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Research paper

Field application of safe chemical elicitors induced the expression of some resistance genes against grey mold and cottony rot diseases during snap bean pods storage

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ABSTRACT

Phaseolus vulgaris is subjected to serious post-harvest diseases such as grey mold and cottony rot diseases caused by *Botrytis cinerea* and *Pythium aphanidermatum*, respectively. In current study, potassium silicate (KSi), potassium thiosulfate (KTS) and potassium sulfate (KS) suppressed moderately the growth of *B. cinerea* and *P. aphanidermatum in vitro*. The applied treatments significantly suppressed grey mold and cottony rot of Xera and Valentino snap beans varieties' pods stored at 7 ± 1 °C and 90–95% RH for 20 days. Ethylene responsive factor (*ERF*), polygalacturonase inhibitor protein (*PGIP*), phosphatase associated to defense (*PA*) and pathogenesis-related protein (*PR1*) defense genes were over-expressed in leaves tissue of both bean varieties responding positively to potassium salts field application. The expression of these genes was influenced by plant genotype and environment as it varied by snap bean varieties. Accumulation of *ERF*, *GIP*, *PA* and *PR1* genes transcript under KTS at 4000 ppm treatment were the highest in Xera tissues (3.5-, 4.8-, 4- and 4.8-fold, respectively). In conclusion, pre-harvest potassium salt *in vivo* application could be used as effective safe alternatives to fungicides against grey mold and cottony rot diseases of snap beans during storage for up to 20 days at 7 ± 1 °C.

1. Introduction

Snap bean (*Phaseolus vulgaris*) is one of the most world's important economic vegetable crops for direct human consumption. It comprises about 50% of the vegetable legumes consumed worldwide (Broughton et al., 2003; Graham et al., 2003). In Egypt, snap bean production for local consumption and export increased considerably in recent years and reached about 251,000 Mg (mega grams) produced from about 24,300 ha with average production of 10.33 Mg ha^{-1} (Min., Agric., ARE., 2012). All over the world, post-harvest losses of fruits and vegetables have been estimated to range from 5% to 50% from harvested amounts (Statistics, FAO., 2012). Snap bean pods of Xera and Valentino varieties are attacked by many fungi causing several diseases during growth in the field, harvest, storage and marketing. Under the Egyptian environmental conditions, economic post-harvest losses in snap beans occurred due to development of grey mold and cottony rot post-harvest diseases caused by *Botrytis cinerea* and *Pythium aphanidermatum*, respectively, affecting snap bean productivity as well as its exportation competitiveness (Snowdon, 1992; Suslow and Cantwell, 1998). They cause serious problems to the harvested snap bean pods during transportation, exportation and storage. Although the use of chemical fungicides gave satisfactory control against fungal infections, the pre-harvest interval and fungicide residues have harmful effects on human health and the environment resulting frequently in rejection of fungicides treated pods for human consumption (Eckert and Ogawa, 1988; Farouk and Osman, 2011).

Fungicides are becoming increasingly used but are less acceptable in the national and international markets. Therefore development of environment friendly methods for disease control is an important goal to be achieved. Treatment of plants with a variety of abiotic and biotic resistance elicitor's agents, including cell wall fragments, plant extracts and synthetic chemicals can be induced to develop enhanced resistance to subsequent pathogen attack both locally and systemically as systemic acquired resistance (SAR) (Walters and Fountaine, 2009). Systemic acquired resistance (SAR) is the readiness of plant to repel subsequent pathogen attacks spread throughout the whole plant (Vallad and Goodman, 2004). Introduction of safe chemical elicitors for resistance gene expression into agricultural practices could manage post-harvest

Abbreviations: cDNA, DNA complementary to RNA; dNTP, deoxyribonucleoside triphosphate; Ksi, potassium silicate; KTs, potassium thiosulfate; KS, potassium sulfate; *ERF* gene, ethylene responsive factor; *PGIP gene*, polygalacturonase inhibitor protein; *PA gene*, phosphatase associated to defense; *PR1 gene*, pathogenesis-related protein; *EF-1α* gene, elongation factor; qRT-PCR, quantitative real time polymerase chain reaction; PDA, potato dextrose agar; mRNA, messenger RNA; gDNA, genomic DNA; NTC, non-template control.

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diseases of snap bean pods as fungicides alternatives (Farouk and Osman, 2011; Picone and Tassel, 2002). Data obtained by Reuveni and Reuveni (1998) indicated that foliar sprays of phosphate and potassium salts can induce systemic protection against foliar pathogens in various crops such as cucumber, maize, rose, grapevine, apple, mango and nectarine. Over 2440 studies concerning the relationship between potassium, (alone or combined) with other elements, and plant health have been reported by (Perrenoud, 1990) where more than 400 diseases and pests were included in this report. Two mechanisms for Silicon-enhanced resistance to diseases have been proposed by (Ma and Yamaji, 2006). First, Si acts as a physical barrier, where it is deposited beneath the cuticle such that the Si layer mechanically impedes penetration by fungi, thereby disrupting the infection process. Second, soluble Si acts as a modulator of host resistance to pathogens.

Applications of several inorganic salts were found to reduce infections of various fungal diseases of different plant organs, foliage, stem, ear; fruits and tubers, pre and post-harvest (Diliopoulos et al., 2010). Abiotic resistance elicitors include chemicals which act at various points in the signaling pathways involved in disease resistance. Induced resistance by these abiotic elicitors may lead to direct activation of defense-related genes which is broad spectrum and long lasting (Walters et al., 2013; Duan et al., 2014). These elicitors are often associated with various cellular defense responses, such as synthesis of pathogen related protein (PR proteins), phytoalexins, accumulation of active oxygen species (AOS), rapid alterations in cell wall, and enhanced activity of various defense related enzymes resulting from expression of many defense genes (Vallad and Goodman, 2004). The development of genomic techniques for profiling gene expression allowed significant progress in characterization of plant response to different abiotic elicitors (Xin et al., 2010).

A number of defense-related genes show increased transcript accumulation in resistant tissue which provided a useful molecular marker correlated with the development of SAR response (Herbers et al., 1996; Xin et al., 2010). Guerrero-Gonzalez et al. (2011) identified a group of defense genes and studied their regulation by means of quantitative real time PCR (qRT-PCR) analysis. An important group of the identified genes, including a receptor-like kinase (*PvRK20-1*), an acid phosphatase associated to defense (*PA*), a pathogenesis related protein (*PR1*), an ethylene responsive factor (*ERF*), a polygalacturonase inhibitor protein (*PGIP*), and an alphadioxygenase (*α-DOX*) are related to different elicitors responses, and some of them have already been reported in many plants (Mahalingam et al., 2003; Zhou et al., 2007; Lehtonen et al., 2008; Guerrero-Gonzalez et al., 2011).

Hence, this work aimed to evaluate the impact of pre-harvest spraying of three potassium salts, KSi, KTS and KS, as safe inducers for resistance, with different concentrations on protecting snap bean pods from grey mold and cottony rot diseases during storage; evaluating its effect on the level of some defense genes transcript in snap bean plants, as promising compounds for controlling post-harvest storage diseases.

2. Materials and methods

2.1. Source of plant materials and safe (GRAS) salts

This study was carried out using Xera and Valentino varieties (*P. vulgaris*) obtained from Nivex Company for Agricultural Investment and Export. KSi, KTS and KS salts were obtained from Elgomhoria Chemical Co. (ARE). Snap bean plants of two summer seasons 2013 and 2014 were facilitated in a farm at Qalyubia Governorate for pre-harvest salt application and samples of plant leaves as well as a source for pods selected for storage and post-harvest disease evaluation. The plants were treated by those chemicals as well as non-treated plants were subjected to similar agricultural practices except for test salt treatments during two seasons 2013 and 2014.

2.2. Source of fungal isolates

B. cinerea (EMCC583), Cairo Microbiological Resources Centre (Cairo MIRCEN), and *P. aphanidermatum* (PPFT183), which isolated from decayed snap bean pods. Both isolates proved their pathogenic activity were used for such disease control studies. The purified isolate of *P. aphanidermatum* was identified according to its morphological features using the descriptions of (Jarvis, 1977; Barnett and Hunter, 1998). Identification of the isolated fungus was confirmed in Fungal Taxonomy Dept., Plant Pathology Institute, ARC, Giza Governorate, Egypt. Cultures of seven days old of both fungal isolates grown on potato dextrose agar medium (PDA) were used for *in vitro* studies as well as the artificial inoculation of snap bean pods in disease control experiments.

2.3. In vitro evaluation of potassium salts on growth of B. cinerea and P. aphanidermatum

Three potassium salts, KSi, KTS and KS were tested to study their effects on growth of *B. cinerea* and *P. aphanidermatum*. Each salt was applied at four concentrations, i.e. 1000, 2000, 4000 and 8000 ppm. PDA warm medium was amended with each tested concentration just before medium solidification, and then poured into Petri-dishes. After solidification, 3 mm mycelial discs cut from the periphery of seven-day-old cultures of the two tested pathogens were placed in the middle of medium surface in Petri dishes, and then incubated at 20 ± 2 °C. Four replicates of Petri dishes containing PDA were prepared for each salt concentration as well as other 4 replicates without salts kept as a control. The linear growth of the tested pathogens was measured for such treatment and control when fungal mycelium covered one plate in the control treatment.

2.4. Effect of pre-harvest treatment of snap bean plants with three potassium salts on incidence of pod rots during storage

Under field conditions for two successive seasons in 2013 and 2014, the effect of pre-harvest spray of snap bean plants of Xera and Valentino varieties at blooming, 10 days and 20 days from 1st spray with the tested salts to control rot diseases during harvesting and storage was studied. The last spray was given 5 days before harvesting as well as getting leaf samples for detecting defense genes differential expression. The three salts were tested for their capability to control post-harvest snap bean pod rots via activation of related defense genes expression in treated plants. The experiment was carried out at Qalyubia governorate. The experimental design was a split-plot, each plot consisted of three rows (one row is 3 × 0.7 m) was used as an experimental unit. Plots, containing snap bean plants, not treated with salt solutions, but treated with water, were used as control. After harvest, harvested pods from each treatment were divided into 3 groups prior to fungal infection. The first group was used as naturally infected pods. The second group was artificially inoculated with *B. cinerea*, while the third group was artificially inoculated with *P. aphanidermatum*. The artificial inoculation was achieved by spraying spore and propagule ((mycelium + Oospore) suspension for *B. cinerea* and *P. aphanidermatum*, respectively, at about 4×10^6 spore or propagule/ml on the pods, and artificially inoculated pods were incubated at 18–20 °C overnight. Three replicates were used for each treatment (25 pods for each) and packed in polyethylene bags. The naturally and artificially inoculated snap bean pods were stored at 7 ± 1 °C and 90–95% RH for 20 days. Severity of infection was estimated as percentage of the external rotten area in proportion to the total area of the pods (Morcos, 1984).

$$\textit{Efficiency}\ (\%) = (\textit{disease severity of the control treatment} - \textit{disease severity of the treatment} / \textit{disease severity of the control treatment}) \text{ X } 100$$