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Identification of Phenolic Substances and Their Seasonal Changes in Sultana and Perlette Grape Cultivars

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Abstract
Eleven phenolics compounds were detected in grape cultivars Sultana and Perlette on two dimensional TLC. No differences were found in the phenolic content of the cultivars. The phenolic substances in the shoot tips and leaves showed some differences. Spots belonging to the flavonoids and chlorogenic acids changed in size and intensity in some stages of the annual growth cycle of the vine.

Introduction
Phenolic substances, the secondary metabolites which are widely distributed in plant kingdom are formed in various organs at different concentrations as a response to environmental stimuli and exert effects on plant growth. Presence of phenolics in vegetative and reproductive parts of plants may actively inhibit or stimulate growth and development. Phenolic substances are found to be related with root formation in callus culture (Feucht and Dausend, 1972), union of graft combinations (Treutter *et al*., 1990) and tolerance to light intensity, drought or cold conditions. Significant positive correlations were determined between phenolics and pest and disease resistance (Howell *et al*., 1976).

It is possible that some physiological changes occurring in plants may be revealed by studying the changes in phenolics. Relationships between phenol metabolism and seasonal development of plant organs and tissues have been shown repeatedly (Treutter *et al*., 1990). The qualitative changes in phenolic substances at some developmental stages were determined in various organs of strawberry and olive (Ozeker, 1994; Akillioglu, 1994).

Since there is only limited data available on the role of phenolics in the processes of growth and development of the vine, it is important to study the physiological changes of phenolic substances in the shoots and leaves. The aim of this investigation is to identify phenolic substances in the shoot tip and leaf of Sultana and Perlette grape cultivars and study the relationship between phenolic content and various physiological events .

Materials and Methods
In the experiment, mature leaves at middle nodes of shoots and shoot tips were sampled from Sultana and Perlette grape cultivars (Anonymous 1983). All samples were collected weekly from bud burst (first week of April) to the middle of harvest (last week of August).

Leaf and shoot tip samples of 19 were extracted with 96 percent ethanol (25 ml). The phenolic substances were analysed through thin layer chromatography (TLC) method. 10*10 cm sized Merck 5577 plates were used in qualification. 10 µl of samples were spotted on the right corner of each plate and developed in two dimensions. The first development was carried in a solvent of butan-1-ol: acetic acid: water (4:1:5 v/v) and the second dimension in acetic acid: water (5: 95 v/v). Chromatographic plates were sprayed with Naturstoff (NS) and examined under long wave (366 nm) UV light. After the second development Rf value of each spot was determined. The color intensity of each spot was investigated, as well (Tanrisever, 1982a). Although there are more delicate methods such as HPLC, TLC was used in this study since it is more practical and easy to apply in the analyses of phenolic substances.

Results and Discussion
The phenolic substances in the shoot tips and leaves of Sultana and Perlette cultivars were analysed from the beginning of April till the end of August qualitatively at weekly intervals by means of TLC. Totally eleven phenolic spots possessing different Rf values and colors were identified in both cultivars of organs and stages. The main chromatogram possessing these spots is shown in Fig. 1.

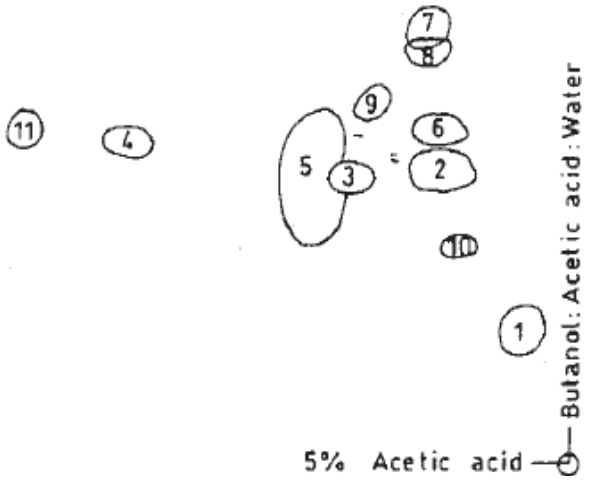

Fig. 1: The main chromatogram of spot patterns on Sultana and Perlette cultivars

Table 1: Rf values, colors and identification of the spots obtained for Sultana and Perlette grape cultivars

Spot No.	Rf value		Color	
	BAW	AA	NS+UV	Identification
1	0.27	0.05	l. blue	Chlorogenic acid
2	0.59	0.12	d. orange	Flavonoid
3	0.56	0.25	l. orange	" "
4	0.60	0.60	d. blue	Chlorogenic acid
5	0.62	0.40	l. yellow	Flavonoid
6	0.64	0.14	d. orange	" "
7	0.79	0.20	l. orange	" "
8	0.75	0.19	" "	" "
9	0.65	0.30	" "	" "
10	0.36	0.10	" "	" "
11	0.63	0.84	l. blue	Chlorogenic acid

BAW: Butan-1-ol: acetic acid: water (4:1:5); AA: 5% Acetic acid; NS: Naturstoff; UV: Ultraviolet lights; l: Light; d: Dark

Table 2: Distribution of spots in shoot tip and leaf of grape cultivars

Spot No.	Sultana		Perlette	
	Shoot tip	Leaf	Shoot tip	Leaf
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	-	+	-
6	+	+	+	+
7	+	+	+	+
8	-	+	-	+
9	-	+	-	+
10	-	+	-	+
11	-	+	-	+

+: Detected; -: Not detected

The identification of phenolics is made by the Rf values of the spots in different solvents and their colors under long wave (366 nm) UV light (Tanrisever, 1982b). In respect, orange and yellow colored spots (2, 3, 5, 6, 7, 8, 9 and 10) were accepted to represent flavonoids and blue colored spots (1, 4 and 11) chlorogenic acid (CA). The Rf values and colors of the spots obtained after two- dimensional development of the chromatograms are given in Table 1.

No differences were found between the two cultivars in terms of phenolics as a result of the investigation of the two dimensional chromatograms (Table 2). In the Vitis roots, shoots, stems and leaves no cultivar specific phenolic substances were observed (Schaefer, 1985). Similarly, it was determined that there were no differences in the phenolics of cherry, peach, almond and strawberry cultivars (Tanrisever, 1982a; Misirli *et al.*, 1993; Ozeker, 1994). But still phenolic compounds have a value in the identification of some fruits species and cultivars (Ulubelde and Lester, 1982).

Between shoot tips and leaves there were some difference concerning phenolics. Flavonoids numbered as spot 8, 9, 10 and 11 were found only in leaves and 5 flavonoids we found only in shoot tips. It was reported that phenolics the root, stem, shoot and leaf of vine were greatly differe from each other both quantitatively and qualitative (Schaefer, 1985).

Table 3: Distribution of spots at different stages of annil growth cycle of the vine

Spot No.	I	II	III	IV
1	+++	+++++	++++	++
2	++++	+++	+++++	++++
3	+	++	+++	+++
4	+	+++	++	+
*5	+++	++++	+++++	++++
6	+++	++++	++++	++++
7	+++	++	+++	++++
**8	+++	++	+++	+++
**9	++	+	++	++
**10	+	+	++	++
***11	++	+++	++	+

+; Relative amount of phenolic substances; +: very scare +++++: very dense; *5: Detected only in shoot tip; **8, **9, **10 and **11: Detected only in leaf I, II, III and Stages

It was determined as a result of the investigation of two dimensional chromatograms that there were four different stages in the phenolics of the shoot tips and leaves of the cultivars. First stage (during April) (I), the spot size and intensity of CA 1, 4 and 11 were less than the other stages (Table 3). The bud burst occurred at this stage of the vin annual growth cycle (Winkler *et al.,* 1977). In the second stage (between the first week of May and second week of June) (II), spots belonging to CA continued to increase flavonoid spots of 2, 3, 6, 7, 8, 9 and 10 were reduced

gradually (Table 3). Blooming occurred and the shoots continued to elongate rapidly during this stage (first week of June) (Winkler *et al*., 1977). The period of rapid vegetative growth of the vine was correlated with high CA content in the shoots and leaves (Lilov and Angelova, 1987). During the third stage (from the second week of June to the first week of August) (III), flavonoids numbered as 2, 5, 6 and 7 were greater in size and intensity than the others and CA began to decrease to its minimal level (Table 3). During last stage (between the second week and last week of August) (IV), spots of the flavonoids increased, whereas, CA decreased gradually (Table 3). The shoots stopped elongating and fruit initiation started during the last period. CA content was low and flavonoid content was high in the shoots and leaves of the vines with intense flower- and fruit formation (Lilov and Angelova, 1987).

Some phenolics could not be detected in certain periods or their concentrations varied. Considering the above mentioned changes in phenolics, it can be concluded that the phenolic substances have a special significance in the grapevine metabolism.

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