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Developmental stimulation of total phenolics and related antioxidant activity in light- and dark-germinated corn by natural elicitors

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Abstract

The phenylpropanoid pathway (PPP) was stimulated in dark-germinated corn sprouts and light-germinated seedlings through the pentose phosphate and shikimate pathways during sprouting and subsequent growth phase. Overall trends indicated that natural elicitors such as fish protein hydrolysates (FPH), lactoferrin (LF) and oregano extract (OE) did not stimulate phenolics, antioxidant and seed vigour properties of the corn sprouts and seedlings significantly, but were stimulated in certain stages for specific treatments. The general trend of developmental regulation of total phenolics in corn sprouts was a steady increase with germination. The total phenolic content of control increased almost three-fold from days 1–8 (4 mg/g FW) of dark germination. In the case of 5 ml/l FPH and 2 ml/l OE treatment, the highest phenolic content of 4.5 mg/g FW on days 8 and 7 of dark germination was observed. The FPH and OE primed light-germinated seedlings had 29 and 22% higher phenolic levels, respectively, compared to control on day 3, which was associated with a 33 and 22% increase, respectively, in growth on the same day. In general, the antioxidant activity also increased with germination and growth for control and all treatments. The highest antioxidant activity was observed in the case of 2 ml/l OE treatment for sprouts on day 8 with 81% 1,1-diphenyl-2-picrylhydrazyl (DPPH) inhibition. This higher activity coincided with higher phenolic content, showing that total soluble phenolics were contributing to high antioxidant activity. In the case of light-germinated corn seedlings, the antioxidant activity was high during early growth, suggesting that activity was linked to free soluble phenolics as GPX activity indicates that phenolics were not being partitioned to polymerization or lignification at this stage. In dark-germinated sprouts, higher glucose-6-phosphate dehydrogenase (G6PDH) activity was observed during early germination possibly due to the carbohydrate mobilization from the cotyledons directed towards the high nutrient requirements of the growing sprout. In light-germinated seedlings, the G6PDH activity only in FPH primed treatment peaked on day 3, and this increased activity was maintained throughout late stages. A positive correlation between G6PDH activity, phenolics and growth was observed for all treatments and control. Both in the case of dark-germinated corn sprouts and light-germinated seedlings, a steady increase in the GPX activity was observed as the germination progressed, reflecting the plants' need for phenolics for lignification and structural development during growth. This study supports the developmental and partial short-term influence of elicitors on the pentose phosphate pathway (PPP), and its mobilization of enhanced phenolics and antioxidant activity.
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Keywords: Corn (*Zea mays*); PPP (pentose phosphate pathway); Developmental stimulation; Elicitors; FPH (fish protein hydrosylates); Lactoferrin (LF); Oregano extract (OE); Phenolics; G6PDH (glucose-6-phosphate dehydrogenase); GPX (guaiacol peroxidase); Antioxidant activity; Seed priming; Seed vigour

1. Introduction

Corn is a major domesticated cereal (*Zea mays* ssp. *Mays*) of Mexican origin. The plant is used to produce grain and fodder that are the basis for a number of foods, feed, pharmaceutical and industrial products. Due to its adaptability and productivity, it is the third most cultivated field crop after wheat and rice. It dominates American agriculture with production of more than double than any other. It is cultivated on roughly 70–80 million US acres annually, with an annual production of about 9 billion bushels [1]. Research has shown that solid-matrix priming with moistened vermiculite in corn improved germination, reduced lipid

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peroxidation, enhanced antioxidative activities and increased seedling growth [2]. Osmotic priming with chloride salts has been shown to induce salt tolerance in corn [3]. Priming is a technique for enhancing seed vigour and thus improving overall germination and seedling development. The seeds are soaked in aerated osmotic solutions at a concentration diluted enough to permit seeds to imbibe and initiate pre-germination metabolism, but concentrated enough to prevent emergence of the radicle. This practice is known to improve seed vigour, synchronize and accelerate germination, confer stress resistance, antioxidant activity, and improve plant growth and productivity [4–6].

Seed vigour is defined as those seed properties that determine the activity and performance of the seed during germination and seedling emergence [7]. Among the aspects of performance are biochemical processes and reactions during germination such as enzyme reactions and respiration activity, rate and uniformity of seed germination and seedling growth, rate and uniformity of seedling emergence and growth in the field, and emergence ability of seedlings under favorable environmental conditions. Factors that influence the level of seed vigour include the genetic constitution of the seed, environment and nutrition of the mother plant, stage of maturity during harvest, seed size, weight, specific gravity, mechanical integrity, deterioration, aging, pathogens, and priming. Priming treatment advances the embryonic root and shoot tip cells into S and G2 phases of the cell cycle as measured by the increase in the DNA content [8]. Research has shown that early vegetative growth was greatly influenced by improved seed vigour in corn [9].

Phenolics are plant secondary metabolites, primarily synthesized through the pentose phosphate pathway (PPP), shikimate and phenylpropanoid pathways (Fig. 1). The oxidative PPP provides precursor erythrose-4-phosphate for the shikimate pathway. The shikimate pathway converts these sugar phosphates to aromatic amino acids like phenylalanine, which becomes the precursor for the phenylpropanoid pathway that synthesize phenolics. In the plant cells, simple phenolics are believed to be scavengers of free radicals, protecting the cells from free radical damage. Phenolics are also involved in strengthening the plant cell walls during growth by polymerization into lignans and lignins. Plant phenolics have potential health benefits mainly due to their antioxidant properties such as reactive oxygen species scavenging and inhibition, electrophile scavenging, and metal chelation [10]. Epidemiological studies support a relationship between the consumption of phenolic-rich food products and a low incidence of coronary heart disease, atherosclerosis, certain forms of cancer and stroke [11–14]. They have also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity [15,16]. Earlier research has showed that the phenylpropanoid pathway can be stimulated through the pentose phosphate and

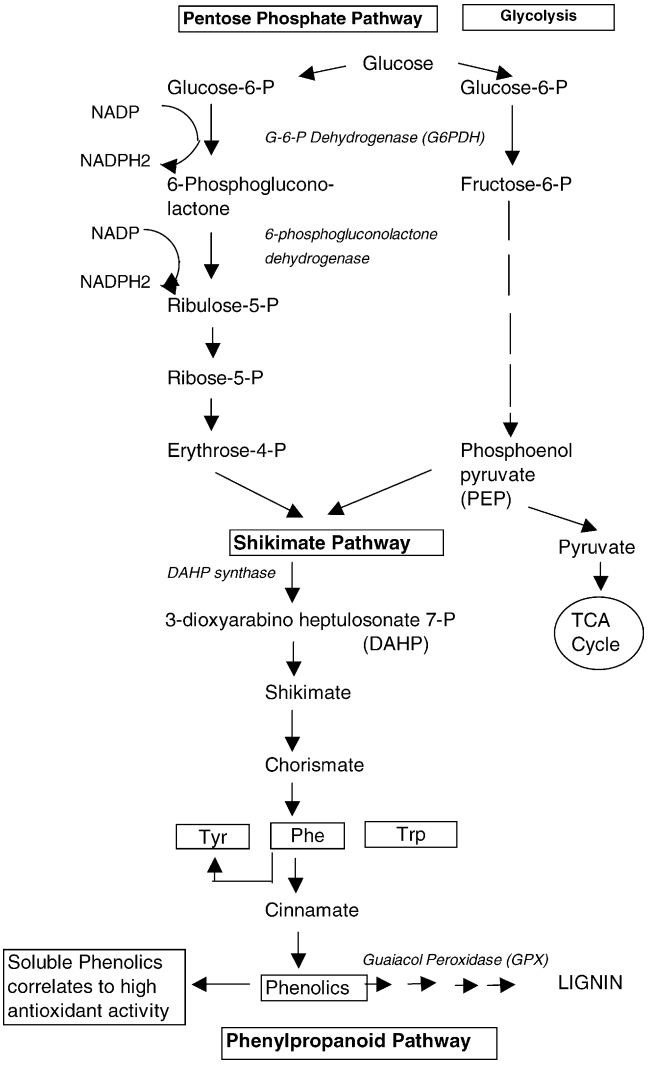

Fig. 1. Pentose phosphate pathway for synthesis of phenolic compounds in corn.

shikimate pathways by phytochemical and small protein elicitors [16–20].

Fish protein hydrolysates (FPH) are small hydrophobic peptides, rich in proline and glutamic acid [17], obtained from seafood waste processed with papain and acid treatment. They have a wide spectrum of applications from high-value peptones, food ingredients and fertilizer production. Earlier research demonstrated that FPH being rich in proline elicits the proline-linked pentose phosphate pathway, shikimate and phenylpropanoid pathways, and therefore increase the phenolic synthesis in peas, mung and fava bean [18–20]. Lactoferrin (LF) is an iron-binding glyco-protein found naturally in milk, saliva mucosal surfaces and within white blood cells. Research has shown LF to be a natural antibiotic, antioxidant, antifungal, antiviral, antitumour and immune booster. Other unique functions attributed to LF include protection from iron-induced lipid peroxidation, immunomodulation, cell growth regulation, DNA and RNA binding, RNAse activity and as a transcriptional factor

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