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Nitrophenolates spray can alter boll abscission rate in cotton through enhanced peroxidase activity and increased ascorbate and phenolics levels

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SUMMARY

Field studies were conducted from 2002 to 2005 to evaluate foliar spray of Atonik (a plant growth regulator (PGR) containing nitrophenolates) on cotton boll abscission rate by assessing various reactive oxygen species (ROS) contents, antioxidant content and antioxidant enzyme activity from 1 to 9 days after anthesis (DAA). The result indicated that the nitrophenolate spray reduced hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) accumulation, lipid peroxidation (malondialdehyde – MDA), lipoxygenase (LOX) activity and membrane permeability relative to the control. Antioxidant enzyme activity (superoxide dismutase, SOD; ascorbate peroxidase, APX; peroxidase, POX; glutathione peroxidase, GSH-Px) was significantly increased by the nitrophenolate spray. The POX (217%) and GSH-Px (242%) activities were enhanced compared with APX (7.7%) activity at 9 DAA. Enhanced accumulation of ascorbate (245%), phenol (253%) and proline (150%) was observed in nitrophenolate-sprayed plants compared with control at 9 DAA. Because ascorbate content is increased by higher dehydroascorbate reductase (DHAR) enzyme activity, the ascorbate was able to replenish reducing equivalents to phenoxyl radicals, resulting in an increase of phenolic compounds. The increased phenolic acid content may be involved in scavenging the ROS produced in developing cotton boll. The role of DHAR and glutathione reductase (GR) in keeping higher levels of reduced ascorbate and low levels of endogenous H_2O_2 in the developing cotton boll may be the prerequisite for boll retention. Based on the present work, we conclude that nitrophenolate-sprayed plants counteracted the deleterious effects of ROS by the peroxide/phenolics/ascorbate system, which causes reduced boll abscission and increased yield.

Introduction

In India, cotton is the premier commercial fiber crop, with a cultivated area of 9 million hectares. Cotton plant development follows an indeterminate fruiting cycle, with excessive production of fruiting structures by individual plants. All fruiting structures produced cannot mature, and excess structures abscise from the plant. Kennedy et al. (1991) found that only 24–36% of flowers produced on a plant during a growing season mature to harvestable bolls. Abscission is a developmentally controlled process associated with the large-scale degradation of cellular and macromolecular components (Rubinstein, 2000). A considerable volume of research has been devoted to identify the enzymes that bring about cell separation. The culmination of abscission is the physical detachment of the target organ, and thus much work has been focused on the phenomenon of cell wall dissolution at the site of abscission. The contemporary view of abscission is that it is mediated through oxidative damage (Roberts et al., 2002; Muller et al., 2007, Prochazkova and Wilhelmova, 2007). Oxidative damage is associated with several biochemical changes, such as increased membrane permeability, leakage of ions, lipid peroxidation, increased reactive oxygen species (ROS), nuclear fragmentation as well as expression/activation of nucleases, proteases, lipases and other cell wall hydrolases leading to loss of cell structure (Paliyath and Droillard, 1992; Rubinstein, 2000; Wagsta et al., 2002).

Plant cells need protective mechanisms through which they respond to oxidative stress: (a) non-enzymatic antioxidants, *viz.*, ascorbate (AA) and glutathione (GSH) and (b) enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX; Navabpour et al., 2003; Zimmermann and Zentgraf, 2005). However, during senescence, the general antioxidant status diminishes and the levels of ROS are enhanced (Srivalli and Khanna-Chopra, 2004), and many

Abbreviations: AA, ascorbate; APX, ascorbate peroxidase; CAT, catalase; DAA, days after anthesis; DAS, days after sowing; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GSH-Px, glutathione peroxidase; H_2O_2, hydrogen peroxide; LOX, lipoxygenase; MDA, malondialdehyde; MSI, membrane stability index; O_2^-, superoxide radical; POX, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase

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antioxidant enzymes reduce their activity (Prochazkova et al., 2001). Antioxidant enzymes such as SOD, CAT and peroxidase (POX) are involved in the scavenging of ROS (Srivalli and Khanna-Chopra, 2004). The O_2^- produced in the cell will be dismutated to O_2 and H_2O_2 by SOD (Bowler et al., 1992). Peroxidases catalyze the dehydrogenation of structurally diverse phenolic and endiolic substrates by H_2O_2 and are thus often regarded as antioxidant enzymes (Shigeoka et al., 2002). CAT removes the H_2O_2 produced, preventing an increase in cytosolic H_2O_2, which is potentially toxic to the plant cell, leading to oxidative stress and cell death (Prochazkova et al., 2001).

Plant growth regulators (PGRs) have been used to improve yield. One such PGR is Atonik (Asahi Co., Ltd.; Nara Prefecture, Japan), a commercially available product containing the active ingredients 1.25 g L^{-1}sodium 5-nitroguaiacolate ($NaC_7H_6NO_4$) ,2.5 g L^{-1} sodium ortho-nitrophenolate ($NaC_6H_4NO_3$) and 3.75 g L^{-1}sodium para-nitrophenolate ($NaC_6H_4NO_3$). These active ingredients, termed nitrophenolates, can stimulate plant growth by altering the activity of specific antioxidant enzymes such as SOD, CAT and POX (Djanaguiraman et al., 2004; Djanaguiraman et al., 2005a, b). Atonik has been used on various crops in more than 20 countries and was registered for pesticide use in cotton (*Gossypium hirsutum*), rice (*Oryza sativa*) and soybeans (*Glycine max*) in 1995 as ARYSTA-Exp-NP321 (Asahi Co., Ltd.; Nara Prefecture, Japan). ARYSTA-Exp-NP321 has the trade name Chaperone, which was registered by the Environmental Protection Agency (EPA) with the patent pending in 2000. Chaperone was introduced into the cotton market in 2004 as a protein transport enhancer and is currently the only agrochemical containing these nitrophenolates registered for cotton (Bynum et al., 2007). Phenolics are able to act as radical scavengers or radical-chain breakers, strongly extinguishing oxidative free radicals such as the hydroxyl-radical-yielding products with much lower oxidative capacities compared with the parent compounds (Grassman et al., 2002). Thus, the ability of phenolics to ameliorate or lessen the impact of ROS has been explored.

With this background, the present study was conducted to examine whether applied nitrophenolates can alleviate oxidative damage by reduced ROS accumulation in the developing boll by enhancing the antioxidants and antioxidant enzymes; we also examined consequent yield improvement.

Materials and methods

Crop management

Cotton (*G. hirsutum*) variety MCU 12 was sown in a clay loam textured soil with a pH of 7.6 and electrical conductivity (EC) of 0.31 dS m^{-1} from 2002 to 2005 in the experimental field of Tamil Nadu Agricultural University, Coimbatore (11°N; 77°E; 426.7 m amsl), India. At the experimental site, the maximum and minimum temperature and relative humidity ranged between 33 and 20 °C and 80% and 60%, respectively. The minimum and maximum irradiances were 800–1100 μM m^{-2} s^{-1} (PAR). The soil of the experimental field was low in available N (180 kg ha^{-1}), medium in available P (6 kg ha^{-1}) and high in available K (376 kg ha^{-1}). The experiment was designed to evaluate the foliar spray of nitrophenolates on yield improvement in cotton by assessing the production of various oxidants, antioxidants, antioxidant enzymes and membrane damage. The experiment utilized two treatments :(i) foliar spray of nitrophenolates at 0.8 g ai ha^{-1} at square formation stage [35 days after sowing (DAS)] and initiation of flowering stage (58 DAS) and (ii) an untreated control with six replications. Twelve plots with a size of 25 m^2 were prepared. The crop was sown during August with a spacing of 75 cm × 30 cm. Pendimethalin @ 3.3 L ha^{-1} was applied 3 DAS, using a hand-operated sprayer fitted with a deflecting-type nozzle. Sufficient moisture was present in the soil at the time of herbicide application and one hand weeding was performed 45 DAS. Gap filling was performed on the 10th day of sowing and thinning of the seedlings was done on the 15th day of sowing. 50 fifty per cent of N and K and a full dose of P_2O_5 was applied as basal in the form of urea, single super phosphate and KCl. The remaining 50% of the recommended doses of N and K were applied as top dressing 40–45 DAS. Recommended plant protection measures were followed. At early stages of square formation, Endosulfan 35 EC was sprayed @ 2 L ha^{-1} using a gooseneck nozzle to cover the under surface of the foliage to get good control of sucking pests. During the boll formation and maturation stage**,** Phosalone 35 EC was sprayed @ 2.5 L ha^{-1} to control bollworms.

The variation in the production of oxidants, *viz*., hydrogen peroxide (H_2O_2) and superoxide radical (O_2^-), lipoxygenase (LOX) enzyme activity, malondialdehyde (MDA) content, membrane stability index (MSI) and antioxidant enzymes namely SOD, CAT, APX, POX and glutathione peroxidase (GSH-Px) activities were recorded from 61 to 69 DAS in the developing bolls. Likewise, various metabolites, *viz*., ascorbate, dehydroascorbate (DHA), soluble phenol and proline contents and activities of enzymes such as dehydroascorbate reductase (DHAR) and glutathione reductase (GR) were recorded 61 DAS (1 day after anthesis – DAA), 63 DAS (3 DAA), 65 DAS (5 DAA), 67 DAS (7 DAA) and 69 DAS (9 DAA) in the developing bolls. The tenth main stem node was chosen as a representative fruiting branch to quantify various oxidants, antioxidants and antioxidant enzyme activity, since it typically possesses the greatest leaf area and contributes to the majority of the yield on a per node basis (Oosterhuis and Wullschleger, 1988).

Oxidant production and lipid peroxidation

Lipid peroxidation was determined by malondialdehyde content produced by thiobarbituric acid (TBA) as described by Behra et al. (1999). A 200 mg boll sample was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000*g* for 5 min at 4 °C. An aliquot of 0.3 mL supernatant was mixed with 1.2 mL of 0.5% TBA prepared in 20%, TCA and incubated at 95 °C for 30 min. After stopping the reaction in an ice bath for 5 min, samples were centrifuged at 10,000*g* for 10 min at 25 °C. The absorbance was measured at 532 nm. After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using on extinction coefficient of 155 mM^{-1} cm^{-1}. Lipid peroxidation was expressed as MDA content in nM g^{-1} FW. The H_2O_2 level was colorimetrically measured as described by Okuda et al. (1991). H_2O_2 was extracted by homogenizing 0.5 g of tissue with 4 mL of perchloric acid (200 nM). The homogenate was centrifuged at 12,000*g* for 10 min. Oxidation of ferrous ions to ferric ions by H_2O_2 in acidic pH was monitored and a stable complex of ferric ions with Xylenol orange dye at 560 nm was measured. H_2O_2 was expressed in nM g^{-1} FW. Superoxide anion was estimated according to Chaitanya and Naithani (1994) and expressed as change in OD min^{-1} g^{-1} FW. Weighed bolls were homogenized in ice-cold sodium phosphate buffer (0.2 M, pH 7.2) containing diethyldithiocarbamate (10^{-3} M). The homogenate was immediately centrifuged for 1 min at 3000 rpm. In the supernatant, superoxide anion was measured by its capacity to reduce nitroblue tetrazolium (NBT, 2.5×10^{-4} M). The absorbance of the end product was measured at 540 nm.