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Polyphenol Oxidase Activity during Rooting in Cuttings of Grape (*Vitis vinifera* L.) Varieties

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Abstract: Polyphenol oxidase (PPO) activity was investigated during rooting in cuttings from three different grape cultivars (*Vitis vinifera* L. cvs. Muscat, Cardinal and Perlelte), and the enzyme activity and rooting ability were compared. Rooting was observed on the Muscat and Perlelte cuttings, but not on the Cardinal cuttings. PPO activity started to increase in the early stage of the experiment, and decreased after root emergence in the Muscat and Perlelte cuttings. However, enzyme activity started to increase in the early stages and continued throughout the experiment in the Cardinal cuttings. No apparent correlation was found between PPO activity and rooting ability in the cutting.

Key Words: *Vitis vinifera*, polyphenol oxidase, rooting

Farklı Asma (*Vitis vinifera* L.) Varyetelerinin Çeliklerinde Köklenme Sırasındaki Polifenol Oksidaz Aktivitesi

Özet: Farklı asma (*Vitis vinifera* L. cvs. Muscat, Cardinal ve Perlelte) çeliklerinde köklenme sırasındaki polifenol oksidaz (PPO) aktivitesi incelenmiş ve enzim aktivitesi ile köklenme yeteneği arasındaki ilişki karşılaştırılmıştır. Bu çalışmada, Muscat ve Perlelte çeliklerinde köklenme oluşurken, Cardinal çeliklerinde oluşmadı. Muscat ve Perlelte çeliklerinin PPO aktivitesinde başlangıçtan itibaren artış görülmüş ve köklenmeyle birlikte azalma görülmüştür. Cardinal çeliklerinde ise PPO aktivitesi sürekli artmaya devam etmiştir. Sonuç olarak, çeliklerin PPO aktivitesi ile köklenme yeteneği arasında belirgin bir ilişki bulunamamıştır.

Anahtar Sözcükler: *Vitis vinifera*, polifenol oksidaz, köklenme

Introduction

Polyphenol oxidase (PPO) is probably present in all plants (Whitaker, 1972). The enzymes, in the presence of oxygen, catalyse the oxidation of phenolic compounds to form corresponding quinone intermediates which polymerise to form undesirable pigments. This enzyme catalyses two types of oxidative reactions: the hydroxylation of monophenols to o-diphenols, and the oxidation of o-diphenols to o-quinones. PPO enzymes extracted from different plant tissues have shown varying degrees of utilisation of phenolic substrates. Mushroom PPO has been shown to display activity with both mono-

and dihydroxyphenols, while PPO extracted from other sources utilised only dihydroxyphenols (Cash et al., 1976).

Extensive research has been carried out on the rooting of cuttings. Many factors have been reported to influence adventitious root formation on cuttings. The activities of a number of enzymes change during the rooting process, and it has been suggested that some of these enzymes are involved in root formation (Dalet et & Cornu, 1988; Gonzales et al., 1991). PPO can indirectly regulate the synthesis of phenolics and plays a role in the organisation and development of root primordia (Hahlbrock & Grisebach, 1979). It has been reported that

PPO plays an important role in cell division, differentiation and primordium development (Huystee & Cairns, 1982).

In this study, PPO enzyme activity during rooting in cuttings from different grape cultivars (*Vitis vinifera* L. cvs. Muscat, Cardinal and Perlelte) was studied, and the relationship between enzyme activity and rooting ability was investigated.

Materials and Methods

Rooting process of cutting

Grape stem cuttings (*Vitis vinifera*) were obtained from the vineyards at the Agriculture Faculty, Dicle University. Ninety cuttings (20 cm in length, with 4-5 nodules) were taken from three cultivars (Muscat, Cardinal and Perlelte). The cuttings were placed in distilled water in a beaker. The rooting and growth of the cuttings took place in darkness at 25 °C. The percentage of cuttings showing root and primordium formations was recorded every 5 days, and sections from the basal end of the cuttings were taken in order to investigate enzyme activities.

Purification of polyphenol oxidase

For extraction, 0.5 g of the sample was homogenised in 5 ml of 0.5 M phosphate buffer (pH 6.3) containing 0.5% polyethylene glycol and 10 mM ascorbic acid using a Fisher Model-300 homogeniser for 3 min. The crude extract was filtered, and centrifuged at 20,000 g for 15 min at 5 °C. The supernatant was brought to 80% $(\text{NH}_4)_2\text{SO}_4$ saturation with solid $(\text{NH}_4)_2\text{SO}_4$. The precipitated PPO was separated by centrifugation at 20,000 g for 30 min. The precipitate was dissolved in a small amount of 5 mM phosphate buffer (pH 6.3) and dialysed at 5 °C in the same buffer for 12 h with three changes of buffer during the dialysis. The

dialysis material remaining in the tube was used for measuring PPO activity.

Determination of polyphenol oxidase activity

PPO activity was determined by measuring the increase in absorbance at 420 nm with a recording spectrophotometer (Shimadzu UV 120-02). The sample cuvette contained 0.2 ml of enzyme solution and 2.8 ml of 10 mM 4-methylcatechol (0.2 M phosphate buffer, pH 6.3). The blank sample contained only 3 ml of 4-methylcatechol solution. The enzyme activity was calculated from the linear portion of the curve (Wong et al., 1971). One unit of PPO activity was defined as the amount of enzyme which caused an increase in absorbance of 0.001/min.

Results

The root formation and the number of roots in the grape cuttings (*Vitis vinifera*) according to time and variety are shown in Table 1. Rooting was observed on the Muscat and Perlelte cuttings, but not on the Cardinal cuttings (Figure 1).

In the Muscat cuttings, primordium formation appeared by the 30th day and roots emerged from the basal parts on the 35th day. The rooting rate and the number of roots were higher than in Perlelte. In the Perlelte cuttings, primordium formation appeared by the 30th day. The emergence of the first roots was observed on the 35th day and the root number increased on the following days. In the Cardinal cuttings, primordium formation and rooting did not take place, and the cuttings started to crack from the basal parts after the 45th day.

The PPO activities in the grape cuttings (*Vitis vinifera*)

Table 1. Primordium and root formation in cuttings (*Vitis vinifera* cvs. Perlelte and Muscat).

Days	Perlelte			Muscat		
	Primordium numbers	Root numbers	Rooting (%)	Primordium numbers	Root numbers	Rooting (%)
30	2.6 ± 1.4	--	--	5.4 ± 3.8	--	--
35	7.3 ± 3.8	8.4 ± 3.9	10.0 ± 7.8	9.8 ± 6.6	8.1 ± 5.9	30.1 ± 20.4
40	4.9 ± 1.9	9.8 ± 6.6	15.2 ± 12.3	7.0 ± 4.6	12.6 ± 10.4	40.4 ± 26.8
45	2.8 ± 1.1	10.3 ± 5.4	23.3 ± 13.1	3.8 ± 1.4	15.5 ± 10.4	50.3 ± 23.3
50	1.8 ± 0.8	11.6 ± 5.9	24.9 ± 15.2	2.2 ± 0.7	18.8 ± 13.3	55.4 ± 25.4



Figure 1. Root formation in the cuttings (*Vitis vinifera* cvs. Cardinal, Perlelte and Muscat).

during the experiment are shown in Table 2. The enzyme activity showed a similar increase in all three grape varieties from the early stages. PPO activity increased from the outset in the Muscat cuttings, reached its highest level on the 40th day and then decreased in the following days. In the Perlelte cuttings, the enzyme activity started to increase from the early stages and the highest increase was observed on the 35th day. The enzyme activity increased continuously from the 5th to the 50th day in the Cardinal cuttings (Figure 2).

Discussion

In this study, PPO enzyme activity was investigated during the rooting of *Vitis vinifera* cv. Muscat, Perlelte and Cardinal, and enzyme activity and rooting ability were compared.

Table 2. Polyphenol oxidase activity (EU/ml) during rooting in the cuttings.

Days	Perlelte	Muscat	Cardinal
0	169.7	218.2	130.3
5	339.4	296.9	233.3
10	615.2	530.3	278.8
15	824.2	551.5	223.3
20	860.6	766.6	266.6
25	852.3	714.3	257.7
30	881.8	805.3	409.1
35	1175.6	1075.8	642.4
40	954.5	1303.2	657.6
45	909.1	1242.4	709.1
50	872.7	1136.4	781.8

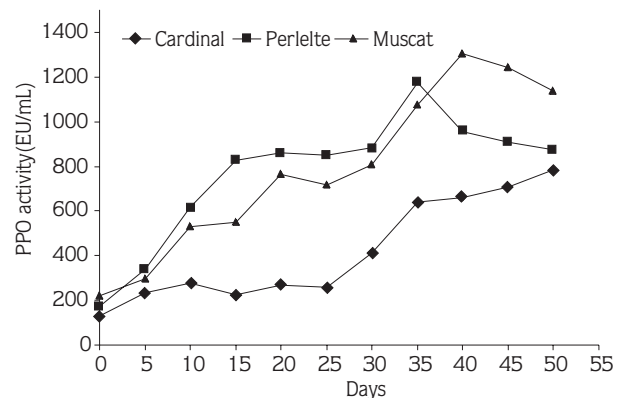


Figure 2. Polyphenol oxidase activity during rooting in the cuttings.

PPO activity started to increase from the early period of growth, and reached its maximum level during primordium and root development. As shown in Table 1, in the Muscat and Perlelte cuttings we detected root formation after the 35th day. When we focused on the PPO activity corresponding to those days, it appeared that there was a relationship between PPO activity and the formation of rooting. In the following days, a constant, gradual decrease in enzyme activity was observed (Figure 2). Results from other studies support this inference. For example, it was reported that PPO plays an important role in cell division, differentiation and primordium development (Huystee & Cairns, 1982). The decrease in PPO activity in the same period led us to assume that root growth in cuttings starts with the decrease in PPO activity. This has also been indicated by

Vaughn & Duke (1984), who determined that PPO plays a role in polyphenol synthesis and inhibits lignin biosynthesis, consequently affecting cell division, cell differentiation and root emergence.

In the Cardinal cuttings, however, there was no indication of root growth. PPO activity was always lower than in the Muscat and Perlelte cuttings. Since the level of PPO activity was unable to reach the next level, it was impossible to distinguish Cardinal root cells. The reason

for is that in our lab Cardinal grew its roots on the 75th day, when PPO activity was about 1000 EU/ml.

In conclusion, it can be stated that PPO does not affect the after- formation of the root, but it does affect cell division, cell differentiation and the development and organisation of root primordia. Our results are in agreement with the earlier findings. The conclusion of this study is supported by the researchers mentioned above.

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