

Full Length Research Paper

Leaf phenolic content of some squash rootstocks used on watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) growing and phenolic accumulation on grafted cultivar

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In this study, phenolic compounds were identified in the leaves of grafted and non-grafted watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) plants and squash rootstocks. The watermelon cultivar 'Crispy' was grafted onto hybrids of *C. maxima* × *C. Moschata*, 'TZ-148' and 'RS-841', and *Lagenaria siceraria* cv. '64 -18'. The experiments were conducted in a randomized complete blocks design in two time periods. Non-grafted plants were used as the control. Some important differences were detected for the contents of the phenolic compounds during the growing periods such as kaempferol accumulates in the leaves of grafted and non-grafted watermelon plants and rootstocks. Kaempferol contents of the rootstocks were found to be higher than the grafted and non-grafted watermelon plants. Plants grafted on 'TZ-148' or 'RS-841' had higher phenolic content than the others.

Key words: Watermelon, squash rootstocks, grafting, phenolic compounds.

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] belong to *Citrullus* genus of the *Cucurbitaceae* family, which originates from Central Africa. It is a popular vegetable in the world as well as in Turkey. The latest reports showed that watermelon production has significantly increased in the world related to increase in growing areas and yield. Whereas the growing area and production were 2.3 million ha and 41 million mt, respectively in 1995, they were increased to 3.5 million ha and 96.5 million mt in 2005 (FAO, 2006). Although still the second largest producer after People's Republic of China, Turkish watermelon production has declined both in acreage and quantity contrary to the increase in the world. In 1995, watermelon were grown on 146.000 ha of land and the total production was 3,9 million mt, but in 2005 watermelon production was decreased to 3,8 million mt which was grown on 137.000 ha of land (FAO, 2006).

Soil borne diseases and pests can be very detrimental to watermelon. The conventional solution to this problem is crop rotations for at least 5 years. In greenhouse production methyl bromide, which is a very harmful chemical for human health and environment, was used against this pathogens. Another control strategy is using of resistant varieties. It is effective on reducing yield loss, minimizing pesticide use and eliminating ruin problem, even some fruit characteristics may not meet the grower demands. To overcome such problems, the use of seedlings grafted on *Cucurbita* and *Lagenaria* rootstocks, which have an acquired resistance to soil borne diseases, was suggested by several researchers as an environmentally safe alternative to methyl bromide (Ristanino and Tomas, 1997; Edelstein et al., 1999; Cebolla et al., 2000; Yetişir et al., 2003; Miguel et al., 2004; Boughalleb et al., 2008). It was reported that the resistance of grafted plants to *Fusarium* wilt is related to rootstock's resistance (Biles et al., 1989; Heo, 1991).

The effects of the rootstocks on plant growth, fruit yield and quality were studied by comparing grafted plants with non-grafted ones under low tunnels for early production

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and later production in open field (Alan et al., 2007). It was reported that grafting was affected plant growth positively where control plants had shorter main stems, less lateral vines and low root dry weight. Fruit yield was also positively influenced by grafting in compared to the control under two growing conditions. Detrimental effects were not determined in fruit quality such as fruit index, rind thickness, and soluble solid contents on grafted plants.

In a different study, watermelons are grafted on *Cucurbita maxima*, *C. moschata*, and *Lagenaria siceraria* rootstocks. These rootstocks were influenced resistance to soil borne diseases, plant growth, yield, and fruit quality. Graft incompatibility and decrease in the fruit quality appeared depending on the scion-rootstock combination (Lee and Oda, 2003).

Plants have developed protection strategies against different pathogens and environmental stress. There are morphological and also biochemical factors on resistance to plant pathogens such as phenylpropanoids. Phenols, tannins, lignins, phenolic acids and flavonoids as phenylpropanoids, are secondary metabolites and have several functions in plants. They functions as visual traps and antioxidants and play important roles on enzymatic activities, plant growth, fruit quality, graft compatibility and provide resistance against pathogenic microorganisms, herbivores, UV radiation, oxidative and thermal stress (Kuhnau, 1976; Sommer and Saedler, 1986; Winnard et al., 1986; Hahlbrock and Scheel, 1989; Treutter et al., 1990; Beier and Nigg, 1992; Seigler, 1998; Paolacci et al., 2001; Jaakola et al., 2002; Lorenc-Kukula et al., 2007). These compounds are present in the roots, shoots, flower and leaf buds, woody parts, leaves, phloem and pollen (Mısırlı et al., 1995).

The phenolic contents could be used for the investigation of the resistance to pathogenic fungi and plant viruses (Oertel, 1994). High concentrations of phenols may inhibit spore germination and development of fungi (Boyraz and Sürel, 2004). Investigation on strawberries, it was found that disease resistant cultivars had more flavonoid content than the susceptible ones. Two of these flavonoids were quercetin and kaempferol (Anttonen et al., 2003). It was found that epidermal strips of *Vicia faba* also contain kaempferol and quercetin glycosides (Takahama, 1988). In a similar study, Awad (2000), used five cucumber (*Cucumis sativus*), three zucchini (*Cucurbita pepo*), three cantaloupe (*Cucumis melo var. cantaloupensis*) and two watermelon (*Citrullus vulgaris* [*Citrullus lanatus*]) cultivars and hybrids to analyze for resistance to mildew (*Sphaerotheca fuliginea*) disease. Consequently, it was seen that resistant watermelon, zucchini and cantaloupe plants contained more free and total phenols than the susceptible ones.

Taking into account the above mentioned explanations on phenolic compounds that plays important roles on disease resistance mechanisms, plant growth, and quality, it is important to determine phenolic compounds on grafted and non-grafted watermelon plants because the grafted seedlings

has been used widely on watermelon production today. Accordingly, in the current study phenolic compounds were analysed on grafted and non-grafted watermelon plants and rootstocks.

MATERIALS AND METHODS

This study was carried out at Ege University Ödemiş Vocational Training School research fields and laboratories in 2006. Watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) cultivar "Crispy", which is widely grown in the region, was used as scion and 'TZ-148' and 'RS-841' (*C. maxima* x *C. Moschata* hybrids) commercial rootstocks and '64-18' (*Lagenaria siceraria* cv.) experimental rootstock were used as materials. Non-grafted "Crispy" was included as the control. An experiment was conducted in a complete randomized block design with three replications in two different periods of the year (April and June planting). The non-grafted *Cucurbita* rootstocks were planted only in April. Seedlings were planted on a 2 x 2 m row space with 2500 seedlings per hectare.

The leaves that reached their ultimate size were used for the determination of phenolic compounds. The leaves (10 -15 pieces) were dried in oven at 65°C, granulated and 100 g granulated samples were extracted in 10 ml methanol for ten days. These samples were filtered, and the filtrate was vacuum dried at 50°C, solved in 2 ml methanol and filtered through a 0, 45 µm filter paper. The samples were kept at 4°C until analyzed (Mısırlı et al., 2001; Gunen et al., 2005). The samples were analyzed by using High Performance Liquid Chromatography (HPLC) (Treutter et al., 1990; Martelock et al., 1994). The C -18 column was used for the analyses and the samples were evaluated at the 280 nm wavelength. The phenolic content was detected as total phenolic concentration (µg) by comparing the data obtained from the peak areas of the phenolic standards. Kaempferol, quercetin and ellagic acid were used as standards. The data were analyzed statistically using SAS (SAS, 1996)²⁹.

RESULTS AND DISCUSSION

Although it was stated that species of *Cucurbitaceae* family contains quercetin and ellagic acid besides kaempferol, in this study, quercetin and ellagic acid were not detected in the samples. Kaempferol was determined in all samples. Kaempferol content of grafted and non-grafted watermelon plants and rootstocks is given at Figure 1.

There were considerable differences between treatments, grafted plants and control cvs. Grafted plants in both periods, but no significant differences were detected between rootstocks (Table 1). In the first period, the lowest kaempferol content was determined in non-grafted crispy cultivar (2.14 µg) and crispy onto 64 -18 rootstock (2.16 µg). These applications were in the same statistical group and remained below than the other grafted plants (Table 1).

Similar to the first period, significant differences were detected between grafted and non-grafted plants, in the second period (Table 1). TZ 148 grafted Crispy plants (3.77 µg) had the first place and followed by RS 841 grafted (3.52 µg) Crispy plants. They contained much more kaempferol (3.77 and 3.52 µg) in their leaves than non-grafted crispy

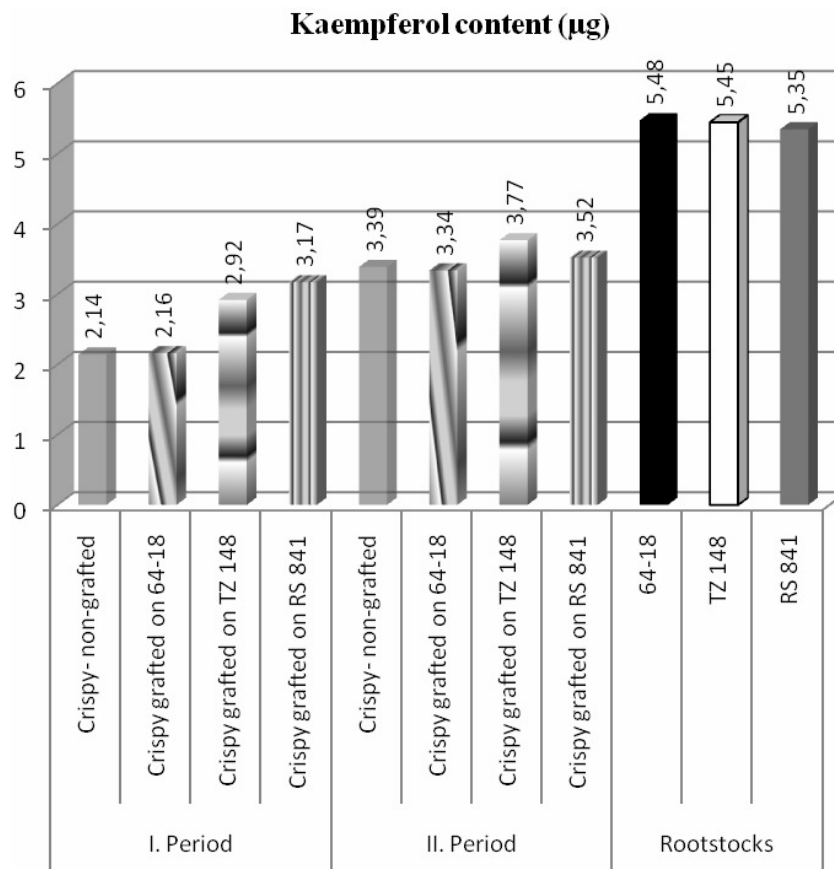


Figure 1. Kaempferol content of grafted and non-grafted plants and rootstocks (μg).

Table 1. Statistical analysis of grafted and non-grafted plants and rootstocks on the first and the second period.

	Treatment	I. Period	II. Period		
rootstocks	64-18	5.48	-		
	TZ 148	5.45	-		
	RS 841	5.35	-		
Grafted	Crispy- 64-18	2.16	3.34		
	Crispy- TZ 148	2.92	3.77		
	Crispy- RS 841	3.17	3.52		
	Control	2.14	3.39		
		ANOVA	ANOVA		
		DF	MS	DF	MS
	Replication	2	0.016	2	0.007
	Treatment	6	7.282**	3	0.111**
	Rootstocks	2	0.014	-	-
	Grafted	2	0.830**	2	0.140**
	Control vs. Grafted	1	0.837**	1	0.053**
	Error	12	0.011	6	0.003
	LSD (%5)		0.19		0.11

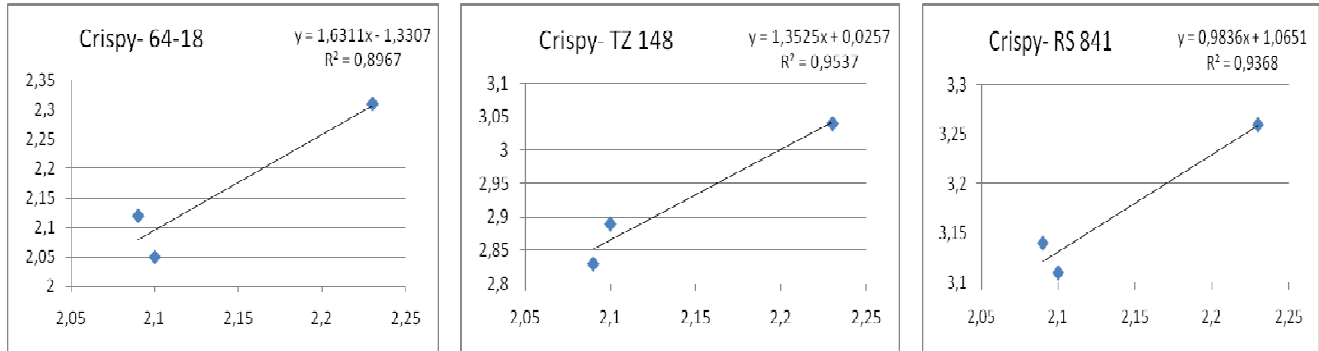


Figure 2. Relationships between non-grafted and grafted plants for the first period.

(3.39 μg), and crispy onto 64 - 18 rootstock (3.34 μg). Watermelon plants grafted on TZ-148 (Boughallep, et al., 2008), RS-841 (Blancard et al., 1991) and *Lagenaria* species (Yetişir et al., 2003; Yetişir and Sari, 2004) are reported as more resistant than non-grafted ones to *Fusarium* wilt. In a similar investigation, it is also mentioned that the *Fusarium oxysporum* resistance of grafted plants comes from the resistance of the rootstocks and the resistance provider compounds that synthesizes in root area than transmits to the stem, so that the plants can tolerate the disease (Biles et al., 1989). The role of phenolic compounds on plant resistance to pathogens is stated by some researchers (Van Etten and Pueppke, 1976; Bell, 1981; Ebel, 1986; Clausen et al., 1992; Nicholson and Hammerschmidt, 1992; Schultz et al., 1992; Dinelli et al., 2006). As mentioned by Alan et al. (2007), grafted plants have longer main stem, more lateral vine and higher root dry weight than the control plants, and fruit yield of grafted plants is superior to the control. One of the reasons of those affirmative effects of rootstocks could be high amounts of the phenolic compounds. Confirming this phenomenon, concerning dwarfing mechanisms of fruit trees, it has been discussed that phenolic compounds are the factors affecting the occurrence of dwarfism and plant growth (Demirsoy and Macit, 2007). It is defined that some phenols promote growing even at low concentrations. Ferulic acid, p-coumaric acid, and o-coumaric acid may induce primer root and hipocotile growing of cucumber at 1,5 ppm concentration, but inhibit plant growth on 150 and 200 ppm (Enu-Kwesi and Dumbroff, 1980).

Statistically significant variations were determined regarding kaempferol accumulation between leaf samples of grafted and non-grafted plants and rootstocks. According to the results, the rootstocks had more kaempferol content than the others (Table 1). However, there were no significant differences between the rootstocks. The 64 -18 rootstock (5.48 μg) had the highest kaempferol content, followed by TZ 148 (5.45 μg) and RS 841 (5.35 μg) rootstocks. High kaempferol content of 64-18 rootstock was not observed in the

leaves of 64 - 18 grafted Crispy plants. Although most *Lagenaria* cultivars are assigned as graft compatible to watermelon cultivars (Yetişir and Sari, 2004; Yetişir et al., 2007), graft-incompatibility may be involved in this phenomenon. The data obtained by Alan et al. (2007) are supported in this result as 64 - 18 rootstock was significantly poor for yield characteristics, than the other rootstocks. After grafting, new vascular tissues which develop from the cambium are permitting the transfer of water, nutrients, and hormones from rootstock to the scion (Masayuki et al., 2005). So, the lowest phenolic compounds in "Crispy grafted on 64 - 18" may due to the low transferring efficiency of the phenolic compounds from the rootstock to the scion.

There were significant differences between two time periods concerning the kaempferol content, and it was increased in the second period compared to the first. In both periods variations were found regarding to phenol accumulation. In the second period, kaempferol content of the plants was increased probably in relation with the rising of temperature (Table 1). Confirming this, phenolic change of hazelnut leaves between April and November is detected by Rodriguez (1988). Thus minimum compound level detected in May and it increased through summer and reached the maximum level.

Variations accounted in non-grafted plants for phenolic content were changed from 89 to 95% for the first period. Correlation between non-grafted plants and kaempferol accumulation was positive and significant for all grafted plants. The rate of increase in grafted plants with the increase in non-grafted plants by 1 μg was 1.35 and 1.63 for TZ 148 grafted and 64 -18 grafted crispy plants, respectively (Figure 2).

Regression analysis showed that variations in phenolic contents of non-grafted plants were changed from 6% to 56% for the second period. The rate of increase in grafted plants with the increase in non-grafted ones by 1 μg was 0, 47 for crispy onto RS 841 rootstock for this period (Figure 3).

No correlations were detected between the number of unknown peaks, grafted and non-grafted plants, and growing season. When it was evaluated total area of

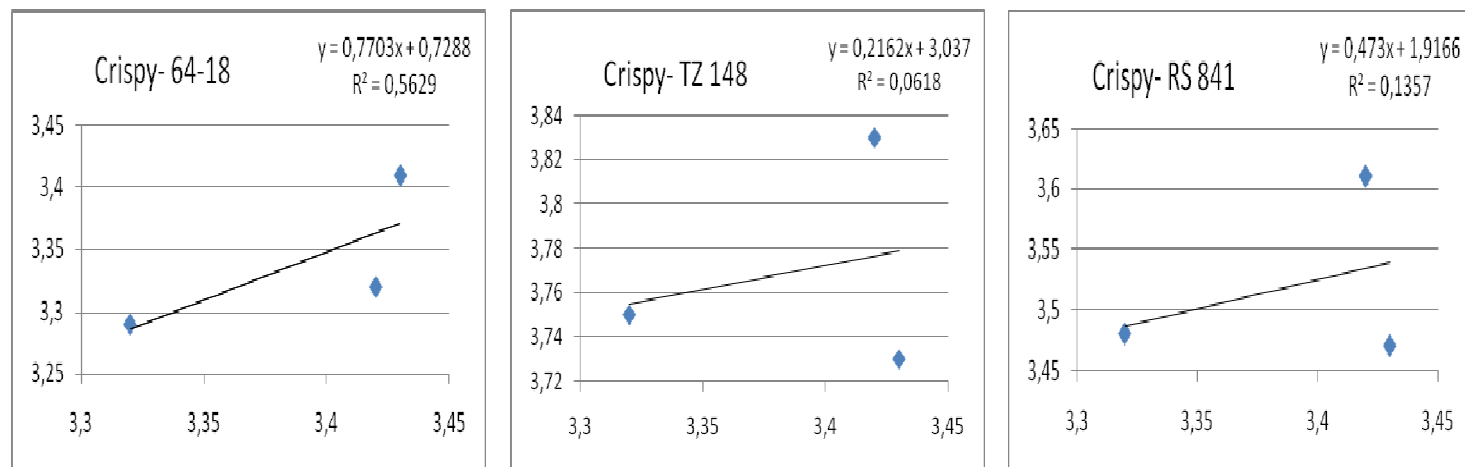


Figure 3. Relationships between non-grafted and grafted plants for the second period.

Table 2. Number and total area of unknown substances in grafted and non-grafted plants and rootstocks.

Application		Number of unknown substances	Total area of unknown substances
i. Period	Crispy, non-grafted	86	38.950.919
	Crispy onto 64-18 rootstock	84	20.405.553
	Crispy onto TZ 148 rootstock	81	38.034.350
	Crispy onto RS 841 rootstock	79	56.494.457
ii. Period	Crispy, non-grafted	83	54.877.240
	Crispy onto 64-18 rootstock	84	40.441.930
	Crispy onto TZ 148 rootstock	79	54.244.668
	Crispy onto RS 841 rootstock	81	46.392.717
Rootstocks	64-18	75	48.631.317
	TZ 148	85	57.469.395
	RS 841	83	46.678.306

unknown peaks, a general increase was observed in the second period. But, only in Crispy onto RS 841 rootstock possessed a decrease in total area (from 56.494.457 to 46.392.717) (Table 2). Similarly, after *Sphaerotheca fuliginea* infection in misc melon, the phenol content of resistant PMR6 cultivar decreases. On the contrary, it increases in susceptible Hara Madhu cultivar. It is found that this characteristic of the resistant cultivar is about high level of flavanol glycosides and chlorogenic acid (Jindal et al., 1979).

In this study, statistically significant differences were determined between kaempferol content of rootstocks and grafted and non-grafted plants. On the contrary, this difference was not observed on the total area of unknown substances. So, it is considered that one of the reasons for the affirmative changes in plant growth and yield, and disease resistance of *Cucurbitaceae* rootstocks can be

related to the high levels of kaempferol.

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