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The Effect of Coumarin Application on Early Growth and Some Physiological Parameters in Faba Bean (*Vicia faba* L.)

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Abstract Many coumarins have been identified from natural sources, especially green plants. These compounds affect many plant activities and can also control growth processes. The effect of coumarin (COU) on germination, early growth, nutrient mobilization, and some physiological parameters of faba bean (*Vicia faba* L.) was researched. Seeds of faba bean were primed with different concentrations of COU (0.5, 1.0, 2.0, and 4.0 mM) to elucidate the effect on germination and nutrient mobilization. Accordingly, a greenhouse pot experiment was conducted to study the effect of 1.0 mM COU, as a seed priming treatment alone or in combination with foliar application, on the growth parameters, some biochemical constituents from primary and secondary metabolism and phytohormones of faba bean. The impact of COU was more pronounced on growth than germination, and was dependent on concentration and the mode of application. Both COU treatments significantly improved the level of primary and secondary metabolites as well as phytohormones. These data suggest that COU can affect the growth and physiology of faba bean either directly, as an active growth substance, or indirectly by its interaction with the metabolism of phytohormones.

Keywords Coumarin · *Vicia faba* · Phytohormones · Germination · Growth · Sugars · Proteins · Phenolics

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Introduction

Plant growth and development are regulated by their endogenous signals and affected by environmental factors (Taiz and Zeiger 2010). Phenolic compounds, which are the most widely distributed secondary metabolites in the plant kingdom, can affect plant growth by their interference with the metabolism of phytohormones and consequently affect their endogenous levels (Einhellig 2004; Cheynier and others 2013). Earlier studies showed the interaction of different types of exogenous phenolic compounds with the biological activities of indole-3-acetic acid (IAA), gibberellic acid (GA_3), and abscisic acid (ABA) (Tomaszewski and Thimann 1966; Ray and others 1980; Datta and Nanda 1985; Li and others 1993; Peer and Murphy 2007).

Among plant phenolics, coumarins are widely distributed in the natural plant communities and are potent allelopathic agents (Razavi 2011). They are well known to interfere with several physiological processes associated with seed germination as well as plant growth and development (Wolf 1974; Murray and others 1982; Li and others 2011). Several studies have focused on the inhibitory effect of coumarin (COU; 2H-chromen-2-one), the simplest member of coumarins, on germination and root growth (Wolf 1974; Kupidlowska and others 1994; Abenavoli and others 2006). However, other investigations reported the growth stimulating activity of COU (Neumann 1960; Lupini and others 2010; Al-Wakeel and others 2013). In addition, the auxin-like behavior of COU and/or its interference with auxin signaling pathways has been suggested (Neumann 1959; Letham 1978; Abenavoli and others 2001; Tartoura and others 2004; Lupini and others 2010). Thus, the impact of COU on plant growth seems to be specific to species, application method, and concentration (Abenavoli and others 2004; Pergo and others 2008).

Foliar application of different nutrients is a common technique used to enhance plant growth (Mohammad Ali 2011; Garde-Cerdán and others 2014). Seed priming is a technique for improving seed germination and vigor under a broad range of environments; however little is known about the physiological processes associated with priming (Manonmani and others 2014). As far as we know, there is no information about its influence on levels and biological activities of endogenous phytohormones (Doğramaci and others 2014; El-Yazal and others 2014).

The present study focused on germination and early growth of faba bean (*Vicia faba* L.) affected by the application of different levels of COU, to improve seedlings establishment and enhance growth, while simultaneously assessing the enhancement of endogenous phytohormones (IAA, GA_3, and ABA). Furthermore, after seed priming alone or in combination with foliar application, different metabolites from primary (carbohydrates and proteins) and secondary (phenolics) metabolism were also investigated in a greenhouse pot experiment.

Materials and Methods

Germination Experiment

Seeds of faba bean (*Vicia faba* L.) cultivar Giza 40 were surface sterilized using 0.1 % (w/v) $HgCl_2$, washed several times with distilled water, and then primed with different concentrations (0.5, 1.0, 2.0, and 4.0 mM) of COU (Sigma Chemical Co., St. Louis, USA) by soaking them in the appropriate concentration for 6 h. COU was dissolved in the least amount of ethanol then water was added to the final volume. Some seeds were soaked in the same amount of ethanol and water to serve as controls. After that, 20 uniform seeds from each group were placed in each of five Petri dishes (15 cm diameter) which had been lined with two layers of filter paper and moistened with 10-ml distilled water. The Petri dishes were incubated at 22 °C in the dark for 7 days. The emergence of a 1 mM radicle was used as the criterion for germination. At the end of the incubation period, the lengths of the plumule and radicle were measured in five seedlings picked up randomly; the seedling fresh weight was determined and followed by oven-drying for dry weight measurements. Some seedlings were ground under liquid nitrogen and kept at −20 °C for amylase and protease activity determination.

Greenhouse Pot Experiment

Based on its impact on germination and early seedling growth, the concentration of 1.0 mM was selected to study the effect of COU on the growth parameters and biochemical constituents of bean plants. Faba bean seeds were soaked in 1.0 mM COU for 6 h or in water to provide a control; thereafter seven seeds were sown in plastic pots (15 cm diameter × 20 cm depth) filled with mixture of clay/sand (1:1, w/w). Before sowing calcium superphosphate (0.3 g/kg soil), ammonium nitrate (0.3 g/kg soil) and potassium sulfate (0.15 g/kg soil) were added to the soil mixture. After emergence, the seedlings were thinned to five seedlings per pot. Pots were maintained in a greenhouse with a 13 h photoperiod at 22 °C ± 2. Plants were irrigated every 7 days with tap water to field capacity. We stopped watering when the water began to leak through the bottom of the pot.

Two weeks after sowing, plants grown from COU-treated seeds were divided into two groups with ten pots each. Plants of the first group were sprayed once (5 ml per plant) with 1.0 mM COU (dissolved in least amount of ethanol then filled to the final volume with distilled water). Plants of the second group as well as the control were sprayed with the same amount of solvent (ethanol plus distilled water) without COU; the soil surface was covered with polyethylene sheets to avoid absorption by the roots. Samples for morphological and biochemical analyses were taken on the 14th day after foliar spraying. Lengths and fresh weights of roots and shoots as well as number of leaves and leaf area were recorded. Plant samples were dried in an oven at 60 °C to provide a constant weight for dry weight measurements. The net assimilation rate (NAR) was calculated according to Alvim (1960). Some fresh leaves were washed with water and blotted then quickly frozen and ground to fine powder in liquid nitrogen and stored at −20 °C until used.

Amylase and Protease Assays

Protease was extracted by homogenizing 7-day-old seedlings in 20 mM phosphate buffer, pH 7.6, with a pre-chilled pestle and mortar. The homogenate was centrifuged at 10,000×*g* for 10 min at 4 °C. For amylase extraction, 100 mM acetate buffer, pH 6.0, was used instead of the phosphate buffer. The supernatants were kept at −20 °C until use.

Proteolytic activity was assayed using bovine serum albumin (BSA) as substrate. The reaction mixture contained 0.5 ml of the crude extract and 2 ml of the substrate solution (20 mM phosphate buffer, pH 7.0, containing 10 mg/ml BSA). After 60 min of incubation at 40 °C, the reaction was stopped by adding 2.0 ml of 10 % trichloroacetic acid and heating briefly in boiling water to precipitate undigested albumin. After centrifugation, the concentration of the resulted soluble peptides was measured by the modified Folin-Lowry method adopted by Hartree (1972).

Amylase activity was measured by mixing 0.5 ml of the crude extract with 0.5 ml of 0.5 % soluble starch prepared in 0.1 M of acetate buffer, pH 6.0, containing 5 mM $CaCl_2$. The reaction was terminated by $HgCl_2$ after 30 min of incubation at 40 °C. The resulting reducing sugars were estimated by the Nelson's method (Clark and Switzer 1977).

Hormone Analysis by Gas Chromatography

Analyses of IAA, GA_3, and ABA were determined according to the method outlined by Baydar and Harmankaya (2005) with some modifications. Two grams of powdered leaf tissues was homogenized in 14 ml of 80 % aqueous methanol then 4 ml of chloroform was added. The slurry was maintained for 1 week at −20 °C with occasional shaking. Each extract was filtered through a Whatman No. 3 filter paper and the residue re-homogenized with the same solvent. The combined extracts were adjusted to pH 8.5 with 1 N NaOH and transferred through a separating funnel to separate the aqueous phase from chloroform. The chloroform phase was discarded, and the aqueous phase was concentrated under reduced pressure in a rotary evaporator at 40 °C. The aqueous phase was then adjusted to pH 2.5 with 1 N HCl and partitioned three times against ethyl acetate to extract acidic free hormones, then dried under vacuum at 40 °C. The residue was dissolved in 1 ml absolute methanol and transferred into an Eppendorf tube.

IAA, GA_3, and ABA levels were estimated with a Fisons 8560 HRGC Mega 2 series equipped with a flame ionization detector (FID) and using a SPB-1 (30 m × 0.32 mM, ID) capillary column. Injection and detector temperatures were 200 °C and 300 °C, respectively. Samples (1 µl) were injected into the column at 80 °C, and the temperature was programmed to 5 °C min^{-1} until the column was at 280 °C. Helium flow rate was 1 ml min^{-1}, and inlet pressure was 22 psi. IAA, GA_3, and ABA were quantified using peak areas. The ratio of detector response to putative IAA, GA_3, and ABA peaks in faba bean leaf samples was compared to the response ratio of the detector for authentic standards (Sigma).

Extraction and Determination of Sugars

Water-soluble carbohydrates were extracted by boiling a known weight of dry powdered leaf tissues in distilled water for 1 h in a water bath (El-Tayeb and others 2006). The extract was cooled and centrifuged at 5,000×*g* for 10 min, after which the supernatant was made up to a known volume. For hydrolysis of the non-reducing sugars, 1 ml of the extract was mixed with 1 ml 6 N HCl and heated for 12 min at 70 °C followed by neutralization with NaOH (Radwan and others 2007).

Total carbohydrates were extracted by boiling a known weight of dry tissue in 1 N HCl for 1.5 h. The extract was cooled and centrifuged at 5,000×*g* for 10 min. The supernatant was neutralized with 1 N NaOH and made up to a known volume with distilled water. Reducing value of each sugar extract was determined according to the method adopted by Clark and Switzer (1977).

Extraction and Determination of Proteins

Extraction of water soluble and insoluble proteins was carried out according to the method described by El-Tayeb and others (2006). Soluble protein was extracted by incubating 100 mg of dry powdered tissues in 10-ml distilled water for 2 h at 90 °C. After cooling, the mixture was centrifuged at 5,000×*g* for 10 min. For the extraction of water-insoluble protein, the remaining residue was homogenized with 10 ml of 1 N NaOH for 2 h at 90 °C. The mixture was centrifuged at 5,000×*g* for 10 min and the clear supernatant neutralized with HCl. Each extract was filled to known volume with distilled water. Proteins determination was carried out according to the modified Folin-Lowry method adopted by Hartree (1972).

Extraction and Determination of Phenolics

Phenolic compounds were extracted according to the method outlined by Sauvesty and others (1992). A known weight of the dried powdered tissues was extracted three times with 70 % ethanol at 40 °C for 4 h. Each extract was centrifuged for 15 min at 3,000×*g*. The clear supernatants were combined, then reduced under low pressure at room temperature, and made up to a known volume with distilled water, then used for determination of phenolic aglycones. This extract contained phenolic aglycones and glycosides. One milli liter of this extract was hydrolyzed with 1 ml of 2 N HCl in a boiling water bath for 1 h to cleave the glycoside linkage into sugar and aglycone. The mixture was neutralized and filled to a known volume with distilled water, then used for the determination of total phenolics. The Folin–Ciocalteau phenol method (Lowe 1993) was used for phenolic aglycone determination.

Statistical Analysis

Experiments were carried out following a randomized complete block design. Data normality and the homogeneity of variances were checked using the Kolmogorov–Smirnov test and Levenés test, respectively. All the data were subjected to one-way analysis of variance (ANOVA). Duncan's Multiple Range Test ($p \leq 0.05$) was carried out as the post hoc test for mean separations. Where needed,

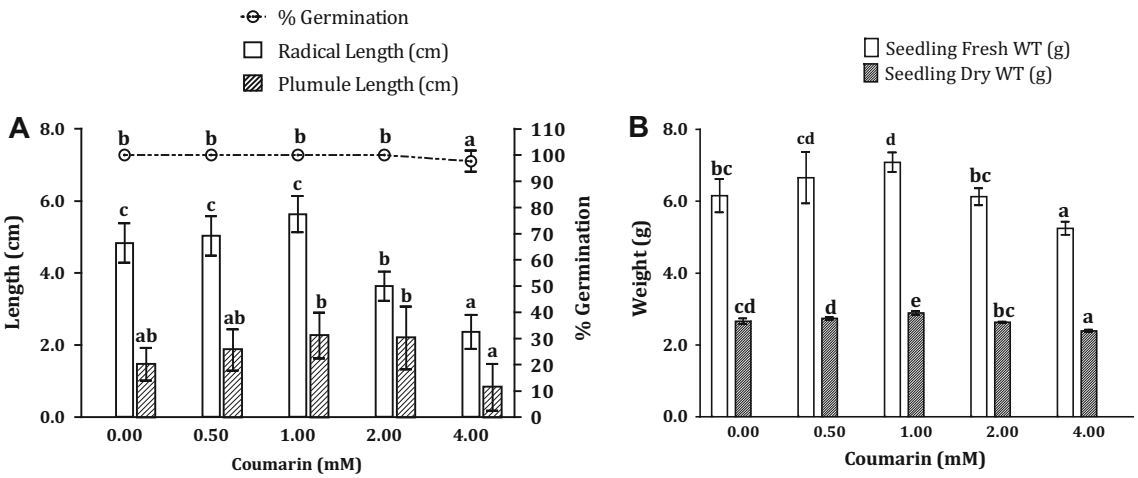

Fig. 1 Effect of different coumarin seed pre-treatments on **a** germination and organ elongation and **b** biomass of *Vicia faba* seedlings. Different letters indicate significant difference among the treatments within each parameter ($P < 0.05$) as given by Duncan test

data were transformed by log (x+1) before statistical analysis. All statistical tests were performed using the computer program PASW statistics 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Germination Experiment

Germination Percent, Radicle, and Plumule Growth

The effect of different concentrations (0.5, 1.0, 2.0, and 4.0 mM) of COU as seed priming treatment on germination and nutrient mobilization in faba bean seeds was studied. The results presented in Fig. 1 showed that COU treatment does not affect the percentage of germination of *Vicia faba* seeds except for the highest concentration (4.0 mM), which significantly inhibited the germination process. Nevertheless, germination speed was clearly affected by COU application, with a strong reduction in the 2.0 and 4.0 mM treatments, 16.7 and 28.3 %, respectively ($P \leq 0.05$). Concerning the radicle and plumule growth, increasing the concentration of COU up to 1.0 mM had no significant effect on the radicle elongation, whereas concentrations of up to 1.0 mM significantly increased the plumule length (Fig. 1). The highest dose of COU (4.0 mM) severely retarded the elongation of radicle and plumule, where the radicle was more sensitive than the plumule. Both fresh and dry masses of seedlings were significantly increased by 1.0 mM COU. The 4.0 mM COU treatment significantly decreased the fresh and dry masses by about 15 and 10 %, respectively, compared to the control.

Nutrient Mobilization

The activities of amylase and protease as well as the content of soluble sugars and proteins were estimated to explore the effect of COU treatment on the nutrient mobilization process in the germinating seeds. The modest dose of COU (1.0 mM) significantly improved the activities of both amylase and protease, where it offered about a 17 % increase in the activity of both enzymes, compared to the control (Fig. 2). On the other hand, the highest concentration (4.0 mM) was inhibitory for amylase and 2.0 and 4.0 mM for protease (14 and 33 % respectively). Soluble proteins were significantly accumulated at 0.50 and 1.00 mM COU (16 and 25 %, respectively), whereas soluble sugars were significantly stimulated at 1 mM COU (24 %).

Greenhouse Pot Experiment

Based on the results of the germination experiment, the concentration of 1.0 mM was selected to study the effect of COU on the growth parameters and biochemical constituents of bean plants. COU treatments were applied as seed priming (COU-P) alone or in combination with foliar application (COU-PF).

Growth Criteria

The data in Table 1 show that both treatments, COU-P and COU-PF, had no significant effect on root elongation, although fresh and dry masses increased significantly. The enhancement in root fresh and dry masses was more pronounced as a result of the combined treatment COU-PF. On the other hand, both treatments significantly stimulated the

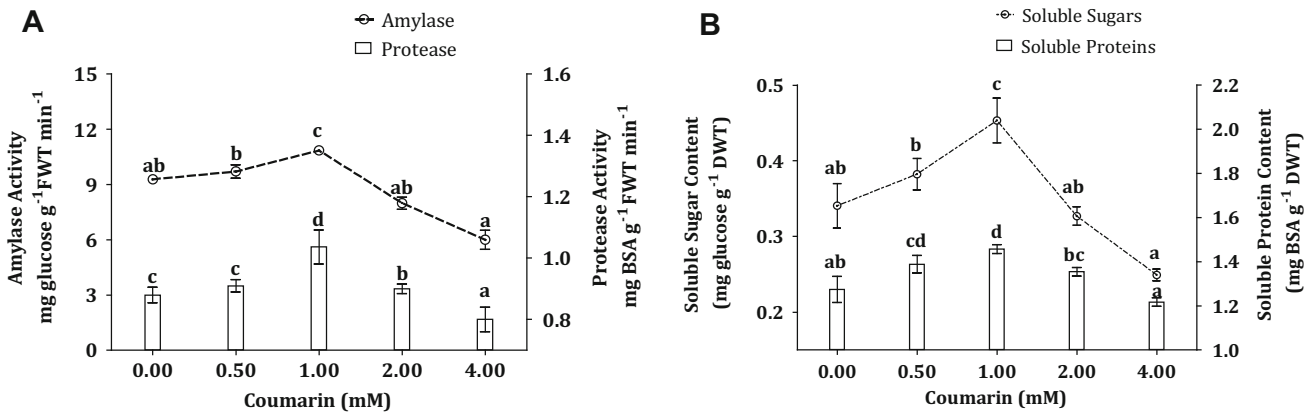

Fig. 2 Effect of different coumarin seed pre-treatments on **a** activities of amylase and protease and **b** contents of soluble sugars and proteins in *Vicia faba* seedlings

Table 1 Effect of different coumarin seed pre-treatments upon growth criteria of *Vicia faba*

Growth parameters	Coumarin treatments		
	Control	COU-P	COU-PF
Root length (cm)	15.76 ± 1.12[a]	017.60 ± 0.87[aw]	15.92 ± 0.81[a]
Shoot length (cm)	34.74 ± 1.04[a]	040.38 ± 1.47[bw]	48.30 ± 0.61[c]
Root fresh weight (g)	01.73 ± 0.04[a]	002.41 ± 0.14[bw]	02.69 ± 0.10[b]
Shoot fresh weight (g)	04.82 ± 0.12[a]	005.80 ± 0.22[bw]	05.81 ± 0.33[b]
Root dry weight (g)	00.13 ± 0.00[a]	000.14 ± 0.01[ab]	00.16 ± 0.00[b]
Shoot dry weight (g)	00.37 ± 0.01[a]	000.43 ± 0.02[bw]	00.46 ± 0.02[b]
Total leaf area (cm^2)	86.16 ± 0.13[a]	100.59 ± 0.57[bw]	89.40 ± 0.40[a]
NAR (g cm^{-2} day^{-1})	00.63 ± 0.04[a]	000.65 ± 0.05[aw]	00.76 ± 0.06[b]

Data in *a row* followed by different letter are significantly different at the 0.05 level by *Duncan's* test

fresh and dry mass production in shoots. COU-P resulted in a significant increase in the shoot length of bean plants (16.2 %) and the largest enhancement was observed in shoot elongation for COU-PF-treated plants (39.0 %). This increase in length was matched with shoot fresh and dry weight enhancement.

The total leaf area per plant significantly increased in response to COU-P treatment, whereas COU-PF had no pronounced effect (Table 1). On the contrary, the NAR was significantly improved (20.1 %) by COU-PF but unchanged as a result of COU-P treatment.

Endogenous IAA, GA_3, and ABA

The results of GC-FID analyses are summarized in Fig. 3. Both COU treatments significantly increased the levels of endogenous IAA and GA_3, although the level of ABA was stimulated only in response to COU-PF treatment. The most affected plant hormone was GA_3 and the most effective treatment was COU-PF. COU-PF treatment also improved the accumulation of GA_3, ABA, and IAA by about threefold, twofold and 30 %, respectively, as compared to control levels.

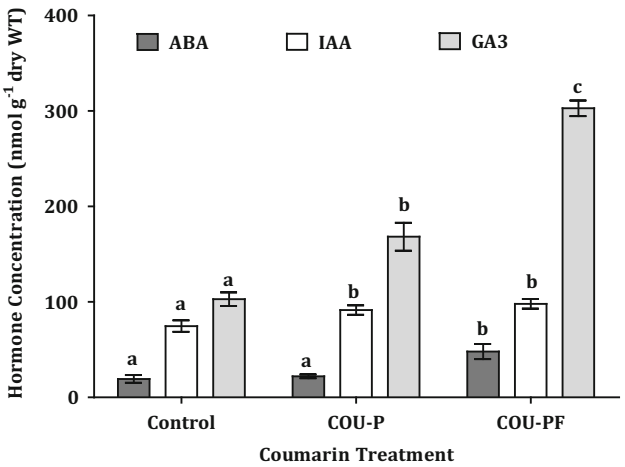

Fig. 3 Effect of different coumarin treatments on the levels of endogenous IAA, GA_3 and ABA in the leaves of *Vicia faba*

Carbohydrate Content

Figure 4 shows the effect of COU treatments on the accumulation of carbohydrates in faba bean leaf tissues. Both treatments significantly increased the level of total

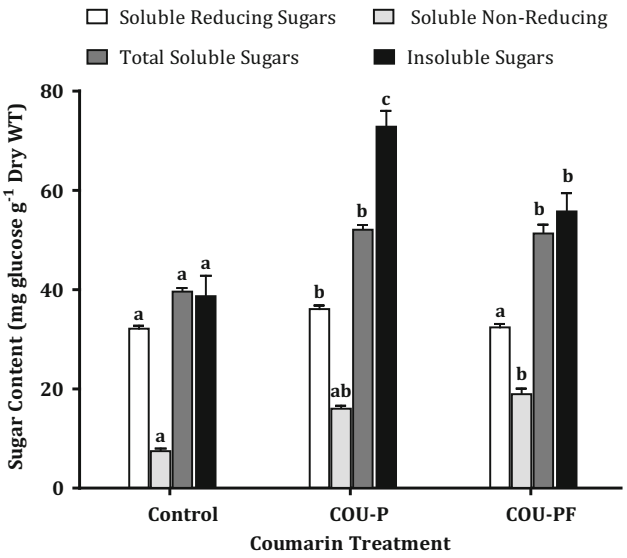

Fig. 4 Effect of different coumarin treatments upon the levels of sugar fractions (mg glucose g^{-1} dry WT) in leaves of *Vicia faba*

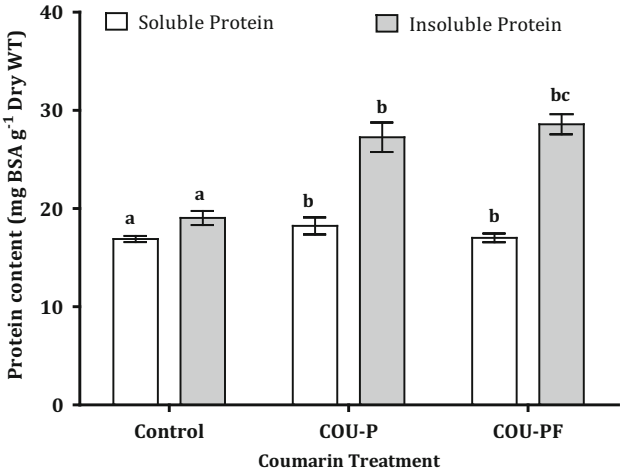

Fig. 5 Effect of different coumarin treatments upon protein content (mg BSA g^{-1} dry WT) in leaves of *Vicia faba*

soluble sugars which accompanied more than twofold accumulation in the levels of non-reducing sugars over the control. Moreover, both treatments caused a significant increase in the level of insoluble sugars, where COU-P and COU-PF treatments caused about 68 and 36 % increases in the accumulation of insoluble sugars, respectively, as compared to the control.

Protein Contents

The effect of COU treatments on the accumulation of soluble and insoluble proteins in the leaf tissues is illustrated in Fig. 5. Both COU-P and COU-PF significantly improved the accumulation of soluble and insoluble protein fractions. However, the effect was more pronounced in the level of insoluble protein which increased by more than 40 % after COU treatments.

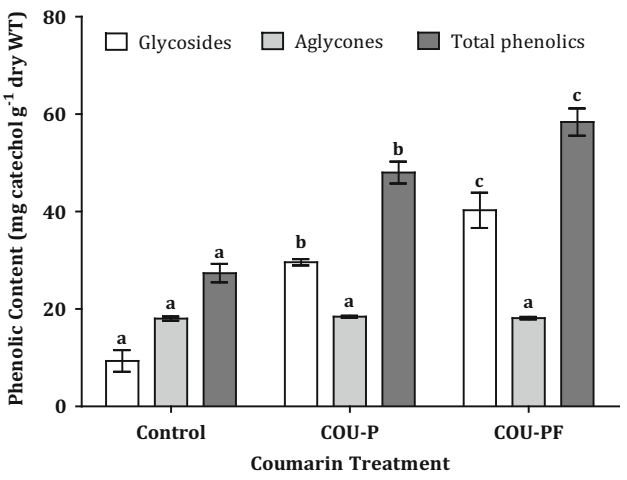

Fig. 6 Effect of different coumarin treatments upon phenolic content (mg catechol g^{-1} dry WT) in leaves of *Vicia faba*

Phenolic Content

Figure 6 shows the effect of COU treatments on the accumulation of phenolic compounds in leaf tissues of faba bean. Both COU-P and COU-PF treatments had no significant effect on the accumulation of phenolic aglycones. However, the situation is different for phenolic glycosides, where COU-P and COU-PF treatments caused about three and fourfold increases in the level of phenolic glycosides. Such accumulation of phenolic glycosides is reflected significantly in the level of total phenolic compounds.

Discussion

Generally, the effect of phenolic compounds on germination and early seedling growth may be stimulatory or inhibitory depending on the compound utilized, concentration, and the test plant (Reigosa and others 1999). The results presented here demonstrate that the effect of COU is more pronounced on growth than germination (Mata and others 1998) of faba bean and such effect is dependent on the concentration and mode of application. Concerning the germination of faba bean seeds, COU up to 2 mM has no significant effect; however, COU affects seedling growth in a concentration-dependent manner, similar to other studies with different species (Razavi 2011). In accordance with our results, Ahrabi and others (2010) reported that

germination of canola was slightly affected by COU, but seedling growth was affected variably depending on the concentration. The inhibitory effect of COU on germination of radish and durum wheat was also reported (Aliotta and others 1993; Abenavoli and others 2006). Moreover, Zhou and others (2013) reported that COU at concentrations above 0.1 mM reduced the fresh mass of shoots and roots of alfalfa seedlings. In addition, Li and Gao (2011) reported that growth of the primary roots of *Arabidopsis thaliana* exhibited a dosage-dependent inhibition in response to the coumarin derivative, 4-methylumbelliferone, whereas the growth of hypocotyls was not significantly changed.

Nutrient mobilization is essential for successful germination and very early growth. Protein and starch are the major reserve foods in cotyledons of faba bean seeds (Duenas and others 2006). During germination they are degraded by amylolytic and proteolytic enzymes to provide nutrient for respiration and growth of the growing embryo. The significant increase in the level of soluble sugars and proteins in response to 1.0 mM COU treatment and their depletion in case of 4.0 mM treated seeds was a logical consequence of amylase and protease activities. In this context, Abenavoli and others (2006) demonstrated that 1.0 mM COU inhibited the α-amylase activity. Moreover, ferulic acid at concentrations of up to 5 mM increased amylase and protease activities of 2-day-old maize seedlings, after which the activities were retarded (Devi and Prasad 1992). The influence of other phenolic compounds on the activities of amylases and proteases was also reported (Kato-Noguchi and Macías 2005; Batish and others 2008).

According to the results of the greenhouse pot experiment, coumarin treatments had no significant effect on elongation of the main root, but improved shoot length, mass production of roots and shoots as well as NAR. In this regard, other investigations reported that COU either promotes or inhibits plant growth depending on its concentration and on the tested plant species (Murray and others 1982; Brown and Zobel 1990; Kupidlowska and others 1994). Similarly to our results, Ahrabi and others (2010) reported that length and mass production of roots and shoots of maize seedlings were either stimulated or unaffected by lower concentrations of COU (0.05–0.5 mM), whereas the higher concentrations (5–10 mM) were inhibitory. In addition, some derivatives of coumarin enhanced the root and shoot growth of pea, cucumber, and wheat (Alexieva and others 1995). At a cellular level, Burström (1957) deduced that COU increases the plasticity of cell walls by its influence on the synthesis of cell wall materials, especially pectins. Moreover, Lupini and others (2010) reported that COU induced cell wall elongation in roots by enhancing plasma membrane H^+-ATPase activity and proton extrusion. They assumed that COU may have an auxin-like behavior and/or an interaction with the auxin signaling pathways.

In light of the present results, the observed improvement in growth of faba bean after COU treatments may be attributed to the elevated levels of endogenous phytohormones (IAA, GA3, and ABA). These results suggest that COU may up-regulate biosynthesis of these phytohormones, decrease their conjugation and/or decrease their degradation. In accordance with our results, Tartoura and others (2004) reported that treatment of cuttings from *Vigna radiate* seedlings with 1.0 mM COU caused a significant increase in endogenous levels of free and conjugated IAA. Moreover, exogenous salicylic acid-induced changes in the levels of endogenous phytohormones (IAA, GA_3, ABA, and cytokinin) of tomato, cucumber, and sweet pepper (Raskin 1992; Abou El-Yazeid 2011; Hao and others 2011). In addition, ferulic acid treatment increased the endogenous ABA levels in wild-type tomato and cucumber (Holappa and Blum 1991). The interaction of other phenolic acids on the metabolism of phytohormones was investigated (Ray and others 1980; Li and others 1993).

The present results revealed that levels of total soluble and insoluble sugars were improved by COU treatments. The enhanced accumulation of the different carbohydrate fractions may be attributed to the increase in the contents of photosynthetic pigments and stimulation of Rubisco activity (Khodary 2004). In previous work, we observed a similar accumulation of carbohydrates in sunflower leaves after seed priming with different concentrations (0.3, 1.0, and 3.0 mM) of COU (Al-Wakeel and others 2013). Similarly, Dhawan and Nanda (1982) demonstrated that exogenous COU increased carbohydrate content in cuttings of *Impatiens balsamina* L. Furthermore, exogenous application of phenolic acids such as SA and ferulic acid affected the accumulation of soluble and insoluble sugars in soybean and sunflower (Ferrarese and others 2001; El-Tayeb and others 2006).

The observed accumulation of soluble sugars was related to the non-reducing sugars rather than the reducing ones. This result could be interpreted by the enhanced rate of dissimilation of glucose and fructose and/or the elevated rate of sucrose synthesis. In this regard, Knypl (1964) reported that exogenous COU accelerates dissimilation of glucose and fructose in sunflower hypocotyl and barley coleoptile sections. Moreover, the high level of soluble non-reducing sugars under COU treatments, especially COU-PF, may be a logical consequence of the elevated level of GA_3. It is well known that GA_3 promotes sucrose synthesis within the leaf through its stimulatory effect on fructose-1,6-biphosphatase, sucrose synthase, and sucrose phosphate synthase (Cheikh and others 1992; Chen and others 1994; Kaur and others 2000; Iqbal and others 2011).

The results presented here show an enhanced accumulation of soluble and insoluble proteins in the leaf tissues of faba bean as a result of COU treatments. In accordance with this result, Dhawan and Nanda (1982) reported that exogenous COU increased the protein content in cuttings of *Impatiens balsamina* L. However, Zhou and others (2013) observed that COU at concentrations of up to 1.0 mM has no statistically significant effects on the soluble protein content in roots of alfalfa seedlings. In addition, the effect of the phenolic acids, *p*-coumaric, ferulic, and vanillic acids on protein synthesis in the isolated leaf cells of velvet-leaf was also reported (Mersie and Singh 1993).

We observed an enhanced accumulation of phenolic compounds in bean leaves as a result of COU treatments, which is related to the increase in the glycoside moiety, regardless of the aglycones. This accumulation may be due to the enhancement in activities of the regulatory enzymes of the phenylpropanoid pathway, especially of phenylalanine ammonia lyase (PAL). In this connection, we previously demonstrated that COU treatment increased the activity of PAL in the leaves of sunflower plant and this increase was accompanied with an enhanced accumulation of phenolic compounds (Al-Wakeel and others 2013). Also, we reported that COU treatment greatly increased the endogenous level of coumarin and its derivatives scopoletin, scopolin, and ayapin. Moreover, exogenous COU increased the total phenolic content in cuttings of *Impatiens balsamina* L. (Dhawan and Nanda 1982).

As we expected, COU has a different effect depending on concentration and physiological parameter studied in faba bean. Seed priming is a controlled hydration process that involves exposing the seeds to low water potentials, meanwhile priming can modulate many physiological aspects in the germinating seeds. These physiological changes permit the plant to cope with environmental stress factors or to respond differently to foliar applications. This assertion is justified by the findings presented in this paper where COU-P and COU-PF showed different results. Despite this, it could be concluded that COU is a powerful growth substance that can affect growth and physiology of faba bean in both seedling and vegetative stages. The beneficial effect of priming might be related to the physiological changes that occur during the soaking in COU.

Modulation in the biosynthesis of primary (carbohydrates and proteins) and secondary (phenolics) metabolites as well as phytohormones (IAA, GA_3, and ABA) indicates that COU can affect the growth either directly, as an active growth substance, or indirectly by its interaction with the metabolism of phytohormones, especially IAA and GA_3. However, other modes of COU action could not be excluded. Consequently more research is necessary to elucidate the interaction of COU with the metabolism and physiological functions of phytohormones.

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