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Effect of calcium soil amendments on phenolic compounds and soft rot resistance in potato tubers

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ABSTRACT

Nutrients such as boron, nitrogen and calcium stimulate the production of phenols. This research focuses on the role of calcium in increasing phenol metabolism in potato peels and the ensuing tuber resistance to soft rot pathogens was investigated. Two field experiments were conducted at the University of Zimbabwe campus plots in 2008 and 2009 summer seasons. Sprouted tubers of cv. BP1 were planted in plots treated with different fertilizer combinations. The treatments were: 1) compound S (7N: 21P: 8K) + ammonium nitrate (34 %N) 2) compound D (7N: 14P: 7K) + calcium nitrate (19 Ca: 15.5N) 3) compound S + calcium nitrate and 4) compound D + ammonium nitrate. The harvested progeny tubers were inoculated with *Pectobacterium carotovorum* subsp. *brasiliense*. Calcium amendment increased the activities of enzymes (phenylalanine ammonia-lyase, polyphenol oxidase and peroxidases) involved in the metabolism of phenolics and total soluble phenols. Calcium amendment significantly reduced ($P < 0.05$) the maceration effect of *P. carotovorum* subsp. *brasiliense* in tuber tissues. Chlorogenic acid, caffeic acid and calcium, but not ferulic acid content were significantly higher ($P < 0.05$) in plants grown in calcium treated plots. Calcium positively and significantly correlated with polyphenol oxidase (PPO), phenylalanine (PAL) and peroxidase (POD), while chlorogenic and caffeic acid showed a positive relationship with POD and PPO. Calcium amendment significantly reduced maceration symptom caused by the bacteria ($P < 0.05$), resulting in smaller decayed zone diameters on inoculated tubers from calcium-treated plots. This study shows that soil amendments of calcium increase concentration of calcium, caffeic and chlorogenic acid in tuber peels and also reduces maceration effect of pectinolytic pathogens. Reduced maceration could be due to increased levels of caffeic and chlorogenic acid which have antimicrobial properties.

1. Introduction

In Zimbabwe, potato growers face the challenge of significant post harvest losses of potato tubers (20–60%) due to soft rot (Manzira, 2010). The causal agents were identified as *Pectobacterium carotovorum* subsp. *brasiliense* (*Pcb*), *P. carotovorum* subsp. *carotovorum* (*Pcc*), *Pectobacterium atrosepticum* (*Pa*) and *Dickeya dadantii* (*Dd*) (Ngadze et al., 2010, 2012b). The pathogens produce large quantities of pectolytic enzymes which macerate plant tissues (Collmer and Keen, 1986). *Pectobacterium* spp. secrete pectinases that induce polyphenol oxidase activity in the hosts, which subsequently oxidizes phenols, forming a black margin around the infection site. The margin restricts the pathogen from spreading (Lovrekovich et al., 1967). Most of the potato cultivars grown in Zimbabwe have some level of susceptibility to soft rot pathogens (Ngadze et al., 2012a).

Calcium is an essential nutrient and considered one of the most important nutrients associated with plant defense (Datnoff et al., 2007). It has been linked to interactions between plant pathogenic bacteria and their host plants. Calcium confers some resistance to pests and diseases in plants via its influence on growth pattern, anatomy, morphology and chemical composition of the plant. Increased plant calcium has been shown to enhance resistance to plant tissue macerating bacterial phytopathogens (Pérombelon and Kelman, 1980; McGuire and Kelman, 1984, 1986; Schöber and Vermeulen, 1999). Calcium increased resistance of witloof to soft rot pathogens, while high concentrations of nitrogen increased susceptibility of the same plants to soft rot pathogens (Schöber and

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Vermeulen, 1999). Calcium is involved in eliciting signal transduction pathways and in membrane and cell wall integrity (McGuire and Kelman, 1984; Busse and Palta, 2006; Datnoff et al., 2007). It also enhances the structural integrity of cell walls and membranes. Adjustments in mineral nutrition could therefore reduce disease severity (Marschner, 1995; Datnoff et al., 2007).

Metabolism of phenolics in plants has been associated with injuries, thermal stress, and tolerance against exposure to UV rays, biotic stress and ozone. The role of Ca^{2+} in phenolic metabolism has been described by several authors; Castañeda and Pérez (1996) demonstrated a direct role of calcium in the synthesis of phenols. They observed that foliar application of 10 μM of $CaCl_2$ increased PAL activity and caused subsequent accumulation of phenols, increasing resistance of citrus to infection caused by *Alternaria alternata*. Given the essential role played by phenol metabolism in many resistance responses to different stresses, rapid and effective manipulation of the metabolic process could enhance resistance in plants to adverse conditions. The objective of this study was to determine the effect of calcium soil amendments in increasing calcium levels in the plant and the subsequent effect thereof on metabolism of phenolic compounds.

2. Materials and methods

2.1. Experimental site

Two experiments were conducted at the University of Zimbabwe (UZ) campus in the 2008 (experiment 1) and 2009 (experiment 2) summer seasons (August–December). The UZ campus is situated in Harare (17°50′ South and 31°30′ East) at an altitude of 1500 m above sea level. The area is characterized by fersiallitic red clay soils with more than 40% clay and receives an annual rainfall of 800–1000 mm. Average maximum temperatures during the growing season ranged from 20 to 25 °C. The fields were planted to Brassicae prior to the experiments. Compound S [7N: 21P: 8K] and compound D [7N: 14P: 7K] fertilizers were used in this experiment because compound S is the fertilizer recommended for growing potato in Zimbabwe, but some smallholder farmers opt for compound D which is cheaper. The crop was hoe weeded when necessary and pests were controlled with carbaryl used at the recommended rate.

2.2. Experimental design

The experiment was laid out as a randomized complete block design with four treatments. The treatments were: 1) compound S [7N: 21P: 8K] + ammonium nitrate [34.5% N]; 2) compound D [7N: 14P: 7K] + calcium nitrate [19Ca: 15.5N]; 3) compound S + calcium nitrate and 4) compound D + ammonium nitrate. The combination of fertilizers in these treatments ensured that calcium was present only in treatments 2 and 3. The different fertilizer treatments were applied to the appropriate plots. Three blocks were used in the experiment and the treatments were replicated three times in each block for each year (that is in experiments 1 and 2). Certified potato seed of cultivar BP1 was used in the experiment.

2.3. Agronomic practices

Compounds D (7N: 14P: 7K) and S (7N: 21P: 8K) were applied as basal fertilizers at a rate of 1000 kg ha^{-1} in the relevant treatments. Calcium nitrate at a rate of 250 kg ha^{-1} was mixed with basal fertilizer for treatments 3 and 4. The first application of calcium nitrate was applied after opening the furrows, and then slightly covered with soil before planting the tubers at a depth of 10 cm. Ammonium nitrate and the second calcium nitrate application were applied as top dressing at a rate of 250 kg ha^{-1} 6 weeks after crop emergence. The fertilizer was placed about 5 cm away from the plants to avoid scorching. The fields were irrigated when necessary and 300 mm of water was applied to the field as a supplement for the whole season. The total amount of precipitation was 650 mm measured for the whole season.

2.4. Plant mineral analysis

2.4.1. Extraction and quantification of total Ca^{2+}

For determination of calcium in the leaf tissue, the top fully expanded leaf was collected from each of 10 plants per treatment at the flowering stage and these were pooled for each treatment. For tuber analysis, five tubers were randomly selected at harvest from each treatment per block and taken as the representative sample. Total Ca^{2+} in the peels was determined by atomic-absorption spectrophotometry (Hocking and Pate, 1977), after digestion of dry and milled material with 12 N H_2SO_4 and H_2O_2. The content of calcium was expressed as m mol g^{-1} dry weight (DW).

2.4.2. Extraction of phenolic compounds

Five daughter tubers from each treatment were randomly selected at harvest. The tubers were washed under running water and peeled with a potato peeler. Tuber peels were freeze-dried for five days and then ground to a fine powder. Two hundred milligrams of this fine powder was passed through a 1 mm sieve and placed in a 1.5 ml micro centrifuge tube for extractions. Aliquots of 1 ml of a cold mixture of methanol: acetone: ultra-pure water (7:7:1; v:v:v) were added, vortexed and ultrasonified for 5 min. After sonification, samples were shaken for 20 min at 160 rpm while on ice. Samples were centrifuged for 5 min at 5000 rpm and the supernatant of each sample was transferred to a 20 ml centrifuge tube. This process was repeated three times on the same sample and supernatants finally evaporated in a laminar flow cabinet at room temperature. The residue was dissolved in 1 ml sterile, ultra-pure water. Finally, samples were filtered through 0.45 μm, 25 mm, Ascrodise, GHP, syringe filters (Separations, South Africa). Samples were stored at 4 °C until analysis using reverse phase – high performance liquid chromatography (RP – HPLC).

2.4.3. Reverse phase – high performance liquid chromatography

For identification and quantitative analysis of samples, 10 μl of purified extract per sample was analyzed using RP - HPLC (Hewlett Packard Agilent 1100 series) with a UV diode array detector, at 325 and 340 nm. Separation was achieved on a Gemini 3 μ, C18, 110A (Phenomenex®) reverse phase column (250 mm length, 5 μm particle size, 4.6 mm inner diameter). A gradient elution was performed with water (pH 2.6 adjusted with H_3PO_4) and acetonitrile (ACN) and consisted of 0 min, 7% ACN; 0–20 min, 20% ACN; 20–28 min, 23% ACN; 28–40 min, 27% ACN; 40–45 min, 29% ACN; 45–47 min, 33% ACN; 47–50 min, 80% ACN. Solvent flow rate was 0.7 ml min^{-1}. Identification of the phenolic compounds was done by comparing their retention times and UV apex spectrum to those of standards: chlorogenic acid, caffeic acid and ferulic acid. The column was re-equilibrated for 10 min, after each run.

2.5. Enzymatic analysis

The extraction of phenylammonia lyase (PAL) in the leaves was carried out following the method proposed by Lister et al. (1996). Five plants were randomly selected from each treatment per plot at 10 WACE and 2 young leaves were taken from each plant. The leaves were pooled to make a composite sample. PAL activity was assayed according to the method described by McCallum and Walker (1990), and determined from the yield of cinnamic acid, estimated from