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L-DOPA and total phenolic stimulation in dark germinated fava bean in response to peptide and phytochemical elicitors

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Abstract

Fava bean sprouts are a rich source of levo dihydroxy phenylalanine (L-DOPA) the precursor of dopamine and is used in the treatment of Parkinson's disease. Its phytopharmaceutical value was improved by priming the seeds with natural elicitors like fish protein hydrolysates (FPH), lactoferrin (LF) and oregano extract (OE). The elicitors in general stimulated the phenylpropanoid pathway through the pentose phosphate and shikimate pathway and enhanced the production of phenolics. Among the different FPH elicitor concentrations, 2 ml/l elicited the highest phenolic content of 3.4 mg/gFW on day 2, which is three times higher than that of control. LF proved to be a better elicitor at a low concentration of 50 ppm producing 5.2 mg of phenolics/gFW on day 3. The response of fava bean treated with 5 ml/l of OE showed the highest phenolic stimulation of 2.9 mg/gFW on day 3. Control, FPH and OE elicitors showed a higher antioxidant activity in germinating fava bean sprout on day 1 and 2 which correlated with higher phenolic content. In fava bean primed with FPH, the glucose-6-phosphate dehydrogenase (G6PDH) and guaiacol peroxidase (GPX) activity peaked on day 5 followed by a concurrent increase in phenolics on day 6 demonstrating the mobilization of carbohydrates from the cotyledons towards the phenylpropanoid pathway in response to the elicitors. In the case of both LF and OE the peak activity of G6PDH and GPX was seen just prior to the boost in phenolics on day 3 and drops to a minimal on day 4 suggesting that the products may allosterically regulate the enzyme. For all elicitors and control the L-DOPA content was high on day 1 and steadily declined with germination. The L-DOPA content in fava bean elicited by LF showed a 40% increase, where as FPH and OE showed a 20% increase compared to that of the control. The maximal stimulation of L-DOPA content was seen on day 2 for fava bean treated with 2 ml/l of FPH, which was 100% higher than that of control.

Keywords: Fava bean (*Vicia faba*); Pentose phosphate pathway; Elicitors; Fish protein hydrolysates; Lactoferrin; Oregano extract; Phenolics; Glucose-6-phosphate dehydrogenase; Guaiacol peroxidase; Antioxidant activity; Levo dihydroxy phenylalanine

1. Introduction

Fava bean (*Vicia faba*) commonly called the broad bean is cultivated widely in most countries of the old world. It plays a vital role in the traditional diets of the Mediterranean, Indian, Chinese, English, Middle Eastern, African and South American people. It is a unique legume because of its extremely rich nutrient content and is now gaining popularity in the new world. It is an excellent source of protein, complex carbohydrates, dietary fibre, choline, lecithin, minerals and secondary metabolites (phenolics and levo dihydroxy phenylalanine (L-DOPA) [1]. L-DOPA the precursor of the neurotransmitter dopamine is naturally found in its seedlings, green pods and beans. The beans are used in the treatment of Parkinson disease, hypertension, renal failure and liver cirrhosis when natriuresis and diuresis are beneficial [1,2]. Parkinson's is a progressive neurodegenerative disease caused by imbalance of dopamine and acetylcholine in the brain. This is usually due to degeneration of the cells that produce dopamine thus altering the brain neurotransmitters that control fine motor movement, gait, memory, intelligence, cognition, mood, sleep, appetite, and gastrointestinal function. L-DOPA is the standard therapy for Parkinson's and is now being supplemented by fava bean ingestion

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[3]. Fava bean consumption increased levels of L-DOPA in the blood with a marked improvement in the motor performance of the patients without any side effects. [1,4]. Its biosynthesis starts with the amino acid tyrosine, a byproduct of the pentose phosphate pathway (PPP) (Fig. 1). An additional hydroxyl group is added to the aromatic ring of tyrosine by the enzyme tyrosine hydroxylase. Tyrosine hydroxylation is the committed step in the synthesis of catecholamines and is subject to feedback inhibition by the end products. This forms L-DOPA, which is decarboxylated by aromatic-L-amino acid decarboxylase to form dopamine [5].

Phenolics are secondary metabolites and in part are produced as a result of the plant's interaction with the environment [6]. They have been reported to exhibit medicinal properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant [7–9]. They are gaining popularity because of their demonstrated effects to reduce the risk of certain cancers, stroke and heart disease [10]. Recent evidence suggests that phenolics also play an important role in the regulation of plant metabolism. For example, flavonoids have been shown to be regulators of auxin transport [11]. Phenolics are primarily produced through the PPP, shikimate and phenyl propanoid pathway. Fig. 1 is a schematic of the major components in their biosynthesis. In oxidative PPP, glucose-6-phosphate dehydrogenase (G6PDH) catalyses the first committed and rate limiting step and its activity is controlled by the ratio of NADP to $NADPH_2$. It regulates the flux of carbon through the PPP providing

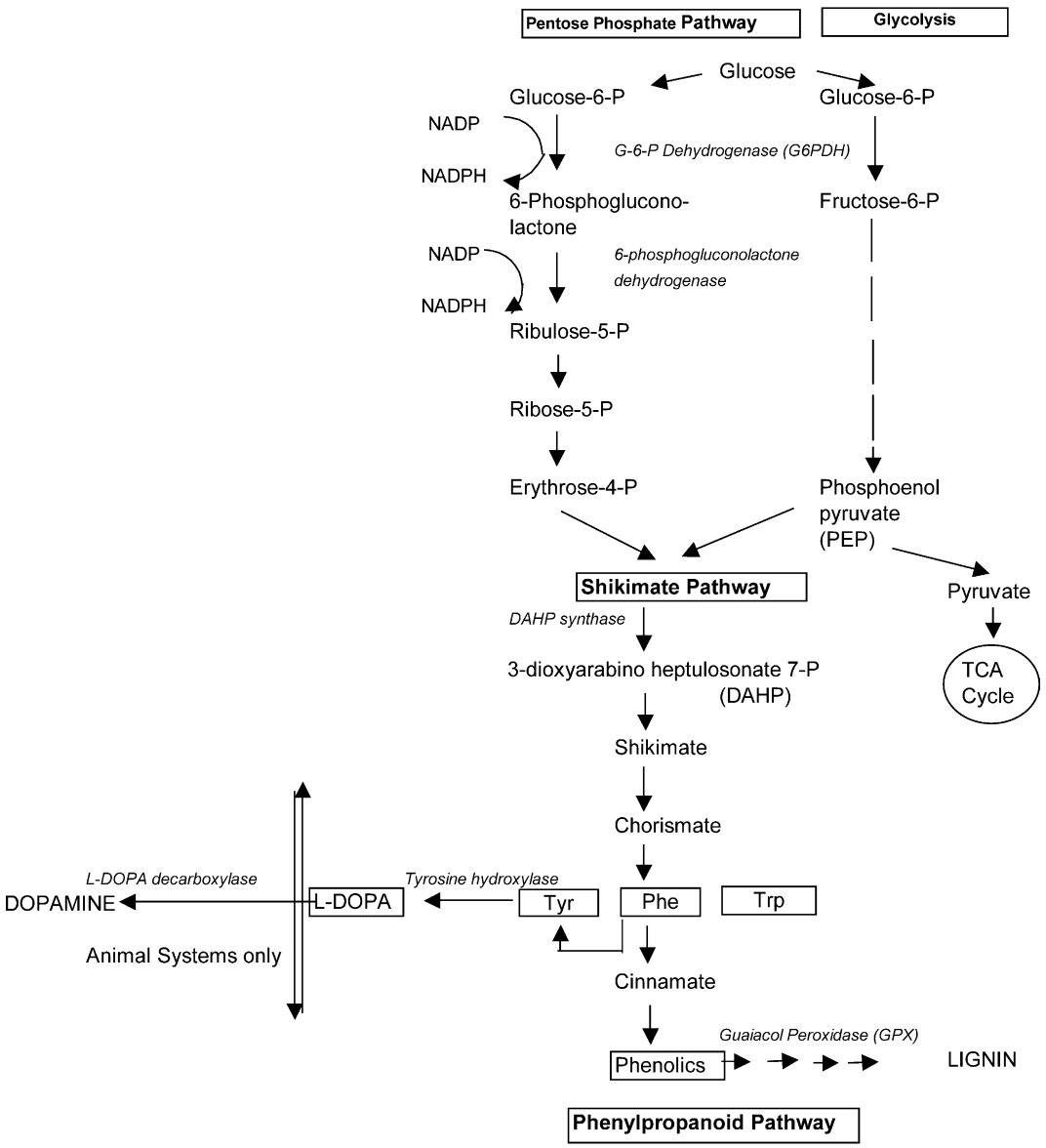

Fig. 1. PPP for synthesis of phenolic compounds and L-DOPA.

precursors or cofactors for other biosynthetic routes. This step provides the conversion of glucose-6-phosphate to 6-phosphoglucono lactone and the concurrent reduction of NADP to $NADPH_2$. This enzyme exists in two isoforms in the plant cell namely the cytosolic form, which is active in the dark and the plastidic form in the chloroplast, which is active in the light [12].

The shikimic pathway gets its substrates erythrose-4-phosphate from PPP and phosphoenol pyruvate from glycolysis to produce phenylalanine. The shikimate pathway converts these simple carbohydrates to aromatic amino acids like phenylalanine, which is the starting material for phenylpropanoid pathway for the synthesis of various secondary metabolites such as rosmarinic acid, flavonoids, phenolic acids, phytoalexins, and lignins. In the phenylpropanoid pathway, phenylalanine ammonia lyase catalyses the conversion of phenylalanine to cinnamic acid, the precursor of phenolics. The enzyme guaiacol peroxidase (GPX) then mediates lignin biosynthesis from phenolic compounds.

This research focused on improving the phytopharmaceutical and functional value of fava beans by the use of natural elicitors. The aim was to stimulate the phenylpropanoid pathway through the pentose phosphate and shikimate pathways thus enhancing the production of secondary metabolites like phenolics and L-DOPA. The natural elicitors chosen for this study were fish protein hydrolysates (FPH), lactoferrin (LF) and oregano extract (OE).

FPH are small hydrophobic peptides rich in proline and glutamic acid [13] obtained from seafood waste processed with papain and acid treatment. The nutritional value of FPH is comparable to other protein sources including meat, milk and eggs. They have a wide spectrum of applications from high value peptones, food ingredients and fertilizer production. These hydrolysates have directed functional characteristics that are used to increase the functional properties of many food products such as emulsification capacity, gelification and coagulation. Earlier research has demonstrated that FPH being rich in proline elicits the proline linked-PPP, shikimate and phenylpropanoid pathways and therefore increase the phenolic synthesis in peas [14–16]. They also have been used to improve organogenesis in in vitro tissue culture of plants [17].

LF is a 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, antitumor 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 [18]. Research shows that LF enters the cell and is transported to the nucleus where it binds to specific DNA sequences and induces transcription of the reporter gene [19]. Since it plays a vital role in primary defence against microbial infections it could be a natural elicitor of phenolics in fava bean.

OE is high in phenolic compounds such as protocatechiuic acid and its phenyl glucoside, caffeic acid, gallic acid, tocopherol and rosmarinic acid [20,21]. It has significant antibacterial, antiviral, antimutagenic and antioxidant activity. They are effective in the inhibition of all phases of the peroxidative process: first neutralizing free radicles, then blocking peroxidation catalysis by iron and finally through interruption of lipid-radicle chain reactions [22]. Elite clonal lines of oregano that produce higher amounts of phenolics and rosmarinic acid in response to *Pseudomonas* inoculation have been developed previously [23]. In this current research we investigated if this OE can stimulate the phenolic pathway in fava bean.

The hypothesis of this research is that the total phenolics and L-DOPA content of germinating fava beans sprouts can be increased by priming seeds with natural elicitors of the PPP, thereby improving the nutraceutical value. Currently there is no research about the use of only fava bean sprouts as a functional food to treat Parkinson's patients. We hope that this research will throw light on the possible use of natural L-DOPA sources as fava sprouts with no side effects in its treatment. The natural elicitors FPH, LF and OE and the different combinations of these elicitors were studied. The parameters measured to characterize the effect of these elicitors were total phenolics, antioxidant activity, G6PDH, GPX and L-DOPA.

2. Materials and methods

2.1. Elicitors and treatments

The three elicitors used in this study were FPH, LF and OE. In order to determine the ideal concentration for maximum elicitor response various dilutions were used. FPH emulsion, a byproduct of mackerel processing was obtained from Conolly Seafood, Gloucester, MA. The FPH dilutions used were 1, 2, 5 and 10 ml/l. LF was obtained from Sigma Chemical Co., St. Louis, MO. Stock solutions were prepared by dissolving LF in distilled water. The LF dilutions used were 10, 25, 50, 100, 250 and 500 ppm. The OE was prepared by soaking 1 g of crushed dried oregano leaves in 50 ml of 95% ethanol for three days. The mixture was pulverized in a blender and centrifuged at 13 000 rpm for 10 min. The supernatant was transferred to a beaker and the ethanol was allowed to evaporate. The residue was then dissolved in 50 ml of distilled water. The OE dilutions tried were 1, 2, 5 and 10 ml/l. After the best elicitor concentrations was determined for phenolic stimulation it was used in combination treatments: FPH + LF,

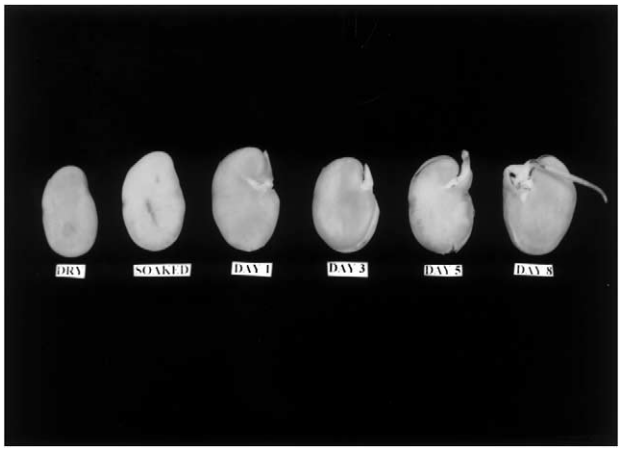

Fig. 2. Stages of Fava bean germination (left to right: Dry seed, Soaked, Day 1, Day 3, Day 5 and Day 8).

FPH + OE, LF + OE and FPH + LF + OE. The best elicitor concentration determined was further used in the antioxidant activity, G6PDH, GPX and L-DOPA determinations.

2.2. Seed treatment and dark germination

Dry seeds of fava bean (*Vicia faba*) were purchased from Stop and Shop Supermarkets, Hadley MA. The seeds were soaked in distilled water for the control and in distilled water plus FPH/LF/OE. Approximately 50 seeds were placed in 500 ml of the soak solution in 1000 ml conical flasks. The flasks were then placed in a rotary shaker at 150 rpm for 24 h. The pre-soaked seeds were washed in distilled water and germinated in flats lined with moist paper towels. The flats were covered with aluminum foil and the seeds were germinated in the dark (Fig. 2). The germinating seeds were kept moist with distilled water and the assays were performed daily for the next 8 days. After 8 days of germination the hypocotyls etiolate and the first pair of leaves emerge. The bean sprouts are thus marketable during the first week of dark germination. Three replications were made for each treatment and the average of each treatment represented each data point on the graphs. In all assays only the hypocotyls of the fava bean was used because earlier research showed that the hypocotyls contained more phenolics than the cotyledons [24].

2.3. Total phenolics assay

Total phenolics were measured as gallic acid equivalents [25]. The assay was developed by Chandler and Dodds [26] and modified by Shetty [27]. Approximately 50 mg of the hypocotyls was immersed in 2.5 ml of 95% ethanol and kept in the freezer for 48–72 h. The sample was homogenized using a tissue tearer (Biospec Products, Bartleville, OK) and centrifuged at 13 000 rpm for 10 min. One millilitre of the supernatant was transferred to a test tube and 1 ml of 95% ethanol, 5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent (Sigma Chemical Co.) were added. After an incubation period of 5 min 1 ml of 5% Na_2CO_3 was added mixed well and kept in the dark for an hour. Then the samples were vortexed and the absorbance was measured at 725 nm using a UV spectrophotometer (Spectonic Genesys 5; Milton Roy Company, Rochester, NY).

2.4. Antioxidant assay

The antioxidant activity of fava bean phenolic extract were determined by the β-carotene oxidation model system as described by Miller with some modifications [28–30]. The β-carotene solution was prepared by dissolving 10 mg of β-carotene in 50 ml of chloroform in amber coloured flask to prevent light oxidation. One ml of this solution was pipetted to a flask covered with aluminum foil. Chloroform was then evaporated under vacuum at 40 °C for 5 min. Then the β-carotene was dissolved in 20 μl of linolenic acid and 184 μl of Tween 40 emulsifier. Then added 50 ml of H_2O_2 solution (176 μl H_2O_2 in 100 ml distilled water) and mixed thoroughly till the β-carotene was completely dissolved. To 100 μl of the phenolic extracts 5 ml of this prepared β-carotene solution was added. Control tubes had 100 μl of 95% ethanol. As soon as the emulsion was added the zero time absorbance was read at 470 nm. Subsequent absorbance readings were recorded after a 30 min incubation period in a 50 °C water bath. The protection factor was used to express antioxidant activity as a ratio of sample absorbance at 30 min to that of the control.

2.5. Total protein assay

A cold pestle and motor was used to thoroughly grind 100 mg of the fava bean sprouts in cold enzyme extraction buffer (0.5% polyvinylpyrrolidone, 3 mM EDTA. 0.1 M potassium phosphate buffer of pH 7.5). The sample was centrifuged at 13 000 rpm for 15 min at 2–5 °C and stored on ice. The supernatant was used in the estimation of total protein, G6PDH and GPX enzyme assays.

The protein content was measured by the Bradford method [31]. The Bradford dye reagent was prepared by diluting the commercial dye concentrate in a 1:4 ratio with distilled water. To 100 ml of the sample and blank (extraction buffer only) in test tubes 5 ml of the dye was added and incubated at room temperature for 5 min. The samples were mixed and the absorbance was read at 595 nm using a UV spectrophotometer.

2.6. Glucose-6-phosphate dehydrogenase assay

A modified version of the assay described by Deutsch [32] was followed. The enzyme reaction mixture containing 5.88 μmol B-NADP, 88.5 μmol $MgCl_2$ and 53.7 μmol glucose-6-phosphate, 0.77 mmol malemide was prepared. This mixture was used to obtain basal blank of the spectrophotometer at 339 nm wavelength. Then 1 ml of this mixture was taken in 1.5 ml plastic cuvettes and 50 μl of the enzyme extract was added. The change is absorbance was monitored for over a period of 5 min. The rate of change in absorbance per minute was used to quantify the enzyme in the mixture using the extinction co-efficient of $NADPH_2$ (6.22/M/cm). The enzyme was quantified in nanomoles per minute per milligram of protein.

2.7. Guaiacol peroxidase assay

The assay followed was a modified version developed by Laloue and George [33,34]. The enzyme reaction mixture containing 0.1 M potassium phosphate buffer (pH 6.8), 50 mM guaiacol solution and 0.2 mM hydrogen peroxidase was prepared. Used this mixture to blank the spectrophotometer at 470 nm. Then 1 ml of this reaction mixture was taken in a 1.5 ml plastic cuvettes and 50 μl of the diluted enzyme extract (1:10) was added. The change in absorbance was monitored for a period of 5 min. The rate of change in absorbance per minute was used to quantify the enzyme in the mixture using the extinction co-efficient of the oxidized product tetraguaiacol (26.6m/M/cm). The GPX enzyme was quantified in nanomoles per minute per milligram of protein.

2.8. High performance liquid chromatography analysis of L-DOPA

Fava bean sprouts (200 mg) were immersed in 95% ethanol and kept in the freezer for 48–72 h. They were then homogenized in a tissue tearer (Biospec Products) and centrifuged at 13 000 rpm for 10 min. The supernatant was transferred to a 25 ml beaker and the ethanol was evaporated. The residue was dissolved in 10 ml of buffer solution (32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM Na_2EDTA, 0.215 mM octyl sulphate pH 4). This solution was filtered through 0.45 μm disposable syringe filters (Schleicher and Schuell, Keene, NH).

High performance liquid chromatography (HPLC) was performed using a Hewlett-Packard 1090 liquid chromatograph equipped with a 1040 diode array detector. The analytical column was a reverse phase Supleco Discovery C18, 250×4.6 mm^2 with a packing material of 5μm particle size. The detector was set at 280 nm with an optical bandwidth of 4 nm. Absorption at 550 and 4 nm bandwidth was used as a reference wavelength. Total composition of the mobile phase was 18% methanol and 82% buffer at the flow rate of 1ml/min. Tyrosine and catecholamines standards (L-DOPA, dopamine, norepinephrine and epinephrine) standards (Sigma Chemicals) were chromatographed separately and in mixture. The sample was chromatographed under the same conditions. Retention time and spectrum was compared with that of the standard L-DOPA. The amount of L-DOPA in the fava bean hypocotyls was measured from the peak height obtained at 280 nm and was expressed in terms of milligrams per gram fresh weight.

2.9. Statistical analysis

Statistical significance was determined using analysis of variance data analysis software that is standard on Microsoft Excel 97.

3. Results and discussion

The total phenolic content of the fava bean hypocotyls was estimated for 8 days of dark germination. The different FPH concentrations tested were 1, 2, 5 and 10 ml/l of the soak solution. Among the different FPH elicitor concentrations tested 2 ml/l gave the highest phenolic content of 3.4 mg/gFW (Fig. 3) on the second day, which is four times higher than that of control. The phenolic content reduced the next few days and peaked again to 2.6 mg/gFW on day 6. Based on this observation we predicted that the glutamate and/or proline present in FPH accumulates in the seeds during the soaking period [35] and this stimulates the PPP (Fig. 1) triggering the increased production of polyphenols. The different LF concentrations tested were 10, 25, 50, 100, 250 and 500 ppms. LF proved to

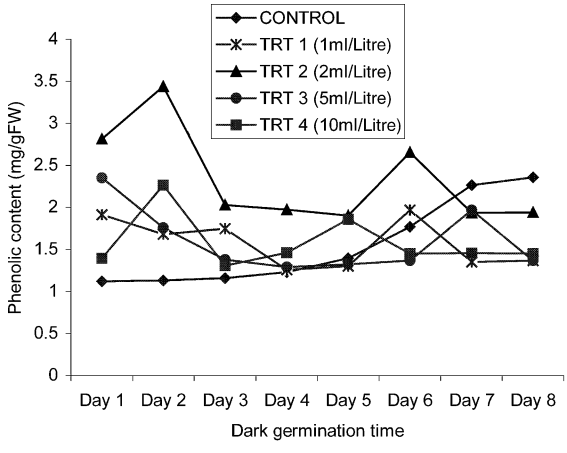

Fig. 3. Effect of FPH on the phenolic content of dark germinating fava bean.

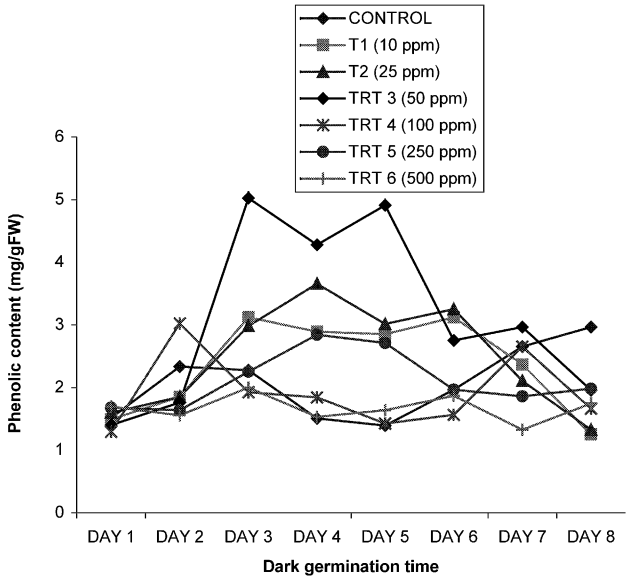

Fig. 4. Effect of LF on the phenolic content of dark germinating fava bean.

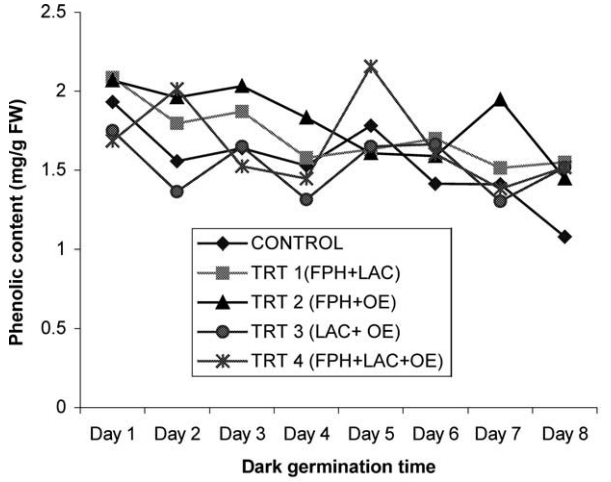

Fig. 6. Effect of the combination of FPH, LF and OE on the phenolic content of dark germinating fava bean.

be a better elicitor at a low concentration of 50 ppm producing 5.2 mg of phenolics/gFW on the third day (Fig. 4). Seed treatment with LF also kept the germinating fava bean healthy with minimal contamination by bacteria and fungi. For all elicitor concentrations the phenolic content increased on days 3 and 4 and slowly declined as the germination progressed. The different OE concentrations tested were 1, 2, 5 and 10 ml/l of the soak solution. The response of fava bean treated with 5 ml/l of OE showed the highest phenolic content of 2.9 mg/gFW on day 3 (Fig. 5). The antimicrobial effect of OE and LF was obvious as they reduced bacterial contamination of the germinating fava bean by 50 and 47%, respectively. FPH did not have antimicrobial action. Surprisingly none of the combination treatments of the three elicitors were cumulative in stimulation of total phenolics as expected and was very similar in comparison with the control (Fig. 6).

Antioxidant activity in relation to total phenolic content was also followed. In fava bean treated with 2 ml/l FPH the antioxidant activity was higher on day 2 (Fig. 7) in correlation with the higher phenolic content and decreased as germination proceeded. Control, FPH and OE elicitors showed a higher antioxidant activity in germinating fava bean sprout on day 1 and 2 in correlation to higher phenolic content. This shows that initially phenolics are antioxidant in nature and lower proportions of phenolics are being partitioned for lig-

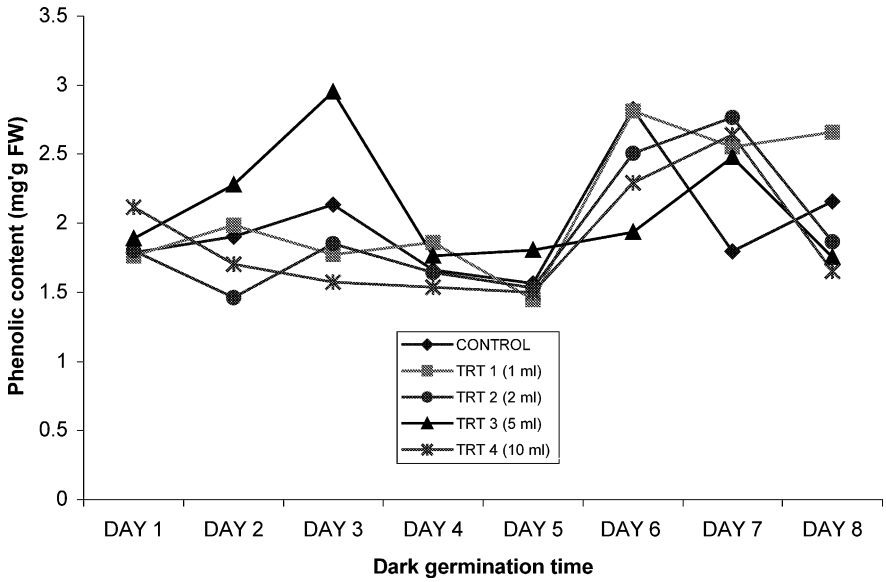

Fig. 5. Effect of OE on the phenolic content of dark germinating fava bean.

nification. This may be due to early stages of germination requiring higher demand for oxygen and therefore phenolics might be protecting the cells from potential oxidation-induced deterioration. The antioxidant activity reduced the next few days and slightly increased on day 7 and 8 in the case of OE treatment. In control treatment antioxidant activity was high on day 1 and in the following days was comparatively lower than the treatments.

G6PDH is the first committed enzyme in driving the PPP towards the production of phenolics and L-DOPA. Germinating seeds in the dark activates the G6PDH isoform present only in the cytosol [36]. For all elicitors and control treatments a high G6PDH activity was recorded during early germination which may be due to the general mobilization of carbohydrates to the growing hypocotyls [37]. When fava bean was treated with 2 ml/l of FPH, the G6PDH activity slowly decreased with germination reaching the lowest activity on day 4 and

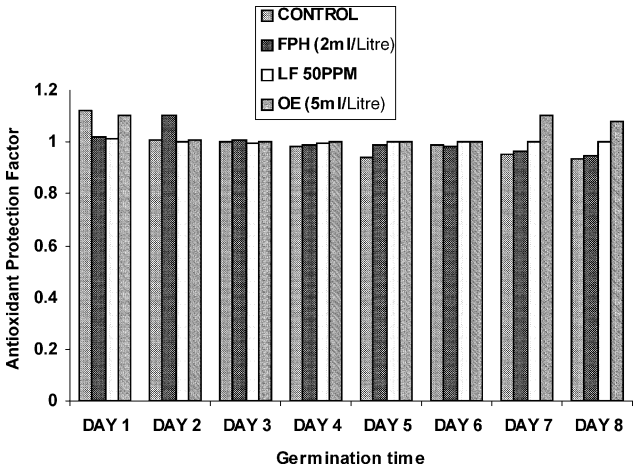

Fig. 7. Effect of FPH, LF and OE on the average antioxidant activity in dark germinating fava bean.

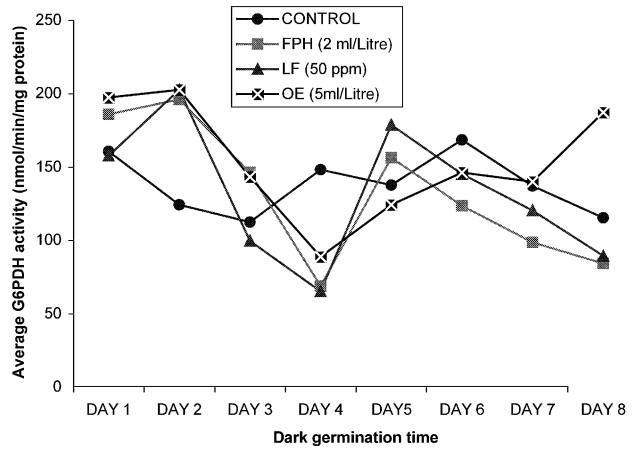

Fig. 8. Effect of FPH, LF and OE on G6PDH activity in dark germinating fava bean.

then peaked on day 5 (Fig. 8). This was followed by a concurrent increase in phenolics on day 6. This indicated that G6PDH likely mobilized carbohydrates in the cotyledons, directing them towards the phenylpropanoid pathway in response to the elicitor. It is also possible that G6PDH was instantaneously stimulated by the elicitor during the soaking period, which is reflected in high levels of phenolics and L-DOPA during the second day of germination. As mobilization occurred an allosteric feedback inhibition by sugar-phosphates was possible thus G6PDH activity was low on days 2 to 4. In the case of fava bean stimulated with 50 ppm of LF the G6PDH activity was high on day 2 and declined to a minimal on day 4 and slowly increased again. In fava bean treated with 5 ml/l OE the G6PDH activity was high with the advent of germination, reached the minimal point on day 4 and slowly increased after day 4. In the case of both LF and OE the peak activity of G6PDH was seen just before the boost in phenolics on day 3 and drops to a minimal on day 4 suggesting that the products may allosterically regulate the enzyme. When its activity increased on day 5 the increase in phenolic activity was seen on day 6. This indicates a pattern of increased G6PDH activity followed by higher phenolics, then lowered activity due to potential feedback inhibition and concurrent reduced phenolics.

GPX an isoenzyme of peroxidase which catalyses the conversion of phenolics from the phenylpropanoid pathway to lignin and lignans [38]. Peroxidase is known to be responsible for the cross linking of phenolic moieties during the biosynthesis of lignins in the plant cell wall [39]. It is also involved in numerous processes such as ethylene production, wound healing, aromatic compound degradation, pathogen defence, stiffening and degradation of indole-3-acetic acid [40]. Increase in peroxidase activities in plants following treatments with pathogens, elicitors or biotic and abiotic stress is well documented [41]. In fava bean treated with FPH a steady increase in the GPX activity was observed as the germination progressed reaching a maximum on day 5, which was followed by higher levels of phenolics on day 6 (Fig. 9). In the case of fava bean stimulated with LF and OE the GPX activity was high on day 3 in correlation with high levels of phenolics on day 3 and then declined slightly with germination. This response is very similar to a stress response where peroxidase activity is triggered accompanied by an accumulation of phenolics [42]. The antioxidant activity of the phenolics being minimal on the subsequent days suggests that most phenolics are being polymerized to form lignin required during germination. Lignin is also known to have antioxidant properties but other more relevant experimental antioxidant assays have to be undertaken to investigate the true antioxidant potential. The GPX activity of the control was comparatively stable having

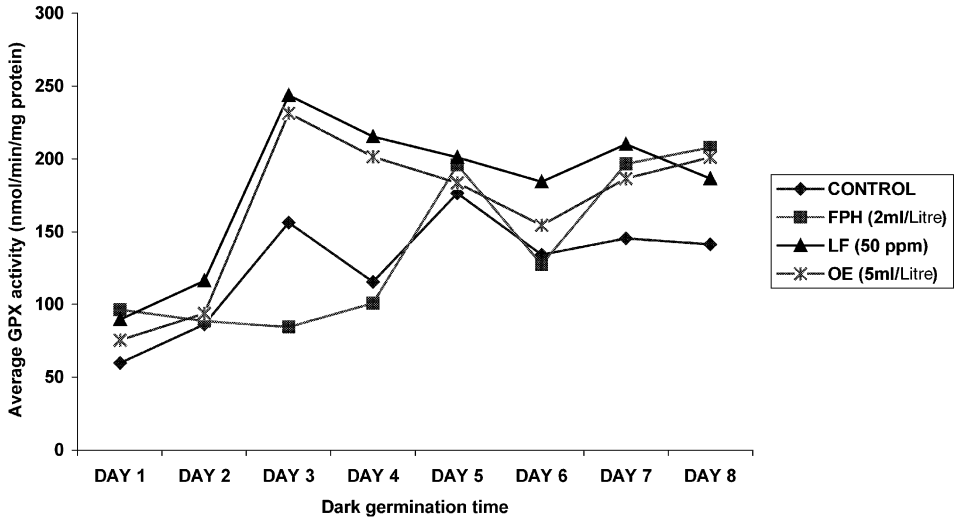

Fig. 9. Effect of FPH, LF and OE on GPX activity in dark germinating fava bean.

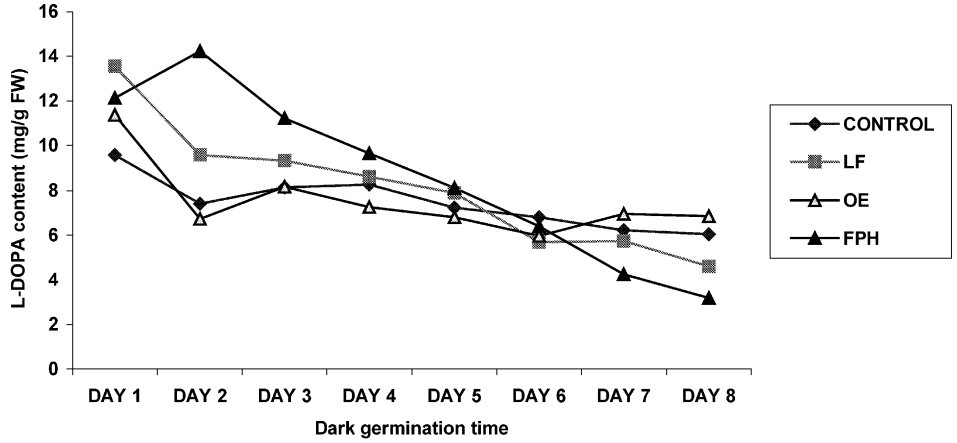

Fig. 10. Effect of FPH, LF and OE on L-DOPA content in dark germinating fava bean.

a steady rise as the germination proceeded reflecting the plants need for lignification.

The L-DOPA content in the phenolic extracts of the dark germinated fava bean sprouts was measured using HPLC. HPLC of the phenolic extract from all three elicitor treatments showed only one major peak in the chromatogram, which was identified to be L-DOPA. For all the elicitors studied and control the L-DOPA content was high on day 1 and slowly declined with germination (Fig. 10). The L-DOPA content in fava bean elicited by 50 ppm LF showed a 40% increase over that of the control on day 1. The elicitors FPH (2 ml/l) and OE (5 ml/l) showed a 20% increase over that of the control. The maximal amount of L-DOPA content was seen on day 2 for fava bean treated with 2 ml/l of FPH, which was 100% higher than that of control. The L-DOPA content was high initially and slowly declined, but was immediately followed by increased phenolic content. This suggests that the precursor metabolites were potentially diverted from L-DOPA production towards the total phenolics, which are required for lignification during germination. This was also evident from the increase in levels of G6PDH and GPX as germination proceeded. The higher levels of L-DOPA correspond to higher antioxidant activity in initial stages of germination suggesting that perhaps L-DOPA was partially antioxidant in nature. During the initial stages of germination most phenolics were diverted toward antioxidant and L-DOPA production when the need for lignification is minimal. It was also evident from L-DOPA and total phenolics data that as L-DOPA levels were reduced over the germination period the phenolic content in general increased in later stages. Further as phenolic content increased in later stages there was a corresponding increase in GPX activity indicating a potential polymerization to lignin or lignan structures.

Plant phenolics, an important group of secondary metabolites are intermediates in the phenylpropanoid metabolism. In plants they are essential to the plant

physiology, morphology, growth and reproduction, defense, and stress response [43,44]. The phenolic levels are affected by many internal and external factors such as phytohormones and growth-regulating substances [45]. They play a vital role in plant development during seed germination, plant-microbe recognition and signal transduction [46]. In recent years food biotechnologists are keen to harness the nutritional benefits of these phenolics namely its: antioxidant or free radicle scavenging ability by donating hydrogen atoms to free radicals, chelating of metal ions that catalyze lipid peroxidation, food preservative, therapeutic and pharmaceutical properties [16,47]. They have tremendous application in the prevention and treatment of highly prevalent human diseases such as cardiovascular disease and cancer, gastric and duodenal ulcer, allergy, vascular fragility, viral and bacterial infections and Parkinson's disease [38]. In this context the phenolic stimulation by FPH, LF and OE elicitors was studied. Clinical studies show that in the treatment of Parkinson's disease the consumption of the unsprouted fava bean in large quantities in order to get the desired amount of L-DOPA caused flatulence in the patients. Hence, perhaps the consumption of just the elicited and sprouted fava bean with high L-DOPA and phenolic content, which is cheap and readily available would be a better option. The above experiments give an understanding and some insight into metabolic interconversions between phenolics and L-DOPA. Understanding the behavior of natural and food-grade elicitor responses is an important step towards the future development of value-added foods with elicited phytochemicals. This study lays the basis for future research on improving the nutraceutical value of functional foods using natural elicitors.

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