International Journal of Fruit Science
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/wsfr20

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Published online: 18 Feb 2014.

To cite this article: Satisha Jogaiah , Ajay Kumar Sharma & Pandurang G. Adsule (2014) Rootstock Influence on the Biochemical Composition and Polyphenol Oxidase Activity of 'Thompson Seedless' Grapes and Raisins, International Journal of Fruit Science, 14:2, 133-146, DOI: 10.1080/15538362.2013.817767

To link to this article: http://dx.doi.org/10.1080/15538362.2013.817767

International Journal of Fruit Science, 14:133–146, 2014

ISSN: 1553-8362 print/1553-8621 online
DOI: 10.1080/15538362.2013.817767

Rootstock Influence on the Biochemical Composition and Polyphenol Oxidase Activity of 'Thompson Seedless' Grapes and Raisins

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'Thompson Seedless' grapes grafted on five rootstocks and own-rooted vines were evaluated for yield, fruit composition, and raisin recovery percentage. Biochemical constituents were analyzed in fresh grapes before drying into raisins and also in raisins. Among different rootstocks, raisin recovery was highest in 'Thompson Seedless' grapes grafted on 110 R followed by those on 'Dogridge', 1103 P and 99 R. Significant differences were observed in total proteins, reducing sugars, phenol contents, and polyphenol oxidase (PPO) activity in both fresh grapes and raisins made from all rootstock scion combinations. Increase in the content of proteins and reducing sugars was observed in raisins compared to fresh grapes. However, there was a reduction in phenolic concentrations in raisins compared to fresh grapes. PPO activity was highest in 'Thompson Seedless' grapes grafted on 'Dogridge', while it was least in 110 R.

KEYWORDS grapes, phenols, polyphenol oxidase, proteins, raisins, rootstocks, 'Thompson Seedless'

INTRODUCTION

Thompson Seedless is one of the commercially grown table grape cultivars in India and it is also processed into raisins. Traditionally, 'Thompson Seedless' grapevines were grown on their own roots and were mostly trained to bower or pergola system and largely relied on a flood system of irrigation.

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Over the past 20 years, many cultural practices have been modified in grape cultivation and a wider variety of new management practices have been introduced in present day viticulture. Some of these practices include the use of drought and salt tolerant rootstocks, a change in the training systems from the bower system to the Geneva Double Curtain (GDC), and use of drip irrigation and fertigation techniques to improve both water and fertilizer use efficiency.

In India, raisins are mostly produced in the Sangli, Solapur, and Nasik districts of Maharashtra State and the Bijapur district in Karnataka State, as these districts have favorable climates for raisin making. Out of the total production of 1,878,000 tons of table grapes, around 22.5% of the fresh produce is dried to raisins. In 2003, the raisin production reached to 65,000 tons, which was third in the world after the United States and Turkey. Raisins are mostly produced from the Thompson Seedless cultivar and its clonal selections, such as Manik Chaman and Tas–A–Ganesh (Adsule et al., 2008).

Antioxidant properties of grapes are attributed at least partly to their phenolic content. The polyphenolic constituents of fruits, vegetables, and beverages are important contributors to color quality and sensory properties (Macheix et al., 1990) and juice stability (Beveridge, 1997). Interest in these compounds has intensified in recent years because of their possible health benefits, including anticancer and antiviral activities (Hertog et al., 1992). Grapes have been shown to be an excellent source of phenolic antioxidants, which ranges from about 115–361 mg kg^{-1} total phenolics (Teissedre et al., 1996; Cantos et al., 2002). Polyphenols act as excellent antioxidants, which quench free radicals, inhibit UV radiation induced peroxidation activity, and protect human body cells from aging and damage. Raisins also seem to have a considerable concentration of phenolic compounds. Raisins are important processed products in many parts of the world where grapes are grown (Dudman and Grncarevic, 1962). The brown color of raisins is a combination of pigments produced by polyphenol oxidase (PPO) activity and non enzymatic reactions (Ramshaw and Hardy, 1969). Raisins are also considered to be an important source of dietary fiber (Valiente et al., 1995). The predominant enzymatic browning occurs when PPO comes into contact with phenolic compounds (principally caftaric acid in grapes) when the cell integrity is lost. Polyphenol oxidase is a generic term for the group of enzymes that catalyze the oxidation of phenolic compounds to produce a brown color in exposed or disrupted plant tissues.

The brown color results from the formation of quinones, which undergo oxidative polymerization to produce brown-black melanin pigments. Oxygen (O_2) and water must be present if the reaction is to take place. Most of the PPO activity is found in plastids, including chloroplasts, in the skin and in the seeds or seed traces. Thus, browning begins at the periphery

and center of the berry, but rapidly progresses throughout the pulp as the substances come into contact with one another. Non enzymatic browning, known as the Maillard reaction, is a much slower process caused by a reaction of reducing sugars with protein amino groups (Harrison and Dake, 2005).

Moisture stress and soil salinity are common abiotic stresses in the major grape growing regions of India. Several drought and salt tolerant rootstocks are in the process of evaluation for major table and wine grape cultivars. Studies on various aspects, such as the influence of rootstocks on the biochemical composition of a particular variety after harvest and its processed products like raisins and wine, are very meager under Indian conditions. Hence, the present investigation was conducted to study the influence of rootstocks on biochemical composition of fresh grapes and raisins made from 'Thompson Seedless' grapes grafted on different rootstocks. This investigation is an offset of the project on evaluation of grape rootstocks for 'Thompson Seedless' grapes.

MATERIALS AND METHODS

The present investigation was undertaken at the experimental vineyard of the National Research Centre for Grapes, Pune, India during the years 2005–06 and 2006–07. Pune is situated in the Midwest of Maharashtra State of India at an altitude of 559 m above sea level; it lies on 18.32°N latitude and 73.51°E longitude. The soil of this region is black clay having a slight alkaline pH. Five-year-old 'Thompson Seedless' vines grafted on five different rootstocks viz., 'Dogridge', St. George, 110 R, 99 R, and 1103 P as well as the ungrafted 'Thompson Seedless' vines as control were selected. The vines were planted at a row × vine spacing of 3.3 × 1.8 m accommodating about 1850 vines per hectare and were drip irrigated as per the irrigation schedule developed for this region. The experiment was a randomized block design with four replications. Each replication consisted of six vines. The vines were pruned twice in a year, once after harvest of the previous crop (usually during April) to encourage development of canes and shoots popularly known as back pruning. The other pruning was done after 5 to 6 months (usually during October) on the developed canes popularly referred to as forward pruning to encourage cluster development. The grapes were harvested when they attained total soluble solids (TSS) of more than 20 °Brix. After the fruit yield was recorded from individual vines, a random sample was drawn from each replication for analysis of fruit quality parameters like TSS, acidity, juice pH, and berry diameter. The same sample was used for analysis of some biochemical parameters like reducing sugars, total phenols, and total proteins.

Reagents and Chemicals

The standard reference chemicals viz., (+) catechol (98% purity) and gallic acid (98% purity) were obtained from Sigma (St. Louis, MO, USA). All other solvents and chemicals used in this study were of HPLC grade and obtained from Merck (Mumbai, India).

Sampling Method

The berry samples were collected randomly from six vines grafted on different rootstocks. After harvesting, the samples were washed thoroughly with distilled water, air-dried, and stored at –20°C. Each sample was subsequently lyophilized using a freeze drier (Model Benchtop 4 K VIRTIS, SP Scientific Gardiner, NY, USA) at –78°C. The lyophilized samples were blended thoroughly and sieved through 40-mesh size sieve and stored at –20°C until further processing.

Extraction of Samples

One gram of different berry samples was lyophilized in three replications and each were extracted by overnight shaking at room temperature on a mechanical shaker in the dark. The solvent used was 80% aqueous methanol as it is reported to be a better solvent for polyphenol extraction (Bonilla et al., 2003). The mixture was centrifuged at 12,000 rpm for 15 min at 4°C. The residue was re-extracted (three times, 3 h each) under similar conditions. The completeness of the extraction for berries and leaves was ensured by a qualitative Folin Ciocalteu negative test on filter paper. The filtrates were pooled and concentrated to one-third volume using Turbovap concentrator under a gentle stream of nitrogen. The extracts were filtered through 0.45-μm filters and stored at 0°C until further analysis (Ju and Howard, 2003).

Total Phenolic Content

Total polyphenol content of the extract was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965) using gallic acid as the standard. The concentration of the total phenolics was expressed as gallic acid equivalent (GAE mg g^{-1}) of the lyophilized sample.

Reducing Sugars

The reducing sugar content was estimated by the Dinitro salicylic acid (DNSA) method using glucose as a standard. The results were expressed as mg g^{-1} fresh weight.

Total Proteins

The total proteins were estimated by the Lowry's method using bovine serum albumin (BSA) as a standard and expressed as mg g^{-1} fresh weight.

Analysis of PPO Activity

Five grams of the fresh or raisin sample was homogenized by mixing 10 mL of 0.2 M potassium phosphate buffer (pH 7.0) for PPO extraction. Homogenates were centrifuged for 10 min at 7244× *g* under cold conditions. PPO activity was measured as per the methods of Haplin and Lee (1987). McIlvaine buffer (0.2 M Na_2HPO_4/0.1 M citrate monohydrate in a proportion of (2.3:1)) was adjusted to pH 6.5 for the substrate preparation, and 1.3764 g catechol (Sigma Aldrich) were dissolved in 25 mL McIlvaine buffer. The prepared substrate solution was added to 250 mL McIlvaine buffer (1 + 10) and stirred for 30 min to equilibrate. Two hundred μL of enzyme extract was added to 2.8 mL of substrate solution in the tube and mixed thoroughly, after which the changes in absorbance at 420 nm were measured over time using a spectrophotometer. One unit of PPO activity was expressed as the change in absorbance of 0.1 per minute per mL of the enzyme extract.

Preparation of Raisins

The technique of raisin production in India is mostly based on the dipping of the berries in an Australian dip emulsion, which contains 2.4% potassium carbonate and 1.5% ethyl oleate and subsequent drying in an open tier system. In the present investigation, a known quantity of freshly harvested grapes from each treatment and replication were brought to the laboratory and washed thoroughly with distilled water and were treated with dipping oil consisting of (ethyl oleate and potassium carbonate) at the rate of 2.5% for 5 min and later dried under shade in the laboratory. The total time needed for drying was about 15–18 days.

The raisins were analyzed for the composition of reducing sugars, total phenols, total proteins, and PPO activity as per the procedures mentioned above.

Statistical Analysis

The results were analyzed using statistical software SAS (Version 9.1.3, service pack 3, SAS Institute, Cary, NC, USA) and the comparison of treatment means was done using least square difference at $p < 0.005$.

RESULTS

Yield Parameters and Berry Composition

The yield and fruit composition parameters of 'Thompson Seedless' grafted on different rootstocks and on their own roots are shown in Table 1. The rootstocks imparted significant differences in yield components in 'Thompson Seedless'. Among the rootstock-scion combinations, a significant difference was observed for the number of clusters, average cluster weight, and yield per vine. 'Thompson Seedless' grafted on 110 R rootstock produced a maximum number of clusters followed by ungrafted 'Thompson Seedless' and those on 99 R and 1103 P rootstocks. In contrast to the number of clusters, the average cluster weight was the greatest on 'Dogridge' rootstocks, where the number of bunches were fewer indicating an increase in berry size in terms of berry diameter and berry weight. Similarly, although the ungrafted 'Thompson Seedless' produced a maximum number of clusters, the average bunch weight and yield per vine was the least due to its small berry size in terms of berry weight and diameter. In rootstocks 110 R, 1103 P, and 99 R, though the number of clusters were more than 'Dogridge', their average cluster weight was in order next to those on 'Dogridge', which could be attributed to the moderate berry characters like berry length and diameter.

The rootstocks displayed a significant difference only with respect to total soluble solids and berry diameter. However, no significant difference was recorded for juice pH and titratable acidity.

Raisin Yield and Biochemical Composition of Fresh Grapes and Raisins

The data on raisin yield and biochemical composition of both fresh grapes and raisins is given in Table 2. Maximum raisin recovery was recorded in 'Thomson Seedless' grapes grafted on 110 R rootstock, which was on par with 'Dogridge' rootstock. The lowest raisin recovery of 21% was recorded on St. George, which was statistically on par with other rootstocks and on own-rooted vines.

As far as biochemical composition of raisins was concerned, there was a drastic increase in the composition of a few biochemical constituents in raisins compared to fresh grapes after harvest except for phenols. The raisins made from ungrafted 'Thompson Seedless' had the highest phenol content followed by those grafted on 99 R, 1103 P, and 110 R. The least was on St. George. The protein content was greatest in 'Thompson Seedless' on 110 R followed by those on 99 R, 1103 P, and 'Dogridge'. The least was in 'Thompson Seedless' grafted on St. George and in ungrafted 'Thompson Seedless'. Similarly, reducing sugar content was highest in raisins made from

TABLE 1 Yield Parameters and Fruit Composition of 'Thompson Seedless' (Fresh Grapes and Raisins) Grafted on Different Rootstocks (Mean of Two Years)

Rootstocks	No. of clusters	Avg. bunch wt (g)	50 berry wt (g)	Yield per vine (Kg)	Berry diameter (mm)	TSS (°B)	Acidity (%)	Juice pH
'Dogridge'	40bc[z]	229.4a	160.80a	8.51de	16.90a	21.35a[z]	0.96	3.66
110 R	60a	226.6ab	152.95ab	13.59a	16.07b	20.90ab	0.92	3.64
1103 P	46b	217.1ab	151.65ab	9.79cd	16.15ab	19.57ab	0.92	3.67
99 R	59a	186.4bc	146.60ab	11.10bc	15.45b	19.67ab	0.98	3.69
St. George	37c	175.9c	133.50b	7.11e	14.95b	21.17a	0.89	3.61
Own rooted	59a	191.5abc	134.82b	12.07ab	15.05b	18.87b	0.90	3.72
Significance	<0.0001[y]	0.001	0.011	<0.0001	0.001	0.009[z]	0.445	0.743

[z]Means followed by one or more identical letters do not differ significantly at $\alpha = 0.05$ by Tukey's studentized range test.
[y]Values below 0.05 indicate significant differences, while values above indicate non significant difference.

TABLE 2 Raisin Recovery and Quality Parameters of 'Thompson Seedless' Grafted on Different Rootstocks (Mean of Two Years)

Rootstocks	Raisin recovery (%)	Proteins (mg g^{-1})		Reducing sugar (mg g^{-1})		Phenols (mg g^{-1})		PPO activity (EU ml^{-1} min^{-1})	
		Fresh	Raisin	Fresh	Raisin	Fresh	Raisin	Fresh	Raisin
'Dogridge'	28.50ab[z]	1.65b	30.19b	18.92cd	43.02ab	4.20ab	1.63b	0.34a	0.010
110 R	29.50a	2.42a	46.76b	21.58a	47.35a	4.52a	2.73a	0.18c	0.001
1103 P	24.5bc	1.94ab	38.76ab	18.66cd	35.65c	3.91bc	1.82b	0.23bc	0.002
99 R	23.5c	1.69b	35.00b	19.78bc	39.15bc	4.32a	1.16c	0.22bc	0.006
St. George	21.0c	2.15ab	38.76ab	17.87d	40.99abc	3.86bc	1.61b	0.25b	0.007
'Thompson Seedless'	22.5c	1.85b	38.47ab	20.78ab	38.42bc	3.59c	1.89b	0.21bc	0.018
Significance	<0.001[y]	0.0001	0.005	<0.001	0.004	<0.001	<0.001	<0.001	NS

[z]Means followed by one or more identical letters do not differ significantly at $\alpha = 0.05$ by Tukey's studentized range test.
[y]Values below 0.05 indicate significant differences, while values above indicate non significant differences.

'Thompson Seedless' grafted on 110 R rootstocks followed by those on 1103 P, 99R, and 'Dogridge'. The least protein was recorded in raisins made from ungrafted 'Thompson Seedless' grapes.

DISCUSSION

It has been noted that among different rootstocks, the yield of 'Thompson Seedless' was highest on 110 R. Rootstock 110 R is known for its ability to control the vigor of 'Thompson Seedless' and thereby increase uniform bud break. Hedberg et al. (1986) recorded higher yields on all grafted cultivars than on own-rooted vines, especially on Ramsey and 'Dogridge' rootstocks. Ferree et al. (1996) reported increased yield of grafted 'Cabernet Franc' and 'White Riesling' than on own-rooted vines. The effect on a particular cultivar is scion specific as evidenced from the studies of Foott et al. (1989), who observed improved yield of 'Cabernet Sauvignon' and 'Chardonnay' on AXR 1, while 1202 C imposed more vigor in the same cultivars.

The increased raisin yield in 'Thompson Seedless' grafted on 110R and 'Dogridge' rootstock may be due to their high TSS content and the individual berry size in terms of berry weight and diameter. The TSS content of fresh grapes is directly related to raisin yield in 'Thompson Seedless' grapes (Chadha and Shikhamany, 1999).

Similar results were recorded by Vasauez et al. (unpublished data) in their studies on nematode resistant rootstocks for early ripening raisin varieties where 'Dogridge' and Ramsey produced the largest berry size in fresh grapes. Raisins made from 'Fiesta' grapes on 'Dogridge' also recorded the lowest percent of substandard raisins in terms of size, appearance, and color.

Although raisin recovery was highest on 'Dogridge' due to increased berry size and TSS, the total raisin yield per unit area was not on par with 110 R (data not shown), which is attributed to its reduced fruit yield per vine. 'Dogridge' conferred lower fruit bud differentiation in 'Thompson Seedless' due to its high vigor inducing characteristic. This is in accordance with findings of Sommer et al. (2001) who recorded lower fruitfulness of 'Sultan' on *Vitis champinii* rootstocks. Clingeleffer and Emmanuelli (2006) recorded similar results wherein 'Sun Muscat' grown on 1103 P rootstock recorded the highest berry weight and sugar level, which produced a good drying ratio. In the same study, they also recorded the smallest berries on own-rooted vines.

The influence of rootstocks on fruit composition has been reported by several workers, especially in wine grapes. There was a close link between fruit quality and wine made from those grapes. Fruit composition parameters, which eventually affect wine quality, include soluble solids, organic acids, pH, phenolics, anthocyanins, monoterpenes, and other components (Jackson

and Lombard, 1993). Hale and Brien (1978) for the first time investigated the influence of Salt Creek rootstock on composition and quality of 'Shiraz' grapes and wine. Their results showed that grafted 'Shiraz' had larger berries with lower soluble solids, higher pH, titratable acidity, malate, and potassium. Cirami et al. (1984) recorded higher juice pH in 'Shiraz' grafted on Ramsey, 'Dogridge', Harmony, Schwarzmann, and 1613C than in own-rooted vines. From long-term studies in light textured soils of the Mildura region, 'Thompson Seedless' grafted on 'Dogridge' and Salt Creek (both of *Vitis champinii*) recorded the highest yields, but those grafted on 110 R and 420 A produced a lesser yield than own-rooted vines (Sauer, 1972).

A significant increase in most of the biochemical constituents, such as proteins and reducing sugars, was recorded in raisins rather than in fresh grapes. There was a reduction in both phenolic content and activity of PPO in raisins compared to fresh grapes. Activity of PPO was almost nil in raisins made from all rootstock scion combinations, which may be attributed to the reduced moisture content in grapes leading to deactivation of enzymes.

Although protein and reducing sugar contents increased during the process of raisin making, the content of phenol was significantly reduced in raisins made from 'Thompson Seedless' grafted on all the rootstocks. This is in correspondence with reduction in PPO activity in raisins compared to their content in fresh grapes (Fig. 1). The enzyme PPO may utilize phenols present in fresh grapes as substrates during the drying process. When the PPO activity of raisins was analyzed after the completion of the drying process, their content was almost negligible, which clearly indicates that the enzyme becomes inactive in the absence of sufficient moisture. Measurement of raisins for phenolic content by Feryal et al. (2000) also showed that raisins are a good dietary source for flavonol glycosides and phenolic acids. Amiot et al. (1992) reported that flavonoids are less susceptible to enzymatic degradation than hydroxyl cinnamic acids and flavan-3-ols. The reduction in the phenolic content of raisins in the present investigation may be attributed to degradation of hydroaxycinaamic acids and flavan-3-ols and the remaining portion of phenols in the finished raisins may be the flavonol groups, which are less susceptible to enzymatic degradation as suggested by Amiot et al. (1992). They also recorded 90% loss in the content of two major hydroxyl cinnamic acids (caftaric and coutaric acids) in all sun dried, dipped, and golden raisins than in fresh grapes. Flavonols were not influenced by processing as much as hydroxycinnamic acids, while procyanidin and flavan-3-ols were completely degraded in all the raisin samples.

The reduction in PPO activity may also be attributed to high concentrations of sugars that inhibit PPO activity and this maintains more cellular integrity during the raisin drying process (Christensen and Peacock, 2006).

Reduction in the enzymatic browning reaction by PPO was observed by Aguilera et al. (1987) where the reduction in moisture content degraded the action of PPO. Use of anti-brown staining agents is also good for inhibiting

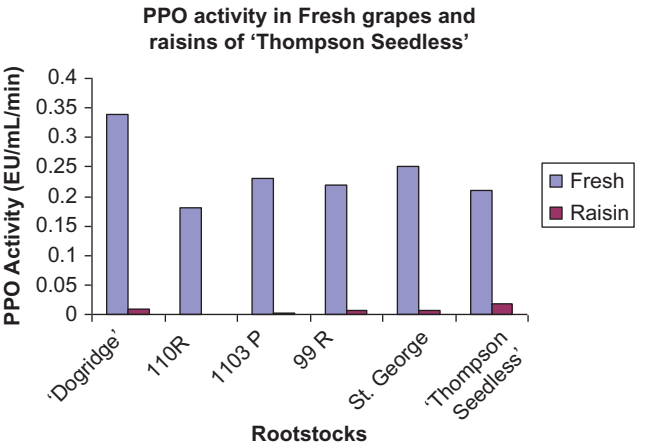

FIGURE 1 PPO activity of fresh grapes and raisins of 'Thompson Seedless' grapes grafted on different rootstocks (color figure available online).

the PPO activity in grapes, thereby preventing seedless raisins from browning. Application of Forchlorfenuron (N-(2-chloro-4-pyridinyl)-N-phenylurea) also resulted in a 36% reduction in PPO activity in 'Flame Seedless' grapes (Carvajal et al., 2001).

Sultana grapes treated with a different concentration of glucose exhibited an inverse relation between glucose concentration and PPO activity (Radler, 1964). The grapes treated with higher concentrations of glucose showed the least PPO activity in the grape skin. In postharvest berries, the rate of water loss induces cell wall enzyme activity, increases respiration and ethylene production, and causes a loss of volatiles and changes in polyphenol levels (Bellincontro et al., 2004). Berry dehydration appears to induce general phenyl proponoid metabolism, which generates precursors for many different categories of phenolic compounds. Zamboni et al. (2008) identified up gradation of some genes involved in hexose metabolism and transport, cell wall decomposition, and secondary metabolites particularly the phenolic and terpenoid compound pathways.

In the present study, there was a drastic reduction in the total phenolic content of raisins compared to fresh grapes. Few previous studies have considered the production of phenolics in grape skins during the postharvest drying process. However, there were some conflicts about the abundance of phenolic compounds, but some of the studies have also shown a general reduction (Servili et al., 2000) and others have shown a general increase of phenols (Tornielli et al., 2005). Shadidi and Nazck (1995) recorded a 50% reduction in phenolic compounds in processed foods than that of fresh products. But, Karakaya et al. (2001) reported significant increases in phenolic compounds in raisins, where fresh grapes recorded total phenols of 1580 mg kg^{-1} while raisins were 3944 mg kg^{-1}. Detailed analysis of phenolic profiles

will help in identifying the phenolic groups, which were lost during drying process and those which were less prone to degradation during the process of drying.

CONCLUSIONS

This investigation on the influence of rootstocks on the yield of 'Thompson Seedless' raisins and their biochemical composition indicated that rootstocks significantly influenced the fruit yield and berry composition in terms of berry size and sugar content. These two factors indirectly influenced the raisin yield. Among the rootstocks, 110 R recorded the highest fruit yield and raisin recovery compared to other rootstocks. Rootstocks are known to influence scion physiology and biochemistry, which can determine final fruit quality. A significant difference was observed for raisin recovery of 'Thompson Seedless' grapes grafted on different rootstocks and also in their biochemical compositions. As raisins are a good source of dietary fiber and antioxidants, a further detailed investigation on phenolic profiles and antioxidant properties of raisins made from different rootstock-scion combination will help in identifying the most suitable rootstock, which can tolerate abiotic stresses in the semiarid tropical climate of India, but also produces fruit with favorable components, such as polyphenols, which have several health benefits.

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