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Profiling of primary metabolites and flavonols in leaves of two table grape varieties collected from semiarid and temperate regions

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ABSTRACT

Cultivation of grapes in West Bank – Palestine is very old and a large number of grape varieties exist as a result of continuous domestication over thousands of years. This rich biodiversity has highly influenced the consumer behavior of local people, who consume both grape berries and leaves. However, studies that address the contents of health-promoting metabolites in leaves are scarce. Accordingly the aim of this study is to assess metabolite levels in leaves of two grape varieties that were collected from semiarid and temperate regions. Metabolic profiling was conducted using GC–MS and LC–MS. The obtained results show that abiotic stresses in the semiarid region led to clear changes in primary metabolites, in particular in amino acids, which exist at very high levels. By contrast, qualitative and genotype-dependent differences in secondary metabolites were observed, whereas abiotic stresses appear to have negligible effect on the content of these metabolites. The qualitative difference in the flavonol profiles between the two genotypes is most probably related to differential expression of specific genes, in particular *flavonol 3-O-rhamnosyltransferase*, *flavonol-3-O-glycoside pentosyltransferases and flavonol-3-O-D-glucoside L-rhamnosyltransferase* by 'Beituni' grape leaves, which led to much higher levels of flavonols with rutinoside, pentoside, and rhamnoside moieties with this genotype.

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1. Introduction

Grape (*Vitis vinifera* L.) is one of the most cultivated plant species worldwide, with more than eight million hectares of vineyards (Vivier and Pretorius, 2000), and its products include wine, juice, fresh berries, and leaves. It is believed that grape is one of the oldest plants to be cultivated with the oldest records dating back to 3500 B.C. (Bowers et al., 1999). The species *Vitis vinifera* (the European or old world grape) is the most cultivated although other species (e.g. *Muscadinia*) are also cultivated at considerable levels.

Grape culture in West Bank is very old, and the ancient Egyptian inscriptions dated the presence of vineyards in historical Palestine to the pre-biblical epoch (around 1800 B.C.); Egyptians wrote 'It was an excellent country… it produced figs and grapes, its wine was more plentiful than water' (Goor, 1966). Nowadays, the annual yield of grapes in West Bank is approximately 80,000 tons, which constitutes a major contribution to the agricultural sector. It is worth to mention here that a large number of grape varieties/genotypes exists, some of which are believed to be very old. Moreover, the long history of grape cultivation and its remarkable biodiversity are tightly coupled with the traditional Mediterranean dishes, in which grape products, including leaves, are widely used among local communities and indigenous people.

Despite the wide use of vine leaves in the Mediterranean region, studies addressed its nutritional value are too limited. Amarowicz et al. (2008) found that the content of total phenolics of grapevine leaves is high (257 mg g^{-1} acetone extract and 232 mg g^{-1} methanolic extract), and that both extracts had strong antiradical activities. Furthermore, Harb et al. (2013) reported that leaves of 'Shami' grape, which is cultivated widely in both semiarid and temperate regions of West Bank, inhibited the proliferation of lung cancer cells. In addition, various studies clearly indicated that vine leaves can be used as an animal foodstuff without any toxic effect, despite the high levels of lignin and condensed tannins that they contain (Gurbuz, 2007).

The nutritional quality of grape is based on the natural compounds found in its products, which include vitamins, minerals, carbohydrates, edible fibers and, in particular, polyphenols that have powerful antioxidant properties (Yilmaz and Toledo, 2004). Among these the phytoalexin resveratrol is believed to have chemopreventative and therapeutic effects (Ndiaye et al., 2011). Moreover, it was reported that juice of 'Concord' grape may

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enhance neurocognitive function in the elderly (Krikorian et al., 2012). In addition there are profound effects of grape products as anticancer agents. Hudson et al. (2007) demonstrated that peel extract led to the apoptosis of prostate tumor cell lines. Furthermore, it was found that proanthocyanidins and catechins retarded breast cancer metastasis (Mantena et al., 2006).

The differences in the nutritional value of grape products are closely related to both primary and secondary metabolism. These two types of metabolism have different roles: primary metabolites are directly involved in normal growth, development, and reproduction of plants, whereas secondary metabolites are mostly involved in defense and other facultative processes. With grapes, various studies show that changes in primary metabolites (e.g. alanine, inositol, and glutamine) may also contribute to an elevated resistance to stress (Figueiredo et al., 2008; Hamzehzarghani et al., 2005). Moreover, a huge number of secondary metabolites has been documented in grape products, in particular in wine and juice. Among these are gallic acid, (+)-catechin, (+)-epicatechin, *p*-coumaric acid and the 3-cafeoylglucosides of peonidin, cyanidin and delphinidin (Ali et al., 2010). Such large diversity in the levels both primary and secondary metabolites is highly influenced by environmental factors including both biotic and abiotic stresses.

In most Mediterranean countries significant part of the vineyards rely solely on rainfall, and are also subjected to heat stress which begins at the end of spring. Consequently, the impact of these abiotic stresses can be considered as the most influential factor for the quality of grape berries and leaves. Such an impact is well studied in various plant species. For example the influence of abiotic stresses on photosynthesis and cell growth is well documented (Chaves et al., 2009), and it is evident that osmotic adjustment is the principal response of stressed plants; such adjustment entails reprogramming and consequently drastic metabolic changes at the whole plant level to attain a new equilibrium in order to cope with stress. In a study with 'Regent' grapes, it was found that several transcripts (e.g. subtilisin-like protease, *phenylalanine ammoina-lyase* (*PAL*), and *S-adenosyl methionine synthase*) are upregulated following stress (Figueiredo et al., 2008), and these transcripts are believed to be involved in the biosynthesis of several compounds important for the defense response. In addition, Shadle et al. (2003) showed that overexpression of *PAL* in tobacco plants led to an elevated level of chlorogenic acid and consequently reduced susceptibility to pathogenic infection. Hamzehzarghani et al. (2005) found also that head blight resistant wheat cultivar contained more *myo*-inositol, glutamine, and phenolic compounds. Taking into account that heat stress is usually combined with drought stress, various studies clearly demonstrate that plant responses are different to stress combinations in comparison to each stress alone (Wang et al., 2010a). Mittler (2006) stated that the metabolic and molecular responses that were observed under the combination of heat and drought stresses are unique. Accordingly, it is hard to discriminate between the influences of each stress in isolation for tissues that are collected from plants grown under natural conditions. However, general trends can be followed and stress combinations may reveal new patterns of resistance. Among these is the finding of Wang et al. (2010b) that both drought and heat stresses induced ultrastructural damage to the chloroplasts and that glycine betaine protects both chloroplasts and thylakoid lamellae from such damage. Concerning the impact of abiotic stresses a large number of studies clearly demonstrates that metabolomics provides functional measures of cellular status and thereby ultimately describes the organism phenotypes (Martins et al., 2014). It is worth noting here that leaf blades are the best plant tissue for such analysis, since they display the greatest metabolic responses to abiotic stresses (Witt et al., 2012).

The aim of the current study is to assess metabolic changes of vine leaves of two grape varieties that were collected from semiarid and temperate regions of West Bank. Such knowledge is likely to be of high value in assessing the nutritional value of these leaves, which are widely consumed by indigenous people in several Mediterranean countries.

2. Results

2.1. Differences in the levels of primary metabolites

Levels of primary metabolites were considerably different most likely reflecting a dramatic metabolic reorganization to cope with abiotic stresses that prevail in the semiarid region (S-AR; Dahria) in comparison to temperate region (TR; Beit Omar). The most noticeable and significant changes are those observed in amino acids (Fig. 1). Levels of branched chain amino acids (valine, and isoleucine) were much higher in both 'Shami' and 'Beituni' leaves that were collected from S-AR. Furthermore, severe differences are observed in the levels of asparagine, lysine, methionine and threonine. Moreover, levels of the aromatic amino acids (tryptophan and tyrosine), which derive from shikimate, were also higher in leaves collected from the S-AR, whereas phenylalanine is considerably higher only in 'Shami' leaves from S-AR. Furthermore, serine that is derived from glycerate follows that same trend, but such trend is not evident with glycine that is derived also from glycerate. Proline follows the same trend described above for most amino acids, whereas GABA was considerably higher in 'Shami' leaves that were collected from the TR. Ornithine and arginine levels show a different pattern of variation. For both varieties, levels in leaves that were collected from S-AR were higher than TR, while 'Shami' leaves contained higher levels compared to 'Beituni' leaves. For putrescine, comparison between varieties reveals that 'Shami' leaves from TR had the highest level.

Differences in sugar levels (Fig. 2) are less dramatic than those in amino acids levels, with almost no differences between varieties and/or locations with respect to glucose, sucrose, galactose (data not shown), and mannose. As for genotypic differences, 'Shami' leaves collected from TR had significantly higher levels of fructose-6-phosphate, glucose-6-phosphate, maltose, trehalose, and isomaltose. In addition to that, it is clear that 'Shami' leaves that were collected from S-AR had higher levels of melezitose, maltitol, and maltotriose.

With organic acids (Fig. 2), changes are obvious with leaves collected from the S-AR. In this respect, comparisons between leaves of the same variety from the two locations reveal that levels of citric, isocitric, and 2-aminoadipic acids are significantly higher in leaves from S-AR. Another obvious trend is that the level of malic acid is higher in 'Beituni' leaves. As for ascorbic acid, its level was the highest by both varieties that were collected from TR. Another difference is related to the level of tartaric acid, which is considered as the main acid in grape berries at full ripeness, as 'Shami' leaves from the S-AR had very low level. In addition, it is worth to notice that there are no significant differences between varieties and locations in the levels of metabolites that may be involved directly in the biosynthesis of polyphenols, namely 4-hydroxycinnamic acid, quercetin, and shikimic acid.

2.2. Differences in secondary metabolites and expression profiles of related genes

Changes in flavonols (Figs. 3 and 4) show that differences between 'Shami' and 'Beituni' leaves are qualitative, and that abiotic stresses had negligible effect. Hochberg et al. (2013) stated in this respect that primary metabolites are significantly more

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