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Nutritional Aspects of Grape (*Vitis vinifera* L.) Clusters Afflicted with SOUR Shrivel Is Related to Functionality of Its Vascular Tissues

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Abstract

Mineral nutrition is essential to growth and development of various plant organs including fruits; consequently deficiency of any element leads to a myriad of physiological disorders, which in fruits are manifested as ripening anomalies. In this study, nutritional composition in tandem with vascular structure of healthy grape clusters and clusters afflicted with SOUR shrivel, a ripening disorder has been examined to unravel its mechanistic basis. The healthy berries without any affliction accumulated the highest amounts of all nutrients, which paralleled with functional xylem and phloem tissues in their cluster framework. Among the macro nutrients, K occurred as the dominant element followed by P, Ca, Mg and S whereas B was the predominant micro nutrient followed by Fe, Cu, and Zn. Although, the SOUR shrivel berries and the healthy appearing berries of afflicted clusters followed similar accumulation patterns, their amounts were significantly lower than the perfectly healthy berries without any SOUR shrivel. The low nutrient levels of SOUR shrivel berries corresponded to necrosis of phloem tissues and a red discoloration of xylem tissues in their cluster framework indicating that a lack of functional vascular tissues provoked the induction of SOUR shrivel by reducing influx of xylem and phloem mobile nutrients into the afflicted berries. Hence, periodic nutritional checks starting before veraison may aid in curbing the incidence of SOUR shriveling.

Keywords

Phloem, Ripening, Xylem

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1. Introduction

The ripening process in fruits persists as the prime topic of discussion and scientific investigation in plant biology as it transforms fruits into various palatable products of high economic importance. Depending upon growth stage and ripening style, occurrence of this phenophase varies in different species. For instance, in the non-climacteric grape berry, it occurs during the second sigmoid growth cycle, consequently, grape growers and wine makers fanatically pay special attention to ripening as it determines fruit composition, yield, and ultimately the time of harvest. These end-of-season targeted events come to fruition only when the highly organized and coordinated events of ripening proceed unperturbed. Accordingly, failure to follow the conserved events of ripening results in dramatic developmental defects marked by a wide range of undesirable physical and quality attributes referred to as physiological ripening disorders [1] [2]. These include bunch stem necrosis, the oldest known affliction [3], sunburn, dehydration, and a relatively recent and lesser known oddity known as SOUR (suppression of uniform ripening) shrivel afflicting both red and white cultivars [4], and hybrids worldwide [5] [6]. Among these, SOUR shrivel, also known as berry shrivel [7] [8] and SAD (Sugar Accumulation Disorder) [9] in viticulture parlance is the most economically detrimental and paradoxical and hence, as of now, its causal factors remain unknown [1] [4]. Typical symptoms include flaccid berries in the form of a deflated soccer ball with reduced levels of color and sugar but remarkably high in acidity resulting increased sourness of the berries, and very often the berries develop an off-flavor [1] [9].

As in other fruit crops, the vascular tissues of xylem and phloem connect the grapes with the parent vine and serve as the major supply route for organic and inorganic substances including all the nutrients [10]. Provided these vascular conduits remain free of any defects, berries are able to import all nutrients normally from the parent vine. For instance, grape berries acquire nitrogen (N), phosphorus (P), potassium (K), sulfur (S), magnesium (Mg), boron (B), iron (Fe), copper (Cu) etc. through the phloem pathway along with other organic solutes whereas calcium (Ca), manganese (Mn), zinc (Zn) etc. are transported through the xylem pathway [11]. Should there be any injury causing an increased resistance in these pathways, various physiological disorders as ripening anomalies loom up immediately. For instance, phloem girdling caused by necrosis of grapevine rachis results in shriveled berries with reduced amounts of K and sugars [2]. Likewise, all fruit crops develop numerous physiological ripening disorders, which are associated with malfunctioning of vascular tissues. Among these, specifically related to nutrient deficiencies include blossom end rot of tomato (*Solanum lycopersicum* L.) [12], split and shattered pits and double fruit of peach (*Prunus persica* L.) [13], albino and malformed fruit of strawberry (*Fragaria* × *ananassa* (L.) Duch.) [14] etc. Although these disorders are physiological in nature, they are manageable to some extent provided they are supplemented with nutrients. For instance, calcium fertilizer application in combination with foliar sprays of ABA reduced the tomato fruit susceptibility to blossom end rot to a lesser extent [15]. On the other hand, measures to curb the incidence of physiological disorders via management practices require an insight into its mechanistic basis. To accomplish this goal, we first need an in-depth analysis of a whole suite of morpho-physiological and nutritional aspects of afflicted fruits. In the case of SOUR shrivel, this was started off with the analysis of symptomatology and compositional attributes of grapes [1] [2] [4]. The present study is a continuation of such an endeavor entailing comparison of nutritional and functional aspects of vascular tissues between healthy and SOUR shrivel clusters. This study will serve as an add-on to previously collected morpho-physiological data, which collectively can lead to devising cultural practices for minimizing the incidence of SOUR shrivel in future.

2. Materials and Methods

2.1. Plant Material

Commercial vineyards (lat. 46˚15'47.48"N, long. 119˚29'16.09"W) with mature own-rooted *V. vinifera* cultivars Cabernet Sauvignon located in Benton City (lat. 46˚15'48.1"N, long. 119˚29'23.5"W), WA were chosen for analyzing nutrient profile of SOUR shrivel disorder. This vineyard was chosen as these vines consistently exhibited incidences of all physiological ripening disorders including SOUR shrivel. The vineyards had vine by row spacing of 1.83 × 2.74 m on a uniformly deep (>1 m) loamy fine sand. Vines were trained to bilateral cordon, which entailed training the vines in both directions along the cordon (an extension of the trunk) wire from the trunk and were drip-irrigated during the growing season. Training in viticulture parlance refers to the design and development of a grapevine framework. The shoots emerging from the cordon were positioned vertically using catch

wires. Vines were spur-pruned during winter, *i.e*., canes (a mature woody and lignified stem from previous season's shoot) were cut back to two count nodes/buds (the readily visible buds on a dormant cane, not including the small base buds); the noncount shoots (shoots arising from base buds of the spur) were removed at the beginning of bloom that approximately equated to 20 shoots/m. Throughout the growing season, the vines were continually monitored for the inception of SOUR shrivel disorder especially during the ripening period. Following the appearance of the disorder after veraison, the symptomatic vines were identified by tagging the vines and their clusters. Thereafter, the progression of the malady was monitored and finally the symptomatic shoots bearing SOUR shrivel clusters from afflicted grapevines and shoots devoid of SOUR shrivel clusters from perfectly healthy grapevines were sampled for comparing vascular structure and nutrient composition between healthy and afflicted clusters.

2.2. Mineral Nutrient Analysis

Healthy and afflicted clusters from the same vineyard were harvested, put in a zip-lock bag and transported to the laboratory. Healthy clusters came from those vine rows that bore absolutely no shriveled clusters. Four replicates of fifty berries from healthy and SOUR shrivel clusters (n = 50) were removed at harvest and de-pediceled using a sharp razor blade. The macro and micro nutrients of whole berries were determined by a commercial laboratory using inductively coupled plasma spectroscopy [16].

2.3. Analysis of Vascular Tissues

The vascular tissues of xylem and phloem from healthy and afflicted clusters (peduncles) were analyzed by a technique previously described for grape clusters [17]. Briefly, a free-hand-sectioning technique was adopted to prepare sections and observed with bright field and epifluorescence microscopy. Images were recorded using a DXM 1200C digital camera (Nikon Instruments Inc., Melville, NY, USA) attached to a microscope (Axioskop 2 plus; Carl Zeiss, Thornwood, NY, USA). The significance was determined using ANOVA and the Fisher's Least Significant Difference (LSD) was used as a post hoc test for separating means. The statistical analyses were performed with SPSS **(**SPSS Statistical Package 11; SPSS, Chicago, IL, USA).

3. Results and Discussion

Grape clusters afflicted with SOUR shrivel are typically flaccid with significant reductions in both volume and weight [1] [2] [4] signifying problems with influx of water, nutrients, and assimilates into the berries, the key prerequisites for berry growth and ripening. Of these, the water balance and assimilate partitioning into berries have been well characterized in previous studies, not the nutritional profile [1] [9]. For that reason, this study performed a comparative analysis of nutrient composition between healthy and SOUR shrivel berries. The healthy berries accumulated high amounts of both the macro and micro nutrients (**Figure 1**, **Figure 2**) in which K accumulation was the highest followed by P, Mg, Ca, S, Zn, B, Fe, and Cu (**Figure 1**, **Figure 2**). An accumulation pattern like this is comparable to the nutrient profile of other grapevine cultivars such as Shiraz [11] emphasizing that mineral nutrition of grape berries including other fleshy fruits are crucial to determining both quality and productivity. Not only that, their amount in ripened fruits reflects vascular functionality and hence mineral composition of fruits can be used as a tool to understand transport properties of xylem and phloem pathways. For instance, grape berries are strong sinks for K, therefore, they accumulate large amounts of K and it is possible only when it is transported through the phloem along with sugars. Conversely, Ca moves into the fruit through xylem; however, the xylem pathway in berries slows down after veraison, consequently Ca levels are much lower than K by harvest [10] [11].

Compared to healthy berries, the SOUR shrivel berries accumulated significantly less amount of all nutrients (**Figure 1**, **Figure 2**). This indicated that various metabolic and cellular functions aided by mineral nutrients did not proceed well in the afflicted berries. As mentioned before, K being a macro nutrient is required in large quantities by grape berries, especially during ripening as berries use it as a major active solute to maintain turgor, develop color, perform various metabolic processes, and to drive irreversible and reversible changes in cell volume [18]. Accordingly, plant organs low in K are likely to undergo a reduction in cell viability and an increase in tissue collapse induced by programmed cell death [19] and the ultimate consequence of these events is flaccidity of mesocarp cells, the hallmark of SOUR shrivel in grape berries [1] [2] [4]. Since accumulation of mineral

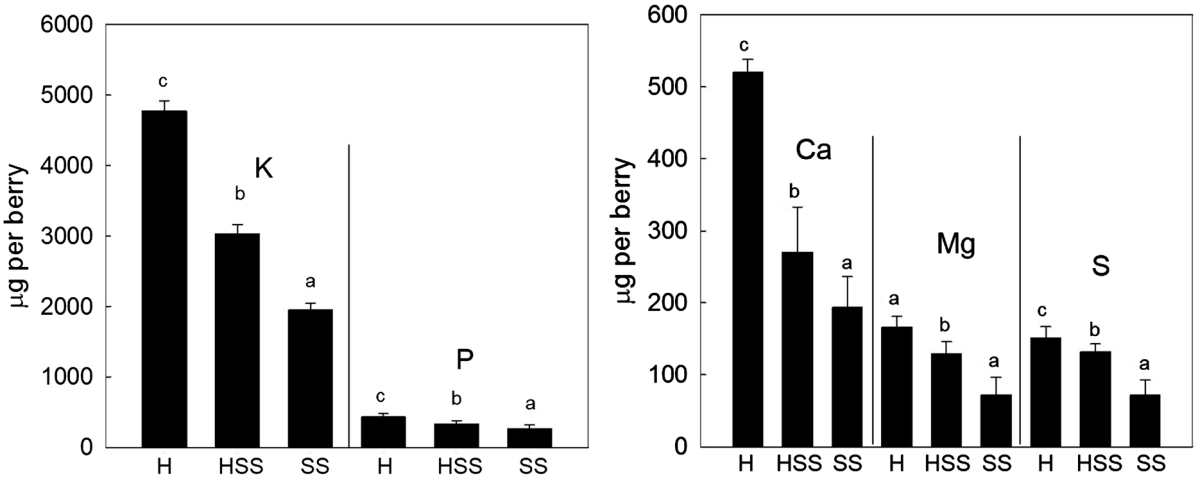

Figure 1. Amount of K, P, Ca, Mg, and S per berry in perfectly healthy clusters and afflicted clusters with healthy appearing and SOUR shrivel berries. Within a nutrient, bars (Mean ± SE) with different letters are significantly different by Fisher's least significant difference test at $P \leq 0.05$. (n = 50 vines). H—Berries of perfectly healthy clusters, HSS—Berries from SOUR shrivel cluster that appear healthy, SS—SOUR shrivel berries. Long vertical lines distinguish one nutrient from the other.

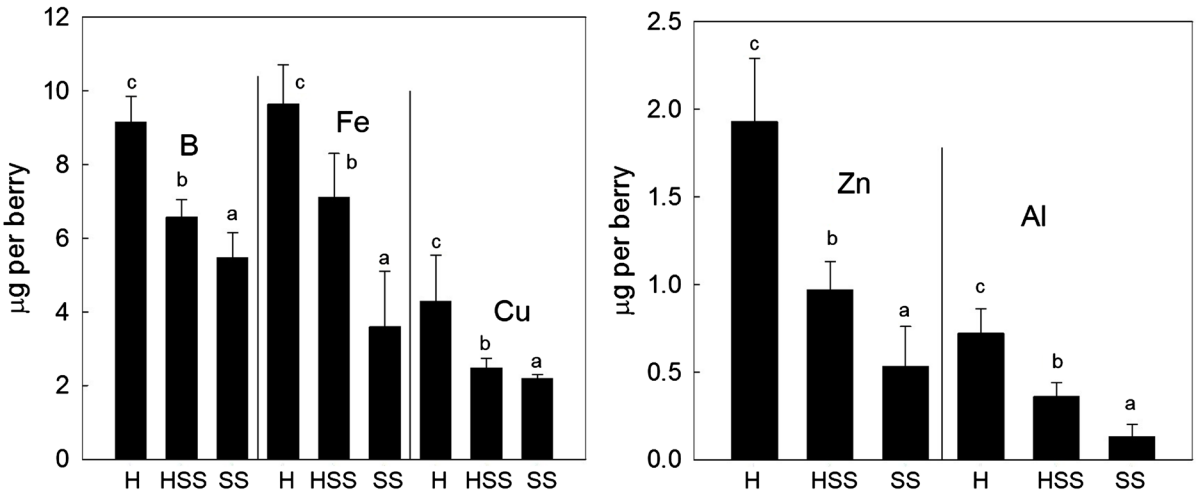

Figure 2. Amount of B, Cu, Fe, Zn, and Al per berry in perfectly healthy clusters and afflicted clusters with healthy appearing and SOUR shrivel berries. Within a nutrient, bars (Mean ± SE) with different letters are significantly different by Fisher's least significant difference test at $P \leq 0.05$. (n = 50 vines). H—Berries of perfectly healthy clusters, HSS—Berries from SOUR shrivel cluster that appear healthy, SS—SOUR shrivel berries. Long vertical lines distinguish one nutrient from the other.

nutrients is an integrated process including interaction between nutrients, the deficiency of other macronutrients such as P, Ca, and Mg might have also contributed to flaccid berries as these stabilize membranes and cell walls maintaining fruit firmness in addition to functioning as key elements for various metabolic processes [20] [21]. Of these Ca is unique in that its deficiency need not be accompanied by other nutrient deficiencies to inflict physiological disorders in any fruit crop including SOUR shrivel in grapes [4] [22]. The same could be true for sulfur deficiency, which may promote SOUR shrivel by inhibiting protein synthesis involving cysteine, and other sulfur-containing primary metabolites such as methionine, glutathione, sulfur-rich peptides, co-enzymes and co-factors, and secondary metabolites [23] [24].

Among the micronutrients, Zn, Cu, and Fe are of particular importance because of their role in the protection of plant cells from oxidative stress mediated by reactive oxygen species [25] [26], which form in plant cells in response to abiotic stress and programmed cell death [27]. One of the factors inducing SOUR shrivel happens to

be abiotic in nature [1], hence it is possible that clusters deficient in Zn might have succumbed to SOUR shrivel. On the other hand, boron is involved in the formation of cell walls; it does so first by synthesizing and then stabilizing it by cross-linking the two rhamnogalacturonan II molecules [28]. Furthermore, B contributes significantly to the absorption and mobility of Ca in the plant controlling cell wall porosity and tensile strength [29], so its deficiency during ripening is likely to induce SOUR shrivel by inducing premature softening. Mn is also an important trace element, but its levels were found to be very low (not detectable) in all berries, hence it may not be involved in the ripening process of grape berries; however, in Shiraz berries, it increased throughout berry development [11] indicating that its requirement is cultivar dependent. Unlike the nutrients discussed thus far, aluminum (Al) is not an essential element, rather it is toxic to plants. Surprisingly, the healthy berries accumulated high amounts of aluminum (Al) but it also coincided with the highest amount of Mg, which is known to alleviate Al toxicity [30].

In order for berries to accumulate mineral nutrients, they need not only intact xylem and phloem tissues within them, but also a viable vascular connection with the parent vine [10] [31] [32]. The healthy clusters met both of these criteria, consequently they accumulated not only high amounts of sugars [1] [4] but also the phloem mobile macronutrients such as P, K, S, and Mg and the xylem mobile Ca (**Figure 1**, **Figure 2**). This was not the case with the afflicted clusters wherein the phloem became dysfunctional ensuing primarily from necrosis of phloem tissues seen as brownish discoloration in the cluster framework (**Figure 3**). Such phenomenon is a specific

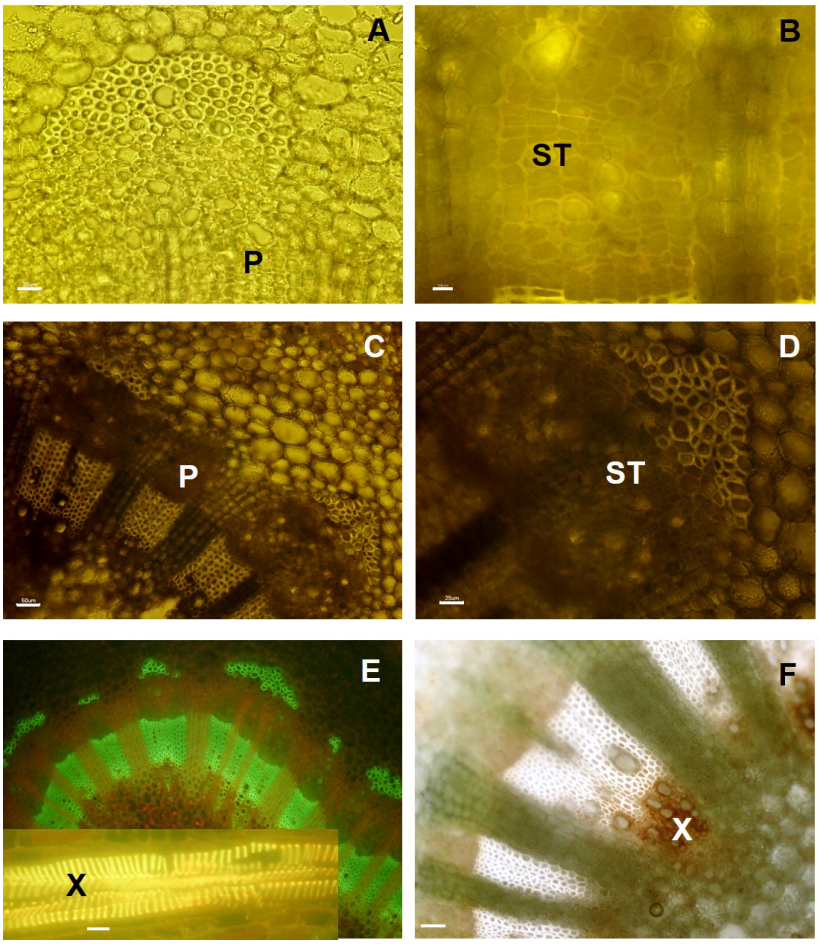

Figure 3. Transverse light micrographs of (A) healthy "Cabernet Sauvignon" peduncle showing phloem tissues, (B) higher magnification of phloem (A) with phloem sieve tubes, (C) necrotic phloem tissues of peduncle from clusters afflicted with SOUR shrivel, (D) higher magnification of phloem (C) with dead phloem sieve tubes, (E) xylem of peduncles in healthy clusters, and (F) xylem of afflicted clusters showing reddish brown discoloration. Scale bars: 50 µm (A), 25 µm (B), 50 µm (C), 25 µm (D), 10 µm (E) and 50 µm (F). P = Phloem, ST = Sieve tubes X = Xylem.

form of programmed cell death, which plants employ during stressful conditions as a defense mechanism to avoid the death of whole plants [1] [33]. Similar to phloem, the xylem in the inflorescence framework developed brownish red discoloration (**Figure 3**), but how that might have affected the accumulation of xylem mobile elements such as Ca could not be ascertained as the vessel lumens appeared to be open. Only when there is an embolism in xylem vessels, does the flow into fruit get impeded [34]. Even if the xylem remained embolism free, the afflicted berries might not have accumulated xylem-mobile nutrients. This is because, the xylem flow in healthy grape berries normally slows down after veraison, thereafter its contribution to berry growth progressively goes down compelling phloem to be responsible for all the growth during ripening [10] [31]. Accordingly, the berries that were predisposed to be the victim of SOUR shrivel (usually the distal berries in the cluster) might have lost their xylem functionality much before ripening started causing a reduction in the accumulation of xylem mobile elements.

4. Conclusion

In conclusion, SOUR shrivel berries showed the least accumulation of all nutrients followed by healthy appearing berries in the afflicted clusters and berries of healthy clusters without any affliction. Their accumulation pattern coincided with the proportion of functional vascular tissues in perfectly healthy clusters whereas structural variations occurred in vascular tissues of the afflicted clusters. Hence, the dysfunctionality of vascular tissues caused by structural modifications in the afflicted clusters reduced the movement of xylem and phloem mobile nutrients into the berries thereby lowered their amounts at harvest.

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