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Polyphenol Oxidase and Related Isoenzymes in Crown-Gall Tumors of Pea

By

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With 1 Figure

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Summary

KADIOĞLU A. & ÖZBAY O. 1990. Polyphenol oxidase and related isoenzymes in crown-gall tumors of pea. – Phyton (Horn, Austria) 30 (2): 283–290, 1 figure. – English with German summary.

The activity of polyphenol oxidase (PPO) and PPO isoenzymes in the crown gall tumors occured in vivo have been examined in pea. It has been observed that PPO activity in crown gall tumor tissues has been increased more than in the normal tissue. This activity of PPO is similar to increase that was observed in auxin treatments. In addition, it has been observed that PPO isoenzymes number in tumor tissue have also been increased in comparison with the number observed in the control application.

Zusammenfassung

KADIOĞLU A. & ÖZBAY O. 1990. Polyphenoloxidase und deren Isoenzyme in Crowngall-Tumoren der Erbse. – Phyton (Horn, Austria) 30 (2): 283–290, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Die Aktivität der Polyphenoloxidase (PPO) und der PPO-Isoenzyme ist in den Crowngall-Tumoren auf *Pisum sativum* höher als im normalen Gewebe. Die Aktivitätserhöhung entspricht der nach Behandlung mit Auxin (IAA). Die Zahl der Isoenzyme ist in den Tumoren höher als im Vergleichsgewebe.

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Introduction

Crown gall tumors are generally induced on a wide range of dicotyledoneus plants species by virulent strains of *Agrobacterium* (NESTER & al. 1984, KAERLAS & al. 1989). Crown gall tumor tissues have a higher level of auxin and cytokinin, and opines (amino-acid derivates) than normal tissue (AMES 1974, WEILER & SPANIER 1981, BRAUN 1981). There are many reports on the biosynthesis of indoleacetic acid (IAA) in tumor tissue (SCHRÖDER & al. 1984, THOMASHOW & al. 1984). They have demostrated that the enzymes in the pathway of IAA synthesis from tryptophan are codding by integrating of transferred DNA (T-DNA) in *Agrobacterium tumefaciens* to the nuclear DNA of plant. They have also reported that the level of IAA in the tumor tissue has been increased by mechanism above mentioned. In addition to such enzymes involved with IAA synthesis, there is a lot of reports about another enzymes in the tumor tissues (JASPARS & VELDSTRA 1965, REAM & GORDON 1982, RAO & al. 1982. DESSAUX & al. 1986). It has also been suggested that polyphenol oxidase (PPO) could be effective in the formation of callus and tumors in plants (GORDON & PALEG 1961). But it is a little known about PPO in crown gall tumors. In this study, PPO activity and its isoenzymes of pea crown gall tumor tissues, in this reason, investigated.

Materials and Methods

Growth of plants

Pea seeds *(Pisum sativum* L. cv. KELVEDON) were used. They were washed for 20 minutes in 1% sodium hypochloride, rinsed throughly and allowed to imbibed overnight in distilled water. The imbibed seeds were sown in moist mixture of sterilized sand and bran (3 : 1) and grown at 25 to 28° C and at 70 to 80% relative humidity in darkness for 8 days. Under dim green light, these epicotyls with third internode 2 to 5 cm long were decapitated, i. e. the plumule and hook were detached (FAN 1967). The internode was marked with ink 10 mm from the cut apex and lanolin paste was applied to prevent loss of water from the cut apex. Then the marked epicotyls were wounded by a sterile needle (LANGLEY & KADO 1972).

Preparation and application of the bacterium and hormone suspensions.

Agrobacterium tumefaciens B6 strains were used.The bacteria were grown LB agar medium (HEMSTAD & REISH 1985). For inoculations of bacteria to plants a liquid medium containing 10 g Bacto-peptone (Difco) and 5 g NaCl per liter were used (AMRHEIN 1974). Inoculations were made with a syringe needle (Hamilton Comp.Reno) 24 h after wounding at marked section of pea seedlings. The plants were incubated for 2 weeks at 25 to 28° C.

Applications were made not only for the bacteria (AB) but also indoleacetic acid (IAA), indolebutyric acid (IBA), ascorbic acid (AA), tryp-

tophan (Trp) and triiodobenzoic acid (TIBA) prepared at 0,5% concentrations. These chemical substances were applied to the each cut stump 50 μM per seedling. The segments were allowed to grow while still attached to the plant. When they were removed for analysis, lanolin paste was wiped off from the segments. These segments were rinsed briefly in diluted hypochloride and distilled water, then wiped dry and stored at 2° C in a refrigerator prior to fractionation.

Extraction and determination of PPO activity

Extracts were made by grinding segments at top speed for 5 min in a Virtis „45" homogenizer in 3 ml of cold 0.1 M sodium-phosphate buffer (pH 6.0) per g fresh weight. The homogenate was centrifuged at 15.000 g for 15 min in a refrigerated centrifuge (IEC/B-60 Model) and the sediment was discarded (De Morrow & Henry 1978). Polyphenol oxidase activity was measured by spectrophotometric method at 480 nm. The other processes for the determination of PPO activity were carried out as described in the earlier studies (Constantinides & Bedford 1967, De Morrow & Henry 1978). Duplicate samples were used for each PPO activity and each experiment was performed at least 5 replications.

Electrophoretical analysis

Polyacrylamide gel electrophoresis was performed as described by Davis 1964 and, Constantinides & Bedford 1967. Tris-glycine buffer (pH 8.3) and 7.5% (w/v) gel were used. Enzyme solution (0.2 ml) containing 10% (w/v) sucrose and a small amount of bromophenol blue (marker dye) was introduced on the top of the vertical polyacrylamide slab gels. An electric current of 100 volt was applied initially until the marker dye had reached the running gel, then increased to 200 volt for the remainder of the electrophoretic run. The run was stopped when the marker dye was about 8 cm from the top of slab gel. All electrophoretic runs were carried out for 3 to 3.5 h at 4° C. Subsequently the gels were incubated in 0.3 M dopa (3,4-dihydroxy-dl-phenylalanine) for 6 to 7 h at 37° C. The location of PPO bands was measured by a ruler.

To determine the statistical significance of differences among the means was used Duncan's multiple range test.

Results

Polyphenol oxidase activity

In this study the interaction between auxin and polyphenol oxidase in pea crown gall tumors has been investigated. The results were evaluated statistically in Table 1.

Table 1

Effect of the various treatments on PPO activity.

Applications	PPO activity*) (A 480/min · g fresh weight)	grouping**)	PPO activity per mg protein
Control	0.52	b	0.43
AB	1.01	ad	0.42
IAA	1.50	fgh	0.59
IBA	1.22	aef	0.54
Trp	0.66	bcd	0.33
AB + IAA	1.43	efg	0.50
AB + IBA	1.09	a	0.44
AB + Trp	0.96	acd	0.43
AB + AA	0.64	bc	0.41
AB + TIBA	1.11	ae	0.42
IAA + TIBA	1.59	gh	0.58
AB + IAA + TIBA	1.87	h	0.64

*) n = 5

**) Means with the same letter are not significantly different at $\alpha = 0.05$ (Duncan's multiple range test).

In the tumor tissues, PPO activity increased significantly comparing to that is in the control. Then auxin hormones were applied to plants for compare PPO activities in auxin applications and in the tumor tissues.

We observed that indoleacetic acid and indolebutyric acid had increased PPO activity. However PPO activity resulted from tryptophan application was not significant in comparison with that in the control.

When both IAA and AB are applied together, PPO activity was found very high in comparison with AB application but not with IAA application. The enzyme activity in AB + IBA applications was not found higher than if AB and IBA have been applicated separately. It has not been observed an important difference in PPO activity between AB + AA and AB applications.

The enzyme activity in AB + TIBA applications has been found to be slightly higher than AB applications. A similar result has been observed between IAA + TIBA and IAA applications.

Polyphenol oxidase isoenzymes

We have observed that the number of PPO isoenzyme bands has been changing from one to four in the various applications (Fig 1).

In the tumor tissue, we observed one more band than the control. In addition, four similar bands were obtained in the applications of IAA and

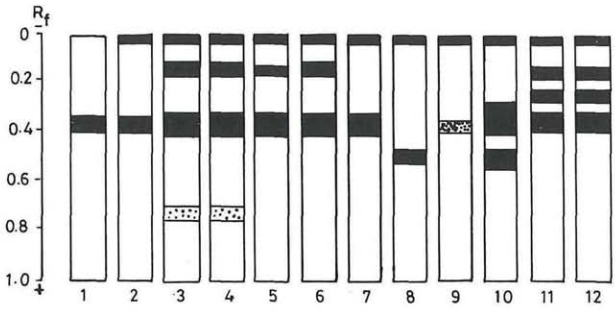

Fig. 1. Polyphenol oxidase isoenzymes.

■ High activity ▨ Medium activity ▩ Low activity

1 Control; 2 AB; 3 IAA; 4 IBA; 5 Trp; 6 AB+IAA;
7 AB+IBA; 8 AB+Trp; 9 AB+AA; 10 AB+TIBA; 11 IAA+TIBA;
12 AB+IAA+TIBA.

Fig. 1. Polyphenol oxidase isoenzymes.

IBA. It was observed a single band in applications of AB with IBA, Trp and AA separately.

In the applications with TIBA the bands were observed in between three and four.

Discussion

There is not enough report about PPO in the tumor tissue as reported previously. However some workers (GORDON & PALEG 1961) concluded that the lysis which occurs after wounding of plant tissue causes the activation of phenolase complex. That may then produce high quantities of growth hormone via the phenol-tryptophan reaction, and callus and gall formation could be histogenic consequences of such hyperauxiny. In accordance with these findings, we have observed that the activity of PPO increased in pea tumor tissue. Some workers (STAFFORD & GALSTON 1970, VERNON & STRAUS 1972) have been reported that IAA can increase PPO activity in plants. This enhancement is explained by increased nucleic acid synthesis (STAFFORD & GALSTON 1970). According to these results we can suggest that the increased PPO activity in pea crown gall tumor tissue is caused by IAA synthesized by the bacterial T-DNA. This suggestion has also been supported by exogenic supply of IAA to plant tissues.

In addition, IBA and Trp have been used to determine the effect of auxin on the PPO activity in the tumor tissue. The results of these applications suggest that the enhancement of PPO activity in tumor tissue may be occured by above caused the mentioned mechanism.

The results of interactions between double and single applications might be indicating that enhancement of the enzyme in the tumor tissue is considerably affected by IAA.

It is known that ascorbic acid is a natural inhibitor of PPO (WEAVER & CHARLEY 1974, WALTER & PURCELL 1980). It has been shown that inhibitory effect of ascorbic acid could be due to either be reducing quinones to phenols (WALTER & PURCELL 1980) or by directly inactivating PPO (WEAVER & CHARLEY 1974). In accordance with these findings we have observed that PPO is inhibited by ascorbic acid in the tumor tissue.

In addition TIBA has also been used with the other applications to verify the effect of IAA on PPO activity. Since TIBA inhibits effectively auxin transport (NIEDERGANG-KAMIEN & LEOPOLD 1957), IAA transport has been inhibited at marked segments of seedlings. Therefore it has been found very high the enzyme activity in TIBA applications.

On the other hand, in all applications (except AB + AA application) the bands of PPO isoenzymes were most active as indicated by the highest color intensity (Fig 1). But a weak band appeared for AB application as different from the control. Probably, this band may be affected by IAA synthesized by *Agrobacterium tumefaciens* T-DNA. Because the similar band with the same of value has also been observed in IAA and IBA applications. Two of the bands in IAA and IBA applications were resembling to the bands formed by the bacterium. This case can also be supporting the fact that the increasing of PPO activity is stimulated by IAA in the tumor tissue.

So far we could not see any investigation on the PPO isoenzymes in pea but the existence of some new isoenzyme bands resulted from IAA application has been reported in various plants. For example, in an earlier study it has been shown that the existence of some isoenzymes which not formed in the control, by the application of IAA in wheat embryos (TANEJA & SACHAR 1977). When IAA was applied to *Nicotiana* tissue cultures, same active bands were appeared in comparison with the control (STAFFORD & GALSTON 1970). These results are in accordance with our findings.

The number of bands has been decreased in double applications as compared to IAA and IBA applications. This situation was probably resulted from the inhibitory effect of high level of IAA. In AB + AA application, we observed that ascorbic acid has been inhibiting the PPO.

According to the all of our findings, PPO activity and isoenzymes in crown gall tumors of pea have been increased. It might be tought that this increment was occured by IAA which synthesized by the bacterium.

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