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Rootstocks Influence the Growth, Biochemical Contents and Disease Incidence in Thompson Seedless Grapevines

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Authors' contributions

The research work was carried out at the research and developmental plot of NRC Grapes, Pune. Authors RGS, SJ and SDS designed the experiment, wrote the protocol and recorded the observations. Authors PBT and DDB done the field work and collected samples for study and author PI performed biochemical and statistical analysis. Author RGS prepared the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The variations in growth, powdery mildew and anthracnose incidence and biochemical changes in Thompson Seedless grapevines grafted onto Dog Ridge and 110-R rootstocks in comparison to the own rooted grape vines was investigated. Bud sprouting after pruning was achieved earlier in case of own-rooted vines. However, the vine growth pattern studied in terms of the shoot length, inter nodal length, shoot diameter and leaf area was highest in case of vines grafted onto Dog Ridge. Foliar powdery mildew and anthracnose incidence was highest in vines grafted onto Dog Ridge and own-rooted vines, respectively. The disease incidence was recorded least in vines grafted onto 110-R. The biochemical analysis was also influenced by the rootstocks. The leaves of vines grafted onto Dog Ridge recorded the highest reducing sugars and protein contents and the least total sugars and phenolic contents. The vines grafted onto 110-R topped in total phenolic contents and other phenolic derivatives.

Keywords: Grape; rootstocks; canopy architecture; diseases; phenolics.

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ABBREVIATIONS

DNSA - Dinitrosalicylic Acid; BSA - Bovine serum albumin-Fraction V; DMACA - p-dimethylaminocinnamaldehyde.

1. INTRODUCTION

Grape (*Vitisvinifera* L.) is one of the major important commercial fruit crops of India with1,11,000 ha area with an annual production of 12,35,000 tones [1]. Table grapes occupy more than 90% of the area under grape cultivation in India. Amongst the table grape varieties, Thompson Seedless is a popular cultivar for both fresh consumption (local and export market) and raisin making. Traditional grape cultivation in India involves growing commercial varieties of grapes on own roots. Due to increasing problems of soil salinity, bad quality water and drought, the decline in the productivity of own rooted vines in Maharashtra and neighboring states has been reported. Owing to the adverse conditions, use of rootstock for the establishment of grape vineyards became important. As a result, the use of rootstock increased for enhancing grape production in India. The primary use of rootstocks is for pest resistance [2,3]. The major functions of the grapevine root system are vine water relations, the uptake and translocation of nutrients, the synthesis and metabolism of plant growth substances and the storage of carbohydrates [4]. Reynolds and Wardle [5] outlined seven major criteria for choosing rootstocks in the order of importance. Numerous reports have also proved that rootstock affect vine growth, yield and fruit quality. These effects take place due to the interaction between environmental factors and the physiology of scion and rootstock cultivars. Grape rootstocks have a primary effect on vine size measured in terms of pruning weights, as indicated by [2,3,6,7]. An increase in vine size when the canopy length is fixed, results in crowding of shoots and internal canopy shading [8]. Most secondary effects of rootstocks are mediated through their influence on vine size and internal canopy shading. The difference in performance of vines grafted on rootstock and own rooted vines is experienced by the grape growers. In the tropical parts of the country where the grapes are being grown for table as well as wine purpose, canopy development is an important aspect for the production of quality grapes and also to minimize the disease incidence.

For sustainable viticulture, it is important to know the interactions among rootstocks, different soil characters and scion productivity [9]. A rootstock found to be beneficial for one cultivar may not be universally advantageous for others, as the interaction of stock and scion influences the vine performance more than the stock or scion alone [10]. With increased awareness about the use of rootstocks in overcoming the adverse effects of drought and salinity, growers started using Dog Ridge rootstock for the cultivation of Thompson Seedless grapes. However, in the tropical and subtropical climate of India, Dog Ridge induced more vegetative vigour in the scions, which reduced the bud fruitfulness of Thompson Seedless in the long run. In this context, there was an increased demand for an alternative rootstock that would be suitable for Thompson Seedless in the tropical and subtropical climate of the Indian subcontinent. Another grape rootstock i.e., 110-R which is alternative rootstock to the present Dog Ridge [11] was introduced to overcome these problems.

Use of Dog Ridge and 110-R rootstocks is gaining importance in Indian viticulture and the majority of vineyards are being established on these rootstocks to overcome the adverse effects of abiotic stresses such as drought and salinity. In addition, rootstocks are known to influence the growth pattern of scions after grafting and some may even induce resistance to diseases by various physiological and biochemical changes in grafted vines [12]. Grape species and their cultivars differ in their susceptibility to powdery mildew infection [13]. The rootstocks alter the canopy architecture of a grapevine thereby creating favorable or unfavorable microclimate for the development of various diseases. This also alters the biochemical composition of a grapevine as a whole and of final harvest. Considering this, the present study was initiated with the objective to evaluate the changes in growth behaviour, biochemical composition and disease incidence of Thompson Seedless grafted onto Dog Ridge and 110-R rootstocks, with own rooted Thompson Seedless as a control.

2. MATERIALS AND METHODS

The trial was conducted at the farm of National Research Centre for Grapes, Pune during 2008-2010. The grape rootstock Dog Ridge and 110-R along with own-rooted vines of Thompson Seedless were planted during the year 1999 with 3.0 m distance between the rows and 1.8 m between the vines and the grafting of table grape variety Thompson seedless was performed during October, 1999. The experimental site is situated in Mid-West Maharashtra at an altitude of 559 m above mean sea level; it lies on 18.32°N latitude and 73.51°E longitudes. The climate in this region is mild to slightly dry. The soil is heavy black with pH 7.75 and EC 0.46 dS m^{-1}.The vines were trained on 'extended Y' system of training with four cordons (H shape – Height = 1.20m from surface, Cross arm width = 0.60m) developed horizontally with vertical shoot positioningon the cordons. The distance from the fruiting wire to the top of foliage support wire was 0.60 m.

2.1 Growth Parameters

The investigation was carried out after foundation pruning on nine year old Thompson Seedless vines grafted onto Dog Ridge and 110-R rootstock and was compared with own rooted Thompson Seedless. The canes were pruned during mid-April leaving only a single visible bud at the base on a cordon. The days taken for sprouting were recorded on day to day basis by visual observations. The shoot length was measured at 30, 60 and 90 days after pruning (DAP) with the help of measuring tape. Inter nodal length, shoot diameter and leaf area was also measured after 90 DAP with the help of measuring scale, vernier calliper and leaf area meter (CID, Inc), respectively. Days taken for cane maturity were also recorded as the time taken for complete browning of cane from the date of foundation pruning inspecting visually of proper lignification.

2.2 Disease Assessment

Powdery mildew and anthracnose incidence were assessed in-situ by visual observations as the percent number of the leaves and canes infected by each disease at 90 days after pruning. The infection of each disease on leaf as well as on cane was recorded using the 0-5 scale and was then mentioned as percent infection.

2.3 Biochemical Analysis of Leaves

The total sugar content was measured by hydrolysing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides by Anthrone method [14]. Quantity of the carbohydrate was expressed as glucose equivalent as determined from linear regression obtained by plotting absorption against known standard glucose concentration. Reducing sugars were estimated by using Dinitrosalicylic Acid (DNSA) method [15]. The results were expressed as glucose equivalent as determined from linear regression obtained by plotting the absorption against known standard glucose concentration.

Proteins were estimated by using standard procedure as suggested by Lowry et al. [16] method. The blue colour developed by the reduction of the phosphomolybdic-phosphotungistic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in Lowry's method. The blue colour developed was read at 660nm on UV-visible spectrophotometer (Shimadzu- 1601). The quantity of protein was determined from linear regression obtained by plotting the absorption against known standard BSA (Bovine serum albumin-Fraction V) concentration. The proteins were expressed as mg g^{-1} fresh weight of sample.

Total phenolic content was estimated by using one gram fresh samples of leaf homogenized in 80% ethanol repetitively and final volume made up to 10.0ml. The mixtures were sonicated for 15min for complete extraction and centrifuged at 9000rpm for 10min. The supernatants were utilized for analysis of total phenolic contents and other phenolic derivatives. The total phenolic contents were determined by Folin-Ciocalteu method [17], using gallic acid as the standard. The total polyphenolic concentration was calculated from a calibration curve using gallic acid as a standard (0-10 mg L^{-1}). Data were expressed in mg g^{-1} GAE. Total flavan-3-ol content was estimated using DMACA(*p*-dimethylaminocinnamaldehyde) method [18]. The concentration of total flavan-3-ols was expressed as the CE (mg g-1) of the fresh sample. Flavonol content was estimated by measuring the absorbance of the extract at 360 nm after the addition of 2% HCl [19] and was expressed as the quercetin equivalent (QE mg g-1). Total flavonoids were determined calorimetrically, following the procedures proposed by Kim et al. [20]. The amount of total flavonoids was expressed as the catechin equivalent (CE, mg g-1).

2.4 Statistical Analysis

The experiment was conducted in randomized block design consisting of three treatment grafted onto Dog Ridge, 110-R and own root vines of Thompson Seedless. Each treatment consisted of 20 plants and was replicated five times totaling 45 plants under each treatment. The data was analyzed statistically using Sigma Stat version 3.5.

3. RESULTS AND DISCUSSION

The results on days taken for sprouting (Table 1) revealed late sprouting in grafted Thompson Seedless vines (13-14 days) after pruning while the early sprouting was reported in own-rooted vines (10 days) after pruning. However, the shoot growth lead in case of grafted vines and the vines grafted onto Dog Ridge exhibit more vigour. The results on shoot length measurements made it clear that at all the stages of investigation i.e. 30, 60 and 90 DAP, the shoot lengths were in the sequence of own-rooted Thompson seedless vines < vines grafted on 110R < vines grafted onto Dog Ridge. The internode length, shoot diameter and leaf area measurements at 90 DAP (Table 1) exhibited the same pattern. The days taken for cane maturity were significantly more in vines grafted onto Dog Ridge (84.51 days) followed by 110-R (79.80 days) and was least in case of own rooted vines (74.59 days). The effect of rootstocks on days taken for sprouting was highly significant. The results of the present investigation are in accordance with the reports of several workers in the past, who established the influence of rootstocks on bud burst. Satisha et al. [21] reported that

Thompson Seedless grafted onto Dog Ridge take significantly more time to sprout after pruning. Prakash and Reddy [22] reported the effect of different rootstocks on bud break in the grape cultivar Anab-e-Shahi, with a significant effect of rootstocks on bud burst. These results are similar to the current findings of delayed bud sprouting on Dog Ridge rootstock. However, Tangolar and Ergenoglu [23] reported that time required for bud break was not significantly affected by rootstocks. The rootstock Dog Ridge is imparting more vigour compared to other rootstock under tropical condition. This has also expressed in terms of increase in shoot length in grafted vines than that of own rooted vines. Sommer et al. [24] also reported the increase in shoot length on grafted vines. Satisha et al. [21] while working on performance of Thompson Seedless grafted onto different rootstock also reported increase in shoot length and cane diameter in the vines grafted onto Dog Ridge rootstock compared to own rooted vines. Bica et al. [25] found significant effect of use of rootstock on higher leaf area. The rootstock Dog Ridge is performing better under Indian condition considering the yield and quality requirement. However, 110-R is another addition which is an alternative to present Dog Ridge, looking into the soil and water problem in grape cultivation [11]. The vines grafted onto 110-R rootstock are early to sprout than Dog Ridge grafted vines in addition to uniform sprouts is also achieved. The cane maturity is an indication of proper lignification in the shoot. The early cane maturity helps to store enough reserve food material in shoot in addition to the healthy vine. The vines grafted onto 110-R rootstock were early to mature indicating proper vine health.

The biochemical composition of the leaves at 90 DAP (Fig. 1) revealed that the total sugars were significantly more in case of own-rooted and 110-R grafted vines than the vines grafted onto Dog Ridge. Reducing sugars and protein contents were significantly higher in case of the vines grafted onto Dog Ridge. The results on phenolic compounds in the leaves (Fig. 2) indicated that the total phenols, flavan-3-ols, flavonols and flavonoids contents were significantly high in the vines grafted onto 110-R rootstock. Own-rooted vines were at second position and the vines grafted onto Dog Ridge had the least phenolic contents. The results on biochemical composition exhibited significant difference due to grafting of Thompson Seedless onto Dog Ridge and 110-R rootstocks. This might be due to the alterations in the growth pattern of the vines by rootstocks as well as the differences in their uptake of nutrients and water from soil solution, as root development patterns vary with the rootstocks. Most secondary effects of rootstocks are mediated through their influence on vine size and internal canopy shading. Previous research has found a relationship between canopy structure and sunlight exposure and subsequent phenolic contents [26]. In addition, a relationship between variations in vine growth and differences in total phenolic levels (measured as absorbance at 280 nm) has been reported [27,28]. Influence of rootstocks on changing pattern of phenolic compounds in Thompson Seedless reported by [29] confirms the results of the present investigation. Grape berries act as typical sink organ which rely on the use of available carbohydrate resource produced by photosynthesis to support their growth and development. In addition to the number of factors assessing to monitor ripening, berry sugar concentration is mainly due to water loss from the berries at maturity [30]. The effect of rootstock on fruit composition has been reported earlier by several workers, especially in wine grapes, with a close link between fruit quality and wine prepared from those grapes. Soluble solids, organic acids, pH, phenolic and anthocyanins, monoterpenes and other components [31] are fruit quality parameters that affect the quality of wine. Hale and Brien [32] first investigated the influence of Salt Creek rootstock on the composition and quality of Shiraz grapes and wine.

Table 1. Effect of Dog Ridge and 110-R rootstocks on growth, powdery mildew and anthracnose disease incidence in Thompson Seedless grapes

Parameters	Vines grafted on Dog Ridge	Vines grafted on 110R	Own-rooted Thompson seedless	*Significance*
Days taken for sprouting	13.48 ±1.12 a	12.64 ±0.93 a	10.08 ±0.59 b	**
Shoot length at 30 DAP (cm)	39.71 ±0.74 a	35.03 ±1.27 b	31.44 ±1.66 c	**
Shoot length at 60 DAP (cm)	72.01 ±1.78 a	66.50 ±1.74 b	59.30 ±1.27 c	**
Shoot length at 90 DAP (cm)	104.21 ±2.78 a	93.34 ±2.40 b	85.01 ±2.25 c	**
Internode length (cm)	6.60 ±0.20 a	5.28 ±0.22 b	4.90 ±0.24 c	**
Shoot diameter (mm)	8.98 ±0.25 a	8.00 ±0.16 b	6.71 ±0.12 c	**
Leaf area (cm2)	8411.14 ±424.29 a	6836.35 ±348.92 b	4727.22 ±252.50 c	**
Days required for cane maturity	84.51 ±0.86 a	79.80 ±0.71 b	74.59 ±1.02 c	**
Foliar powdery mildew incidence (%)	3.19 ±0.62 a	1.57 ±0.41 b	2.08 ±0.65 b	*
Foliar anthracnose incidence (%)	1.30 ±0.54 a	1.14 ±1.22 a	1.96 ±0.55 a	ns
Anthracnose incidence on canes (%)	0.80 ±0.12 c	1.20 ±0.13 b	2.00 ±0.39 a	**

The data are the means of five replicates ±standard deviation. '', '**' and 'ns' represent significance $P<0.05$, $p<0.01$ and non-significance, respectively by one-way-ANOVA statistics. Values followed by different letters differ significantly by DMRT at $P=0.05$.*

Their results showed that grafted Shiraz had larger berries with lower soluble solids and higher pH, titratable acidity, malate and potassium. Kubota et al. [33] on his study on Fujimori grapes grafted onto seven different rootstocks found that the glucose and fructose content of the pulp was higher in berries grafted onto 3309 C, 3306C and 8B. However, some researchers reported no significant effects of rootstock on fruit composition in grapes. Reynolds and Wardle [34] studied nine grape cultivars and four rootstocks (3309C, 5BB, 5C, SO4) and they reported non-significant differences in titratable acidity as well as pH.

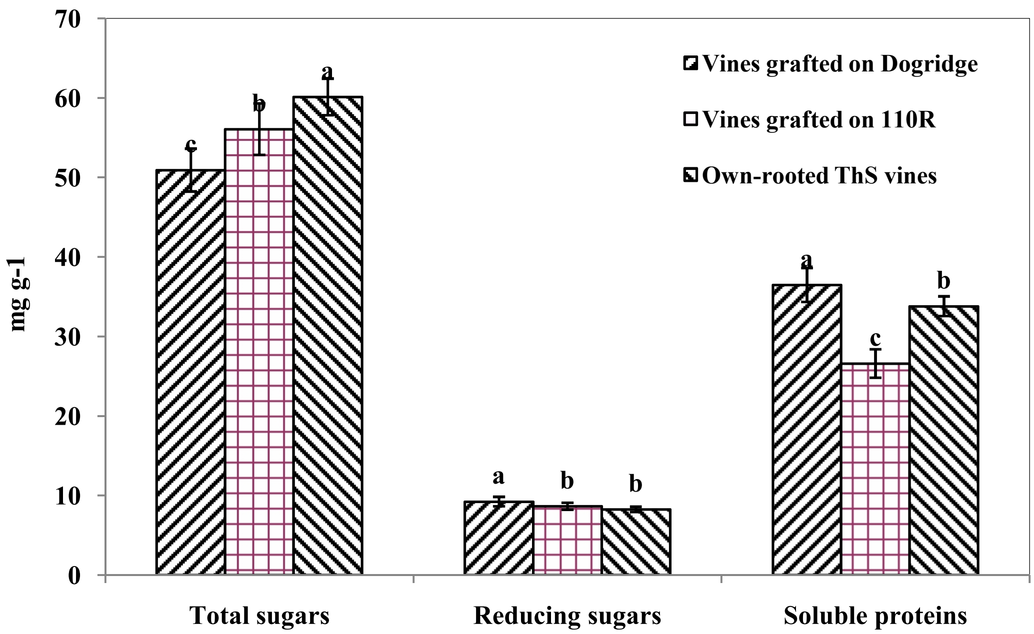

Fig. 1. Effect of dog ridge and 110-R rootstocks on biochemical composition of Thompson seedless grapes as compared to own-rooted vines

The data on disease incidence on Thompson Seedless grapevines (Table 1) revealed that the powdery mildew incidence in case of vines grafted onto Dog Ridge (3.19%) was significantly higher than the vines grafted onto 110-R (1.57%) and own-rooted Thompson Seedless vines (2.08%). There was no significant difference in anthracnose infection on leaves. However, the anthracnose infection of the canes was the highest in own rooted (2.00%) Thompson Seedless followed by vines grafted onto 110-R (1.20%) and the least in case of the vines grafted onto Dog Ridge (0.80%). Rootstocks not only perform a critical function in water and nutrient assimilation [35], but also provide increased tolerance to insect pest and diseases of grapes [36]. In the present investigation, use of Dog Ridge rootstock found increase in foliar powdery mildew disease susceptibility. However, the vines grafted onto 110-R rootstock exhibited significantly low powdery mildew incidence. The finding of the present investigation confirms the results of [29] who reported less powdery mildew infection in vines grafted onto 110-R rootstocks. The species of genus Vitis that contain high polyphenols content (V. rotundifolia) are more resistant to infection caused by *Uncinulanecator*, and when infected they produce larger amounts of polyphenols than varieties that are more susceptible [37]. Rootstocks may influence the biochemical composition of the scion leaves grafted onto them, which in turn affects the degree of resistance or susceptibility to disease [29].Variation in the incidence of disease in Thompson Seedless grafted onto different rootstocks may have been due to the genetic makeup of the rootstock, which have indirectly contributed to the synthesis of various secondary metabolites. The results on reduced anthracnose incidence on Thompson Seedless grafted vines were similar with the finding of Ren and Lu [36] where they reported increased resistance of Florida hybrid grapes to anthracnose disease. Pospisilova [38] rated the severity of mildew on both the fruit and leaves for 926 cultivars of V. vinifera and found that disease severity on the fruit generally corresponded to that on the leaves for a given cultivar.

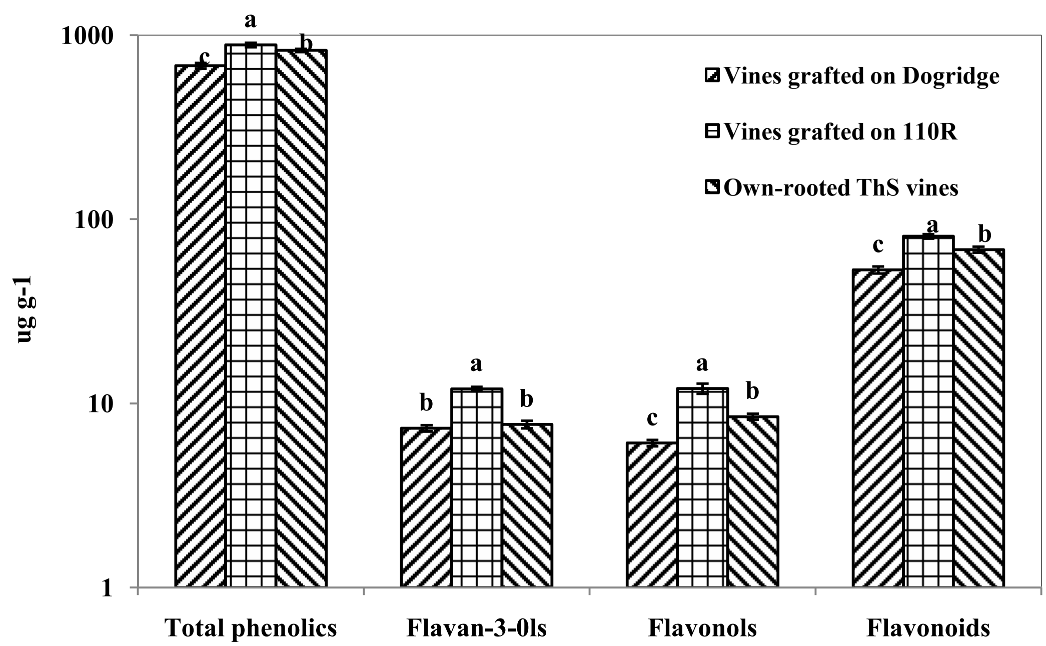

Fig. 2. Effect of dog ridge and 110-R rootstocks on phenolic contents of Thompson seedless leaves as compared to own-rooted vines

The correlation coefficients (Table 2) between the growth parameters, biochemical composition and disease incidence of the vines revealed that the days taken for sprouting after pruning had no significant impact on further growth, biochemical composition as well as disease incidence of the vines. The vine vigour measured in terms of shoot length, inter nodal length and leaf area were significantly inter-related and had positive correlation with foliar powdery mildew incidence and anthracnose infection of canes with mixed influence on biochemical composition. Disease incidence of Thompson Seedless vines had no significant correlation with biochemical composition of the leaves at 90 DAP.

Table 2. Correlation between vine growth, disease incidence and biochemical composition in own-rooted and grafted Thompson seedless grape

Parameters	Shoot length	Inter nodal length	Leaf area	Foliar PM	Foliar anthracnose	Cane anthracnose	Total phenolics	Reducing sugar	Total sugars	Protein
Days taken for sprouting	-0.19ns	-0.13ns	-0.29ns	-0.19ns	0.15ns	0.41ns	0.10ns	-0.05ns	0.31 ns	0.03ns
Shoot length	0.89**	0.89**	0.88**	0.70**	-0.29ns	-0.84**	-0.72**	0.57*	-0.86**	0.26ns
Inter nodal length			0.86**	0.80**	-0.24ns	-0.79**	-0.83**	0.68**	-0.74**	0.50*
Foliar PM incidence					-0.39ns	-0.63**	-0.66**	0.79**	-0.41ns	0.47*
Foliar Anthracnose						0.33ns	-0.02ns	-0.38ns	0.05 ns	0.10ns
Cane Anthracnose							0.50*	-0.51*	0.80**	-0.08ns
Total phenolic								-0.38ns	0.69**	-0.75**
Reducing sugars									-0.19ns	0.38 ns
Total sugars										-0.10ns

'', '**' and 'ns' represent significance of the correlation coefficients at P<0.05, p<0.01 and non-significance, respectively.*

4. CONCLUSION

The rootstock Dog Ridge and 110-R were found to alter the growth of the Thompson Seedless grapevines as compared to the own-rooted vines. The rootstock Dog Ridge imparted more vigour to the scion followed by 110-R. Although the own rooted vines sprouted earlier, they were less vigorous. The results on biochemical composition, powdery mildew and anthracnose disease incidence of vines were also influenced due to use of rootstocks. These traits may have significance in selection of rootstock considering the local soil and climatic conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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