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Seed vigour studies in corn, soybean and tomato in response to fish protein hydrolysates and consequences on phenolic-linked responses

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Abstract

Seed priming with fish protein hydrolysates (FPH) has been studied for the enhancement of seed vigour of corn, soybean and tomato. The influence of FPH at 2.5 mL/L and 5.0 mL/L on traditional agronomic parameters for seed vigour (germination percentage, seedling weight, seedling height) and potential new vigour-associated parameters (phenolic content, antioxidant activity, guaiacol peroxidase (GuPX) activity, chlorophyll content) was investigated. FPH treatment preferentially stimulated seedling vigour in the following order: soybean > tomato > corn. For soybean, FPH at 2.5 mL/L and 5 mL/L improved the majority of the seed vigour parameters (seedling weight and height, phenolic content, antioxidant activity and chlorophyll content, and lignification-associated GuPX activity). Similarly, for tomato, FPH at 2.5 mL/L stimulated seedling weight and height, GuPX activity and chlorophyll content. However, FPH did not stimulate corn seed vigour. Our results suggest an ability of proline precursor-rich FPH to improve of plant growth and development (e.g., seed vigour) in phenolic-rich plant species through modulation of phenolic and chlorophyll metabolisms.

Keywords: Seed vigour; Germination; Plant growth; Fish protein hydrolysate; Proline; Chlorophyll; Phenolics

1. Introduction

Strategies for improving the growth and development of crop species have been investigated for many years. Seed priming is a pre-sowing strategy for influencing seedling development by modulating pre-germination metabolic activity prior to emergence of the radical and generally enhances germination rate and plant performance (Bradford, 1986; Taylor and Harman, 1990). A number of commonly used priming solutions are available, such as polyetheylene glycol (Pill et al., 1994), inorganic salts (Guedes and Cantliffe, 1980), fertilizer and plain water. However, optimization of priming solutions is required for each crop species (Bradford, 1986; Taylor and Harman, 1990; Harris et al., 1999; Heydechker et al., 1973).

Our laboratory has been investigating the effect of fish protein hydrolysates (FPH) on seedling vigour. As a common byproduct of the fisheries industry, FPH is known to be rich in amino acids common to proline metabolism (Mackie, 1982). Milazzo et al. (1999) reported that addition of FPH to tissue culture medium stimulated multiple shoot formation in melon (*Cucumis melo* L.). FPH treatment at 2 mL/L has also been shown to enhance seed vigour, phenolic content, guaiacol peroxidase (GuPX) activity, and glucose-6-phosphate dehydrogenase (G6PDH) activity in pea during early stages of germination (Andarwulan and Shetty, 1999), suggesting a potential stimulation of pentose-phosphate pathway (PPP) activity by FPH as a source of sugar-phosphate precursors to support phenolic synthesis and plant development needs. In addition, priming fava bean with 2 mL/L FPH stimulated not only phenolic content, but seedling weight and height (Randhir and Shetty, 2003). In this same study, GuPX activity was observed to increase steadily with germination time (Randhir and

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Shetty, 2003). Together, these reports suggest a potential role for FPH as an elicitor of seed vigour and further support the inclusion of phenolic content and lignification-associated GuPX activity (Gasper et al., 1985) as new parameters of seed vigour.

Based on these studies, we investigated the effect of FPH at concentrations of 2.5 mL/L and 5.0 mL/L on corn, soybean and tomato, using traditional agronomic standards for measuring seed vigour (germination rate, seedling weight and height) and the potential new vigour parameters phenolic content, free radical-scavenging (FRS) antioxidant activity, GuPX activity, and chlorophyll content.

2. Methods

2.1. Plant material and seed treatment

Corn seed (*Zea mays*; Syngenta Seeds Corp., Indianapolis, IN), soybean seed (*Glycine max*; Syngenta), and tomato seed (*Lycopersicon esculentum*) were used for this study. Sixty-four (64) seeds were soaked overnight in each solution on an orbital shaker set at 150 rpm. FPH (Marine Hydrosylates, Sjoland, Ltd., Iceland) was used as a priming solution at a concentration of 2.5 mL/L or 5.0 mL/L. The treatment solutions used were 5.0 mL/L of FPH (Marine Hydrolysates; Sjoland, Ltd., Iceland) or 2.5 mL/L of FPH. The control (C) solution was distilled water. The treated seeds were sown in potting soil and covered with thin layer of fine vermiculite powder. Corn seeds were germinated for 10 d. Soybean seeds and tomato seeds were germinated for 12 d. Samples were taken every 3 d after seedling emergence.

2.2. Germination percentage, shoot weight, and shoot height

Germination percentage was calculated as the total number of seedlings that emerged versus the total number sown. The average shoot height (in mm) from soil to shoot tip and the weight (in grams) of whole seedlings, including roots, were measured. Soil was carefully removed from the roots before weighing.

2.3. Total soluble phenolics assay

The total soluble phenolic content of leaf tissue of germinated seedlings was determined according to McCue et al. (2000). Briefly, 50 mg of leaf tissue was extracted in ethanol at −20 °C. After homogenization, sample supernatants were assayed for total soluble phenolic content using Folin–Ciocalteu's (FC) phenol reagent (Sigma Chemical Co., St. Louis, MO). After incubating the reaction to develop color indicator, absorbance at 725 nm was measured by a UV spectrophotometer. A phenolic standard curve was established using various concentrations (25–200 μg/mL) of gallic acid in 95% ethanol. Phenolic content was reported as mg gallic acid equivalents (GAE)/g FW.

2.4. Free radical-scavenging (FRS) antioxidant activity assay

The FRS antioxidant activity of phenolic extracts was determined as the ability to scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radicals, using the method of Yildirim et al. (2001) with a slight modification. Two-hundred (200) microliters of each phenolic extract was mixed with 800 mL of freshly prepared 0.1 mM ethanolic DPPH, vortexed well, and then incubated for 30 min at RT. The samples were clarified by centrifugation for 30 s at 13,000 rpm at RT. The absorbance of each clarified sample at 517 nm was measured. Ethanol (95%) was used both as control sample and to blank the spectrophotometer. Antioxidant activity was reported as percent (%) DPPH scavenging, calculated as [(control absorbance − extract absorbance)/(control absorbance) × 100].

2.5. Preparation of enzyme and protein extracts

One-hundred (100) mg of leaf tissue was ground in 2 mL of enzyme extraction buffer with glass beads by mortar and pestle chilled on ice. The extraction buffer consisted of 0.5% (w/v) polyvinylpyrrolidone, 3 mM disodium EDTA, and 0.1 M potassium phosphate buffer, pH 7.5. The homogenate was centrifuged at 13,000 rpm for 10 min at 4 °C and was used as the crude enzyme extract. The samples were kept on ice during the experiment. Protein content was measured using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA). A protein standard curve was generated using bovine serum albumin.

2.6. Guaiacol peroxidase (GuPX) assay

GuPX was determined as previously described (McCue et al., 2000). GuPX reaction mixture contained 0.1 M potassium phosphate buffer (pH 6.8), 20 mM guaiacol solution, 0.2 mM hydrogen peroxide and sufficient enzyme in a total volume of 1 mL. At a wavelength of 470 nm, the increase in absorbance (i.e. production) of tetraguaiacol was assayed over a period of 5 min. The millimolar extinction coefficient of tetraguaiacol at 470 nm is 26.6 mM^{-1} cm^{-1}. GuPX activity was reported as nmol tetraguaiacol/min/mg protein.

2.7. Chlorophyll content

Chlorophyll *a*, *b* and total chlorophyll content levels in seedling leaf tissue were measured by the method of Hipkins and Baker (1986). Fifty (50) mg of leaf tissue was added to 3.0 mL of 100% methanol and incubated in the dark for 2 h. Next, each sample was homogenized and centrifuged at 13,000 rpm for 10 min. Absorbance of the samples at 650 nm and 665 nm was measured by UV spectrophotometer. Absolute methanol (100%) was used as blank. The calculation of chlorophyll *a*, chlorophyll *b* and total chlorophyll content was as follows: