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Stimulation of polyphenols production in cell suspensions of cotton (*gossypium hirsutum* l.) By oligosaccharide fraction of fusarium oxysporum f. Sp. Vasinfectum, causal agent of fusarium wilt

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ABSTRACT: Among the natural defense mechanisms that plants develop, we find polyphenols biosynthesis. Thus, in order to increase the natural resistance of cotton to Fusarium oxysporum f. sp. vasinfectum (FOV), we considered possibility of capacities induction of polyphenols production by oligosaccharide fraction (OSF) or fungal filtrate. The use of cell suspensions of cotton has enabled us to demonstrate that the addition of OSF extracted from two different pathogenicities of FOV strains, induce the production of polyphenols. The best polyphenols content were obtained with addition of OSF 10 % in cell suspensions. OSF of FOV14 (low virulent) strain stimulated polyphenols production up to 6.0-fold against 4.0-fold to OSF of FOV 17 (high virulent strain). So, pathogenicity degree of FOV strains is negatively correlated to polyphenols production in cell suspensions of cotton. This study revealed also the existence of a positive correlation between total sugar content in OSF of FOV and polyphenols accumulation in cotton cell suspensions. Thus, the results suggest that natural molecule such as oligosaccharides could be considered as treatment in order to increase the resistance of cotton FOV. This paper chiefly highlights the use of elicitors such as oligosaccharide of fungal origin aiming to limit chemical control in cotton culture.
Key words: cell suspension, cotton, elicitor, Gossypium hirsutum, oligosaccharide, polyphenol.
Abbreviations: OSF, oligosaccharide fraction; FOV, Fusarium oxysporum f. sp. vasinfectum.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is cultivated for its fibers which constitute the main raw material in textile industry (Berti et al., 2006). In West Africa, cotton is regarded as "the white gold" of the economy. . Indeed, it is of considerable economic and social importance, because it does not only provide a livelihood to a substantial part of the population, but it is also a significant source of foreign exchange earnings. However, cotton is susceptible to many diseases and parasites which reduce the production quality of fibers and seeds (Vaissayre, 1994). In West Africa, particularly in Côte d'Ivoire, cotton diseases are usually the main cause of production losses which is estimated between 15 and 25 %. During unfavorable years, a non-riding or mishandled parasitism can cause production losses greater than 50 % and sometimes up to the almost total destruction of potential production (Sayegh, 2009). Fusarium oxysporum f. sp. vasinfectum (FOV), the causal agent of Fusarium wilt causes most damages. The incidence of parasitism is such that chemical control is the dominant strategy of fight. Unfortunately, misuses of pesticides are causing environment pollution, health and biodiversity problems (Leroux et al., 1999; Faurie et al., 2009). Thus, it appears necessary to seek more effective alternatives for the development of a sustainable agriculture. Give plants the means to defend

themselves, or reinforce their defenses, rather than to fight the attacker directly could be a promising solution (Daire et al., 2002; Amari, 2012).

During the interaction cotton-Verticillium or cotton-FOV, the polyphenol content was increased (Xu et al., 2011; 1970; Konan et al., 2014). Resistance mechanisms are induced if plants have recognized the attack by perception of signal molecules or elicitors. The active mechanisms are induced only if plants have recognized the attack by perception of signal molecules or elicitors. Elicitors generally refers to molecules secreted by microorganisms, derived from the cell walls of fungi, bacteria, host plants (Klarzinsky et al., 2000; Korsangruang et al., 2010). Eliciting properties could be attributed to some natural compounds such as salicylic acid, ethylene and methyl jasmonate (Zhao et al., 2004; Faurie et al., 2009). Other molecules, classified as general elicitors, are also able to initiate a defense reaction of the host plant. These are mostly oligosaccharides released by the pathogen (elicitors exogenous) or the plant cell (endogenous elicitors) (Li et al., 2003). These oligosaccharides are divided into four classes: oligoglucanes, oligochitines and oligochitosanes of fungal origin; and oligogalacturonides of vegetable origin. Oligosaccharides are usually composed of residues of compounds whose number is between 4 and 15; and have an eliciting action (Côté et al., 1994; John et al., 1997). Moreover, Fanizza et al. (1995) showed that the elicitor activity may be due to the presence in the culture filtrate of extracellular polysaccharides such as glucanes and rhamno-galacto-mannans. The elicitors' application has caused defensive reactions and increased resistance of many plants to pathogens (Buhot, 2003; Zeneli et al., 2006; Heijari et al., 2008). Furthermore, several studies have already reported the effectiveness of elicitors in plant resistance to pathogens by stimulating the antifungal compounds synthesis like polyphenols (Faurie et al., 2009; Lambert, 2011). Moreover, polyphenols accumulate in adjacent tissues at the necrotic areas, suggesting that these compounds may be defensive (Belhadj, 2005; Ahuja et al., 2012). Their role in plant resistance to fungi was reported by recent studies (Dufour et al., 2013; Yin et al., 2013; Konan et al., 2014).

Cell suspension cultures offer many advantages for secondary metabolites production (Kouakou et al., 2008; 2009). Cells are cultivated under controlled environmental conditions and constitute a homogeneous material. They are at the same physiological stage at some point. Adding substance is easy to perform and make it more efficient absorption. All cells will respond to the contrary where the reaction is limited to a site or a plant tissue. Thus, the molecular events leading to cell response are easier to study. To the secondary metabolism is very active, which facilitates the study of the biosynthesis pathways, including polyphenols.

The aim of this study is the development of an alternative treatment of cotton to chemical control through research for elicitors of natural origin able to induce defense responses that can protect it against FOV. The effect of fungal filtrate or oligosaccharide extract of FOV on polyphenols content using cell suspensions of cotton was investigate.

MATERIALS AND METHODS

PLANT MATERIAL

Seeds of cotton (Gossypium hirsutum L.) were obtained from CNRA (Centre National de Recherche Agronomique, Côte d'Ivoire, West Africa).The cultivar Y764AG3 which has a high sensitivity to Fusarium wilt was used in this study.

In vitro Seed germination

The in vitro seeds germination conditions were those described previously (Kouakou et al., 2007; 2009). Seeds of cotton were delinted with sulphuric acid. Plump and mature seeds were chosen and surface sterilized by dipping in 70 % (v/v) ethanol (1 min) prior to a 20 min exposure to 2.5 % sodium hypochlorite (v/v). After rinsing three times with sterile distilled water for 5 min, sterile seeds dipped and kept in sterile water for one day for coats softening. Sterile seeds were placed on half-strength MS (Murashige and Skoog, 1962) salts with vitamins B5 (Gamborg et al., 1968) medium (MSB), supplemented with 30 g/L sucrose (Sigma Chemical Co.) solidified with 2.5 g/L gelrite (Sigma Chemical Co.) and 0.75 g/L $MgCl_2$ (Sigma Chemical Co.). The seeds were placed in culture tubes and incubated in culture room and seven days after, seedlings were obtained.

Callus and cell suspension cultures

The callus cultures and procedures for maintenance have already been described (Kouakou, 2009; Abeda et al., 2014). Briefly, callogenesis was routinely initiated with hypocotyls of 7-day-old sterile seedlings were cultivated in Petri dishes containing MSB medium including 30 g/L glucose, 0.1 mg/L 2,4-dichlorophenoxyacetic acid (2.4-D), 0.5 mg/L kinetin (KIN) and solidified by 2.5 g/L gelrite plus 0.75 g/L $MgCl_2$ during for weeks. Calli were maintained and stabilized through sub-culturing on the same medium condition for three times with monthly intervals. Friable and well-grown calli were used to initiate cell suspension cultures.

Approximately 2 g of callus were placed into a 250 ml Erlenmeyer flask containing 50 mL of the above medium, without gelling agent. The suspensions were placed on an orbital shaker at 110 rpm during four weeks.

Cell culture conditions

The pH of media was adjusted to 5.8 before autoclaving at 121 °C for 30 min. All cultures were incubated at 28 ± 2°C under a light intensity of approximately 2000 lux. Light was provided by cool white fluorescent lamps with photoperiod (16 h light/8 h dark).

Fungal material

Different strains of Fusarium oxysporum f. sp. vasinfectum (FOV) were provided by the Phytopathology Laboratory of the Superior School of Agronomy of Félix Houphouët-Boigny National Polytechnic Institute, Yamoussoukro-Côte d'Ivoire. FOV strains come from the Centraalbureau voor Schimmelcultures, Baarn-Netherlands. Two FOV strains with different virulence were used:

- FOV strain n° CBS-116623 identified as strain 14 (low virulent) = FOV14
- FOV strain n° CBS 116626 identified as strain 17 (highly virulent) = FOV17

Maintenance and culture of fungal strains

Strains of FOV are kept at 4 °C on PDA medium in test tubes. Approximately, 0.5 cm diameter of agar fragments with mycelia of both FOV strains was taken from test tubes containing inclined potato dextrose agar (PDA) medium in sterile conditions and transferred in Petri dishes containing sterile PDA medium. Each strain was grown at 28 ± 2 °C with a 12 h photoperiod during 14 days to obtain spores (**Konan et al., 2014**). The mycelia fragments taken in Petri dish containing cultures of FOV were crushed under sterile conditions in the presence of 5 mL of sterile distilled water. The mixture was filtered through two layers of sterile cheesecloth to remove mycelia. The filtrate was plated in Petri dishes containing a new PDA medium (1 mL/Petri dish) and incubated under the same conditions as above during seven days. Each Petri dish was flooding with 5 mL of sterile distilled water containing a drop of 0.1 % tween 20. A FOV spore's suspension was prepared dislodging the conidia by gently rubbing the surface of the fungus colony with a Pasteur pipette curved. Conidial concentration was adjusted to 2.5 x 10^4 conidia/mL using a haemocytometer.

Preparation of fungal fractions potentially eliciting

Conidia were placed into Erlenmeyer flask containing Czapek-Dox liquid medium. The FOV spores suspensions were placed on an orbital shaker at 80 rpm during ten days in darkness under a 12h photoperiod at 28 ± 2 °C. Then, cultures are placed in darkness and without agitation for 4 weeks (**Fanizza et al., 1995**). Culture filtrate was collected after mycelium removal by filtration on partial vacuum through a 30 µm nylon mesh and autoclaved 20 min at 121 °C. This stock solution was used as fungal filtrate or oligosaccharide fraction.

Estimation of total soluble sugars

The amount of total soluble sugars was estimated by phenol sulphuric acid reagent method (Dubois et al., 1951). Briefly, 1.0 mL of oligosaccharide fraction was added to 1.0 mL of 5 % phenol solution and mixed. Then 5.0 mL of 96 % sulphuric acid was added rapidly. Each tube was gently agitated during the addition of the acid and then allowed to stand in a water bath at 27-30 °C for 20 min. The OD of the characteristic yellow orange colour thus developed was measured at 490 nm in a spectrophotometer. Simultaneously a standard curve was prepared by using known concentration of glucose. The quantity of sugar was expressed as of mg glucose equivalent.L^{-1} of oligosaccharide fraction.

Addition of FOV filtrates to cotton cell suspensions

In order to test their potential eliciting defense responses in cotton, the both fungal fractions or oligosaccharide fractions are added to cells suspensions where the induction of polyphenols was measured. Six concentrations ranges of fungal fractions were used in this study (2, 5, 10, 20, 50 and 100 %). Fungal fraction or oligosaccharide extract of FOV (OSEF) was dissolved in sterile distilled. Then, 2 mL of each OSEF concentration of both FOV strains was added to cell suspensions. Cells were harvested at 14-days-old cultures by filtration at early stationary phase of growth. The control was received sterile distilled water. Cells were then lyophilized and stored at -20 °C prior to any dosage.

Phenol extraction and quantification in cells

Polyphenols extraction

Polyphenols extraction was performed using the method of Kouakou et al. (2008; 2009). Approximately, 50 mg of freeze-dried cells were dissolved overnight with 10 mL of methanol at 4 °C in a

blender. Sample was centrifuged at 2000 g for 10 min. Supernatant was collected and filtered through a Millipore membrane (0.45 µm) and represents the polyphenols extract.

Polyphenols content

The total polyphenols content (TPC) was determined using Folin-Ciocalteu's reagent according to the method of Siriwoharn et al. (2004). Approximately, Sample extract (0.1 mL) was mixed with 0.9 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent. The mixture added to 1.5 mL of sodium carbonate 17 % was incubated at 25 °C for 20 min in darkness. The absorbance was measured at 765 nm, and the standard curve was prepared using the gallic acid (10–100 µg/mL).TPC was calculated from the calibration plot and expressed as mg gallic acid equivalents/g of freeze-dried cells. The calibration equation for gallic acid was $y = 0.038x + 0.192$, $R^2 = 0.998$, where y is absorbance and x is the concentration of gallic acid in mg/mL. All measures were performed in triplicate.

Statistical Analysis

All Experiments were of complete randomised design and treatments consisted of five replications. All the experiments were performed three times. Treatments were compared to controls by one-way ANOVA using the Duncan test ($P < 0.05$). The statistical analyses were performed with SAS (version 6.0).

RESULTS AND DISCUSSION

To investigate the ability of oligosaccharide fractions (OSF) to trigger responses defenses in cotton, namely the polyphenols production, we looked for a reliable and reproducible in vitro study model which is likely to aptly reflect plant natural reactions (Belhadj et al., 2008; Kouakou et al., 2009). Besides its benefits in the secondary metabolites production, plant cell culture proved to be an excellent tool for plant defense reactions study (Zhao et al., 2001). In general, responses identified in the in vitro cultures can be found in the whole plant. The perception of a pathogen by the plant cell is through the recognition of exogenous elicitors produced by the pathogen, which activate a cascade of signal transduction leading to the establishment of defense reactions such as gene activation defense-related and production of polyphenols (Belhadj et al., 2008; Konan et al., 2014). Studies showed that in the case of interaction plant/fungus, oligosaccharides resulting from the fungal polysaccharides degradation of fungus wall or secreted by the fungus are recognized by receptors on the surface and activate the plant defense systems (Thakur and Sohal, 2013; Verhagen et al., 2010). In vitro experiments have reported that the addition of fungal oligosaccharides in plant cell suspension cultures was capable of inducing characteristic defense reactions and in particular the polyphenols production (Lattanzio et al., 2006; Yamaner et al., 2013). Thus, based on these knowledges, we chose the treatment of cotton cell suspensions by FOV oligosaccharide fraction in the hope that they would be likely to mimic the exogenous elicitors' action on cell suspensions.

Figure 1 shows the results of sugars content analysis of OSF from strains 14 and 17 of FOV. The OSF of FOV 14 has a content of glucose equivalent (45.0 mg/L of oligosaccharide fraction) which is significantly greater than the fraction of FOV 17 (25.0 mg/L of oligosaccharide fraction). The study of the elicitor capacity of OSF resulting these two FOV strains was carried out.

The results presented in Figure 2 reveal that the OSF of FOV are able to increase the polyphenols production in the treated cells compared to control. Accumulation profiles of polyphenols are very different depending on FOV strains. OSF showed little elicitor activity at concentrations of 2 and 5 %, reached an optimum with the concentration of 10 % and then decreases up to concentration of 100 %. Polyphenols production with OSF of FOV14 (185.15 mg/g of cells) is 1.4 times higher than that obtained with OSF of FOV 17. Compared to the control (0 %), the OSF of FOV14 has a polyphenols stimulation factor of 6.0 against 4.0 for OSF of FOV 17. Thus, OSF was able to induce the stimulation of polyphenols accumulation by cotton cell suspensions. FOV14, the less virulent, caused a more significant polyphenols production in cotton cells suspensions than that triggered by virulent strain, FOV 17. These results are in agreement with the work carried out in vivo show that the two strains of Botrytis cinerea (T4 and T8) of different virulence preferentially stimulate the defense response in grapevine (Derkel et al., 1999). The least virulent strain induced more rapidly and with greater intensity accumulation of phytoalexines and PR proteins. Therefore it secretes a greater proportion of elicitor molecules in the filtrate. The elicitor effect of the various fractions could due to the presence in the fractions of composed of polysaccharidic nature be excreted in the culture medium by FOV like revealed it figure 1. Indeed, the results show a correlation between the sugar content of OSF and the elicitor capacity of polyphenols. The fractions of FOV 14 suffer a strong acid hydrolysis can cleave the polysaccharides in more active oligosaccharides compared to the strain FOV 17 (Nita-Lazar et al., 2004). Indeed, the polymerization degree of oligosaccharides is a test that would determine their elicitor effect of defense reactions (Côté and Hahn, 1984). Many studies reported the possibility of using elicitation technique to increase in vitro production of bioactive secondary metabolites by cell suspensions (Belhadj et al., 2008; Faurie et al., 2009; Verhagen et

al., 2010; Yamaner et al., 2013), notably based on the use of fungal elicitors (Dornenburg and Knorr, 1995). Others studies also showed the induction of phenolic phytoalexins by grapevine cells after addition of fungal fractions of Botrytis cinerea (Repka, 2001; Poinsol et al., 2003). High concentrations of FOV fraction (>10 %) showing low accumulation rate of polyphenols seems to induce hypersensitivity reactions leading to cell death, while a minimum concentration is necessary to induction as reported by authors (Mukundan and Hjorsoto, 1990; Roewer et al., 1992). This study revealed the existence of a positive correlation between total sugars content in OSF of FOV and polyphenols accumulation in cotton cell suspensions. In addition, the pathogenicity or virulence degree of FOV strains is negatively correlated to the stimulation of polyphenols production in cell suspensions of cotton.

This study is the first report showing the stimulatory effects of fungal filtrate or OSF on the production of polyphenols in cotton cell suspensions. Our results show that production of polyphenols can be increased by elicitors such as OSF of FOV. Polyphenols increase in response to OSF of FOV demonstrate that these elicitors can be evaluated in control against Fusarium wilt caused by FOV in cotton.

This study is the first report showing the stimulatory effects of fungal filtrate or OSF on the production of polyphenols in cells suspensions of cotton. Our results show that production of polyphenols can be increased by elicitors such as OSF. Polyphenols increase in response to OSF of FOV demonstrate that these elicitors can be evaluated in control against Fusarium wilt caused by FOV in cotton. This type of elicitor can be a significant and promising alternative to chemical control. Indeed, the ease of obtaining and preparing of the fungal filtrate is a major asset for its use by farmers. However, experiments in fields must be realized to consolidate these results. Their introduction into agricultural practice could minimize the scope of chemical control, thus contributing to the development of sustainable agriculture.

REFERENCES

Abeda ZH, Kouassi KM, Yapo KD, Koffi E, Sié RS, Koné M, Kouakou TH. 2014. Production and Enhancement of Anthocyanin in Callus line of Roselle (Hibiscus sabdariffa L.). Int J Rec Biotechnol 2 (1):45-56.

Adrian M, Jeandet P, Bessis R, Joubert JM. 1996. Induction of phytoalexin (resveratrol) synthesis in grapevine leave treated with aluminium chloride ($AlCl_3$). J Agric Food Chem 44 (8) : 1979-1981.

Belhadj A, Telef N, Saigne C, Cluzet S, Barrieu F, Hamdi S. 2008 b. Effect of methyl jasmonate in combination with carbohydrates on gene expression of PR proteins, stilbene and anthocyanin accumulation in grapevine cell cultures. Plant Physiol Biochem 46(4): 493-499.

Belhadj A. 2005. Stimulation des défenses naturelles de la vigne par le méthyl jasmonate: Impact sur la biosynthèse des polyphénols et sur la résistance aux champignons. Thèse de doctorat, Université de Bordeaux 2, France.

Bell AA, Stipanovic RD. 1978. Biochemistry of disease and pest resistance in cotton. Mycopathologia 65: 91-106.

Benedict JH, Altman DW. 2001. Commercialization of transgenic cotton expressing insecticidal crystal protein. In: Jenkins JN, Saha S (ed) Genetic improvement of cotton. USDA- ARS. Oxford & IBH, New Delhi.

Bouarab K, Potin P, Correa J, Kloareg B. 1999. Sulfated oligosaccharides mediate the interaction between a marine red alga and its green algal pathogenic endophyte. Plant Cell 11 : 1635-1650.

Buhot N. 2003. Rôle des élicitines et des protéines de transfert de lipides dans l'induction de la résistance des plantes à leurs agents pathogènes. Thèse de doctorat, Université de Bourgogne, Dijon, France.

Côté F, Hahn MG. 1994. Oligossaccharins : structures and signal transduction. Plant Mol Biol 26: 1379-1411.

Dornenburg H, Knorr D. 1995. Strategies for the improvement of secondary metabolite production cell cultures. Enzyme Microb Technol 17: 674-684.

Dubios MK, Gilles JK, Robers PA, Smith F. 1951. Calorimetric determination of sugar and related substance. Anal Chem 26 : 351-356.

Ebel J, Cosio EG. 1994. Elicitors of plant defense responses. International J Rev Cytol 148: 1-36.

Fanizza G, Bissignano V, Pollastro S, Miazzi M, Faretra F. 1995. Effects of polysaccharides from Botryotinia fulkeliana (Botrytis cinerea) on in Vitro culture of table and wine grapes (Vitis vinifera). Vitis 34: 41-44.

Jeandet P, Bessis R, Sbaghi M, Meunier P. 1995. Production of the phytoalexin resveratrol by grapes as a response to Botrytis attack under natural conditions. J Phytopathol 143: 135-139.

John M, Rohrig H, Schmidt J, Walden R, Schell J. 1997. Cell signalling by oligosaccharides. Trends Plant Sci 2(3): 111-115.

Kamoen O. 1984. Secretions from Botrytis cinerea as elicitors of necrosis and defence. Rev Cytol BiolVég Bot 7: 241-248.

Klarzinsky O, Plesse B, Joubert JM, Yvin JC, Kopp M, Kloareg B, Fritig B. 2000. Linear beta-1,3glucans are elicitors of defense responses in tobacco. Plant physiol 124(3): 1027-1038.

Konan YKF, Kouassi KM, Kouakou KL, Koffi E, Kouassi KN, Sékou D, Koné M, Kouakou TH. 2014. Effect of Methyl jasmonate on phytoalexins biosynthesis and induced disease resistance to Fusarium oxysporum f. sp. vasinfectum in Cotton (Gossypium hirsutum L.). Int J Agron 2014: Article ID 806439, 11 pages. http://dx.doi.org/10.1155/2014/806439.

Korsangruang S, Soonthornchareonnon N, Chintapakorn Y, Saralamp P, Prathanturarug S. 2010. Effects of abiotic and biotic elicitors on growth and isoflavonoid accumulation in Pueraria candollei var candollei and P. candollei var mirifica cell suspension cultures. Plant Cell Tiss Org Cult 3: 333-342.

Kouakou TH, Due EA, Kouadio NEJP, Niamke S, Kouadio YJ, Waffo TP, Decendit A, Mérillon JM. 2009. Purification and Characterization of Cell Suspensions Peroxidase from Cotton (Gossypium hirsutum L.). Appl Biochem Biotechnol 157: 575-592.

Kouakou TH, Koné M, Koné D, Kouadio YJ, Amani NG, Waffo TP, Decendit A, Mérillon JM. 2008. Trans-resveratrol as phenolic indicator of somatic embryogenesis induction in cotton (Gossypium hirsutum L.) cell suspensions. Afr J Biochem Res 2: 15-23.

Kouakou TH, Kouadio YJ, Waffo TP, Valls J, Badoc A, Decendit A, Mérillon JM. 2009. Polyphenol levels in cotton (Gossypium hirsutum L.) callus cultures. Acta Bot Gallica 152: 223-231.

Kouakou TH, Waffo TP, Kouadio YJ, Valls J, Tristan R, Descendit A, Mérillon JM. 2007. Phenolic compounds and somatic embryogenesis in cotton (Gossypium hirsutum L.). Plant Cell Tiss Org Cult 90: 25-29.

Kouakou TH. 2009. Embryogenèse somatique chez le cotonnier [Gossypium hirsutum L. (malvaceae)] : variation des composés phénoliques au cours de la callogenèse et de la culture des suspensions cellulaires. Thèse d'état, Université d'Abobo-Adjamé, Abidjan-Côte d'Ivoire.

Langcake P, Pryce RJ. 1976. The production of resveratrol by Vitisvinifera and other members of the Vitaceae as a reponse to infection or injury. Physiol Plant Pathol 9: 77-86.
Lattanzio VMT, Cardinali A. 2006. Phytochemistry: Advances in Research. In: Imperato F. (ed) Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Research Signpost, 37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India.
Lepoivre P. 2003. Les mécanismes de résistance et la spécificité parasitaire. In: Editions de Boeck and Larcier (ed) Phytopathologie, Bruxelles, PP. 161-191.
Leroux P, Chapeland F, Desbrosses D, Gredt M. 1999. Patterns of cross-resistance to fungicides in Botryotinia fuckeliana (Botrytis cinerea) isolates from French vineyards. Crop Prot 18: 687-697.
Li MY, Lan WZ, Chen C, Yu LJ. 2003. The effects of Oligosaccharides and spores from Aspergillus niger on the defense responses of Taxus chinensis leaves in vitro. J Phytopathol 151: 540-545.
Liwidowati F, Melchior F, Hohmann F, Schwer B, Kindl H. 1991. Induction of stilbenes synthase by Botrytis cinerea in cultured grapevine cells. Planta 183: 307-314.
Martinez C. 1997. Rôles des peroxydases dans la résistance du cotonnier à la bactériose causée par Xanthomonas campestris Pathovar Malvacearum. Thèse de doctorat, Université de Montpellier 2 (France).
Mukundan U, Hjorsoto MA. 1990. Effect of fungal elicitor on thiophene production in hairy root cultures of Tagetespatula. Appl. Microbiol Biotechnol 33: 145-147.
Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15: 473-497.
Nita-Lazar M, Heyraud A, Gey G, Braccini I, Lienart Y. 2004. Novel oligosaccharides isolated from Fusarium oxysporum L. rapidly induce PAL activity in Rubus cells. Acta Biochim Pol 51(3): 625-634.
Poinssot B, Vandelle E, Bentejac M, Adrian M, Levis C, Brygoo Y, Garin J, Sicillia F, Coutos-Thevenot P, Pugin A. 2003. The endopolygalacturones 1 from Botrytis cinerea activates grapevine defense reactions unrelated to its enzymatic activity. Mol Plant-Microb Inter 16(6): 553-564.
Repka V, Fischerova I, Silharova K. 2001. Methyl jasmonate induces a hypersensitive-like response of grapevine in the absence of avirulent pathogens. Vitis 40(1): 5-10.
Roewer IA, Colutier N, Nessier CL, De Luce V. 1992. Transient induction of tryptophane decarboxylase and strictosidine synthase in cell suspension culture of Catharanthus roseus. Plant Cell Rep, 11: 86-89.
Siriwoharn T, Wrolstad RE, Finn CE, Pereira CB. 2004. Influence of cultivar, maturity, and sampling on blackberry (Rubus L. hybrids) anthocyanins, polyphenolics, and antioxidant properties. J Agric Food Chem 52: 8021-8030.
Soulard-Saigne C. 2005. Stimulation des défenses naturelles de la vigne par des éliciteurs d'origine fongique. Intérêts phytopathologiques des stilbènes. Thèse de doctorat, Université Bordeaux 2 (France).
Thakur M, Sohal BS. 2013. Role of elicitors in inducing resistance in plants against pathogen infection: A Review. ISRN Biochem, 2013: Article ID 762412, 10 pages. http://dx.doi.org/10.1155/2013/762412.
Vaissayre M. 1994. Dix années d'expérimentation pour la protection du cotonnier en Côte d'Ivoire : 1981-1990. Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), 34 - Montpellier (France). Dept. des Cultures Annuelles. CIRAD-CA-DT-94-03, 63p.
Verhagen BWM, Trotel-Aziz P, Couderchet M, Hofte M, Aziz A. 2010. Pseudomonas spp.-induced systemic resistance to Botrytis cinerea is associated with induction and priming of defence responses in grapevine. J Exp Bot 61(1): 249-260.
Wiese MV, Devay JE. 1970. Growth regulator changes in cotton associated with defoliation caused by Verticillium alboatrum. Plant Physiol 45: 304-30.
Yamaner O, Erdag B, Gokbulut C. 2013. Stimulation of the production of hypericins in in vitro seedlings of Hypericum adenotrichum by some biotic elicitors. Turk J Bot 37: 153-159.
Zhao J, Fujita K, Yamada J, Sakai K. 2001. Improved beta-thujaplicin production in Cupressus lusitanica suspension cultures by fungal elicitor and methyl jasmonate. Appl Microbiol Biotechnol 55: 301-305.
Zhu L, Tu L, Liu L, Yuan D, Jin L, Long L, Zhang X. 2011. Lignin metabolism has a central role in the resistance of cotton to the wilt fungus Verticillium dahliae as revealed by RNA-Seq-dependent transcriptional analysis and histochemistry. J Exp Bot 62(15): 5607-5621.

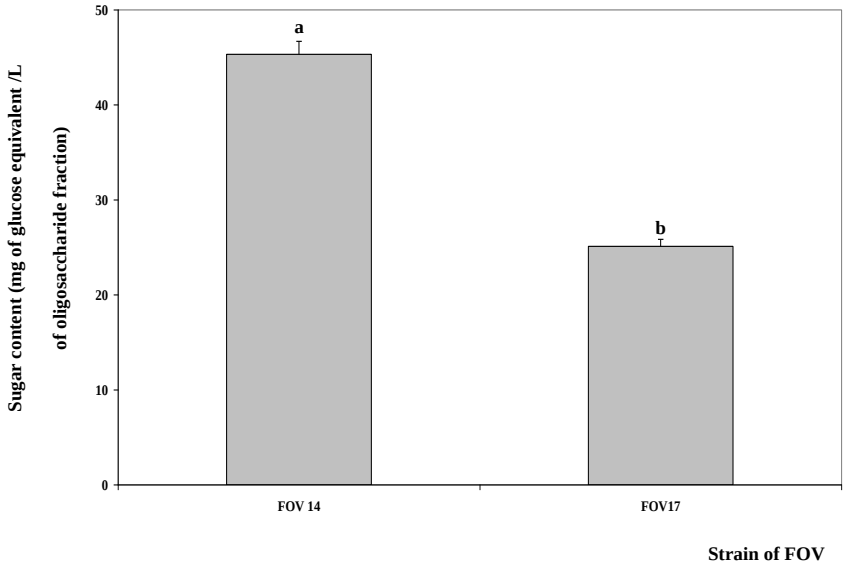

Figure 1. Sugar content in oligosaccharides fraction from two strains of *Fusarium oxysporum* f. sp. vasinfectum

FOV, *Fusarium oxysporum* f. sp. vasinfectum; bar represent standard deviation; values are the mean of three replicates; values followed of a different letter are significantly different (test of Duncan at 5%).

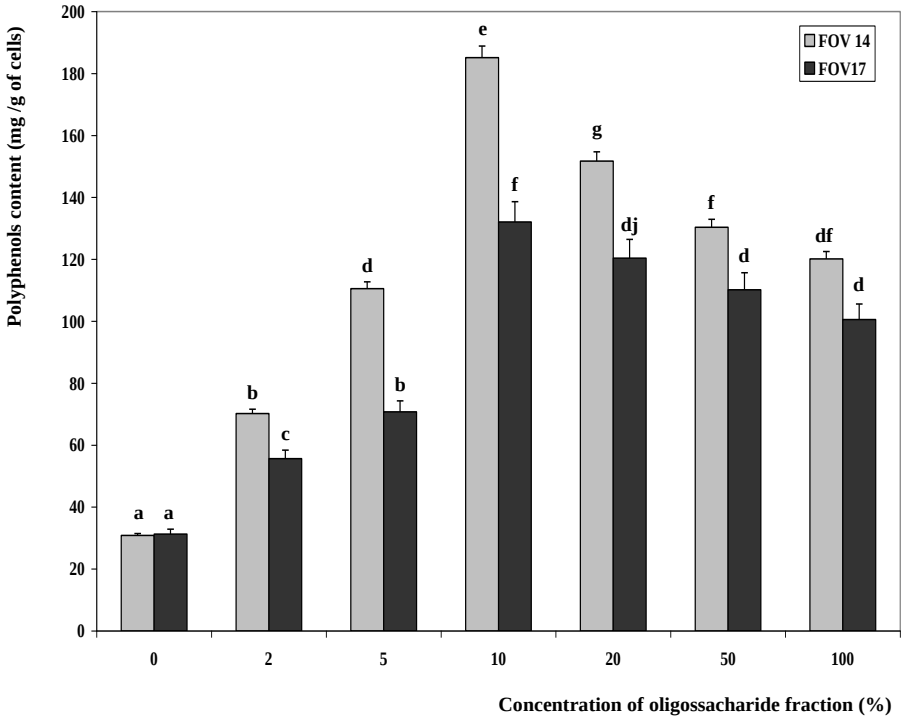

Figure 2. Effect of oligosaccharide fraction from two strains of FOV on polyphenols production in cell suspensions of cotton

FOV, *Fusarium oxysporum* f. sp. vasinfectum; bar represent standard deviation; values are the mean of three replicates; values followed of a different letter are significantly different (test of Duncan at 5%).