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ORIGINAL ARTICLE

Effects of Methyl Jasmonate And Cytokinin on Biochemical Responses Of Maize Seedlings Infected By *Fusarium moniliforme*

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Aim of this study is to explain the role of exogenous application of methyl jasmonate and cytokinin in improvement of Zea mays (cv.KSC 704) resistance against Fusarium moniliforme. Seed treatment with 500 µM methyl jasmonate and cytokinin individually, was found to improve plant defense responses against F. moniliforme. Fusarium inoculation resulted in chlorophyll and protein content reduction and increase in catalase activity and soluble sugar content as compared to control seedlings. Available data suggest that the protective effect of cytokinin and methyl jasmonate was accompanied with increase in chlorophyll and protein content and reduction in catalase activity and soluble sugar content in comparison with infected seedlings with no treatment we propose that methyl jasmonate and cytikinin seed treatment can be used as bio protection agent against the parasite F. moniliforme.

KEYWORDS: *cytokinin, Zea mays, methyl jasmonate, fusarium moniliforme, fumonisin*

INTRODUCTION

Phytohormones may be part of signaling pathways or their presence may stimulate signaling reactions or molecules that are responsible for plants to response stresses. Cytokinins stimulate water uptake and increase cell division. They are responsible for apical dominance, organ formation and regeneration, vascular development, nutrient mobilization, and senescence [1, 2]. It has been implicated that cytokinin is involved in the plant defense signal transduction pathway [3]. There is a close correlation between the content of endogenous cytokinins and the degree of resistance in plants [4].

Jasmonic acid and its methyl ester, methyl jasmonate, are naturally occurring plant growth regulators, which play pervasive roles in several physiological and biochemical processes in plants. The octadecanoic pathway represents the series of metabolic steps through which jasmonates are synthesized following oxidation of linolenic acid. This pathway has long been proposed a part of the signaling cascade that mediates plant defense responses after several insect and pathogen invasions [5, 6]. It was reported that exogenous application of jasmonates induced different physiological responses to various abiotic stresses [7, 8, 9]. It has been found that jasmonate induce the expression of plant-defense genes in response to different pathogen attacks [10]. Exogenous application of methyl jasmonate to Arabidopsis plants reduced disease development after infection by several fungi [11].

It was showed that exogenous application of cytokinin induced resistance against white clover mosaic virus and TNV in *Phaseolus vulgaris* plants [12, 13]. Cytokinin, zeatin, reduced virus replication of potato virus Y (PVY) in infected potato callus [14]. It was reported that cytokinin regulates endogenous levels of JA after wounding [15]. Cytokinin increased Arabidopsis resistance against *Pseudomonas syringae pv. tomato* [16].

It is now known that plant inducible defense pathways are regulated through a complex network of signaling cascades that involve three main molecules: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), enabling the plant to fine-tune its resistance reaction depending on the micro-organism encountered . Induced systemic resistance (ISR)

acts through the JA and ET signaling pathways, but it is independent on SA [17, 18, 19].

Fusarium moniliforme Sheldon is a widespread facultative endophyte, and is one of the most important pathogens of maize (*Zea mays* L.) blight. *F. moniliforme* can be toxigenic, the carcinogenic fumonisin being accumulated predominantly when the fungus colonizes corn plants. The pathogen transmitted both through contaminated seeds and environmental inoculums [20]. In this study we have shown that the exogenous application of the cytokinin and methyl jasmonate to maize (*Zea mays*) led to reduction of fungi infection.

MATERIALS AND METHODS

Cultivation of Plants and Treatments

Seeds of *Zea mays* (var KSC.704) were surface sterilized by using 20-min incubation in 5% (w/v) sodium hypochlorite. After three washes with distilled water, seeds were germinated on moist filter paper for 48 h at 24°C and then transferred to pots containing vermiculate and watered with 1/2 strength Hoagland nutrient solution. Methyl jasmonate and cytokinin were applied at concentration of 500µM at fifth day. The seedlings were grown for 14 days in a greenhouse. Growth conditions were 16 h light (maximum intensity of full sunlight was about 2000 $\mu mol\ m^{-2}\ s^{-1}$) and 8 h dark, temperature of 25/30°C day/night and relative humidity of 70% .The treatments were: (1) nutrient solution alone (Control), (2) 500µM methyl jasmonate, (3) 500µM cytokinin, (4) Fusarium, (5) Fusarium + 500µM cytokinin , (6) Fusarium + 500µM cytokinin .

Inoculums Production and Inoculation

Isolate *Fusarium moniliforme* (F10) originally isolated from infected maize. The isolate was grown on potato dextrose agar (PDA) at 25°C for 7 days. Spores were harvested by adding 5ml distilled water to the petri dish, rubbing the surface with sterile glass rod. The suspension was diluted with distilled water. 10^4 $sporeml^{-1}$ were determined with a hemocytometer [21]. Roots were inoculated by irrigating the spore suspension (concentration of $5\text{-}10\times10^4$ spores ml^{-1}) into vermiculate (10 ml $plant^{-1}$, 5 days after germination) and the infected seedlings were harvested at 14 days post-inoculation and stored at -20°C for subsequent analysis.

Chlorophyll content assay

Chlorophyll content was extracted with 80% acetone and determined according to Arnon's methods [22].

Catalase activity assay

Catalase activity was measured by the methods described according to Aebi's method [23].

Protein content assay

Protein contents were assayed by Bradford's method [24].

Total soluble sugar content assay

Total soluble sugar content in maize seedling was extracted according to Nelson's method [25].

Statistical Analyses

The experimental designs were randomized complete block and each value reported is the average of three replicates. Raw data were imported to Microsoft Excel 2003 program for calculations and graphical representation. SPSS version 17.0 program was used for analysis of variance. Quantitative changes of different parameters were analyzed through analysis of variance (ANOVA), with Duncan's multiple range test at $P < 0.05$ being used to determine significant differences among treatments.

RESULTS AND DISCUSSION

Reduction in chlorophyll, protein content and increase in total soluble sugar and catalase activity in maize shoot and root as a result of Fusarium blight disease may be a consequence of the fungal effect on the liberation of reactive oxygen species. It was reported that endophytic hyphae of *F. moniliforme* produced mycotoxin fumonisin early in maize seedlings [20]. Mycotoxins may cause lipid peroxidation of membrane structures and initiate production of reactive oxygen species [26]. Inhibition of ceramide synthase is one of the action of fumonisin on plant cells specially in roots [27]. Fumonisin elicits an apoptotic form of PCD (Programmed Cell Death) in plants tissue culture cells [28, 29] most probably through competitive inhibition of ceramide synthase, a key enzyme in sphingolipid biosynthesis (30). Sphingolipids function as anchors for membrane proteins [31] and as second messengers regulating various cellular processes, including differentiation, growth, and apoptosis [32]. The adverse effects of fumonisin on the plasma membrane H^+-ATPase (EC 3.6.1.34) from germinating maize embryos, and on the fluidity and lipid peroxidation of these membranes was reported [33].

Shoot and root catalase activity increased by about 70.96% and 63.62% after fungi infection respectively. Methyl jasmonate and cytokinin treatments were found to reduce catalase activity by about %17.7% and 9.6% in shoot and 48% and 21.55% in root as compared to infected seedlings.

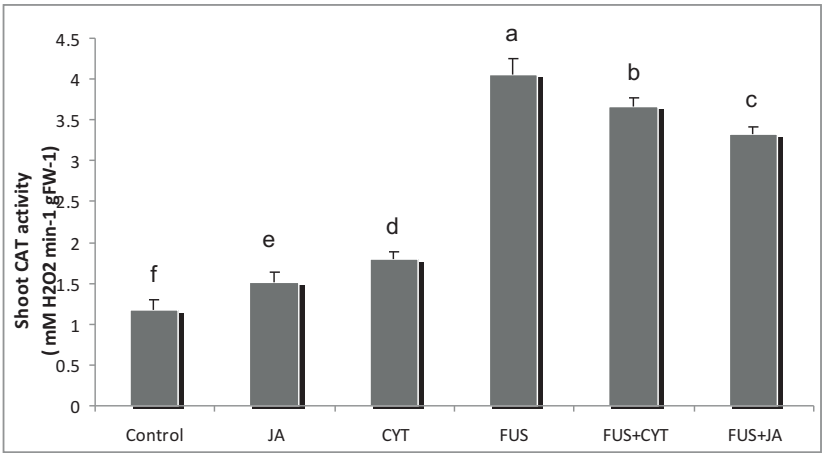

Figure 1. Effect of methyl jasmonate and cytokinin seed treatment with the inoculation of the root with zoospores of *F. moniliforme* on shoot catalase activity 14d after inoculation. Columns are means ± standard deviation of 3 replicates .Columns with different letters are significantly different at P<0.05 (Duncan's test).
(JA= jasmonate, CYT= cytokinin, F= *Fusarium*)

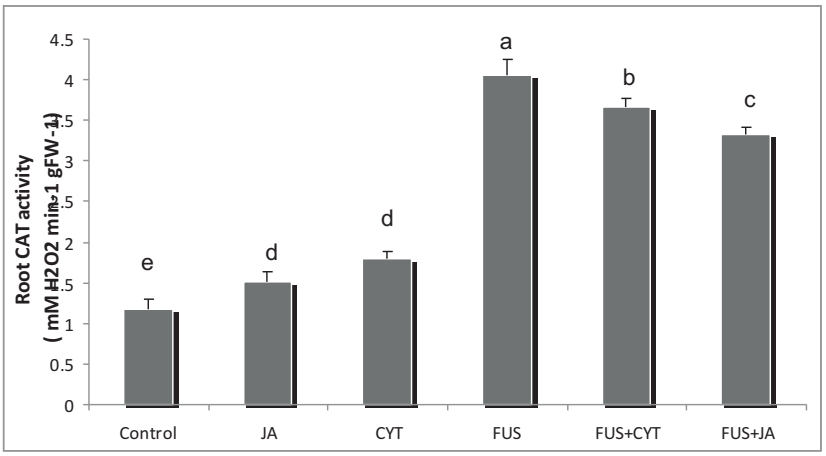

Figure 2. Effect of methyl jasmonate and cytokinin seed treatment with the inoculation of the root with zoospores of *F. moniliforme* on root catalase activity 14d after inoculation. Columns are means ± standard deviation of 3 replicates .Columns with different letters are significantly different at P<0.05 (Duncan's test).
(JA= jasmonate, CYT= cytokinin, F= *Fusarium*)

pathogen- induced reactive oxygen species including H_2O_2, are known to participate in the hypersensitive reaction (HR). Two enzymes, ascorbate peroxidase and catalase have crucial role for determining the steady state level of superoxide radicals and hydrogen peroxide and modulate oxidative stress [34]. Although H_2O_2 is essential for signaling pathogen invasion and defense, excess of H_2O_2 amount results in oxidative stress that can damage plant tissues. Although higher H_2O_2 levels might enhance disease resistance, they also make the plant more susceptible to abiotic stresses [35]. We found that the Fusarium infection caused chlorophyll two decrease during the plant-pathogen interaction, reaching 26.90% of the control values.

It might result from high levels of lipid peroxidation mediating membranes structure damage in plant tissues [36]. Other reports also described the negative effect of fungal infection on fluorescence parameters. Chlorophyll

fluorescence measurements can help in determining the health status of plant before any disease symptom appear [37, 38]. In contrast seed treatment with methyl jasmonate and cytokinin increased chlorophyll content by about 13.5% and 7.45% in comparison with infected seedlings.

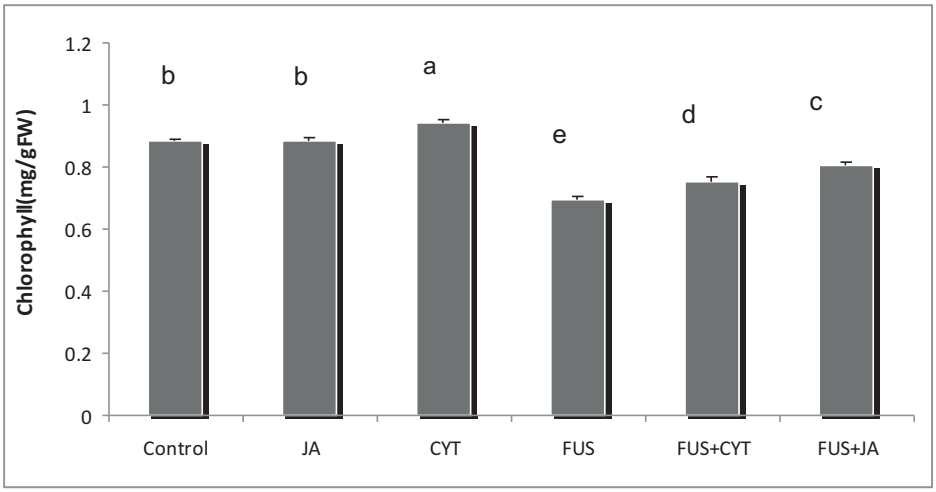

Figure 3. Effect of methyl jasmonate and cytokinin seed treatment with the inoculation of the root with zoospores of *F. moniliforme* on shoot protein 14d after inoculation. Columns are means ± standard deviation of 3 replicates .Columns with different letters are significantly different at P <0.05 (Duncan's test).
(JA= jasmonate, CYT= cytokinin, F= *Fusarium*)

F.moniliforme exhibited significant reduction in protein content of shoot (about 9.7%) and root (30%). The significant decrease in the protein content of maize shoot and root tissues as a result of pathogen infection may be due to some activities related to a hypersensitive response [39]. Methyl jasmonate and cytokinin application increased protein content by about 14.13% and 6.33% in shoot (about 12.97%) and in root (about 6.87%) respectively in comparison with infected seedlings.

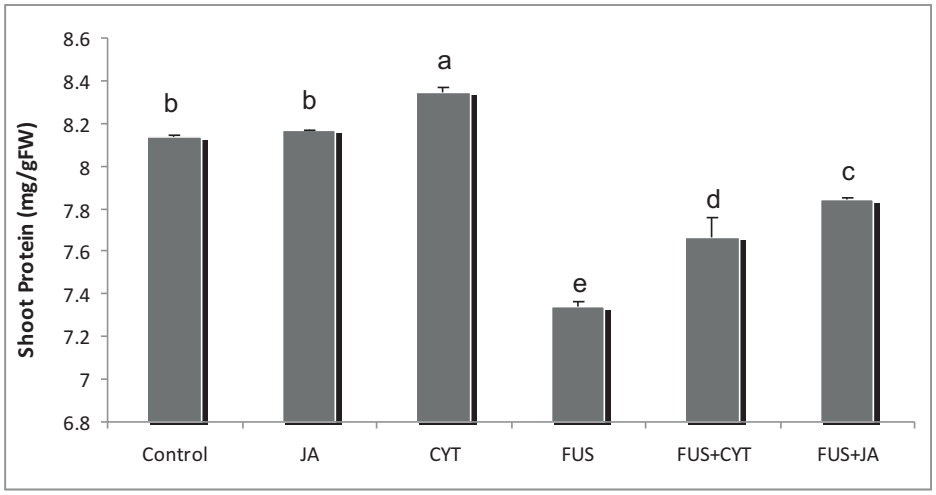

Figure 4. Effect of methyl jasmonate and cytokinin seed treatment with the inoculation of the root with zoospores of *F. moniliforme* on chlorophyll 14d after inoculation. Columns are means ± standard deviation of 3 replicates .Columns with different letters are significantly different at P <0.05 (Duncan's test).
(JA= jasmonate, CYT= cytokinin, F= *Fusarium*)

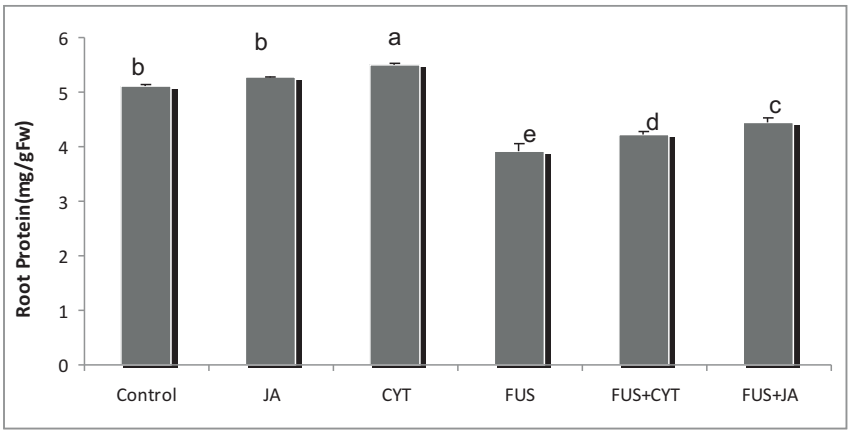

Figure 5. Effect of methyl jasmonate and cytokinin seed treatment with the inoculation of the root with zoospores of *F. moniliforme* on root protein 14d after inoculation. Columns are means ± standard deviation of 3 replicates .Columns with different letters are significantly different at P <0.05 (Duncan's test).
(JA= jasmonate, CYT= cytokinin, F= *Fusarium*)

Soluble sugar content increased after pathogen treatment (about 21.50%). It has been proposed that plants switch off photosynthesis and other assimilatory metabolism to initiate respiration and other processes required for defense [40]. The increase in sugar concentration may be a result from the degradation of starch [41]. Starch may play an important role in accumulation of soluble sugars in cells. Starch depletion may occur in response to drought stress [42]. The concentrations of soluble sugars increased at the same time as a decrease in the starch concentration was observed. It means that the raised soluble sugar fraction was accompanied by a sharp decrease in the starch fraction as the water potential dropped. This change increased the soluble sugar/starch ratio in roots and shoots. The tolerance mechanism in water-deficit may be associated with accumulation of osmoprotectants such as soluble sugars. The accumulation of soluble sugars is strongly correlated to the acquisition of drought tolerance in plants [43].While the mycelium spreads to vascular bundles of the immature stem and blocks the vessels that eventually leads to growth distortions and plug the water conducting vessels, causing wilting. The wilting due to the plugging of vessels with gels and gums and the tyloses barriers may occur after fusarium infection [44].

Methyl jasmonate and cytokinin treatments caused a significant reduction in soluble sugar content in order 10.59% and 5.14% as compared to infected seedlings.

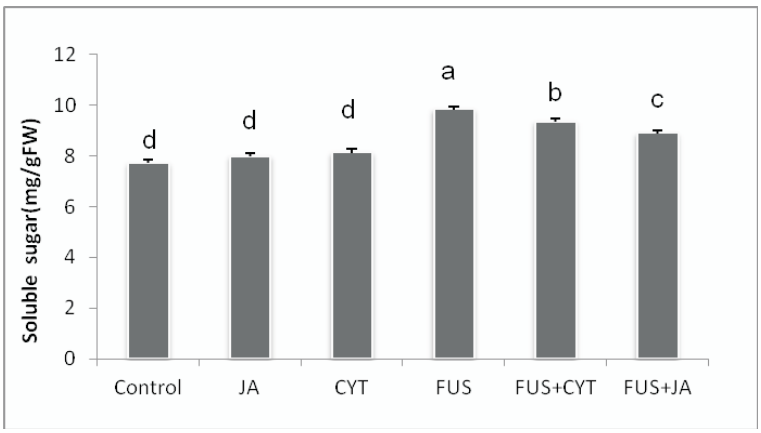

Figure 6. Effect of methyl jasmonate and cytokinin seed treatment with the inoculation of the root with zoospores of *F. moniliforme* on soluble sugar 14d after inoculation. Columns are means ± standard deviation of 3 replicates .Columns with different letters are significantly different at P <0.05 (Duncan's test).
(JA= jasmonate, CYT= cytokinin, F= *Fusarium*)

According to the figures, the inhibitory effect of methyl jasmonate against fusarium blight was more significant than cytokinin. Upon pathogenic fungal attack (especially in necrotrophic fungi) jasmonic acid production induced in infected plant cells to initiate defense mechanisms surrounding cells to limit pathogen spread [45]. It is now known that exogenous application of JA is associated with an increased level of endogenous JA [46, 47]. It was reported that cytokinins modulate the SA signaling to augment resistance against pathogen [16]. It was observed that cytokinin could act as a protector by activation of the antioxidant defense systems and caused an increase in GSH and hydrogen peroxide content. Hydrogen peroxide and glutathione participate in the regulation of the cell antioxidant systems [48]. Results indicated that seed treatment of methyl jasmonate and cytokinin can effectively alleviate the infection of Fusarium on maize seedlings through increase in both the chlorophyll and protein content and reduction in catalase activity and soluble sugar as compared to seedlings with no treatment.

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