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Potassium-induced freezing tolerance is associated with endogenous abscisic acid, polyamines and soluble sugars changes in grapevine

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ABSTRACT

Crop nutrition is a practical method against freezing injury which may improves grapevine freezing tolerance (FT) potential by increasing bud nitrogenous and carbohydrates storages. In this study the effect of foliar application of potassium sulfate (PS; K_2SO_4; 0, 1, 2 and 3%) on some physico-chemical properties of fruit and leaf mineral contents of 'Sultana' grapevine (*Vitis vinifera* L.) were studied. Moreover, to further elucidate the efficiency of foliar PS on FT and some relevant morpho-physiological changes were evaluated at four sampling dates: Nov., Jan., Mar., and Apr. following exposure to artificial freezing using electrolyte leakage and bud browning bioassay. In summer 2013 and 2014, PS was sprayed at fruit set, pea-sized berry, verison and maturity on 16 years old grapevines located in Malayer Grape Research Station (Iran) under a randomized complete block design. Based on results, foliar PS significantly affected yield, cluster weights, and berry weight and some fruit quality indices including soluble solids, titratable acidity, pH and phenolic compounds. Foliar PS significantly changed the leaf N, K, P, Mg, Zn and Fe concentrations. The effect of nutrition treatments was also significant on FT of grapevines at four sampling stages. In Jan., the highest FT (LT_{50} = −25.97 °C) and the lowest FT (LT_{50} = −20.16 °C) was found in 3% PS- treated and control untreated vines, in respectively. Moreover, PS especially at 3% resulted in higher increments in abscisic acid, total phenol, soluble sugars, and polyamines concentrations. Potassium spray increased membrane stability and decreased electrolyte leakage. The ability of Potassium in FT improvement (lower electrolyte leakage) was found to be related to change in endogenous abscisic acid concentration as a stress hormone in preliminary and subsequent changes in other metabolites such as accumulation of soluble sugars, polyamines, and phenolic acids.

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the economically important fruit trees with several valuable byproducts. Grapes are the world's first and fourth largest fruit crop in terms of cultivated areas and fresh weight (FW) production, respectively. In terms of grapevine area and production, Iran ranks 8th (215,000 ha) and 10th (2.15 million tons) in the world, respectively, under the diverse ecosystems subject to irrigated, rainfed and deep water conditions (FAO, 2012).

Low temperature is one of the most severe environmental stresses, which not only affects the growth and distribution of plants, but also causes serious damage to a number of crops such as grapevine. As temperatures decline in the early fall, grapevine tissues begin acclimating to low freezing temperatures (Salzman et al., 1996; Karimi and Ershadi, 2015). Increases in some soluble sugars or free amino acids, synthesis of certain proteins, expression of C-repeat binding factor (CBF) genes, and increases in unsaturated fatty acids, phenolic compounds, antioxidant capacity, and hormone levels are some of the most important metabolic defenses against freezing stress (Salzman et al., 1996; Koussa et al., 1998; Jones et al., 1999; Xiao et al., 2006; Liu et al., 2007; Karimi and Ershadi, 2015). Abscisic acid (ABA) mediates plant responses to adverse environmental stimuli since the level of ABA in plants usually increases during abiotic stress conditions, and exogenous application of ABA can enhance plant adaptation to cold stresses (Karimi and Ershadi, 2015; Sah et al., 2016). Soluble carbohydrates accumulated in plants serve as cytoprotective compounds, likely to prevent or slow ice crystal formation (Jones et al., 1999; Karimi

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and Ershadi, 2015; Ershadi et al., 2016). An increase in polyamines (PAs) particularly putrescine (Put) levels after exposure to low temperature stress has been reported in a number of plant species (Bouchereau et al., 1999; Shen et al., 2000). In hardened Scots pines, Put concentration showed a positive correlation with frost resistance, whereas spermidine (Spd) and spermine (Spm) show no correlation. The relationship between phenolic compounds and low temperature tolerance was reported by Cansev et al. (2012) in olive and by Karimi and Ershadi (2015) in grapevine.

In addition to different genetic properties of grapevine cultivars and rootstocks (Ershadi et al., 2016), some cultural practices have been shown to be effective in cold hardiness improvement of vineyards (Zhang et al., 2011; Sarikhani et al., 2014; Karimi and Ershadi, 2015). Although a number of such factors have already been studied, there is little information regarding the potassium nutrition and its physiological effects on freezing tolerance (FT) of grapevine. Fertilization is one of the viticultural techniques with a great effect on the vineyard fruit yield and quality. Foliar nutrition is a useful alternative tool for plants when soil conditions may limit root uptake or during periods of fast growth when needs may exceed root supply (Reickenberg and Pritts, 1996). Potassium is required for cellular osmotic and ionic balance, electrochemical processes, neutralization of organic acids, regulation of stomatal function, cell division, enzyme activation, protein synthesis, as well as the synthesis and translocation of sugars (Ronald, 2008; Keller, 2010). Vines with inadequate potassium are also more drought-prone and less cold-tolerant. When adequate, potassium favors grape quality by enhancing fruit coloration and sufficient acidity (Ronald, 2008). Potassium deficiency also suppresses sugar transport in the phloem and can result in sucrose (but not starch) accumulation in the leaves to substitute the missing K^+ as osmoticum (Cakmak, 2005). It has been shown to be an effective nutrient in the induction of cold hardiness in woody plants (Sarikhani et al., 2014; Karimi et al., 2014). Khristov and Lazaroz (1994) investigated the effects of pre-planting applications of K and P on cold-hardiness in grapevine ('Dimyat') and found a higher ratio of bud survival in fertilized vines. Therefore, foliar application of potassium sulfate (PS) has been proven to play an important role in the development of FT in *Vitis vinifera* (Sarikhani et al., 2014; Karimi et al., 2014).

Experimental measurement of cold hardiness provides a useful means for examination of different hardiness characteristics in plants, which among them bud browning (BB) and electrolyte leakage (EL) tests are the most common ones (Ershadi et al., 2016). The concept of lethal temperature for 50% loss of electrolytes (LT_{50}) has been used as a measure of cold hardiness and is defined as the predicted test temperature resulting in 50% or greater loss of total electrolytes.

Potassium is an essential element in plant nutrition and plays a vital role in many physiologically important processes of grapevine. Nevertheless, even as a major nutrient and the most abundant cation present in grape berries (Keller, 2010; Mpelasoka et al., 2003), the effects of this element on FT have rarely been investigated. It is generally known that cold hardiness can be hampered by insufficient carbohydrate reserves in the cane due to overloading of the plant (Keller, 2010). Up to now, there have not been comprehensive studies regarding to mechanism of PS-induced FT of woody plants, although cold hardiness induced by PS application are known to occur in some plants (Sarikhani et al., 2014; Karimi et al., 2014). In the present study, the effects of the foliar application of PS on some physico-chemical properties of fruit and leaf mineral contents of 'Sultana' grapevine (*Vitis vinifera* L.) were studied. The – aim of this study was, in particular, to assess whether foliar spray applications of PS could affect soluble sugars, PA_S, ABA and phenolic compounds in 'Sultana' grapevine buds in the course of cold acclimation and deacclimation.

2. Materials and methods

2.1. Plant material, vineyard site and treatments

The present investigation was conducted in 2013 and 2014, on 16-year-old 'Sultana' grapevines grown in a commercial vineyard located at Malayer Grape Research Station (lat. 34° 30′ N, long. 48° 85′ E, alt. 1550 m), Iran. The average annual temperature of Malayer is 12.2 °C. Although this region has relatively long and hot summers, but the winter temperatures may plunge down to −20 °C or even lower, causing serious damage to grapevine. The vines were grown on their own root in a clay-loam soil based on the results of soil analysis (data not shown) under drip irrigation system, spaced 1.5 × 3 m, trained to cane pruning under non-trained system and pruned on 5th March to 8 canes with 12 buds besides 8 renewal spurs. A randomized complete block design was adopted, with three replications, and included two grapevine plants per experimental unit. The vines were sprayed with four potassium rates (0, 1, 2 and 3%; w/v) as PS (K_2SO_4, 50% K_2O) to run-off on all leaves four times at fruit set, pea-sized berry, verison and maturity stages during the growth season of 2013 and 2014.

2.2. Fruit yield and quality

At the second week of Sep., clusters per vine were counted and weighted to determine total yield per vine. Representative random samples of 24 clusters per replication were taken to the laboratory to determine cluster weight and number of berries per cluster. A random sample of 100 berries per each replication was taken to determine vine yield, cluster weight, weight of 20 berries, total soluble solids (TSS), titratable acidity (TA) and pH of fruit juice. The TSS of berry juice was measured at 25 °C using a refractometer (Atago, Japan) and was expressed as ◦Brix scale. Total acidity was determined in the same juice by titration with NaOH (0.1 N) up to pH 8.1, using 1 ml of diluted juice in 25 ml distilled H_2O, and results expressed as g tartaric acid equivalent per liter.

Total phenolics of fruit juice were determined colorimetrically using Folin-Ciocalteu reagent as described by Velioglu et al. (1998) with slight modifications. In brief, a volume of 0.3 ml from each diluted methanolic extract (10%) was mixed with 1 ml Folin-Ciocalteau reagent (10%) and vortexed. After 5 min, 1 ml of 7% sodium carbonate solution was added to the mixture. The final solution was shaken for 90 min at room temperature and then the absorbance was spectrophotometerically (Cary Win UV 100, Varian, Australia) measured at 765 nm. The total phenolics were quantified by the calibration curve obtained from measuring the absorbance of a known concentration of gallic acid standard. The concentrations were expressed in terms of gallic acid equivalent (mg g^{-1} of FW). All samples were analyzed in triplicates.

2.3. Leaf mineral contents

Samples of leaves were collected on 25th August 2013 and their N, P, K, Mg, Ca, Zn, Mn and Fe concentrations were measured. Three leaves were taken from the middle of growing shoots at harvest. Petioles were oven dried at 70 °C up to a constant weight, then ground to a powdery texture; and 0.2 g was taken to determine aforementioned elements. Total N was determined by the Kjeldahl. P was determined using a spectrophotometer. K was flame-photometrically determined. The sample extracts were analyzed for Mg, Ca, Fe, Zn and Mn using an atomic absorption spectrophotometer (Varian, 220).