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A Study on Polyphenol Oxidase Activity During Seed Germination

By

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Summary

KOCAÇALISKAN I., DEMİR Y. & KABAR, K. 1995. A study on polyphenol oxidase activity during seed germination. – *Phyton* (Horn, Austria) 35 (1): 37-43. – English with German summary.

In this work, polyphenol oxidase (PPO) activities, when used four different substrates (dopa, catechol, caffeic acid, tyrosine) oxidizing activities, during seed germination of six different plants were studied in the embryos and food tissues of the seeds depending on time.

It was found that during germination, PPO activities of seeds, did not show similar fate but the activities in embryos were higher than in food reserve tissues. Dopa-oxidizing enzyme had the highest activity in general. Relation between seed germination and PPO activity was discussed in point of respiration.

Zusammenfassung

KOCAÇALISKAN I., DEMİR Y. & KABAR K. 1995. Untersuchungen zur Aktivität der Polyphenoloxidase während der Samenkeimung. – *Phyton* (Horn, Austria) 35 (1): 37-43. – Englisch mit deutscher Zusammenfassung.

In dieser Arbeit werden die Polyphenoloxidase-Aktivitäten (PPO) während der Samenkeimung in den Embryonen und Nährgeweben von sechs verschiedenen Pflanzen in Abhängigkeit von der Zeit untersucht, wobei vier verschiedene Substrate (Dopa, Catechol, Kaffeesäure und Tyrosin) eingesetzt werden. Es stellte sich heraus,

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daß während der Keimung die PPO-Aktivitäten von Samen sich nicht ähnlich verhalten, aber die Aktivitäten in Embryonen waren stets höher als in den Nährgeweben. Im allgemeinen zeigen die Dopaoxidierenden Enzyme die höchste Aktivität. Die Zusammenhänge zwischen Samenkeimung und PPO-Aktivität werden aus dem Blickwinkel der Atmung diskutiert.

Introduction

Polphenol oxidase (PPO) enzyme complex which converts some phenols to quinons which play an important role in respiratory chain is one of a number of oxidation systems reported in seeds and seedlings (BIDWELL 1979, BEWLEY & BLACK 1983). The well known characteristic of the enzyme is to lead to enzymatic browning in fruits and potato (MATHEIS 1983, KOCAÇALISKAN & ÖZBAY 1987, SIDDIQ et al. 1992). In addition, it has been studied in relation to plant diseases (MAXWELL & BATEMAN 1967, JENNINGS & al. 1969), wounding (HYODO & URITANI 1966), and hormonal regulation (STAFFORD & GALSTON 1970, JENNINGS & DUFFUS 1977).

According to the international enzyme nomenclature; diphenol oxidase (diphenol oxygen oxidoreductase) has the E.C. number 1.10.3.2 and tyrosinase (monophenol monoxygenase) E.C.1.14.18.1 (Mayer, 1987). Very few studies on the role of this enzyme in seed germination have been done (KRUGER 1976, RAO & DEOSTHALE, 1987, KOCAÇALISKAN & KABAR 1990) and sufficient findings to reach a certain conclusion have not been obtained yet. For this reason, the objective of this work is to try to determine the changes in PPO activities during germination and probable role of this enzyme by using rather a lot of various seeds (six species) and PPO substrates (four).

Materials and Methods

1. Seed Germination

In this work, seeds of barley (*Hordeum vulgare* L. cv. Tokak), wheat (*Triticum aestivum* L. cv. Yayla 305), corn (*Zea mays* L. cv. Karmelci), dwarf bean (*Phaseolus vulgaris* L. cv. Kizilhaç), chickpea (*Cicer arietinum* L. cv. Flip), and soybean (*Glycine max* L. cv. Gelso) were used.

The seeds were surface sterilized with 1.0% sodium hypochloride. 40 seeds were placed in 9 cm (for barley, wheat, and soybean) or 12 cm (for other seeds) petri dishes, furnished with 2 sheets of Whatman No. 1 filter paper moistened with 10 ml of distilled water and were left to germinate in an incubator at 25°C, in continuous dark for 48 h. All the seeds germinated 100% at the end of this period.

2. Enzyme Extraction

Seed fractions- embryo and food tissues (endosperm or cotyledon)-were weighed and homogenized with 10 vol. of chilled phosphate buffer (0.05 M, pH 6.5) for 2 min and filtered. The filtrate was centrifuged at 40 000xg at 2°C for 15 min. The supernatant (crude extract) was used in determination of the enzyme activities.

3. Determination of PPO Activities

For determination of PPO activities four substrates (dopa, catechol, caffeic acid, and tyrosine) were used. 2.5 mM of tyrosine and 10 mM of each of the other substrates were prepared in 0.1 M (pH 6.5) of phosphate buffer solution. For each determination, the reaction mixture containing 4 ml of substrate and 0.2 ml of crude extract was incubated in an incubator at 30°C for 3 min. The absorbances of the mixtures were measured by a spectrophotometer at 490 nm for diphenoloxidase activity using dopa, catechol or caffeic acid substrates and at 430 nm for tyrosinase activity using tyrosine substrate. The mixture without crude extract served as a blank. The absorbance values at mentioned wavelength are expressed as PPO activity units per g seed fraction (A_{490} or A_{430} /g seed) on the Table 1 (JENNINGS & DUFFUS 1977).

The enzyme activities were determined from zero h (i.e. before sowing), for each 12 h up to 48th h. The experiment was repeated 3 times. LSD test was used to determine statistical significance of difference among mean values.

Results

In this work, the role of PPO during seed germination has been tried to be determined by using many species. The results are presented in Table 1. As seen in this table, PPO activities for each of four substrates have been found to be higher in the embryos than in the food tissues of all the studied seeds.

The substrates supplying the highest PPO activity during seed germination have varied according to species. In short, PPO activities of bean, chickpea, barley and wheat were the highest when used dopa substrate, but in corn seed caffeic acid. On the other hand, tyrosinase activity was the highest in soybean. In comparison with the other substrates, dopa was the best substrate for PPO in showing the highest activity in the both embryos and food tissues of the mentioned seeds besides soybean and corn. While tyrosinase and PPO oxidizing caffeic acid had the highest activity in soybean and corn, respectively, dopa-oxidizing enzyme activity was the highest in corn endosperm.

In respect to activity, the PPO substrates occupying the second group are: tyrosine in bean embryo, tyrosine and caffeic acid in both embryo and cotyledon of chickpea, caffeic acid and catechol in soybean embryo, caffeic acid in both embryo and endosperm of barley and wheat and dopa in corn embryo.

The PPO substrates showing the lowest activity are also the following: catechol in both embryo and cotyledon of bean and chickpea, dopa in soybean embryo, tyrosine in embryo and endosperm of barley and corn, and also catechol and tyrosine in wheat embryo and endosperm.

On the other hand, the relationship of PPO activities with germination period is found relatively complicated. For example, in bean embryo in which dopa-oxidizing activity was the highest, this activity increased with

time whereas an important change was not observed in cotyledon. This enzyme activity showed an important increase with time during germination in chickpea embryo and cotyledon while it showed a decrease in barley embryo and endosperm. Also in wheat, it decreased markedly in embryo, but increased in endosperm. However, dopa-oxidizing activity in the mentioned seeds, even at hours in which the decreases are, was higher than that of the other substrates. In corn that caffeic acid-oxidizing enzyme had the highest activity, while this enzyme activity decreased in embryo, it increased in endosperm. In soybean tyrosinase activity was the highest, the activity showed a decrease with time in embryo, but there was no significant activity changing in cotyledon. On the other hand, the lowest activity of tyrosinase observed at 48th h in soybean embryo was higher than those of PPO oxidizing the other substrates. In corn embryo, the lowest activity of PPO oxidizing caffeic acid seen at 36th and 48th h was both relatively higher and more than the other two PPO activities except dopa-oxidizing PPO activity.

Discussion

In this work, we observed that the PPO activities did not show a parallel course of activity to lead to generalization during germination of all the seeds we investigated. However, we found that PPO activities are higher in the embryo of all the seeds than in the food tissues. The finding we obtained is consistent with previously reported works (TANEJA & SACHAR 1974, RAO & DEOSTHALE 1987, KABAR & KOCAÇALISKAN 1990). This result is not surprising. Because food reserves broken down in food tissues will pass to embryo to be used for its grow. PPO, also, probably has a role in degradation of this foods during respiration of embryo. The fact that PPO has a high activity in embryo supports presence of a positive relation between this enzyme complex and respiration (STILES 1960). ATP synthesis in respiratory chain is probably stimulated by supplying quinon production as a result of PPO activity in embryo. These ATPs synthesized, also, are used in processes of synthesis required for germination.

In our present study, we found that PPO substrate which supplies the highest mean activity varies according to species. For example, while dopa-oxidizing PPO had more activity in germination of bean, chickpea, barley and wheat, caffeic acid-oxidizing PPO had more activity in corn, and also, tyrosinase in soybean. These findings may indicate that the PPO substrate which plays an active role in germination may possibly vary according to species. PPO substrates showing high activity may be thought to play a more important role than the others in germination of related species. On the other hand, dopa-oxidizing PPO had the highest activity in germination of seeds except soybean and corn may indicate that, generally, this enzyme plays an important role in seed germination. The fact that

Table 1
PPO activities in embryos and food tissues (cotyledon or endosperm) of the seeds during germination

Seed	Substrates: Hours:	Dopa 0 12 24 36 48	Catechol 0 12 24 36 48	Caffeic acid 0 12 24 36 48	Tyrosine 0 12 24 36 48
Chickpea	Embr Coty	32 32 34 39 51 18 20 26 28 50	15 16 20 20 20 16 17 19 18 18	21 25 27 30 32 19 23 25 29 35	21 27 30 32 29 21 22 26 28 28
Soybean	Embr Coty	26 17 16 15 13 9 8 8 7 6	57 50 45 42 35 5 5 6 7 8	56 52 46 42 33 9 6 6 6 6	88 82 61 55 43 6 6 6 8 8
Bean	Embr. Coty.	50 50 50 52 58 8 8 9 10 10	15 14 12 10 10 3 4 5 5 5	16 15 14 14 15 3 5 8 8 8	22 18 20 19 19 5 5 6 7 8
Barley	Embr. End.	66 54 52 52 52 39 24 25 25 21	9 8 9 10 6 6 6 3 4 3	28 30 28 28 22 22 23 19 19 15	10 8 8 8 5 3 3 2 1 3
Wheat	Embr End.	62 50 43 42 26 9 8 11 18 18	14 12 12 6 4 2 3 4 4 5	28 28 33 30 20 2 4 10 15 16	16 12 10 6 4 1 2 2 2 3
Corn	Embr End.	13 22 40 44 56 14 17 18 20 21	19 23 26 29 38 5 5 5 5 5	66 59 45 40 40 9 10 11 13 17	24 20 18 18 18 5 3 2 2 2
LSD: (0.05)	(Hours)	3.7	2.3	2.4	3.6
	(Seeds)	2.4	1.4	1.5	2.2

soybean and corn are the seeds containing more oil may be a cause of the mentioned difference. VAMOS-VIGYAZO 1981 indicated that PPO activity may be different according to plant species and variety, tissues and even organelles of a cell. Studying of interactions between the enzyme and various factors affecting germination, such as salinity and temperature, will clarify further this subject.

The relation of PPO activities with germination period is, also, rather complex. For example, in the case of chickpea, activities of PPO oxidizing all the substrates have increased with time in both embryo and cotyledon, but decreased in soybean particularly in its embryo. Also, there were activity fluctuations in embryo and endosperm of barley seeds. In earlier works by some workers, similar results are obtained as well. RAO & DEOSTHALE 1987, studied with various legume seeds, reported that catechol-oxidizing activity increased in a species while it decreased in another. KABAR & KOCAÇALISKAN 1990, working with wheat, showed that the activity of dopa- and catechol-oxidizing activities decreased while tyrosinase activity increased. That the changes in the PPO activities during germination differ according to the seeds may be due to chemical and structural differences of the seeds. In addition to differences in embryo size of seeds, size of their food tissues and amount and type of organic material they contain are different as well. All these differences may lead to, that respiration is in different course during germination of seeds. During initial germination, pre-existing substrates found in embryo are used by respiration and then the embryo is benefited from substrates of food tissues. The size of embryo and the amount of food – after imbibition –, may prolong the initial period of respiration, and respiration may slow down in an interphase, until substrates from food tissues are used in embryo. When considering the relation of PPO with respiration, that is its contribution to quinon formation (BEWLEY & BLACK 1983), and when taking into account that changes in respiration during germination are not similar in all the seeds, the fact that PPO activities exhibit differences to seeds seems normal.

That PPO shows a higher activity in embryos of the seeds suggest that this enzyme complex has a close relation with germination events. But the mode and mechanism of this relation have not been exactly revealed yet. PPO studies concerned in germination both are very few and the findings obtained are different and even the opposite of each other. Therefore, time is still too early to arrive an exact decision about the role of this enzyme in germination. Our present work will pave the way for further and more detailed studies.

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