Asian Journal of Chemistry Vol. 19, No. 5 (2007), 4020-4024

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

HARUN ÇOBAN
Alasehir Vocational Training College, Celal Bayar University
45600 Alasehir, Manisa, Turkey
E-mail: harun.cobann@hotmail.com

Poly phenol oxidase (PPO) activity was investigated during rooting in cuttings from six grape cultivars (*Vitis vinifera* L. cvs. Sultana, round seedless, Yalova incisi, Trakya ilkeren, Pembe Gemre and Cardinal) and the enzyme activity and rooting ability were compared. Rooting was observed on Sultana, round seedless, Yalova incisi and Trakya ilkeren cuttings, but not on the pembe gemre and cardinal cuttings. PPO activity started to increase in the early stage of the experiment and decreased after root emergence in the Sultana, round seedless, Yalova incisi and Trakya ilkeren cuttings. However, enzyme activity started to increase in the early stages and continued throughout the experiment in the pembe gemre and cardinal cuttings. In concluded that PPO does not any effect on the after formation of the root. However, it is found that PPO affects cell division, cell differentiation and the development of root primordial considerably.

Key Words: *Vitis vinifera* L., Polyphenol oxidase, Cutting, Rooting.

INTRODUCTION

The oxidation of phenolic substances is carried out by polyphenol oxidase and peroxidase[1]. Polyphenol oxidase (PPO) is probably present in all plants[2]. The enzymes, in the presence of oxygen, catalyze the oxidation of phenolic compounds to form corresponding quinone intermediates which polymerize to form undesireble pigments. This enzyme catalyzes two types of oxidative reactions *i.e.*, 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 utilization of phenolic substrates. Mushroom PPO has been shown to display activity with both mono and dihydroxyphenols, while PPO extracted from other sources utilized only dihydroxyphenols[3,4].

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[5-8]. PPO can indirectly regulate the synthesis of phenolics and plays a role in the organization and development of root primordia[9]. It has been reported that PPO plays an important role in cell division, differentiation and primordium development[10,11].

In this study, PPO enzyme activity during rooting in cuttings of some grape cultivars (*Vitis vinifera* L. cvs. Sultana, round seedless, Yalova incisi, Trakya ilkeren, pembe gemre and cardinal) was studied, and the relationship between enzyme activity and rooting ability was investigated.

EXPERIMENTAL

Rooting process of cutting: Grape stem cuttings (*Vitis vinifera* L.) were obtained from the vineyards at the Viticulture Research Institute of Manisa, 180 cuttings (30 cm in length, with 5-6 nodules) were taken from five cultivars (Sultana, round seedless, Yalova incisi (Hönüsü X Siyah Gemre), Trakya ilkeren (Alphonse Lavalleé X Perlette), pembe gemre and cardinal). The cuttings were placed in distilled water in a beaker. The rooting and growth of the cuttings took place in darkness at 25ºC. The percentage of cuttings showing root and primordium formations was recorded every 7 d 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 Jec Model homogenizer for 3 min. The crude extract was filtered and centrifuged at 20,000 g for 15 min at 5ºC. The supernatant was brought to 80 % $(NH_4)_2SO_4$ saturation with solid $(NH_4)_2SO_4$. The precipitated PPO was separated by centrifugation at 20,000 g for 0.5 h. The precipitate was dissolved in a small amount of 5 mM phosphate buffer (pH 6.3) and dialyzed at 5ºC in the same buffer for 12 h with changes of buffer during the dialysis. The dialysis material remaining in the tube was used for measuring PPO activity[8].

Determination of polyphenol oxidase activity: PPO activity was determined by measuring the increase in absorbance at 420 nm with a recording spectrophotometer (Hitachi U 2000 UV 121-002). 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[12].

RESULTS AND DISCUSSION

The root formation and number of roots in the grape cuttings (*Vitis vinifera* L.) according to time and variety are shown in Table-1.

Rooting was observed on the Sultana, round seedless, Yalova incisi and Trakya ilkeren cuttings, but not on the pembe gemre and cardinal cuttings. In the Sultana, round seedless, Yalova incisi and Trakya ilkeren cuttings, primordium formation appeared by the 28th day roots emerged from the basal parts on the 35th day and the root number and rooting increased on the following days.

TABLE-1
PRIMORDIUM AND ROOT FORMATION IN CUTTINGS OF SOME GRAPE VARITIES (*Vitis vinifera* L.)

Days	Sultana			Round Seedless		
	Primordium numbers	Root numbers	Rooting (%)	Primordium numbers	Root numbers	Rooting (%)
28	4.4 ± 2.1	–	–	4.6 ± 1.8	–	–
35	8.3 ± 5.7	8.0 ± 6.0	26.6 ± 20.0	9.8 ± 6.6	8.0 ± 6.0	27.5 ± 21.2
42	9.8 ± 5.5	13.2 ± 11.0	46.9 ± 27.2	7.0 ± 4.6	13.2 ± 11.0	47.8 ± 28.6
49	11.3 ± 7.4	16.3 ± 9.4	54.3 ± 29.6	3.8 ± 1.4	16.3 ± 9.4	54.3 ± 31.2
56	14.6 ± 10.7	19.1 ± 14.1	53.2 ± 34.4	2.2 ± 0.7	19.1 ± 14.1	66.0 ± 37.2
Days	Yalova Incisi			Trakya Ilkeren		
	Primordium numbers	Root numbers	Rooting (%)	Primordium numbers	Root numbers	Rooting (%)
28	3.4 ± 1.7	–	–	2.6 ± 1.2	–	–
35	6.2 ± 3.1	8.2 ± 3.6	27.6 ± 18.4	7.8 ± 4.6	7.4 ± 3.9	18.8 ± 20.4
42	4.3 ± 1.9	9.5 ± 5.8	33.3 ± 22.6	5.1 ± 2.1	9.9 ± 5.3	36.0 ± 26.8
49	3.0 ± 1.2	11.0 ± 5.1	37.0 ± 23.7	3.8 ± 1.4	10.5 ± 6.2	35.5 ± 23.3
56	2.1 ± 1.1	12.3 ± 6.1	41.4 ± 26.2	1.7 ± 0.7	11.3 ± 4.3	37.5 ± 25.4

The highest values on the 56th day for primordium numbers were found round seedless (4.6 ± 1.8), Sultana (4.4 ± 2.1), Yalova incisi (3.4 ± 1.7) and Trakya ilkeren (2.6 ± 1.2), respectively. However, The highest values on the 56th day for the rooting rate and the number of roots were determined round seedless (66.0; 19.1), Sultana (53.2; 19.1), Yalova incisi (41.4; 12.3) and Trakya ilkeren (37.4; 11.3), respectively.

In the pembe gemre and cardinal cuttings, primordium formation and rooting did not take place and the cuttings started to crack from the basal parts after the 42nd day. The PPO activity in the grape cuttings (*Vitis vinifera* L.) during the experiment are shown in Table-2.

The enzyme activity showed a similar increase in all six grape varieties from the early stages. PPO activity increased from the outset in the Sultana, Round Seedless cuttings, reached its highest level on the 56th day

TABLE-2
POLYPHENOL OXIDASE ACTIVITY (EU/mL)
DURING ROOTING IN THE CUTTINGS

	Grape Varieties					
Days	Sultana	Round seedless	Yalova incisi	Trakya ilkeren	Pembe gemre	Cardinal
0	224.3	218.2	154.5	165.6	143.5	120.8
7	284.5	296.9	313.5	348.4	137.9	228.6
14	522.3	530.3	590.4	605.2	142.2	272.3
21	644.2	551.5	785.5	812.6	232.4	288.5
28	738.3	766.6	874.6	884.8	261.4	298.7
35	732.5	724.3	842.3	886.3	237.8	257.7
42	815.3	825.2	871.3	888.5	388.3	409.1
49	1110.3	1180.4	1083.8	1138.6	583.6	614.3
56	1303.2	1340.5	965.6	983.3	653.4	653.8
63	1221.6	1253.3	894.8	903.5	689.6	710.6
70	1123.7	1143.8	843.7	847.2	798.9	814.8

and decreased in the following days. In the Yalova incisi and Trakya ilkeren cuttings, the enzyme activity started to increase from the early stages and the highest increase was observed on the 49th day. The enzyme activity increased from the 14th to the 63rd day in the pembe gemre and 7th to the 63th cardinal cuttings (Fig. 1).

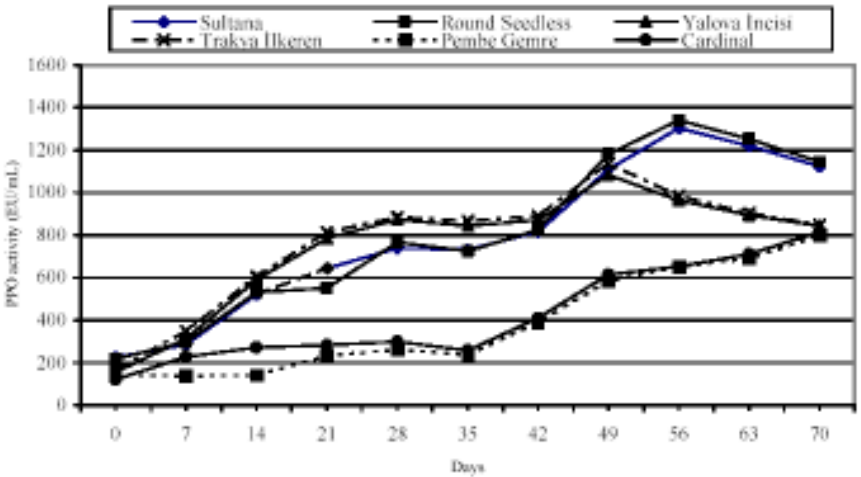

Fig. 1. Polyphenol oxidase activity during rooting in the cuttings

In this study, PPO enzyme activity was investigated during the rooting of *Vitis vinifera* L. cv. Sultana, round seedless, Yalova incisi, Trakya ilkeren, pembe gemre and cardinal, and enzyme activity and rooting were compared.

PPO activity started to increased from the early period of growth and reached its maximum level during primodium and root development.

As shown in the Table-1, in the Sultana, round seedless, Yalova incisi, and Trakya ilkeren 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. The enzyme activity was observed (Fig. 1).

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[8,10]. 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 and Duke[13], 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 pembe gemre and cardinal cuttings, however, they were no indication of root growth. PPO activity was always lower of than in the Sultana, round seedless, Yalova incisi and Trakya ilkeren cuttings. Since the level of PPO activity was unable to reach the net level, it was impossible to distinguish pembe gemre and cardinal root cells. The reason for is that in our laboratory cardinal grew its roots on the 70th day, and in the pembe gemre 56th day, when PPO activity was about 700 EU/mL.

Conclusion

PPO does not effect on the after formation of the root. However, it is found that PPO affects cell division, cell differentiation and the development of root primordial considerably. Our results are in agreement whit the earlier findings.

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(*Received*: 31 October 2006; *Accepted*: 9 March 2007) AJC-5511