Kinetics Properties of Guaiacol Peroxidase Activity in *Crocus sativus* L. Corm during Rooting

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ABSTRACT

Background: Guaiacol peroxidases (GP) are haem-containing enzymes participating in many physiological processes in plants. The expression pattern of these enzymes is organ-specific and developmentally regulated. **Methods:** The presence of GP activity in extract samples, prepared from *Crocus sativus* L. corms that were either dormant or rooting for 3, 6 and 10 days, was investigated. **Results:** Kinetic studies revealed a significant similarity among GP activities detectable in the corm at different stages of development: in all extract samples, the activity was maximal at pH 7.5 and after preincubation at 30-40ºC. When guaiacol was used as the varying substrate, Michaelis-Menten kinetics behavior was observed in all extract samples and resulted in similar K_M values; catalytic efficiencies were also very similar. The corm GP activity was inhibited by cyanide, azide and ascorbate. The GP activities from different extract samples had the same sensitivities to azide, cyanide and ascorbate and the type of inhibition by azide and cyanide was competitive and uncompetitive, respectively, while ascorbate inhibited the GP activity non-competitively. Corm extract samples from different stages of rooting similarly responded to temperature treatment and a biphasic Arrhenius plot resulted for each extract sample studied. When dormant, 3-, 6- and 10-days-rooting corm extracts were submitted to non-denaturing polyacrylamide gel electrophoresis, the GP-specific activity staining revealed one band on the gel, with the same migrating distances. **Conclusion:** This finding in combination with kinetic studies demonstrated that at least one form of GP, with an apparent molecular weight of 68 kDa, was expressed during development of *Crocus sativus* L. corm. *Iran. Biomed. J. 11 (3): 137-146, 2007*

Keywords: Arrhenius plot, *Crocus sativus* L. corm, Development, guaiacol peroxidase (GP), kinetics

INTRODUCTION

Higher plants are known to possess a large set of peroxidases (EC 1.11.1.7) [1]. These enzymes are usually expressed as several isoforms [2] and their expression pattern is tissue-specific and developmentally regulated [3, 4]. Isoperoxidases are thought to participate in a wide range of physiological processes. These include H_2O_2 detoxification [5], cell elongation [6], cell wall construction and differentiation [7] and the plant response to stress [8, 9].

Peroxidases are a single-polypeptide chain, haem-containing enzymes with molecular weights between 28 to 60 kDa [10]. They catalyze the following reaction and exhibit a broad range of reducing substrate (AH) specificity [11].

$$2\ AH + H_2O_2 \rightarrow 2\ A + 2\ H_2O$$
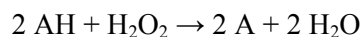
On the basis of their function as well as subcellular localization, peroxidases are categorized into two different groups [12]. Peroxidases which oxidize guaiacol (o-methoxyphenol), as a commonly used reducing substrate *in vitro,* are referred to as guaiacol peroxidases (GP). They are located in cytosol, vacuole, cell wall, apoplast and extracellular medium, but not in organelles and are assumed to be involved in a range of processes related to plant growth and development. Ascorbate peroxidases belong to another group of plant peroxidases that

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show preference for ascorbic acid as reducing substrate. These enzymes are localized in chloroplast, microbody and cytosol and their main function is to scavenge H_2O_2 and defense against oxidative stress in plant cell [13]. In spite of the fact that peroxidases are among the most studied enzymes in plants and are thought to participate in many physiological processes, their role in the physiology and biochemistry of plants has remained to be elucidated.

Saffron, the dried flower stigmas of *Crocus sativus* L., has been used from ancient times as spice, colorant and also for medicinal purposes. However, despite the importance of the plant in food industry as well as medicine, little is known about its biochemistry. *Crocus sativus* L. is a sterile triploid plant that is propagated by corms. Corms are underground, storage stems which provide the necessities during sprouting, after a period of dormancy. Knowledge of the physiological and biochemical processes taking place in the corm is important in order to understand the factors affecting the quality of the plant. Thus in this study, we investigated the presence of GP activity in extracts prepared from *Crocus sativus* L. dormant corm and 3-, 6- and 10-days-rooting corms. Kinetics characteristics, as well as sensitivity to inhibitors and thermostability of the enzymatic activity were investigated and compared in dormant corm and in 3-, 6- and 10-days-rooting corms.

MATERIALS AND METHODS

Materials. Guaiacol, sodium azide, potassium cyanide (Merck Chemical Co., Germany) and sodium ascorbate (Sigma Chemical Co., USA) were all of the highest analytical grade. Water was purified by a Fistreem purification system and all buffers were filtered (0.2 μm) prior to use. Hydrogen peroxide solutions were freshly prepared by dilution of a 30% (v/v) solution (Merck Chemical Co.).

Plant material. The corms used for this study were collected in August from Tehran University field located in Karaj, near Tehran. These corms showed neither shoot, nor root and hereafter are referred to as "dormant corms". Unearthed dormant corms were transferred to the laboratory, depleted from sheathing leaves and cleaned from any dirt particles. Selected corms weighing each between 3 and 6 g were placed in glass jars containing distilled water. Corms were grown at room temperature (~

20-25°C) and were collected after 3, 6 and 10 days.

Extract preparation. Extracts were prepared from dormant corms and 3-, 6- and 10-days-rooting corms, according to the procedure described in reference [14]. Briefly, saffron corms were obtained from Tehran University field located in Karaj, near Tehran (Iran). Dormant corms were homogenized in phosphate buffer 0.01 M, pH 7.00, containing 0.02% phenylmethanesulfonyl fluoride as protease inhibitor. After centrifugation at 3,000 ×g for 10 min, then at 35,000 g for 30 min, a clear, transparent supernatant termed "crude extract" was obtained and used for our studies.

Protein determination. Protein concentration was determined according to the method proposed by Lowry *et al.* [15] using bovine serum albumin as standard protein.

Enzyme assays. Steady-state measurements for peroxidase activity were carried out spectro-photometrically using guaiacol as electron donor substrate. The increase in the absorption as a result of the formation of the oxidized product (tetraguaiacol) was measured at 470 nm using the extinction coefficient of 26.6 mM^{-1} cm^{-1} [16]. Initial rates were calculated from time-dependant absorbance changes and were used for detection of enzyme activity. Assays were carried out at room temperature (~ 20-25°C) using Pharmacia, Biotech Ultrospec 1000 spectrophotometer (Sweden). Reaction mixture contained 0.1 M citrate-phosphate-borate buffer system, pH 7.5, 15 mM guaiacol, 3.3 mM H_2O_2 and 30 μl extract (with final protein amount of 0.273 mg, 0.255 mg, 0.198 mg and 0.204 mg for dormant, 3-, 6- and 10-days-rooting corm extracts, respectively). The total volume of reaction mixture was 3 cm^3 and assays were carried out in a quartz cuvette with 1 cm path length. All reactions were started by addition of H_2O_2 to the reaction mixture.

The pH dependence of the enzyme activity was determined using 0.1 M citrate-phosphate-borate buffer system ranging from pH 3 to pH 10.

Effect of inhibitors including sodium azide, potassium cyanide and sodium ascorbate on GP activity was investigated after 5 min preincubation of extract with selected inhibitor concentrations in 0.1 M citrate-phosphate-borate buffer. The concentration ranges used were chosen as a function of the sensitivity of the extracts towards the various inhibitors and were as follows: 10-200 μM for

sodium azide, 50-400 μM for potassium cyanide, and 5-30 μM for sodium ascorbate.

Effect of temperature on enzyme stability and activity was determined after preincubation of extract aliquots at various temperatures for 15 min. Aliquots were then placed in ice (0ºC) for 15 min and then assayed for GP activity at room temperature. Results were average of three separate experiments.

Gel electrophoresis. Gel electrophoresis was performed in a 2-mm, 10% non-denaturing polyacrylamide gel according to Laemmli [17], but omitting SDS and mercaptoethanol. Samples containing 1 mg protein were subjected to electrophoresis at room temperature at 65 V for 18 h. Peroxidase bands were detected on the gel by submerging the gel in a staining solution containing 50 mM sodium citrate buffer, pH 4.5, 2 mM H_2O_2 and 20 mM guaiacol. Bands corresponding to GP activity emerged after 40-60 min.
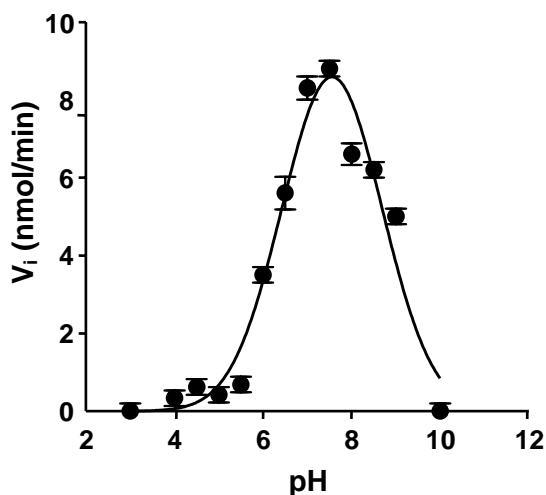

Fig. 1. The pH dependence of guaiacol peroxidase activity of dormant *Crocus sativus* L. corm extract. Guaiacol oxidation was determined in 0.1 M citrate-phosphate-borate buffer system in the presence of 15 mM guaiacol and 3.3 mM H_2O_2.

RESULTS

The pH Dependency of GP activity. The effect of $[H^+]$ on GP activity of dormant corm extract and 3-, 6- and 10-days-rooting corm extracts was investigated. The pH profile curves demonstrated a similar pH dependency for GP activity in all extracts examined. As shown in Figure 1, the maximum GP activity of dormant corm extract was observed at pH 7.5 and the enzyme completely lost its activity at pH lower than pH 4 and higher than pH 10. The GP activity of 3-, 6- and 10-days-rooting corm extracts showed the same peak of activity at pH 7.5 with loss of activity being again observed at pH 4 and pH 10. At this optimum pH (7.5) and at saturating concentration of hydrogen peroxide, steady-state kinetic parameters were determined for GP activity found in dormant, 3-, 6- and 10-days-rooting corm extracts (Table 1). The oxidation of guaiacol by dormant corm extract as well as 3-, 6- and 10-days-rooting corm extracts obeyed Michaelis-Menten kinetics and resulted in the apparent K_M of 10.50 mM, 8.70 mM, 9.70 mM and 11.0 mM, respectively. As for K_M, the apparent V_{max} values were very close in all examined extract samples. Consistent with these data, catalytic efficiency (expressed per mg protein in sample) was also found to be almost identical for GP activity present in dormant, 3-, 6- and 10-days-rooting corm extracts (Table 1).

Effect of inhibitors on GP activity. Azide and cyanide are two typical peroxidase inhibitors [18]. Ascorbate has been shown to have an inhibitory effect on GP [19, 20]. We studied the sensitivity of GP activity found in dormant, 3-, 6- and 10-days-rooting corm extracts to these inhibitors. Our results indicated loss of GP activity of all extracts examined with the three inhibitors. As shown in Figure 2, ascorbate and azide were more efficient than cyanide in reducing GP activity of the extract samples. The

Table 1. Kinetic parameters detected for guaiacol-dependent peroxidase activity in different *Crocus sativus* L. corm developmental stages.

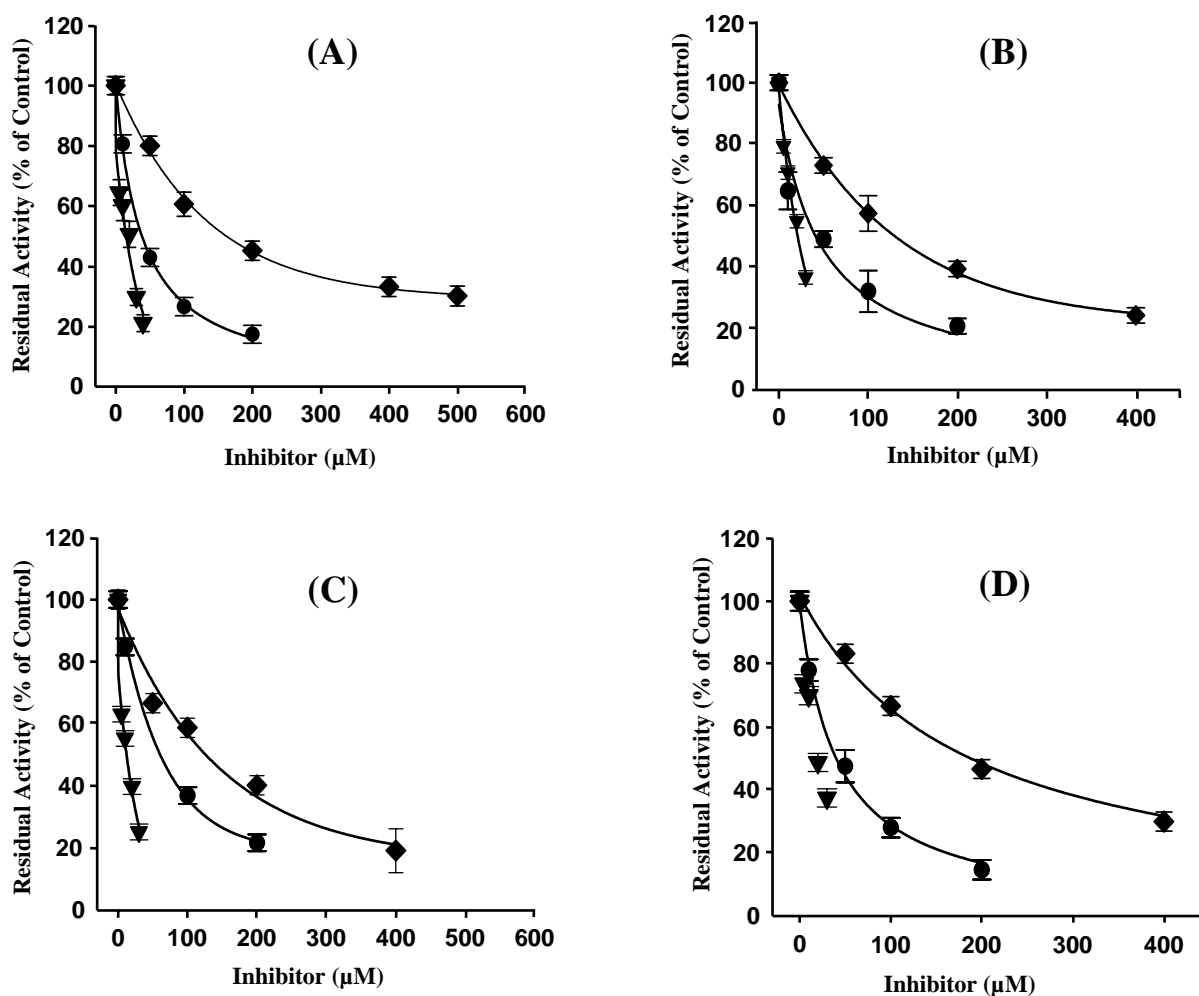| Extract Sample	K_M (mM)	V_{max} (nmol.min^{-1}.mg prot.$^{-1}$)	Catalytic Efficiency (min^{-1}.mg prot.$^{-1}$)
Dormant Corm	10.50 ± 0.00	28.50 ± 1.75	$9.05 \times 10^{-4} \pm 0.55 \times 10^{-4}$
3-Day-rooting Corm	8.70 ± 0.25	20.47 ± 0.10	$7.80 \times 10^{-4} \pm 0.26 \times 10^{-4}$
6-Day-rooting Corm	9.67 ± 0.75	24.60 ± 0.35	$8.51 \times 10^{-4} \pm 0.26 \times 10^{-4}$
10-Day-rooting Corm	11.0 ± 0.25	27.63 ± 0.30	$8.37 \times 10^{-4} \pm 0.11 \times 10^{-4}$

Fig. 2. Effect of cyanide (♦), azide (●) and ascorbate (▼) on guaiacol peroxidase activity in extracts from *Crocus sativus* L. dormant corms (A), 3-days-rooting corms (B), 6-days-rooting corms (C) and 10-days-rooting corms (D). All assays were performed after 5 min preincubation of extract samples with different concentrations of either cyanide, azide or ascorbate. Activities were expressed as percentage of the control.

IC_{50} value (the concentration of inhibitor that causes 50% loss of enzyme activity) obtained for ascorbate effect on GP activity of dormant corm extract was 15.5 μM (Fig. 2a). GP activity of 3-, 6- and 10-days-rooting corms showed the same sensitivity to ascorbate with IC_{50} values of 22 μM, 13 μM and 19 μM, respectively (Fig. 2 b-d). Similarly, azide had an undiscriminating effect on GP activity of the four extracts examined. Fifty percent loss of activity was achieved with 42 μM, 46 μM, 50 μM and 46 μM of azide, respectively (Fig. 2 a-d). When cyanide was applied as inhibitor, the IC_{50} values obtained for dormant, 3-, 6- and 10-days-rooting corm extracts were very close (150 μM, 135 μM, 145 μM and 165 μM, respectively), indicating similar sensitivity of GP activity of those extracts towards cyanide (Fig. 2 a-d).

To further characterize the effect of inhibitors, we explored the type of inhibition of GP activity in dormant corm extract. As presented in Figure 3a, when different cyanide concentrations were used, both K_M and V_{max} values decreased with increasing cyanide concentration, while the value for K_M/V_{max} ratio remained the same, as in the absence of inhibitor, suggesting an uncompetitive type of inhibition. With sodium azide as applied inhibitor, a competitive type of inhibition was observed (Fig. 3b). The K_M increased as the result of increasing azide concentration and the value for V_{max} was not altered when the inhibitor concentration changed.

The effect of sodium ascorbate on GP activity was also studied. As shown in Figure 3c, this specific GP
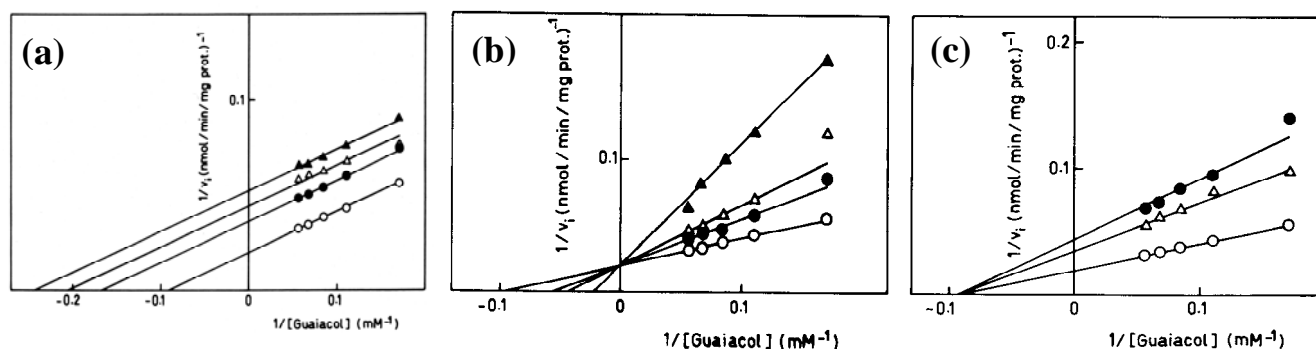
Fig. 3. Inverse plots obtained when guaiacol peroxidase was assayed in *Crocus sativus* L. dormant corm extract in the presence of increasing cyanide **(a)**, azide **(b)** or ascorbate **(c)** concentrations. Uncompetitive inhibition was observed in the presence of cyanide, competitive inhibition was observed in the presence of azide and noncompetitive inhibition was observed in the presence of ascorbate. **(a)**, (○) control, (●) 50 µM cyanide, (Δ) 100 µM cyanide, (▲) 200 µM cyanide; **(b)**, (○) control, (●) 30 µM azide, (Δ) 100 µM azide, (▲) 200 µM azide; **(c)**, (○) control, (Δ) 10 µM ascorbate and (●) 20 µM ascorbate. Control values show guaiacol peroxidase activity in the absence of inhibitor. Guaiacol peroxidase activity was measured after 5 min incubation of extract with various inhibitor concentrations. The inhibitors concentrations were chosen according to the sensitivity of the extracts and in order to establish unequivocally the type of inhibition. Data presented are average of three separate experiments.

inhibitor inhibited non-competitively the enzyme activity. The K_M value was unaffected with increasing ascorbate concentration, while the V_{max} decreased.

Effect of temperature on GP activity. Thermal stability of GP activity present in dormant, 3-, 6- and 10-days-rooting corms was investigated and results are presented in Figure 4. The enzyme activity was measured after a 15-min incubation of extract aliquots at various temperatures and then 15 min in ice to fix the enzyme conformation. As results in Figure 4a show, GP activity from dormant corm extract was maximal after preincubation at

temperatures ranging from 30-40°C for 15 min, and drastically decreased after preincubation at higher temperatures; the activity became undetectable after preincubation at 60°C for 15 min. For GP active in 3-days-rooting corm extract, optimum activity was detectable after preincubation at 30-45°C for 15 min, and loss of activity was observed after preincubation at temperatures higher than 60°C for 15 min (Fig. 4b). For GP activity in 6- and 10-days-rooting corm extracts, similarly to the findings for extracts from dormant and 3-days-rooting corms, maximum activity was observed after preincubation at 30 to 40°C and no activity was detectable after preincubation at 60°C for 15 min (Fig. 4 c and d).
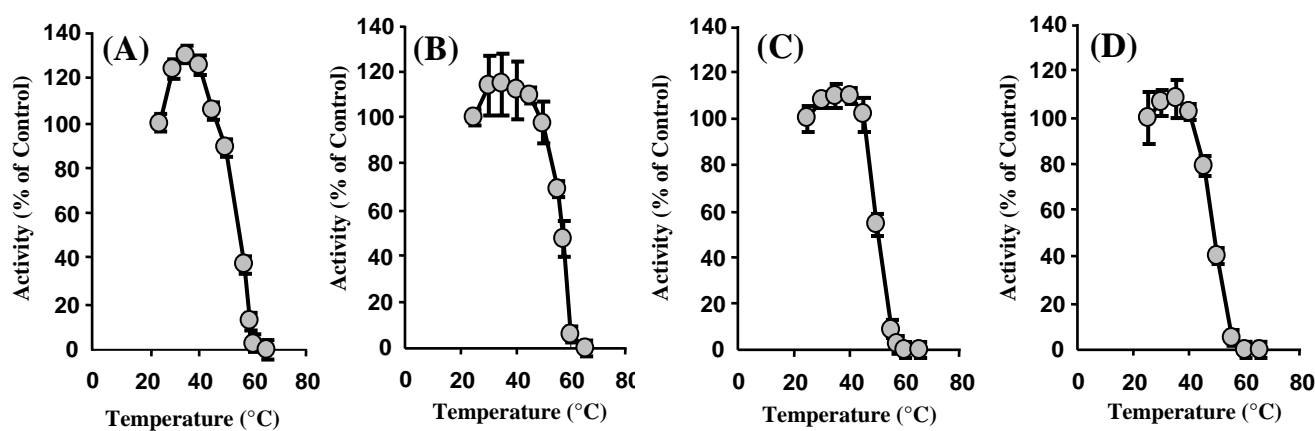

Fig. 4. Dependence of guaiacol peroxidase activity of different *Crocus sativus* L. corm extracts on temperature. **(A)**, dormant corm extract. **(B)**, 3-days-rooting corm extract. **(C)**, 6-days-rooting corm extract. **(D)**, 10-days rooting corm extract. The oxidation rate of guaiacol was measured at room temperature after a 15-min incubation of extract aliquots at various temperatures and then at 0°C for 15 min. Activities were expressed as percentage of control.
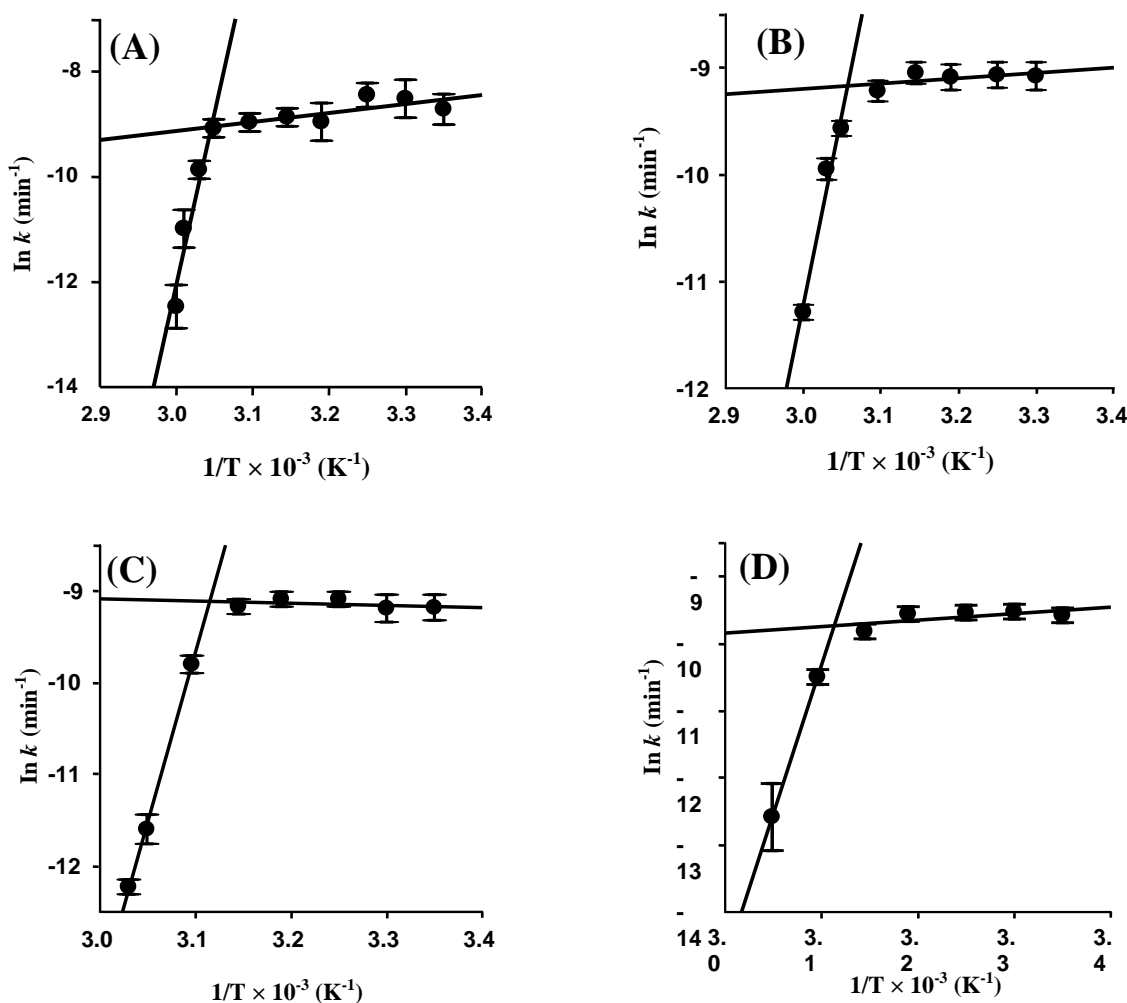
Fig. 5. Arrhenius plots for guaiacol peroxidase activity found in different stages of *Crocus sativus* L. corm development. (**A**), dormant corm extract. (**B**), 3-days-rooting corm extract. (**C**), 6-days-rooting corm extract and (**D**), 10-days-rooting corm extract. Reaction rates were measured at 5ºC intervals. Extract aliquots were incubated at various temperatures for 15 min and then in ice for 15 min, before activity measurement.

Arrhenius plots showed again a similarity between GP active in dormant, 3-, 6- and 10-days-rooting corms (Fig. 5). A biphasic linear plot, with the transition temperature of 55°C, was obtained for GP activity in dormant corm extract (Fig. 5a). As shown in Figure 5 b-d, for GP activity in 3-, 6- and 10-days-rooting corm extracts, this biphasic pattern was again observed. The transition temperature for GP activity found in 3-days-rooting corm was almost unchanged compared to that of dormant corm and was calculated to be 54°C. For GP activity found in 6- and 10-days-rooting corms, the transition temperatures were 48°C, slightly lower than those found for dormant and 3-days-rooting corms.

In order to study the effect of temperature in more details, we investigated any possible alteration in the kinetic behavior of GP activity observed in dormant,

3-, 6- and 10-days-rooting corms under treatment with four selected temperatures in the range of 25-45°C. As results in Table 2 show, in this temperature range, the Michaelis-Menten kinetics was observed for all extracts examined. The K_M values for GP activity in every extract sample did not change as a function of temperature and these values were almost identical when compared to those of the other extract samples. In other words, no preferable temperature over the other was detected in the efficiency of enzyme(s) to oxidize guaiacol.

Non-denaturing polyacrylamide gel electrophoresis. Because several peroxidase isoenzymes might be active in each corm extract and their expression pattern might change during development, we performed a non-denaturing gel

Table 2. Effect of temperature on kinetic behavior of guaiacol peroxidase activity found in extracts prepared from different stages of *Crocus sativus* L. corm development.

Extract Sample	t (ºC)	K_M (mM)	V_{max} (nmol.min^{-1}. mg prot.$^{-1}$)
Dormant Corm	25	12.37 ± 0.18	32.01 ± 3.21
	30	9.87 ± 0.53	34.90 ± 0.30
	35	10.00 ± 0.00	41.72 ± 0.56
	45	10.25 ± 0.00	38.60 ± 0.88
3-days rooting corm	25	11.75 ± 0.35	39.75 ± 0.54
	30	13.00 ± 0.00	42.47 ± 0.00
	35	12.00 ± 0.00	40.47 ± 0.95
	45	10.75 ± 1.06	33.83 ± 2.81
6-days rooting corm	25	13.00 ± 0.71	35.45 ± 1.79
	30	13.25 ± 1.77	36.74 ± 3.61
	35	n.d.	n.d
	45	n.d.	n.d.
10-days rooting corm	25	11.75 ± 0.35	39.80 ± 0.00
	30	10.50 ± 0.00	43.14 ± 0.00
	35	11.12 ± 0.18	25.68 ± 1.18
	45	10.75 ± 1.06	21.57 ± 0.00

n.d.: Not determined

electrophoresis to explore the possibility of isoenzymes existence and or their variation in each stage of corm development. Figure 6 shows the picture of the gel after specific GP activity staining. Only one band was detected on the gel for each extract sample loaded. This confirmed that the observed GP activity in each stage of corm development was due to the presence of at least one form of enzyme in the extract. As shown in Figure 6, the single bands, each corresponding to GP activity in one stage of corm development, migrated the same distance in the gel, indicating that the same enzyme is active in all extracts. The apparent molecular weight of this protein was estimated to be 68.4 ± 1.1 kDa.
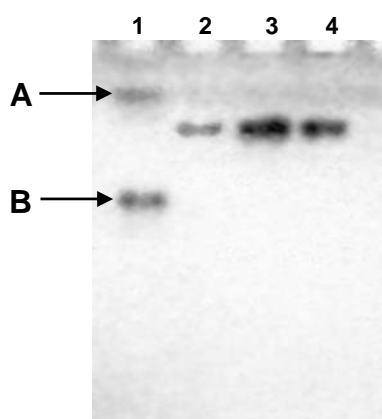

Fig. 6. Guaiacol peroxidase activity staining on non-denaturing gel. *Lane 1:* lactoperoxidase (A) (77 kDa) and horseradish peroxidase (B) (44 kDa). *Lane 2:* GP active in dormant corm extract. *Lane 3:* GP active in 3-days-rooting corm extract. *Lane 4:* GP active in 6-days-rooting corm extract.

DISCUSSION

Peroxidases are the most studied enzymes in plants and the fact that they have been found in all major divisions of plants denotes the functional importance of these proteins. Numerous physiological functions have been attributed to plant isoperoxidases. These include lignification, suberization, cross linking of cell wall proteins, stress response, defense against pathogens, salt tolerance and senescence. The diversification in peroxidase functions is provided by the existence of several forms of the enzyme. It has been suggested that the high number of peroxidases in plants results from various gene duplication events during plant evolution and the formation of new organs such as stems, roots, leaves and flowers favored the appearance and diversification of new peroxidases to the specific needs of new organs [21]. This diversification is also thought to be important in plant response to environmental and developmental conditions [3, 22].

The *Crocus sativus* L. corm is a subterraneous stem that contains reservoirs for sprouting after a dormancy period. Due to the fundamental role of corm in sprouting and vegetative reproduction, we focused our study to investigate developmental expression profile of GP in this organ. Our results demonstrated that at least one form of GP is active during corm development. This activity was detected in a soluble fraction and since the centrifugation procedure we used to prepare extract removed organelles as well as membrane particles, the observed GP activity is postulated to be related to a cytosolic enzyme.

It has been suggested by de Marco *et al.* [7] that variation in pH can change the relative contribution of different isoperoxidases to total activity and this is manifested with the emergence of different pH optima in pH activity curves. When pH activity curves were compared for dormant, 3-, 6- and 10-days-rooting corms, only one peak of GP activity was detected which suggests the presence of one form of enzyme in all extract samples.

When kinetic parameters were measured at the optimum pH, again a similarity among GP active in dormant, 3-, 6- and 10-days-rooting corms was observed. The K_M value for GP of all extract samples was almost the same. K_M values in a millimolar range as obtained in this work are typical for peroxidases with artificial substrates like guaiacol. For instance, soluble peroxidases from kiwi fruit, tomato and garlic GP, as well as corn root plasma membrane GP, had K_M values of 7.4 mM, 10

mM, 9.5 mM and 12.2 mM, respectively [18, 23, 24].

The uniformity of GP activity in different stages of corm development was further evidenced by the similarity in V_{max} and catalytic efficiency values. In addition, GP active in dormant, 3-, 6- and 10-days-rooting corms exhibited the same sensitivity to various inhibitors, namely cyanide, azide and ascorbate. More than 70% loss of activity was indiscriminatingly achieved by 400 μM KCN in all extract samples tested. Azide, in a concentration of 200 μM decreased the activity of GP found in the four extract samples to 80% of its original. These concentrations of KCN and azide were close to those found for the inhibition of other GP [18]. Ascorbate was observed to be a potent inhibitor of GP activity. All extract samples exhibited the same sensitivity to this inhibitor as well: only as low as 40 μM of sodium ascorbate caused more than 80% loss of activity in extract samples from different stages of corm development. Furthermore, KCN inhibited peroxidase activity in an uncompetitive manner. This inhibitory behavior of KCN has been previously observed for other peroxidases and a mechanism for that has been suggested [25]. Azide, seemingly, bound to the same position as guaiacol, as a competitive type of inhibition was resulted. The non-competitive type of inhibition observed with ascorbate suggests the existence of a binding site for ascorbate which is different from that of guaiacol. It should be noted here that the reported inhibitory effect of ascorbate on GP may be due to the utilization of ascorbate as substrate [18, 26].

The effect of temperature on GP activity of dormant, 3-, 6- and 10-days-rooting corms was investigated and results were compared. Analysis of thermal stability curves as well as Arrhenius plots, again, showed uniformity among GP active in different stages of corm development. The optimal temperature for GP activity in dormant corm, 3-, 6- and 10-days-rooting corms was the same, 35°C. The activity of GP from all those extract samples was also abolished at the same temperature. Apoplastic, cytosolic and soluble peroxidases of several plant tissues showed temperatures optima between 30°C and 60°C [18, 24, 27, 28]. The optimal temperature found for GP activity in *Crocus sativus* L. extracts was within that range. Arrhenius plots depicted for GP activity in dormant, 3-, 6- and 10-days-rooting corms showed a similar biphasic pattern with only one transition temperature for each. Linear Arrhenius plots in the temperature ranges 25-54°C (for dormant and 3-days-sprouting corms) and 25-

48°C (for 6- and 10-days-sprouting corms) suggest that the temperature does not affect the conformation of enzyme, at least not at guaiacol binding region. This finding was further confirmed by the results of Table 2 that shows the K_M does not change with temperature in this range. It has been reported that isoenzymes are usually different in their sensitivities to temperatures [29, 30] and therefore at various temperatures, the relative contribution of each isoform to total activity can change. As results showed, temperature had no effect on K_M of GP activity. Thus, the possibility of presence of several isoenzymes within the extracts was again ruled out by this result.

Together, kinetic studies showed that at least one form of active GP is expressed in all stages of corm development. This finding was further confirmed by appearance of one band on gel, with the same mobility for all extract samples, after specific activity staining. The bands represented a high molecular mass peroxidase and this observation might be correlated to the attachment of a massive carbohydrate moiety to this protein [18]. However, purification of the enzyme and its characterization, in combination with other techniques, can ultimately prove or disprove that only one GP exists in saffron corm.

In conclusion, this study showed that a single soluble GP is active in *Crocus sativus* L. during corm development. The expression of this enzyme might have important consequences for normal plant growth and development. Until now, the physiological functions of GP are not completely clear, but several functions such as cell wall lignification [31], cell wall stiffening [32], auxin metabolism [33] and root elongation [19] have been suggested. The single form of enzyme detected in saffron plant corm may be involved in all these functions.

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