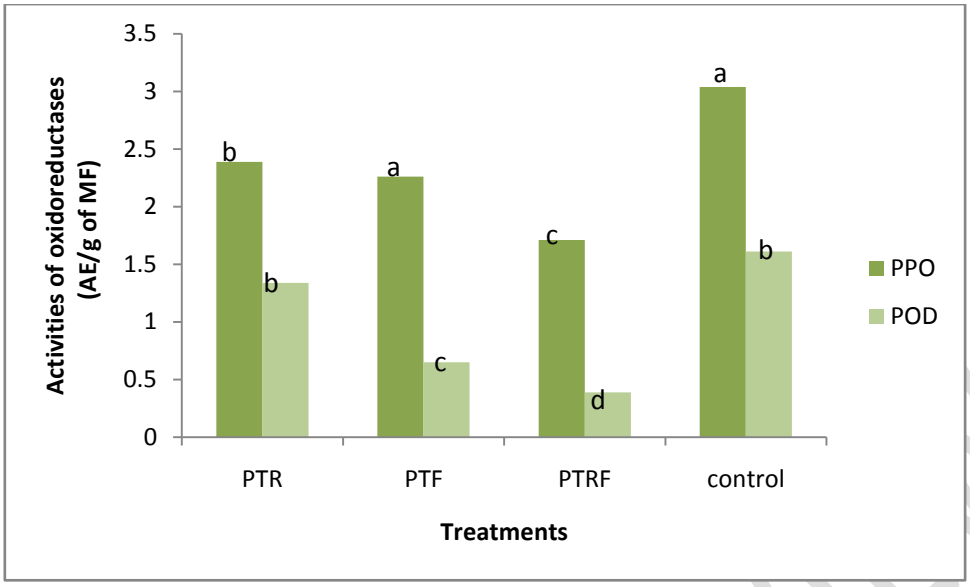

Fig. 2: Evolution of oxidoreductase activity in leaves of cotton plants treated and untreated with FOS from FOV

FOS, oligosaccharide fraction; FOV, *Fusarium oxysporum* f. sp. *Vasinfectum*; PPO, polyphenoloxidase; POD, peroxidase; PTR, root-treated plant; PTF, leaf-treated plant; PTRF, root- and leaf-treated plant; means followed by the same letter are not significantly different (Duncan's test at 5 %); values represent the average of three replicates

3.3. Participation rate of enzymes in phenolic metabolism

The participation rate of phenylalanine ammonia-lyase (PAL) in phenolic biosynthetic activities was 62.05% in control plants and 55.06, 56.83 and 56.47% in root-treated plants (RTP), leaf-treated plants (LTP) and root and leaf-treated plants (RFLP), respectively (Table). There was no significant difference between the PAL participation rates of treated plants. However, there was a significant difference between the LAP participation rate of control and treated plants.

The participation rate of tyrosine ammonia-lyase (TAL) is 37.94 in the control; it shows higher values in PTR, PTF and PTRF with 44.93, 43.16 and 43.52% respectively. The participation rate of TAL in treated plants is not significantly different in any treatment. However, these rates are significantly different with the control.

Concerning oxidoreductases, the participation rate of peroxidases (POD) in phenolic degradation activities is 35.92, 22.36 and 18.57% in PTR, PTF and PTRF respectively, while it is 34.62% in control plants. Furthermore, the participation rate of PODs in the control and PTRs is not significantly different but in comparison to that of PTFs and PTRFs are different. On the other hand, the participation rates of PTF and PTRF are not significantly different from each other. For polyphenoloxidases (PPO),

The participation rate in degradation activities is 64.07%, 77.76% and 81.42% in the RTPs, TFPs and FTRPs, respectively, while it is 65.37% in the control. Furthermore, the participation rates of the control and RTP ODPs are not significantly different but compared to those of the TFPs and FTRPs are different. On the other hand, those of the TFPs and the FTRPs are not significantly different from each other.

Table 1: Participation rate of enzymes in the metabolism of phenolic compounds in cotton plants after application of the FOS of FOV

	Participation rate (%)			
	Control	**PTR**	**PTF**	**PTRF**
PAL	62,05 ± 1,12[d]	55,06 ± 1,02[c]	56,83 ± 1,04[c]	56,47 ± 1,09[c]
TAL	37,94 ± 0,09[b]	44,93 ± 0,07[e]	43,16 ± 0,08[e]	43,52 ± 0,1[c]
PAL / TAL	1,63 ± 0,045[a]	1,28 ± 0,01[a]	1,31 ± 0,01[a]	1,29 ± 0,05[a]
POD	34,62 ± 0,08[c]	35,92 ± 0,09[c]	22,36 ± 0,05[b]	18,57 ± 0,07[b]
PPO	65,37 ± 1,00[d]	64,07 ±1,01[d]	77,76 ± 1,08[e]	81,42 ± 1,09[e]
PPO / POD	1,88 ± 0,03[e]	1,78 ± 0,03[e]	3,47 ± 0,01[a]	4,38 ± 0,01[a]

FOV, *Fusarium oxysporum* f. sp. *Vasinfectum*; FOS, oligosaccharide fraction; PTR, root-treated plant; PTF, leaf-treated plant; PTRF, root and leaf-treated plant; PAL, phenylalanine ammonia-lyase; TAL, tyrosine ammonia-lyase; PPO, polyphenoloxidase; POD, peroxidase; ± S, standard error; within a line, values followed by the same letter are not significantly different (5% Kruskal-Wallis test); Values represent the average of three replicates.

3.4. Evaluation of catalase activity

In the leaves of the control plants, the catalase activity is very low (0.01 AE/g of MF) whereas in the FOS-treated plants, there is a significantly higher catalase activity. It is 0.37, 1.42 and 2.20 AE/g of MF respectively in root-treated plants, leaf-treated plants and root- and leaf-treated plants (Fig. 3). There was also a significant difference between the catalase activities of each treatment and the control. These results reflect an increase in the content of hydrogen peroxide, which is a defence gene inducer, in the treated plants.

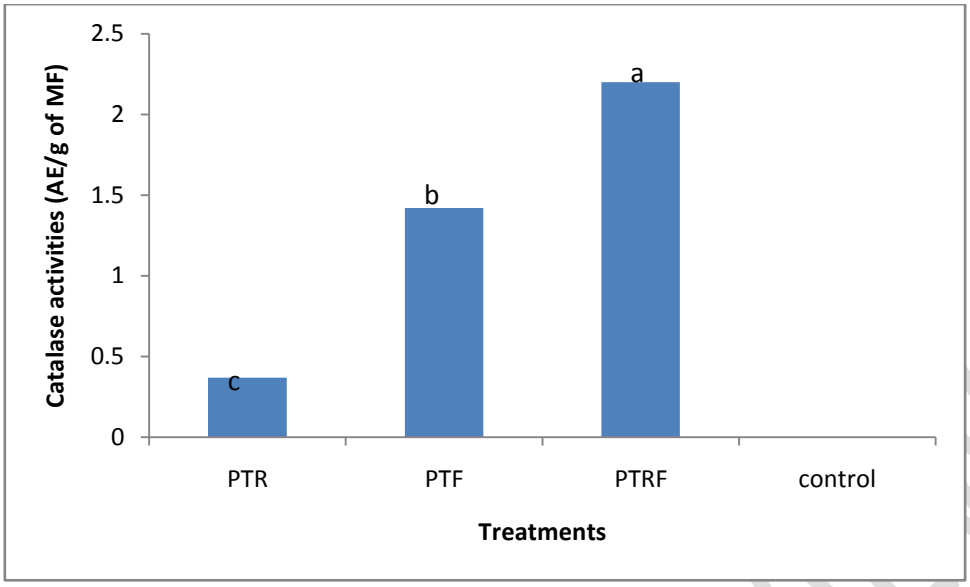

Fig. 3: Catalase activities in leaves of cotton plants treated or not with FOS from FOV
FOS, oligosaccharide fraction; FOV, *Fusarium oxysporum* f. sp. *Vasinfectum*; PTR, root-treated plant; PTF, leaf-treated plant; PTRF, root and leaf-treated plant; means followed by the same letter are not significantly different (Duncan's test at 5%); values represent the mean of three replicates

4. DISCUSSION

The results obtained concerning the activity of the enzyme showed that PAL and TAL have a significant activity in FOS-treated cotton plants compared to controls. As these enzymes are biosynthetic enzymes, their high activity in the treated plants suggests that FOS stimulates the biosynthesis of phenolic compounds. Indeed, N'goran *et al.*[24] showed that FOS from *Fusarium oxysporum* f. sp. *Vasinfectum* (FOV) stimulated the production of phenolic compounds in cotton. Furthermore, our results are similar to those obtained by Navigué et al. [15] using MEJA and ethephon as elicitors in cotton. Similarly, several authors have reported an activation of biosynthetic enzymes under the effect of MeJA in tomato [17]. Moreover, the results obtained showed that the participation of PAL and TAL respectively, in the biosynthesis of phenolic compounds appeared sequentially according to the treatment. In fact, in the FOS-treated plants, although the activity of PAL activity is high compared to that of TAL, the synthesis of new compounds follows the TAL pathway. This is reflected in the increasing rate of TAL participation compared to the decreasing rate of PAL participation. Thus, TAL was the enzyme that participated most in the biosynthesis of phenolic compounds in FOS-treated plants. These results are similar to those obtained by Navigué *et al*. [15] who elicited cotton with MEJA and ethephon.

The biosynthesis of phenolic compounds is therefore directed via tyrosine in treated plants. In contrast, in untreated plants, PAL is the biosynthetic enzyme. Thus, phenylalanine is used for the biosynthesis of phenolic compounds in the control plants. According to Gomez-Vasquez *et al.*[25], this preference for one or the other pathway is due to the availability of the two substrates. The biosynthesis of phenolic compounds via PAL or TAL can be attributed to a simple orientation of metabolism according to the substrate present [26]. The rate of participation of TAL in phenolic syntheses in treated plants is higher than in the control. This result could indicate that the phenolic difference between treated and control plants is related to the involvement of TAL in phenol synthesis. Indeed, N'goran et al. [24] showed that 3-pcoumaric acid represented 218.10, 240.98 and

238.45% of the phenolic pool in root-treated plants, leaf-treated plants and root- and leaf-treated plants by FOV FOS, respectively. This phenolic compound, 3-pcoumaric acid, is synthesised through the tyrosine pathway. In contrast, in control plants, these authors [24] showed a predominance of hydroxycinnamic acid, flavonoids (catechin and gossypetin), resveratrol and pterostilbene. PAL, with a participation rate of 62.05%, was responsible for the synthesis of these compounds in the control plants.

Although the enzymatic activities of biosynthesis play a regulatory role in the accumulation kinetics of phenolic compounds, they are not the only ones. Indeed, the phenol content measured at a given stage is the result of a balance between biosynthesis and, above all, oxidative degradation [27]. In plants, Tognolli *et al.*[28] showed that peroxidase (POD) and polyphenoloxidases (PPO) are involved in these oxidations.

The analysis of the activity of the phenolic degradation enzymes shows a decrease in the activity of POD and PPO in the FOS-treated plants compared to the control plants. This decrease in POD and PPO activities is therefore contrary to the increase in PAL and TAL content, and therefore in phenolic compounds in the treated plants. Navigué *et al*. [15] obtained the same results in cotton after elicitation with MEJA and summerphon. However, Doumbia *et al*. [14] had opposite results. Indeed, they reported that in cotton, treatment with MEJA and ethephon resulted in a decrease in peroxidase activity and an increase in PPO activity.

Concerning the decrease in PPO activity obtained, this result is contrary to the work of Constabel *et al*. [29]who showed that in many species, treatment with methyl jasmonate induced an increase in PPO activity. However, an increase in the rate of participation of PPOs in the degradation of phenolic compounds is observed. Thus, PPOs participate in more than 60% of the degradation of phenolic compounds. According to Ricard-Forget and Gauillard [30], the oxidation of phenols by PPOs leads to the formation of diquinones whose beneficial effect in plant defence has been demonstrated by several authors [16]. Moreover, Nicholson and Hammerschidt [12] have shown that quinones have a remarkable toxicity towards parasites. This intense activity of PPOs in FOS-treated plants suggests a strong increase of diphenols in these plants, as PPO would have a diphenol activity as it has been reported by several authors [30].

The decrease in POD activity, which is contrary to the increase in PAL and TAL content and thus in phenolic compounds in treated plants, is in agreement with those reported by Ziouiti *et al*.[31] Indeed, they showed that the increase of POD activities coincides with the decrease of phenolics contents and vice versa in the date palm root in case of infection. On the other hand, Dai *et al*. [32] indicated a high peroxidase activity in cell walls and intercellular spaces in cotton leaves infected *with Xanthomonas campestris*pv. *Malvacearum*. This increase in peroxidase activity according to Hückelhoven[33] is due to the participation of peroxidases in the production of highly antimicrobial hydroxyl radicals. In this study, the decrease in peroxidase activity in treated plants could indicate a low involvement of peroxidases in the removal of hydrogen peroxide (H_2O_2). Indeed, the main enzymes in H_2O_2 detoxification are catalase and peroxidases [34]. The catalase detoxifies most of the H_2O_2 produced while peroxidases neutralise the H_2O_2 molecules not destroyed by catalase. However, the role of the non-specific POD in H_2O_2 protection is minor [35] and its function is generally less defined as it catalyses the oxidation of phenolic substrates[36] and it may therefore have another physiological function. In contrast to polyphenoloxidases, a decrease in the rate of participation of POD in the degradation of phenolics in treated plants is observed compared to that in control plants. PODs have monophenols as their substrate, so this decrease in POD activity in treated plants implies a decrease in the monophenol content in these plants.

Concerning catalase activity, the results showed an increase in catalase activity in the plants treated with the oligosaccharide fraction (FOS) compared to the control plants. These results are similar to those of [37]. Indeed, they observed an increase in catalase activity in barley following inoculation with an avirulent race of *Blumeria graminis*. Some studies have even shown that water stress could lead to high catalase activities [38]. The increase in catalase activity in treated plants is due to the increase in H_2O_2 content in these plants. Indeed, one of the most rapid biochemical responses identified in elicited plant cells is the production of active forms of oxygen (FAO) such as superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2), which are toxic to the pests but also to the plant [39]. High catalase activity in treated plants means high production of H_2O_2 which is a key signal for the hypersensitive reaction and a defence gene inducer. These results therefore suggest that the application of FOS activates the defence system of the cotton plant. Moreover, according to Tenhaken *et al*.[40], H_2O_2 allows an overexpression of genes coding for phenylalanine ammonia-lyase and chalcone synthase, which could imply an intense biosynthesis of polyphenols, as the results obtained show. There would thus be a positive correlation between phenolic biosynthesis and catalase activity.

5. CONCLUSION

The study of the activity of phenolic biosynthesis and degradation enzymes in FOS-treated cotton leaves revealed the existence of an active phenolic metabolism. Indeed, phenylalanine ammonialyase and tyrosine ammonialyase have a high activity in FOV FOS-treated cotton plants compared to controls. On the other hand, peroxidases and polyphenoloxidases have low activity in FOV FOS treated plants compared to controls. On the other hand, catalase, an antioxidant enzyme, has a higher activity in the treated plants than in the control. This shows a production of active forms of oxygen (FAO) activating the production of defence genes. Thus defence mechanisms are activated in plants treated with the oligosaccharide fraction of *Fusarium oxysporum* f. sp. *Vasinfectum*.

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