

Biochemically Induced Variations During Some Phenological Stages in Thompson Seedless Grapevines Grafted on Different Rootstocks

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Phenological variation in Thompson Seedless grapevines grafted on different rootstocks and own rooted vines was assessed for two consecutive years and the reasons for such variations were studied through biochemical analysis. Uniform and early bud sprouting was recorded in the vines grafted on 110R rootstock and on own roots, which was attributed to increased peroxidase activity and protein content in the buds before bud burst. Increased fruitfulness on 110R rootstock and own rooted vines was attributed to the increased phosphorus and protein content of those vines and reduced vegetative vigour measured in terms of shoot length, cane diameter and pruned biomass. Thompson Seedless grafted on Dogridge rootstock recorded the highest nitrogen content, increased shoot length, cane diameter and increased pruned biomass attributing to reduced fruitfulness. The highest concentration of phenolic compounds and amino acids was recorded in the fruits produced on 110R rootstock, while it was least on St. George and own roots. Significant variation in the accumulation pattern of amino acids (especially proline and arginine) was observed, with the least proline/arginine ratio recorded on 110R rootstocks at the time of harvest, indicating the variation in the days taken for fruit ripening on different rootstocks.

INTRODUCTION

Choosing the rootstock is one of the most important decisions when establishing vineyards. Rootstocks are employed in grape cultivation to overcome several biotic stresses (phylloxera, nematodes, root diseases, etc.), abiotic stresses (soil and water salinity, water scarcity, frost tolerance, etc.) and, to a limited extent, for controlling vegetative growth, precocity and fruit quality. Numerous studies have shown that rootstocks can affect tree growth, flower development, yield and fruit quality in apples (Seeley *et al.*, 1979; Hirst & Ferree, 1995), grapes (Bica *et al.*, 2000; Ollat *et al.*, 2003), pistachio (Turker and Ak, 2010) and other fruit. Differences in flowering have been reported by El-Shammaa *et al.* (2011) in Anna apple grafted on different rootstocks.

The need for using grape rootstock in India is to ensure profitable production under major abiotic stresses such as soil and water salinity, water scarcity, etc. Besides these, rootstocks provide a large number of choices to grape growers to increase fruit quality, ensure uniform and quick bud burst, for increased fruitfulness, to maintain vine vigour, etc. These factors provide a lot of economically important advantages to the growers. Thus choosing a rootstock is an important decision and the selection of a rootstock depends on local soil and climatic conditions. Rootstock influence

may be either advantages, or may have ill effects on vine growth and productivity, particularly when the rootstock induces more vegetative vigour in the scions, thus reducing fruitfulness and yield.

Many studies have been undertaken across several decades in an attempt to improve the knowledge of rootstock effect on scion growth and other yield and quality parameters. Over the years, several hypotheses have been evaluated that attempt to explain the anatomical, nutritional, hormonal or other physiological influences of rootstocks on scion performance. These studies have been reviewed by Tubbs (1973), Lockhard and Schneider (1981) and Jones (1986). The shoot growth of apple trees and other fruit crops has been shown to be closely related to root growth by Kramer and Kozlowski (1979), who argued that each species has a characteristic root:shoot ratio that remains constant in a stable environment but decreases progressively with increasing plant age and size. Rootstocks also generally affect the abundance of blossoms and fruit produced by the scion, which has an indirect effect on the vigour of shoot growth (Webster, 1995). Striegler and Howell (1991) explained the influence of rootstock by dividing their effects into direct and indirect effects. The direct effect is caused by the function of root systems, and indirect effects are due to

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modifications in vine size. Considering the fact that different rootstocks having varied root distribution patterns, root dry weight and root numbers (Williams & Smith, 1991; Morano & Kliever, 1994), rootstocks may have some direct influence on scion cultivars with respect to certain phenomena, such as mineral uptake, water absorption, etc. Several studies in the past have shown that rootstocks are known to influence many physiological and biochemical reactions in the grafted scions. Leaves of Flame Seedless and Sharad Seedless vines grafted on Dogridge rootstock were known to accumulate more ABA at 50% moisture stress, resulting in increased water-use efficiency, than own rooted vines of the same cultivars (Satisha *et al.*, 2007). Rootstocks had a significant influence on stomatal conductance of scions after budding, and this suggested some possible signal (mainly the hormone cytokinin and abscisic acid) from the rootstock, which must have contributed to a reduction in stomatal conductance in response to soil perturbation. Bica *et al.* (2000) observed a significant effect of rootstock on leaf area, chlorophyll content, stomatal conductance and quantum yield in Pinot Noir and Chardonnay grapevines.

Very little information is available on biochemically induced phenological variations in a scion variety grafted on different rootstocks, particularly under tropical and subtropical conditions of grape growing. Hence this study attempted to discover the variations in the phenology of Thompson Seedless grapes grafted on different rootstocks.

MATERIALS AND METHODS

This study was conducted during two seasons – 2010/11 and 2011/12 – in the experimental vineyards of the National Research Centre for Grapes, Pune, India. Pune is located in the Midwest Maharashtra state (India) at an altitude of 559 m above sea level, at latitude 18.32° N and longitude 7.51° E. The vines were grown in calcareous black cotton-type soil (clay content was 44.5%) exhibiting swelling and shrinkage properties. The average bulk density of the root zone up to a depth of 30 cm was 1.25 g/cm³. The average EC of the irrigation water during the experimentation was 1.98 dS/m, with an average pH value of 7.78. The rainfall in 2010/11 and 2011/12 was 484 mm and 540 mm respectively. A uniform fertiliser dose of 160 kg N, 40 kg P₂O₅ and 210 kg K₂O on a per hectare basis was applied during the entire fruiting season. Soil samples were collected from the root zone of the vines at the time of fruit pruning, representing 40 cm soil surface diameter below the emitter and up to 30 cm depth. On average, the soil had a pH (1:2.5 soil:water) of 7.82, an EC (1:2 soil: water) = 0.70 dS/m, organic carbon = 1.035%, calcium carbonate = 9.5%, mineralisable N = 235 ppm and available P (Olson's P) = 162 ppm. The ammonium acetate-extractable K, Ca, Mg and Na content in the soil was 1 356 ppm, 7 872 ppm, 2 977 ppm and 1 225 ppm respectively, whereas the water-soluble chloride content was 115 ppm. Available (DTPA extractable) Zn, Fe, Mn and Cu was 2.52, 3.5, 4.6 and 3.25 ppm respectively.

The experimental block consisted of 10-years-old Thompson Seedless vines grafted on five different rootstocks, namely Dogridge, 110R, 99R, 1103P and St. George, and own rooted vines. The vines were planted with a spacing of 10 ft between rows and 6 ft between vines within

a row. The vines were trained according to the flat roof gable system, and were irrigated as per the irrigation schedule developed for this region based on pan evaporation. All the vines were pruned twice in an annual growth cycle, which is a common practice in tropical viticulture. The first pruning is done immediately after fruit harvest during the summer months to develop fruitful canes, popularly called “back pruning”, and another pruning is done at about five to six months after back pruning on the fruitful canes to encourage cluster development. This is popularly known as “forward pruning”. The forward pruning coincides with the period just prior to the onset of winter and hence budburst is sometimes a problem due to the cold temperatures. This is popularly known as the “double pruning and single cropping system” of grape growing. Within 24 to 48 hours after forward pruning, two to three apical buds on the pruned canes were swabbed with a bud-breaking chemical, hydrogen cyanamide (at 1.5% a.i.), commercially known as “Dormex”, to facilitate quick and uniform bud burst.

The influence of rootstock on variations in some important phenological stages, such as bud burst, vegetative vigour, fruitfulness, biochemical composition of fruits, etc. was recorded during both the years of study.

Bud burst

Days taken for sprouting were measured after forward pruning. The first sprouted bud with a fully expanded leaf was taken as an indicator to measure the days taken for sprouting. The buds were also analysed for total phenols, proteins and activity of peroxidase and polyphenol oxidase (PPO) by collecting buds within 48 hours after the application of Dormex.

Estimation of total phenols and proteins by spectrometry

The total phenol content of the fruit extract was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965), with gallic acid as the standard. The total protein content was estimated as per the procedures of Lowry's method.

Peroxidase (POD) activity

POD activity was estimated spectrophotometrically according to a modification of the method described by Rodriguez and Sanchez (1982). The assay mixture contained 1 mL of 0.05 M phosphate-citrate buffer (pH 4.6), 1 mL of 40 mM guaiacol and 0.5 mL of 26 mM H₂O₂. The mixture was incubated for 15 minutes at 25°C, and finally 0.5 mL of the enzyme extract was added to the cuvette. Changes in the absorbance at 420 nm were measured for three minutes using a UV spectrometer. POD activity was expressed as “ΔA420/min/g fresh weight”.

Polyphenol oxidase (PPO) activity

PPO activity was measured as per the methods of Haplin and Lee (1987). McIlvaine buffer (0.2 M Na₂HPO₄/0.1 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) was 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 475 nm were measured over time using a spectrophotometer. One unit of PPO activity was expressed as the change in absorbance of 0.1 per min per mL of the enzyme extract.

Measurement of vegetative vigour and fruitfulness

Observations of vine vigour measured in terms of number of shoots, average shoot length, cane diameter and stock scion ratio were recorded before forward pruning, and pruning weight was recorded at the time of forward pruning.

Data on fruitfulness was recorded at the time of cluster emergence after forward pruning. Canes were categorised into fruitful and vegetative canes and the percentage of fruitful canes was estimated. Under the double pruning and single cropping system of grape growing, fruit bud differentiation takes place on the canes at about 45 to 60 days after back pruning, hence the biochemical composition during that stage correlates with fruitfulness. The percentage fruitfulness therefore was correlated with phosphorus, proteins and the C/N ratio in the leaves, which were estimated at the time of fruit bud differentiation.

Estimation of mineral nutrients

Recently matured leaves were collected at the time of fruit bud differentiation (45 days after back pruning) and were analysed for nitrogen, phosphorus, potassium, sodium and chloride, as per the common procedures reported elsewhere. Similarly, at the time of harvest the fruit were analysed for potassium and sodium content.

Analysis of fruit composition parameters

After harvesting, representative berry samples were analysed for basic fruit composition parameters such as total soluble solids (TSS), titratable acidity, juice pH, etc. The fruit samples were also analysed for total proteins, total phenols, potassium, sodium and chloride by the procedures explained above. In addition, fruit also were analysed for free amino acids and individual phenolic compounds using mass spectrometry.

Estimation of amino acids and phenolic compounds

Amino acids

The analysis of amino acids was performed using the Perkin-Elmer Series 200 HPLC system (Perkin-Elmer India Limited, Mumbai, India) connected to an API 2000 (ABS Sciex, Ontario, Canada) triple quadrupole mass spectrometer equipped with an electrospray ionisation (ESI+) probe. The separation of amino acids was achieved on a Zorbax eclipse RP-C18 (150 mm × 4.6 mm, 5 µm particle size) using mobile phase A (0.1 % formic acid in water: 0.1% pentadecafluorooctanoic acid (PDFOA) in methanol; 90:10 v/v) and B (0.1% formic acid in water: 0.1% PDFOA in methanol; 10:90 v/v). The oven temperature was set at 30°C and the injection volume was 10 µL. The mass parameters were curtain gas 20 psi, turbo spray voltage 5500, GS1 30°C, GS2 60°C, and the flow rate was 1.0 mL/min. The gradient was as follows: 0-4 min 2% B; 4-7 min 2-80% B; 7-11 min 80% B; 11-13 min 80-2% B and 13-20 min 2% B.

Phenolic compounds

The LC-MS/MS analysis for phenolic compounds was done with an Agilent Technologies 1200 series coupled to an API 4000 Qtrap (ABS Sciex) mass spectrometer equipped with an electrospray ionisation (ESI+) probe. The separation of the phenolic compounds was achieved on a Precenton SPHER-60 C-18 60A (150 x 2 mm x 5 µm), with mobile phase A: 1% formic acid in water, B: 1% formic acid in water:acetonitrile (1:1), and C: acetonitrile. The oven temperature was set at 35°C, with an injection volume of 10 µL. The mass parameters were curtain gas 20 psi, the ion spray voltage 5500 V and the temperature was 50°C, with a flow rate of 0.400 mL/min.

Statistical analysis

The experiment was conducted as a randomised block design with three replications and the data was analysed using SAS Version 9.3. Tukey's test was used for comparing treatment means.

RESULTS AND DISCUSSION

Percentage bud burst, peroxidase and PPO activity

Significant variation was observed in days taken to bud burst (Table 1), with Thompson Seedless on its own roots and those grafted on 110R requiring 11 and 14 days respectively for bud burst, while Dogridge recorded a longer duration until bud burst (22 days). Similarly, the maximum percentage of bud burst was recorded on 110R rootstock (70.13%), followed by St. George (58.49%) and on own roots (54.58%), while it was least on Dogridge (41.66%). The analysis of POD activity in the buds also showed significant variation, with buds on 110R rootstock recording maximum POD activity, followed by those on own roots. The early and increased percentage of bud burst on 110R and own roots may be attributed to the increased activity of POD and fewer growth inhibitors in their buds. The least POD activity in vines on Dogridge rootstock might have resulted in late and uneven bud sprouting. A significant positive correlation was observed between percentage bud burst and peroxidase activity in the buds. However, no correlation could be established between PPO activity and percentage bud burst. Significant differences were observed in the number of days taken until bud burst, which is in accordance with the reports of several workers who established the influence of rootstocks on bud burst. Prakash and Reddy (1990) reported the effect of different rootstocks on bud break in the cultivar Anab-e-shahi, with a significant effect of rootstocks on bud burst. For example, the number of days required for bud burst was shorter with Gulabi (Isabella) as rootstock and was longer in vines grafted on Dogridge. These results are similar to the current findings of delayed bud sprouting on Dogridge rootstock. In contrast, Tangolar and Ergenoglu (1989) found that time to bud break was not significantly affected by rootstock, although it tended to be earlier on 420 A and Rupestris du Lot. The biochemical changes in the different parts of the vine during bud break have been studied by several workers (Kenis, 1979; Marqu^t *et al.*, 1999; Sivaci, 2006). The change in enzyme activity seems to be an indicator of the end of dormancy and the start of growth, as described by a few researchers (Baassuk *et al.*, 1981; Citadin *et al.*, 2011). The activity of peroxidase

TABLE 1

Budburst, enzyme activity and fruitfulness of Thompson Seedless grafted on different rootstocks (values are average of two seasons).

Rootstocks	Days taken to sprout	% bud burst	Bud peroxidase ($\Delta/A_{420}/\text{min/g}$)	Bud peroxidase ($\Delta/A_{475}/\text{min/g}$)	Bud protein ($\mu\text{g/mg}$)
Own roots	11.00	54.58	5.58	0.0323	0.0597
Dogridge	22.60	41.99	4.75	0.0304	0.0426
110R	14.40	70.13	7.07	0.0512	0.0634
1103P	17.00	50.65	5.27	0.0319	0.0553
99R	17.33	58.41	4.84	0.0524	0.0533
St. George	14.66	58.49	3.85	0.0260	0.0626
LSD	2.401	10.831	0.780	0.012	0.007
P<0.05*	0.0002	0.002	<0.0001	0.0017	0.0005

LSD: Least significant difference

*: values below 0.05 are significantly different and above 0.05 are non-significant

in the roots, shoots and trunk of grapevines increases in autumn and reaches a maximum in December, after which it decreases in the winter, as reported by Schaefer (1983). The most phenolic compounds have been isolated from bud scales and they have been described as growth inhibitors, as they increase during dormancy in peach buds, then decrease after dormancy and are completely eliminated at flowering (Jindal & Makotia, 2004). The changes in peroxidase and PPO activity could be an indicator of when important endogenous changes occur, as the enzymes might lead to the scavenging of the accumulation of H_2O_2 in the buds and thus release dormancy, resulting in bud sprouting (Tripathi *et al.*, 2006).

Vegetative vigour and fruitfulness

As mentioned in the Materials and Methods section, grapevines growing in a tropical climate are pruned twice in the annual growth cycle. Hence the biochemical status of the vines was measured at the time of fruit bud differentiation (45 days after back pruning) and the vegetative vigour (number of shoots, average shoot length, cane diameter and stock:scion ratio) of the vines was measured before forward pruning to correlate the percentage of fruitfulness after forward pruning. The correlation between fruitfulness and the biochemical compounds was worked out, and is shown in Tables 2 and 3, respectively.

Most of the vegetative growth parameters, such as average shoot length, cane diameter and stock:scion ratio, were highest in vines grafted on Dogridge rootstock and this was reflected in the increased pruning mass on the same rootstock (Table 2). The least vigour was observed on own rooted vines. Moderate vigour was recorded in vines grafted on 110R and 1103 P rootstocks. The increased vigour of the vines grafted on Dogridge rootstock is attributed to more vigorous growth, which is evident from the increased diameter of the scion girth above the graft joint, and also due to increased uptake of nitrogen (data not shown) by that rootstock.

In the present study, although we could not see any incompatibility problems with the stock:scion ratio, which varied between 0.71 and 1.01 on different stock-scion

combinations, it is clear that there were differences in the growth behaviour of rootstocks in controlling the vigour of scions. This was evident from the highest shoot length, cane diameter and more pruning mass on Dogridge rootstock, which had the lowest stock:scion ratio of 0.71. In viticulture, growth abnormalities of the graft union have been linked to rootstock-scion incompatibility (Bioletti *et al.*, 1921). However, despite some rootstocks showing swelling of the scions relative to the rootstocks at the graft union, no evidence has been found of the incompatibility of Sun Muscat scions with any of the rootstocks (Clingeffer & Emmanuelli, 2006). A significant rootstock effect on biomass distribution between the root system and the shoot was observed, which led to the conclusion that rootstocks can have implication for biomass partitioning by varying the allocation between roots and shoots in vegetative vines, and then between the fruit and the rest of vine (Holzapfel & Smith, 2008). Williams and Smith (1991), working with Teleki 5C, Aramon rupestri Ganzin and St. George rootstocks, concluded that Cabernet Sauvignon vines showed great vegetative growth, expressed in high values of biomass, nitrogen and phosphorous content, when grafted on Aramon rupestri Ganzin, whereas those on St. George recorded smaller values. The same rootstock may have a different effect on the macro-element content of scion varieties; on the other hand, the different rootstock varieties can give rise to different reactions in different scion varieties (Garcia *et al.*, 2001).

Maximum fruitful canes were recorded on 110R and own rooted vines (about 32%), while they were least on Dogridge and St. George rootstock (22.73 and 18.66% respectively) (Table 2). The analysis of biochemical constituents and nutrient elements in the leaves at the time of fruit bud differentiation indicated a significant variation in phosphorus concentration in Thompson Seedless grafted on different rootstocks, and it was positively correlated with percentage of fruitful canes. A significant correlation was observed between the C/N ratio, phosphorus and fruitfulness, while a negative correlation was observed between sodium and chloride concentration and percentage fruitful canes (Table 3). Greater cane diameter (8.85 mm), maximum shoot length (121.43 cm) and pruned mass (2.98 kg/vine)

TABLE 2

Vegetative growth parameters of Thompson Seedless grapes grafted on different rootstocks (values are average of two seasons).

Rootstocks	Stock/scion ratio	No. of shoots	Average shoot length (cm)	Cane diameter (mm)	Pruned mass (kg/vine)	%Fruitful canes
Own roots	1.000	55	93.63	7.05	2.07	32.41
Dogridge	0.715	48	121.43	8.85	2.98	22.73
110R	0.881	52	105.10	8.30	2.27	32.45
1103P	0.839	45	98.13	7.22	1.99	25.77
99R	0.775	48	99.45	7.74	2.43	27.59
St. George	1.013	42	129.00	7.45	2.16	18.66
LSD	0.126	3.726	12.283	0.470	0.483	7.202
P<0.05	0.001	0.048	0.042	<0.0001	0.008	0.0001

LSD: Least significant difference

*: values below 0.05 are significantly different and above 0.05 are non-significant

were recorded in vines grafted on Dogridge rootstock, which agrees with the findings of Sommer *et al.* (1993), namely that the rootstocks Ramsey and Dogridge (*Vitis champinii*) convey high shoot length and vine vigour to the scions, with a tendency to develop dense canopies. These authors also observed lesser penetration of sunlight into the vine canopy and even negligible penetration of sunlight to the location of auxiliary buds in vines grafted onto vigorous rootstocks relative to own rooted vines, and those grafted onto less vigorous rootstocks. This phenomenon also holds good in the present study, as the reduced fruit bud differentiation on the Dogridge and St. George rootstocks may be due to their denser canopies in comparison to canopies on own roots and on 110R rootstock, which have reduced shoot length and vigour, thus allowing more sunlight to reach the fruiting buds during the period of fruit bud differentiation, and hence higher fruitfulness. The protein content in the leaves at the time of fruit bud differentiation is also one of the key indicators determining fruitfulness. The highest protein concentration was recorded in vines grafted on 110R rootstock (data not shown), followed by those on own rooted vines, both of which also recorded increased fruitful canes. An increase in protein content in the apical leaves during fruit bud differentiation may increase fruitfulness, as suggested by Kessler *et al.* (1959), who found that treating leaves with uracil increased nucleic acid and protein synthesis in the leaves. Phosphorus is known to increase bud fruitfulness, as reviewed by Srinivasan and Mullins (1980). When radioactive P was applied to five grape cultivars at the bud burst stage, most of the P^{32} subsequently was found in the nucleic acid fraction of latent buds, which had primordial inflorescences (Srinivasan *et al.*, 1974). The nucleic acid content, RNA/DNA ratio, catalase and peroxidase are all in the young shoots (at bud break) bearing inflorescences, rather than in vegetative shoots (Srinivasan & Rao, 1972). The reduced fruitfulness on Dogridge rootstock in the present study may be due to its higher vigour-inducing capacity along with reduced protein content and increased C/N ratio in the leaves at the time of fruit bud differentiation (Tables 2 and 3). Zhongyan (1992) was of the opinion that flower-promoting rootstocks decrease the level of floral abortion by encouraging the buds of the scions to use a

greater proportion of the reserve carbohydrates for flower development in kiwi fruit.

Fruit composition parameters

Among the several fruit composition parameters analysed, a significant difference was recorded for total proteins, total phenols, total free amino acids and nutrients (Table 4). Thompson Seedless grapes grafted on 110R rootstock recorded the highest phenols, proteins and total free amino acids, followed by those on 1103P and 99R rootstocks. The least phenolic compound was recorded on own rooted vines, while the least free amino acid content was recorded on St. George rootstock. No significant differences were observed between juice pH, TSS, titratable acidity and potassium content among the rootstocks. However, significant differences were recorded for sodium content in the fruit, where fruit on 110R rootstock recorded the lowest sodium concentration while those on Dogridge, own roots and St. George recorded the highest sodium concentration.

Not much work has been reported on how rootstocks influence the fruit composition of table grapes. However, the influence of rootstocks on fruit composition has been reported by several workers, especially in relation to wine grapes, with a close link between fruit quality and wine made from those grapes. Fruit composition parameters that eventually affect quality include soluble solids, organic acids, pH, phenolics and anthocyanins, monoterpenes and other components (Jackson & Lombard, 1993). Vercesi (1987) characterised the rootstock's mineral absorption ability on the grounds of its genetic background. Rootstocks with a *Berlandieri* x *Rupestis* genetic background (140Ru, 110R) generally have a good ability for nutrient uptake, with an increased K uptake and a weaker ability for Mg^{2+} and Ca^{2+} uptake. At ripening, the K concentration in the leaves of Chardonnay and White Riesling on 110R and 140Ru was the highest (Wolpert *et al.*, 2005). Avenant *et al.* (1997) attributed the variation in potassium accumulation rate in different rootstocks to their genetic origin. Cirami *et al.* (1984) recorded a higher juice pH in Shiraz grafted onto Ramsey, Dogridge, Harmony, Schwartzman and 1613C than on own rooted Shiraz vines. Kubota *et al.* (1993) grafted Fujimori grapes onto seven different rootstocks and observed

TABLE 3

Correlation coefficient between biochemical constituents vs. bud burst and fruitfulness in Thompson Seedless grapevines grafted on different rootstocks.

	Correlation coefficient (r)	Significance
Bud burst stage		
% Bud burst vs. peroxidase	0.470	*
% Bud burst vs. PPO	-0.235	NS
% Bud burst vs. proteins	0.749	**
Fruit bud differentiation stage		
% Fruitfulness vs. nitrogen	0.0180	NS
% Fruitfulness vs. phosphorus	0.338	*
% Fruitfulness vs. potassium	-0.425	*
% Fruitfulness vs. sodium	-0.450	*
% Fruitfulness vs. chloride	-0.498	*
%Fruitfulness vs. CN ratio	0.490	*

*: Significant at $p \leq 0.05$; **: Significant at $p \leq 0.01$; NS: Non-significant

TABLE 4

Fruit composition of Thompson Seedless grapes grafted on different rootstocks (values are average of two seasons).

Rootstocks	Total phenols (mg/g)	Proteins (mg/g)	Total free amino acids (ppm)	pH	TSS (°B)	Acidity (%)	Juice potassium (ppm)	Juice sodium (ppm)
Own roots	2.73	138.52	958.73	3.65	21.33	0.606	540.00	20.16
Dogridge	2.80	126.62	968.42	3.70	23.00	0.550	525.00	27.50
110R	3.10	141.44	1146.15	3.59	23.70	0.536	543.33	14.23
1103P	3.09	137.69	945.15	3.81	22.47	0.536	541.33	19.66
99R	2.77	141.14	945.55	3.76	22.83	0.537	534.66	13.00
St. George	2.73	169.80	944.75	3.71	22.20	0.545	574.00	20.66
LSD	0.431	31.402	144.09	0.243	1.71	0.0417	65.015	0.642
P<0.05	0.0787	0.0359	0.0456	0.441	0.147	0.0239	0.654	0.0065

LSD: Least significant difference

*: values below 0.05 are significantly different and above 0.05 are non-significant

TABLE 5

Phenolic compounds (ppm) in Thompson Seedless grapes grafted on different rootstocks at harvest stage.

Rootstocks	Quercetin hydrate	Rutine hydrate	Quercetin	Ellagic acid	Catechin	Kaempferol	Malvidine-3-glucoside
Own roots	0.71	1.09	10.52	9.78	1.01	0.91	0.047
Dogridge	0.38	0.65	9.03	8.46	0.83	0.58	0.069
110R	0.20	0.63	13.37	12.92	0.89	1.60	0.011
1103P	1.28	0.94	7.46	7.52	1.21	0.35	0.027
99R	0.82	0.65	10.48	10.07	1.16	1.04	0.016
St. George	0.83	1.17	11.64	10.86	2.61	1.14	0.015
LSD	1.361	0.718	8.123	7.315	0.843	1.405	0.074
P<0.05	0.6089	0.458	0.700	0.670	0.005	0.497	0.512

LSD: Least significant difference

*: values below 0.05 are significantly different and above 0.05 are non-significant

higher concentrations of glucose and fructose content in berries grafted onto 3309C, 3306C and 88 rootstocks. The highest level of skin anthocyanin was observed in berries from vines grafted onto 3306C. In the present study, the highest potassium concentration was recorded in Thompson

Seedless grapes grafted onto either St. George or 110R and 1103P rootstocks, which have a *Rupestris* and/or *Berlandieri* genetic makeup, thus confirming findings reported in earlier studies.

Phenolic compounds

Among the 14 different phenolic compounds targeted in Thompson Seedless grapes grafted onto different rootstocks, only seven phenolic compounds could be detected in most of the stock-scion combinations, which are shown in Table 5. Among these, quercetin and ellagic acid were the prominent phenolic compounds detected in all stock-scion combinations. Catechin is the only compound that differed among the different stock-scion combinations, with those grafted on 110R recording the highest catechin content, followed by those on St. George. The lowest catechin content was recorded in fruit grafted on 1103P rootstocks. Although it was interesting to detect malvidin-3-O-glucoside (anthocyanin compound) in Thompson seedless grapes, which is a white variety, its concentration varied on the different rootstocks. It was highest in fruits on own rooted vines and on Dogridge rootstocks, while it was least in fruits grafted on 110R rootstock. The literature on phenolic concentration in table grapes is very limited. In one study, Cantos *et al.* (2002) identified caffetarinic acid and P coumaric acid in four red table grape cultivars (Red Globe, Flame Seedless, Crimson Seedless and Napoleon) and three white table grape cultivars (Superior Seedless, Doming and Moscatel Italica). The main flavonols in different table grape cultivars are quercetin-3 glucuronide, quercetin-3-glucoside and quercetin-3-rutinoside (Cantos *et al.*, 2002). These authors also discovered other flavonols in trace amounts, namely kaempferol and isorhamnetin-3-glucoside. Myricetin was not found in any of the seven table grape cultivars tested. However, some workers have obtained minor concentrations of myricetin and its derivatives in table grapes (Fernández de Simon *et al.*, 1992). Studies on phenolic compounds in grapes have gained importance in recent years due to their influence on various health benefits. In view of this, the findings of this study may help in deciding the rootstock for table grape varieties to improve their nutraceutical properties, in addition to their influence on growth and yield parameters, as such information is lacking under Indian conditions.

Amino acids

Significant differences in the concentration of amino acids were observed in Thompson Seedless grafted on different rootstocks at harvest stage (Table 6). The prominent amino acids were arginine, proline, glutamic acid, serine and alanine. The amino acids present in moderate concentrations were aspartic acid, histidine, ornithine, phenylalanine and tyrosine. The amino acids present in the lowest concentration were methionine, leucine and tryptophan. It therefore is clear from this study that the variation in the accumulation of sugar and in the ripening process among different rootstocks may be a function of amino acids, as it has been reported in various literature that some of the amino acids, such as aspartic acid, alanine and glutamic acid, determine sweetness, flavour and taste. Thus, rootstocks play a definite role in altering the fruit composition parameters through several secondary metabolic reactions involved in the synthesis of amino acids and proteins. Arginine and proline were among the major amino acids studied. The ratio of arginine and proline determines fruit maturity and ripening

TABLE 6
Amino acids (ppm) in Thompson Seedless grapes grafted on different rootstocks at harvest stage.

Rootstocks	Arg	Asp	Glu	His	Leu	Met	Norleu	Orn	Phe	Pro	Ser	Try	Tyr	Ala
Own roots	360.82	11.67	81.71	18.41	8.75	3.42	9.64	26.88	12.14	124.66	61.48	3.96	10.80	63.18
Dogridge	311.24	15.37	89.39	15.86	4.64	1.80	7.59	16.10	10.71	122.00	40.57	4.25	9.18	58.25
110R	313.36	13.78	77.49	17.00	6.30	1.93	8.47	15.18	11.03	134.56	47.74	4.54	9.60	62.06
1103P	351.90	15.30	73.95	19.48	8.17	3.57	9.64	25.55	14.07	123.95	52.70	5.92	12.15	52.98
99R	255.00	14.20	83.86	16.56	4.64	2.40	8.67	19.97	11.87	12.32	45.57	4.29	10.48	60.06
St. George	235.00	10.83	67.29	11.39	3.85	1.14	5.45	7.25	7.60	85.85	33.13	3.54	7.11	37.08
LSD	39.393	3.288	13.686	3.967	4.723	1.384	2.633	13.137	5.083	13.371	14.521	2.135	2.708	8.606
P<0.05	0.001	0.049	0.005	0.017	0.243	0.015	0.021	0.059	0.213	0.002	0.018	0.293	0.027	0.0002

LSD: Least significant difference

*: values below 0.05 are significantly different and above 0.05 are non-significant

Arg: Arginine; Asp: Aspartic acid; His: Histidine; Leu: Leucine; Met: Methionine; Norleu: Norleucine; Orn: Ornithine; Phe: Phenylalanine; Pro: Proline; Ser: Serine; Try: Tryptophan; Tyr: Tyrosine; Ala: Alanine

in table grapes (Kliewer & Ough, 1970). The higher the arginine-proline ratio in earlier stages of berry development, the more rapid the ripening process. Accordingly, in the present study, vines grafted on St. George and on their own roots attained the highest arginine-proline ratio very early, followed by those on Dogridge and 99R, while it took longer to attain the highest arginine-proline ratio on 110R rootstock. Hence, the berries need more time to ripen on 110R rootstock (Fig. 1). The variation in TSS content of Thompson Seedless grapes grafted on different rootstocks may thus support the variation in the arginine-proline ratio, which determines grape maturity and ripening.

Several physiological and biochemical reactions in the leaves of scions may result in the production of various primary and secondary metabolites, which will translocate to the root system, thus changing the root morphology and root absorption patterns. Similarly, several of the enzymes and hormones synthesised in the root system may translocate to the leaves, thus bringing about their effect on grafted plants (Ballesta *et al.*, 2010). Thus the biochemical constituents of grape leaves and fruit may be influenced by stock-scion interactions. Rootstocks have preference for the uptake of nutrient elements from the soil system, which may act as coenzymes in the synthesis of several secondary metabolites, including amino acids. This explains the interaction effect of stocks and scions on the concentration of a few amino acids. Findings such as these have been reported by Lee and Steenwerth (2011), who have shown that Cabernet Sauvignon grapes grafted on 110R rootstock recorded a significantly higher concentration of serine, glutamine, threonine, arginine, valine, leucine, isoleucine and yeast assimilable nitrogen (YAN) than those grafted on 420A rootstocks. In another study, Treeby *et al.* (1998) recorded the lowest concentration of free amino acids in Chardonnay grapes grafted on 140 Ru and 101-14 rootstocks, and

the highest on their own roots, Schwarzman and K51-40 rootstocks. The concentrations of leucine, isoleucine, valine, threonine, tyrosine and phenylalanine generally were highest in Chardonnay grapes grafted on K51-40 rather than on other rootstocks. The content of arginine remained stable or declined slightly at fruit ripening (Stines *et al.*, 2000), leading to the conclusion that the accumulation of proline and arginine appears to be developmentally regulated. The role of proline is to act as carbon, nitrogen and energy for cellular metabolism (Hare & Cress, 1997), possibly providing energy for the transport and accumulation of sugars (Kliewer, 1968). In this study, the highest concentration of proline was recorded after véraison and at the ripening stage in all stock-scion combinations, although the concentrations varied significantly. The increased accumulation of proline in the later stages of berry development may also be due to its role in osmotic adjustment, thereby maintaining water potential during berry ripening (Chu *et al.*, 1976). Our results with respect to the accumulation of arginine in developing berries confirm the early findings of Nassar and Kliewer (1966), who reported arginine to accumulate before proline in Thompson Seedless grapes. Essential amino acids like lysine, isoleucine, methionine, valine, phenyl alanine and cysteine contribute significantly to the nutritional qualities of fruit. Some of the free amino acids may influence fruit taste. Best known are L-glutamic acid, which is responsible for delicious taste, alanine and lysine, which are responsible for sweetness, and phenyl alanine and tyrosine, which contribute to bitterness (Hirano *et al.*, 2000). As very limited information is available on the influence of amino acids in determining fruit quality in table grapes, this information on the concentration of different amino acids can be utilised to determine the taste and quality of table grapes grown in different soil and climatic conditions.

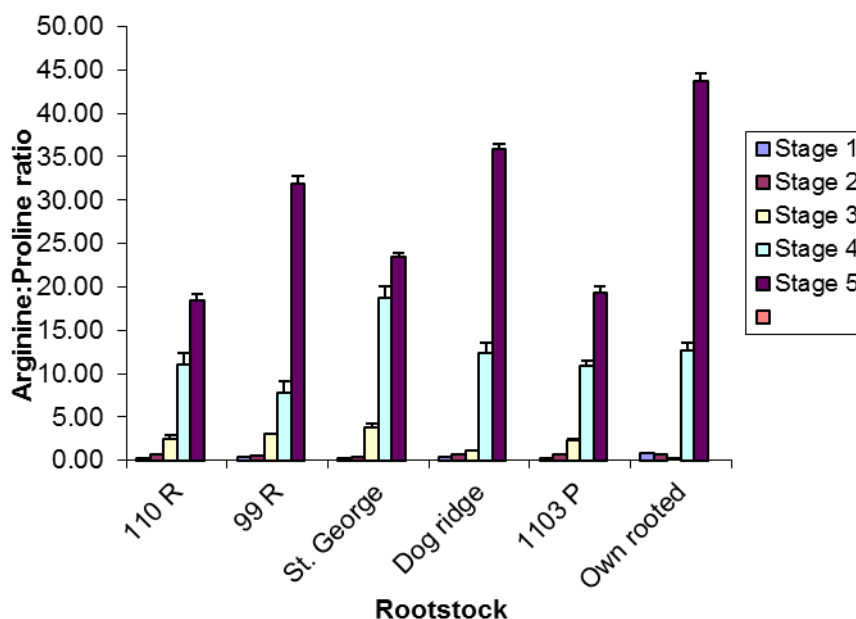


FIGURE 1

Arginine-proline ratio in Thompson Seedless grapes grafted on different rootstocks at various stages of berry development. Stage 1: Fruit set; Stage 2: 3-4 mm berries; Stage 3: 8-10 mm berries; Stage 4: Véraison; Stage 5: Harvesting

CONCLUSION

The use of rootstocks in viticulture in India has gained in importance in recent years, due to their ability to tolerate the adverse effects of abiotic stresses such as soil and water salinity and water scarcity. In addition, the influence of rootstocks on growth, yield and fruit composition parameters in grafted scions was observed in our previous studies. Hence, the present investigation was undertaken to study the biochemical mechanisms by which rootstocks induce variations in scions at a few phenological stages. Quick bud burst after forward pruning was recorded in vines grafted on 110R rootstock and in own rooted vines, which was attributed to the higher protein content of and peroxidase activity in buds before sprouting. Similarly, the highest fruitfulness was recorded in vines grafted on 110R rootstock, indicating better fruit bud differentiation on those rootstocks, owing to increased leaf phosphorus and protein content at the time of fruit bud differentiation and reduced vegetative vigour measured in terms of shoot length, cane diameter and pruned biomass. An indirect relationship was observed between vegetative growth and fruitfulness in vines grafted on Dogridge rootstock. Though rootstocks did not significantly influence basic fruit composition parameters such as TSS, acidity and juice pH, some of the secondary metabolites, such as phenolic compounds, amino acids, total proteins, etc., showed significant variation among different stock-scion combinations. Thompson Seedless grafted on 110R rootstock recorded the highest concentration of phenolic compounds and amino acids. A significant variation was recorded in the accumulation pattern of arginine and proline, indicating their importance during the developmental stages of the berry. The outcomes of this study need to be confirmed further through in depth molecular studies to understand the exact mechanisms induced by rootstocks in scion physiology and biochemistry at different phenological stages of vine growth and development.

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