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Rootstock induced changes in enzymes activity and biochemical constituents during budbreak in 'Thompson Seedless' grapevine

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Summary

The process of budbreak was monitored in own rooted and grafted vines of 'Thompson Seedless' (*Vitis vinifera* L.). Vines grafted on 110R rootstock and own rooted vines took a shorter time to achieve maximum percentage of budbreak as compared to those grafted on Dogridge rootstock. The higher starch degradation with corresponding increase in α-amylase activity was observed in buds of own rooted and 110R grafted vines till 9th day after pruning, while the α-amylase activity was least on Dogridge rootstock. In own rooted and 110R grafted vines, activity of peroxidase and polyphenol oxidase enzymes showed drastic reduction from 0-9th day after pruning. The highest phenolic concentration was observed in the buds of vines grafted on Dogridge rootstock from 0-9th day after pruning. Significant and positive correlation was observed for α-amylase activity and percent budbreak while it was negatively correlated with starch concentration in both buds and canes.

K e y w o r d s : Grapes, rootstocks, α-amylase, peroxidase, polyphenol oxidase, starch, phenols.

Introduction

Budbreak is defined as the first appearance of leaf tip accompanied by rapid growth and development of vegetative and floral meristem (Eichhorn and Lorenz 1977). In tropical climatic conditions like India, grapevines (*Vitis vinifera* L.) are pruned twice in an annual growth cycle. The first pruning referred to as foundation pruning is performed after the harvesting and coincides with summer season (Chadha and Shikhamany 1999). This pruning is performed to develop fruiting canes. The second pruning, called fruit pruning is done on canes developed after foundation pruning and usually coincides with colder months. This is known as "double pruning and single cropping system" of grape growing. In warmer regions, prolonged dormancy of bud is a major obstacle for uniform bud burst in grapevines (Shulman *et al.* 1983; Erez 1987, Botelho *et al.* 2010). In such scenarios, dormancy release needs to be controlled by the use of dormancy breaking compound to compensate for the lack of natural chilling and this practice is inevitable for obtaining economic production of table grapes in such regions (Erez 1995). Growers in these regions use hydrogen cyanamide (HC) to increase and synchronise bud-break (Vergara and Perez 2010).

Majority of the 'Thompson Seedless' vineyards in India are grafted either on Dogridge (*Vitis champinii*) or 110R (*Vitis berlandierii* × *Vitis rupestris*) rootstocks. In some regions, 'Thompson Seedless' vines are still grown on their own roots. Dogridge (*Vitis Champinii*) is the most frequently used rootstock for table grape production in India. It is a highly vigorous rootstock, but performs well in soils and irrigation water having less sodium content. In regions where soil and irrigation water has high sodium concentration, it tends to accumulate more sodium in scions (Sharma and Upadhyay 2008). In black cotton soils it tends to increase vigour of scions and thus reduces bud fruitfulness. Rootstock 110R (*Vitis berlandieri* × *Vitis rupestris*) is gaining popularity in semiarid tropical regions of India in recent years owing to its drought tolerance, and less accumulation of sodium ions in regions having problems associated with soil sodicity (Sharma and Upadhyay 2011). It imparts moderate vigour to scions with increased bud fruitfulness. In addition to their role in overcoming biotic (soil nematodes, phylloxera etc.) and abiotic (soil and water salinity, water scarcity, etc) stresses, rootstocks are known to influence vegetative growth, yield and fruit composition parameters of grape berries (Foott *et al.* 1989, Ferree *et al.* 1996, Cirami *et al.* 1984, Tangolar and Ergenoglu 1989). The anatomical, nutritional, hormonal or other physiological influence of rootstocks on scion performance have been reviewed by Tubbs (1973), Lockard and Schneider (1981) and Jones (1986). Though temperature is clearly a dominant factor in determining the time of budbreak (Pouget 1963, Williams *et al.* 1985, Moncur *et al.* 1989 and Swanepoel *et al.* 1990), other factors also often influence the time of budbreak. According to Boso *et al.* (2008), rootstocks are known to exert their influence basically through plant vigor and consistently have effect on leaf exposure and on water and nitrogen availability at maturation. Apart from external factors such as temperature, light, water supply and nutrition, internal factors such as carbohydrate levels, hormones and enzyme activity are also involved in budbreak and release of dormancy. Many of the researchers have suggested changes in enzyme activity as an indicator at the end of dormancy and start of new growth (Bassuk *et al.* 1981, Marquat 1999, Citadin *et al.* 2001, Marguerit *et al.* 2012). Phenolic compounds have been isolated from buds and identified as possible growth inhibitors during bud dormancy (Jindal and Makotia 2004).

Uniform and quick budbreak is one of the important aspects in table grape cultivation as most of the cultural operations like shoot pinching, shoot thinning, application

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of growth regulators and berry thinning needs to be carried out uniformly. Uneven and delayed bud sprouting delays subsequent cultural operations and thus increases labor expenditures. In our earlier studies, we observed variation in percent budbreak and days taken for budbreak in Thompson Seedless grapevines grafted on different rootstocks (SATISHA *et al.* 2010, JOGAIAH *et al.* 2013). As accumulation of most of the biochemical constituents and/or enzyme activity are indirectly influenced by rootstocks through their variation in uptake of water and mineral elements, synthesis of secondary metabolites etc., these mechanisms in turn may also influence the process of budbreak. Hence, the objective of this study was to understand the influence of rootstocks on the activity of different enzymes, biochemical constituents such as phenols, proteins and starch concentration in buds of 'Thompson Seedless' vines during budbreak.

Material and Methods

Plant material: This study was undertaken during the 2012-13 season in the experimental vineyards of National Research Centre for Grapes, Pune, India. Pune (18.32°N, 73.55°E) is located in Midwest Maharashtra state (India) at an altitude of 559 m above the mean sea level. The average minimum and maximum temperature during the experimental period was 18.7 °C and 30.90 °C respectively and minimum and maximum relative humidity was 39.19 % and 98.14 % respectively. No rainfall was recorded during the period from pruning till bud break. The vines were grown on 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 $g \cdot cm^{3-1}$. The average EC of the irrigation water during the experimentation was 1.98 dS/m with an average pH value of 7.78.

The experiment block consisted of 11 years old Thompson Seedless vines grafted on Dogridge and 110R rootstocks and on their own roots. The vines were planted at a spacing of 3.05 m between rows and 1.83 m between vines within a row. The vines were trained to flat roof gable system. Twenty vines per stock-scion combination were used. Observations were recorded on four replications, each consisting of five vines. Vines 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. After fruit pruning, apical 2-3 buds on pruned canes were swabbed with "Dormex", a commercial formulation of hydrogen cyanamide (@1.5 % a.i.), within 24-48 h after pruning to facilitate quick and uniform budbreak. Immediately after pruning 1 % Bordeaux mixture was sprayed as a preventive measure against fungal diseases. No other fungicide was sprayed during the period of experimentation.

Observation on bud break: Days taken for budbreak was measured after fruit pruning. The first sprouted bud with a fully expanded leaf (EL stage 4) was taken as an indicator to measure the days taken for sprouting. Total number of buds present on a vine and number of buds sprouted were counted. The per cent budbreak was calculated using the formula

$$\% \text{ Budbreak} = \frac{\text{Number of buds sprouted}}{\text{Total no. of buds on vine}} \times 100$$

Per cent budbreak on five vines made one replication. Observations were recorded on three such replications. Data was analysed using SAS 9.3 (SAS institute Inc. Cary, NC, USA).

Sampling of buds and canes: First two buds on a cane were scrapped, frozen in liquid nitrogen and preserved at -20 °C till use. Pooled buds from five vines of each combination were taken as one biological replicate. Four such biological replicates and two technical replicates of each were used for biochemical analysis and enzyme assays. The buds were collected immediately after pruning (before HC application), and on 3rd, 6th and 9th d after pruning (DAP).

Cane samples were collected before pruning and 15 d after pruning and stored at -20 °C for further analysis. Canes from five vines were pooled as one biological replicate.

Biochemical analysis: The total phenol content of the bud extract was determined using the Folin-Ciocalteu method (SINGLETON and ROSSI 1965), using gallic acid as the standard. The total protein content was estimated as per the procedures explained by BRADFORD (1976) using Bovine Serum Albumin as the standard. The starch concentration in the canes and buds was estimated following anthrone reagent method (MORRIS 1948, ROBBIN *et al.* 1991) with glucose as standard. All the chemicals for biochemical analysis were procured from Sigma Aldrich (USA). The data was analysed using SAS 9.3 (SAS institute Inc. Cary, NC, USA).

Estimation of enzyme activity: The enzymes were extracted according to KOUSSA *et al.* (2005). 200 mg of tissue were crushed into 2 mL of ice-cold Tris-HCl buffer (50 mM, pH 7.5) containing $CaCl_2$ (6 mM), Na_2CO_3 (4 mM), 2% (w/v) of insoluble PVPP and ascorbic acid ($1g \cdot L^{-1}$). The extract was centrifuged at 4 °C and 10,000 × g for 10 min, and the supernatant was used to determine enzyme activity. All the chemicals for enzyme extraction and assays were procured from Sigma Aldrich (USA). The data were analysed using SAS 9.3 (SAS institute Inc. Cary, NC, USA).

The α-amylase enzyme activity was assayed based on the modified Fuwa's colorimetric method (FUWA 1954). The enzyme extracts were heated for 10 min at 70 °C to inactivate β amylase and other heat sensitive enzymes (CASTILLO-MICHEL *et al.* 2007). The reaction mixture contained 600 µL of 50 mM Tris-HCl buffer (pH 7.5), 200 µL of 0.1 % starch solution and 200 µL of enzyme extract. The starch degradation was stopped by adding 2 mL of iodine reagent. The absorbance was measured at 620 nm and enzyme activity was calculated based on the standard calibration curve ranging from 0-200 $\mu g \cdot mL^{-1}$ of starch taking into account the dilution factor.

The peroxidase (POD) activity was estimated according to the method described by RODRIGUEZ and SANCHEZ (1982). The assay mixture contained 1 ml of 0.05 M Phos-

phate-citrate buffer (pH 4.6), 1 mL of 40 mM guaiacol and 0.5 mL of 26 mM H_2O_2. The mixture was incubated for 15 min 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 3 min using UV spectrophotometer. POD activity was expressed as "ΔA420/min/g fresh weight".

The polyphenol oxidase (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 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. 200 µL of enzyme extract was added to 2.8 mL of substrate solution in the tube and mixed thoroughly. 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.

Results

Days taken for budbreak and per cent budbreak: 'Thompson Seedless' vines on its own roots and those grafted on 110R rootstock took a shorter period of 7.6 d and 9.4 d respectively for budbreak, while vines grafted on Dogridge required about 14.5 d for budbreak (Fig. 1). On 15th d after pruning, maximum per cent of budbreak was recorded on 110R rootstock (55 %) followed by own rooted vines (50 %) and Dogridge (33 %).

Biochemical constituents

Starch: The dynamics of starch concentration in buds of 'Thompson Seedless' grafted on different rootstocks and its own roots showed gradual reduction during 0 to 9th DAP and there was significant variation in starch concentration among rootstocks on any given day. At the beginning, buds on own rooted vines had highest starch content followed by those on Dogridge while it was least on 110R. However, on 9th DAP, the highest starch concentration was recorded on Dogridge followed by 110R and own rooted vines (Fig. 2). Data in Tab. 1 indicated that the magnitude of reduction during 0-3rd and 3-6th DAP was significantly higher in own rooted and 110R grafted vines as compared to Dogridge grafted vines. During 6-9th DAP also, the magnitude of reduction was less on Dogridge rootstock (50.9 %) as compared to own rooted (72.7 %) vines and vines grafted on 110R rootstock (69.4 %).

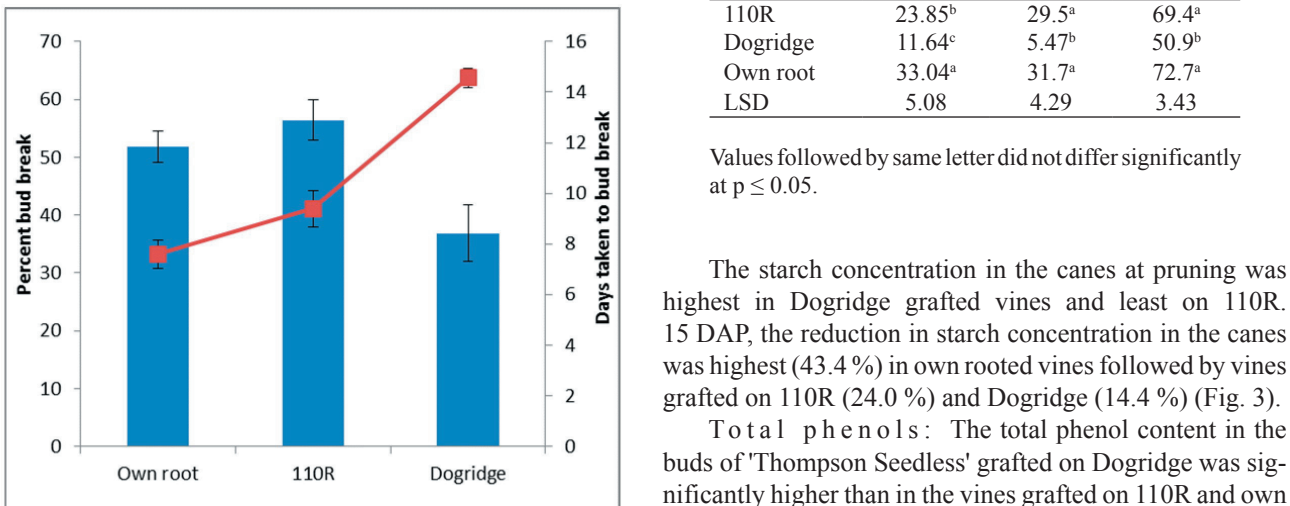

Fig. 1: Days taken for budbreak (line) and per cent budbreak (bar) on 15th d after pruning in 'Thompson Seedless' grapevines grafted on different rootstocks. Bars represents standard error of mean (±) with n = 4.

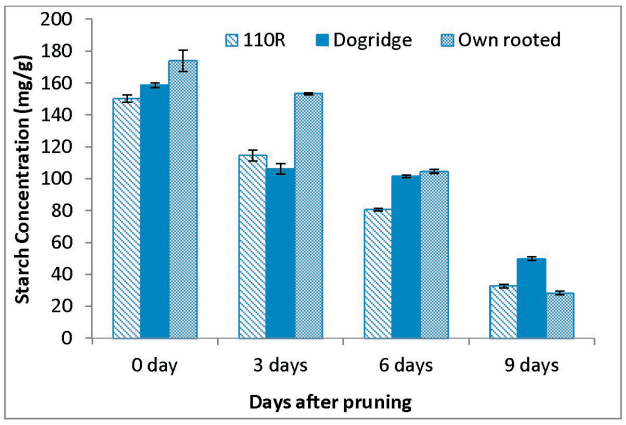

Fig. 2: Concentration of starch (mg·g^{-1}) in buds of 'Thompson Seedless' grafted on different rootstocks during budbreak. Bars represents standard error of mean (±) with n = 4.

Table 1

Percent reduction in starch concentration during process of budbreak in 'Thompson Seedless' grapevines grafted on different rootstocks

Rootstocks	0-3rd day	3-6th day	6-9th day
110R	23.85^b	29.5^a	69.4^a
Dogridge	11.64^c	5.47^b	50.9^b
Own root	33.04^a	31.7^a	72.7^a
LSD	5.08	4.29	3.43

Values followed by same letter did not differ significantly at $p \leq 0.05$.

The starch concentration in the canes at pruning was highest in Dogridge grafted vines and least on 110R. 15 DAP, the reduction in starch concentration in the canes was highest (43.4 %) in own rooted vines followed by vines grafted on 110R (24.0 %) and Dogridge (14.4 %) (Fig. 3).

Total phenols: The total phenol content in the buds of 'Thompson Seedless' grafted on Dogridge was significantly higher than in the vines grafted on 110R and own root on all the days of sampling (Tab. 2A). The phenol content in 110R grafted vines did not change significantly till 6th DAP, followed by marginal increase on 9th DAP; whereas in own rooted vines, phenol content decreased significantly after 3 DAP. In Dogridge grafted vines, a marginal reduction was observed at 3rd and 6th DAP followed by a

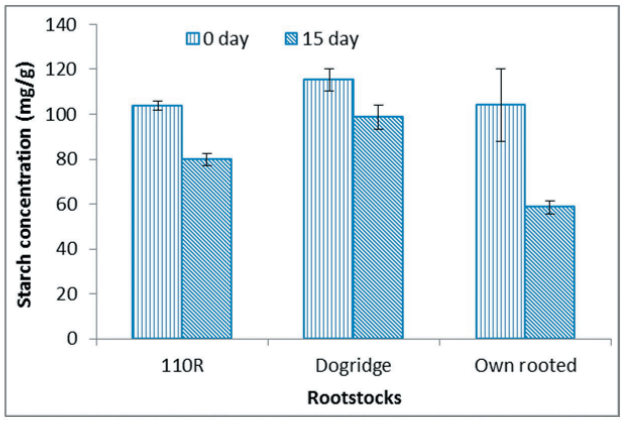

Fig. 3: Concentration of starch (mg·g^{-1}) in canes of 'Thompson Seedless' grafted on different rootstocks before and after budbreak. Bars represents standard error of mean (±) with n = 4.

Table 2A

Total phenols (mg·g^{-1}) concentration in buds during budbreak process (n = 4)

Rootstocks	0 day	3rd day	6th day	9th day	LSD_{days} ($p \leq 0.05$)
110R	8.86^{b}	8.54^{b}	8.35^{b}	9.35^{b}	0.78
Dogridge	14.36^{a}	12.47^{a}	11.24^{a}	19.74^{a}	1.15
Own root	9.15^{b}	9.30^{b}	6.23^{c}	6.31^{c}	0.20
LSD_{RS} ($p \leq 0.05$)	0.535	1.276	0.482	1.167	

Table 2B

Total protein (mg·g^{-1}) concentration in buds during budbreak process (n = 4)

Rootstocks	0 day	3rd day	6th day	9th day	LSD_{days} ($p \leq 0.05$)
110R	3.87	4.24	2.14	2.10	0.421
Dogridge	3.48	3.83	1.83	2.12	0.297
Own root	4.89	4.96	1.45	1.26	0.276
LSD_{RS} ($p \leq 0.05$)	0.415	0.326	0.282	0.663	

sharp increase at 9th DAP. Maximum phenol content was recorded in vines grafted on Dogridge (19.74 mg·g^{-1}) followed by 110R (9.35 mg·g^{-1}) and own root (6.31 mg·g^{-1}) on 9th DAP.

Total proteins: Total maximum protein concentration was recorded in the buds of own rooted vines (Tab. 2B) at 0 d and it was significantly different from protein content in 110R and Dogridge grafted vines. In all the stock-scion combinations, a marginal increase in protein content was recorded on 3rd d followed by a sharp decline on 6th DAP. The protein content did not change significantly after 6th DAP.

Enzyme activity - α-amylase activity: In all the stock-scion combinations, the activity of α-amylase in buds showed an increasing trend from 0-9th DAP, though there was a significant variation among different combinations on a given day. On 0 d, the highest enzyme activity was recorded in the buds of own rooted vines. In these vines, the activity increased very rapidly till 6th DAP, however enzyme activity reduced on 9th DAP. However, enzyme activity in buds of vines grafted on 110R and Dogridge rootstocks continued to increase even on 9th DAP. On 9th DAP, highest activity of α-amylase enzyme was recorded on Dogridge rootstock (Fig. 4).

Peroxidase (POD): On 0 d, the highest POD activity (Fig. 5) was recorded on 110R grafted vines (0.0511), followed by own root (0.046) and the least on Dogridge (0.042). In vines grafted on 110R and own root, activity decreased significantly on 3rd DAP, whereas peroxidase activity in Dogridge grafted vines decreased marginally which was not statistically significant. A sharp reduction in peroxidase activity was observed at 6th DAP and later stage in all the stock scion combinations.

Polyphenol peroxidase (PPO): In all the stock-scion combinations, high PPO activity was observed on the day of pruning, the maximum activity was in vine grafted on 110R (0.0511). In 110R grafted vines, the PPO activity declined gradually till 9th DAP. In own rooted and Dogridge grafted vines, reduced PPO activity was record-

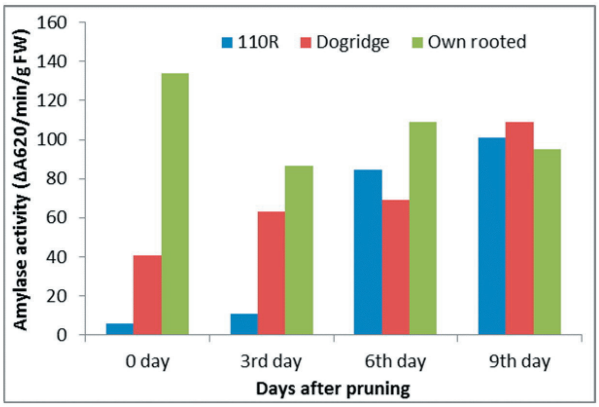

Fig. 4: Changes in amylase activity (ΔA620/min/g fresh weight) in buds of 'Thompson Seedless' vines grafted on different rootstocks during budbreak (LSD values for comparing means among stock scion combination are for 0 d – 12.28; 3rd d – 18.69; 6th d – 15.31 and 9th d – 13.21).

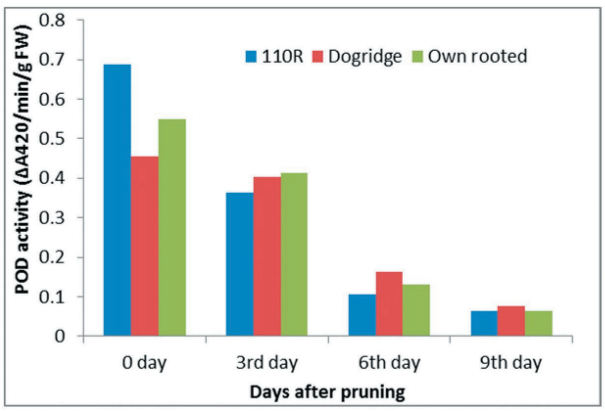

Fig. 5: Changes in peroxidase activity (ΔA420/min/g fresh weight) in buds of 'Thompson Seedless' vines grafted on different rootstocks during bud break. (LSD values for comparing means among stock scion combination are for 0 d – 0.045; 3rd d – 0.010; 6th d – 0.018 and 9th d – 0.035).

ed on 3rd DAP followed by significant increase at 6th DAP and decrease again at 9th DAP (Fig. 6).

Correlation between bud break and enzyme activity: The data in Tab. 3 indicated that per cent budbreak and α-amylase activity (r = 0.763) had a significant positive correlation, whereas activity of PPO and POD enzymes was negatively correlated with per cent bud break. Per cent bud break was negatively correlated with starch content. The activity of enzyme α-amylase was positively correlated with starch content in buds.

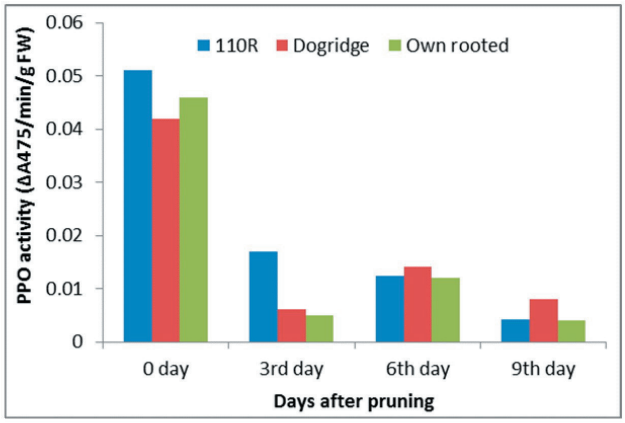

Fig. 6: Changes in polyphenol oxidase activity (ΔA475/min/g fresh weight) in buds of 'Thompson Seedless' vines grafted on different rootstocks during bud break. (LSD values for comparing means among stock scion combination are for 0 d – 0.002; 3rd d – 0.002; 6th d – 0.003 and 9th d – 0.001).

Table 3

Correlation coefficient between budbreak, enzyme activity and starch concentration (n = 12)

	Correlation coefficient (r)	Level of significance
Budbreak v/s starch	-0.676	*
Budbreak v/s amylase	0.763	*
Budbreak v/s polyphenol oxidase	-0.766	*
Budbreak v/s peroxidase	-0.808	*
Starch v/s amylase	0.893	*
Starch v/s polyphenol oxidase	0.932	*
Starch v/s peroxidase	0.946	*
Amylase v/s polyphenol oxidase	0.958	*
Amylase v/s peroxidase	0.964	*
Polyphenol oxidase v/s peroxidase	0.993	*

*: $p \leq 0.05$

Discussion

Rootstocks induce many effects on scion varieties via several mechanisms including translocations of hormones (Reddy *et al.* 1990, Patakas and Noitokas 1999). Rootstocks probably may have a number of primary and secondary effects on chemical, hydraulic and nutritional messages (Tworkoski and 2007). In the present study, significant differences were observed for time taken for budbreak and per cent budbreak among different root systems, and delayed bud break was observed in vine grafted on Dogridge. These results are in accordance with the earlier reports on influence of rootstocks on budbreak. El-Morsi *et al.* 2006 observed that grafting of Superior Seedless grapes on Freedom rootstock gave higher percentage of budbreak and fruitful buds than on Salt Creek. Prakash and Reddy (1990) in their study in cultivar 'Anab-e-shahi' obtained significant difference for budbreak among different rootstocks. The time taken for budbreak was shorter with 'Gulabi' as rootstock as compared to 'Dogridge' rootstock. Similarly, in another study on compatibility of table grape scion varieties and rootstocks, Hamdasn and Salimia (2010) observed earlier bud burst (33-48 d) in grafted vines as compared to own rooted vines (60-66 d). On the contrary, Tangolar and Ergenoglu (1989) from their study concluded that time to budbreak is not significantly affected by rootstocks, although it tended to be earlier on 420A and 'Rupestris du Lot' rootstocks.

Borkowska (1980) found that transition of buds from dormant stage to the breaking is characterised by increase in water content in tissue, mobilization of nutrients, activation of hydrolytic enzymes and intensification of respiration. During budbreak, buds act as strong sink and stored carbohydrates from different vine parts such as canes, cordons, stem and roots are used. Enzyme α-amylase plays an important role in starch breakdown. In present study, a decline in starch concentration and an increase in amylase activity were observed during budbreak. Higher per cent reduction in starch concentration and amylase activity was observed in 110R grafted and own rooted vines with early and higher per cent bud break. Since, buds act as a strong sink at the time of budbreak, they may draw carbohydrates stored in different parts of the vines and this might be the reason for reduction in cane starch content after 15 d of pruning in all stock scion combinations. A significant positive correlation was observed between per cent budbreak and α-amylase activity and negative correlation between per cent budbreak and starch concentration. The present results are in accordance with Mohamed *et al.* (2012), who also observed increased budbreak to faster degradation of starch with increased activity of α-amylase, starch phosphorylase, maltase etc. The released reducing sugars might act as a source of carbon and energy to the cells for synthesis of different biochemical compounds which are essential for budbreak. In some cases, soluble sugars such as glucose and fructose not only function as source of nutrients for sustaining growth but also as signals that regulate the process of bud development (Chao and Serpe 2010). Mohamed *et al.* (2010) in their study on biochemical changes in dormant grapevine tissues found high acid invertase activity, high soluble sugar concentration and a decrease in putricine and spermidine as the indicators of dormancy release and improved budbreak.

The activity of peroxidase in roots, shoots and trunk in grapevines increases in autumn reached maximum in December and then decreased in winter as reported by Schaefer (1983). Peroxidase activity was highest during dormancy and declined during budbreak and again increased after bud expansion. A significant positive correla-

tion between the enzyme activities of these two enzymes at the time of budbreak was observed. Similar results, where change in enzyme activity as an indicator of the end of dormancy and start of growth have been reported by several researchers (Bassuk *et al.* 1981, Citadin *et al.* 2002). Tripathi *et al.* 2006 suggested that the change in POD and PPO activities could be indicator of endogenous changes as these enzymes led to scavenging of the accumulated hydrogen peroxide in buds and thus releasing dormancy resulting in bud sprouting.

Hydrogen cyanamide is the most useful bud dormancy breaking compound for grape vines (Henzel *et al.* 1991). Timely application of HC can advance budbreak (Shulman *et al.* 1983) especially in warmer climate (George *et al.* 1988, Cirami and Furkaliev 1991). The application of hydrogen cyanamide is a common practice to induce bud burst in tropical countries like Brazil (Hawerroth *et al.* 2010) and India (Chadha and Shikhamany 1999). However in countries like USA, this compound has been classified as highest toxicity category (Category I) by environment protection agency and it has been under regulatory review by European Union Authority (Settimi *et al.* 2005). The dissipation of this compound has been studied under Indian conditions and was found to degrade very quickly after its application (Banerjee *et al.* 2000). The application of HC causes disturbances in transient respiration and produces oxidative stress to grapevine buds which helps to release bud dormancy. It is also known to up-regulate transcripts coding enzymes of the respiratory pathway and dormancy breaking related protein kinase (Or *et al.* 2000, 2002) and inhibit the activity of catalase with concurrent increase in hydrogen peroxide (Nir *et al.* 1986, Pérez and Lira 2005, Pérez *et al.* 2008). Pérez *et al.* 2008 reported that increase in H_2O_2 level in HC-treated grapevine buds are due to the inhibition of Catalase activity and enhancement of the respiratory activity of buds. Walton *et al.* (2009) suggested that hydrogen peroxide functions as a chemical messenger by activating the expression of some genes directly or indirectly triggering some metabolic alterations that can be detected by other molecules that would activate or repress the expression of genes responsible for dormancy breaking. In the present study, the significant variations in POD and PPO activities immediately after the application of HC could be an indicator of endogenous changes and suggest their possible protective role along with other components in the defensive mechanism against stress created by HC. The variations in the activity of these enzymes may also be attributed to the influence of rootstocks through uptake and translocation of water, nutrients and plant hormones.

The biochemical changes in the different parts of the vine during budbreak have been studied by several workers (Sivaci 2006, Kenis 1976, Marquat *et al.* 1999). The concentration of phenolic compounds in buds during the process of budbreak showed significant variation among rootstocks. Highest phenolic compound was recorded on Dogridge rootstock on 0 day which increased on 9th DAP. The phenol content increased marginally in 110R rootstock and decreased in own rooted vines during budbreak. Thus the reduced budbreak in Dogridge was concomitant by high phenolic compounds and higher PPO and POD activity on the 9th d after pruning. Significantly negative correlation was observed between per cent budbreak and activity of peroxidase and PPO enzymes. This is in accordance with the reports of Wang *et al*. (1991), who observed high amount of phenolic substance in dormant buds which decreased just before and after budbreak. Phenolic compounds were found to be potent modifiers of catalase, POD and PPO activity as both inhibitors and stimulators. Similarly, Jindal and Makotia (2004) isolated phenolic compounds from bud scales and described them as growth inhibitors as they were increased during dormancy in peach buds, then decreased after dormancy and completely eliminated at flowering. The antioxidant activity of flavonols may be apparently due to their ability to act as free radical acceptors as expressed by Xiao and Parkin (2007). Similarly, El-Mansy and Walker (1969) reported that phenols induced several multi-biological responses which ultimately led to promotion of budbreak and subsequent flowering. Changes associated with protein concentration during dormancy and its release is studied by several workers in different temperate plant species. In peach buds, Lang and Tao (1991) identified several soluble proteins that decreased or increased in dormant flower buds. In blue berry, Muthalif and Rowland (1994) reported expression of dehydrin like proteins to be associated with dormancy release. Jeknic and Chen (1999) in their study on changes in protein profile of poplar tissue during the induction of bud dormancy, observed decreased total protein content in buds and leaves, while it increased in bark tissues during dormancy release. In present study, there was gradual reduction in total protein concentration in buds of 'Thompson Seedless' grapes on different rootstocks. The substantial changes in protein metabolism during the budbreak period have indicated an alteration in gene expression associated with budbreak or dormancy release. Detailed analysis on biochemical and molecular characterization of different polypeptides will help in proper understanding of the molecular mechanisms controlling dormancy and budbreak on different rootstocks.

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