Vitis **60**,93–99 (2021) DOI: 10.5073/vitis.2021.60.93-99

Rootstock affects stress relieving enzymatic activity during bud break in 'Red Globe' grapevine under semi-arid condition

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Summary

The role of stress relieving enzymes during bud sprouting in grapevines has already been established in different varieties. However, data on 'Red Globe' variety under tropical conditions are not reported. The present study was conducted to generate data on stress relieving enzymatic activities during bud sprout in 'Red Globe' on different rootstocks under arid conditions of India. Influence of different rootstocks on stress relieving enzymes (catalase, peroxidase, ascorbic acid oxidase and polyphenol oxidase) involved in bud sprouting under tropical conditions with double pruning and single cropping pattern was evidenced. Positive interactions were observed between enzymatic activities of stress relieving enzymes, increased bud break (64.25 %) and reduction in days taken to bud sprout (8.43 days). Among the rootstocks under study, vines on 110R and own rooted vines have strong impact on stress relieving enzymes that resulted into early and increased bud sprouting. Also, the dynamics of enzymatic activity can be used as biological indicators for forecasting the end of bud dormancy and recommencement of growth.

K e y w o r d s : bud dormancy; bud sprouting; bud burst; enzymes; polyphenols.

Introduction

Grapevines (*Vitis vinifera* L.) originated from temperate regions. Varietal adaptability and technological interventions facilitate successful grape growing under tropical conditions. In India, grape growing is mainly adopted under tropical conditions.The total area under cultivation of grapes accounts to 139,000 ha with the production of 2,958 MT (Anonymous 2018). 'Thompson Seedless' and its clonal selections, 'Sharad Seedless' and its clonal selections as well as 'Flame Seedless' and 'Red Globe' are mainly grown in India (Adsule *et al.* 2012). 'Red Globe' is attracting the consumers due to its affectionate red colour, round, crispy, mild sweet, medium large berries and the variety is gaining demand in Indian markets. Growers are concentrating their efforts to obtain quality grape but several constraints are affecting its production under tropical conditions.

In an annual growth cycle of grapevine, various cultural practices such as pruning, pinching, shoot and berry thinning, application of growth regulators etc. are followed to ensure the balanced growth and to produce quality grapes. Under tropical climatic conditions of India, grapevines are pruned twice *i.e.* first pruning in the month of April (foundation pruning) and a second in October (fruit pruning). Under tropical conditions during foundation pruning, the high temperature (above 40 °C) and low relative humidity (below 30 %) leads to delayed and erratic bud sprouting in the vineyards. Even though climatic conditions are favourable during the fruit pruning period, delayed and erratic bud sprouting is observed in the vineyards. To safeguard economic grape production in a wide range of climatic conditions, use of dormancy breaking chemicals (e.g. hydrogen cyanamide) becomes obligatory to the grape growers (Erez 1995, Vergara and Perez 2010). Due to adverse soil and water qualities, the grapevines are grafted on different rootstocks, such as 'Dogridge', '110R', '140Ru', 'Salt Creek' etc. However, in India, 'Dogridge' and '110R' are popular among the farmers because of their ability to tolerate prevailing drought and salt conditions (Somkuwar *et al.* 2006, Jogaiah *et al.* 2007). Rootstock '110R' is documented for reducing sodium toxicity in sodic soils (Sharma and Upadhyay 2011) as well as biotic stresses (soil nematodes and phylloxera) which leads to uniform and early bud fruitfulness, yield and fruit composition (Cirami *et al.* 1984, Tangolar and Ergenoglu 1989, Foott *et al.* 1989, Ferree *et al.* 1996). Rootstock stimulates plant vigour by improving physiological and metabolic activities of scions by increasing leaf exposure, water and nitrogen availability at the time of its maturity (Boso *et al.* 2008). Apart from outdoor factors (light, temperature, water, nutrients), the inside factors such as sugar level, plant growth regulators and enzymatic activities are also involved in bud break and release of bud dormancy (Marguerit *et al.* 2012). It is also reported that the transition of buds from dormant stage to sprouting is characterised by an increase in water content in tissues, mobilisation of nutrients, activation of enzymes and increase in respiration. Jogaiah *et al.* (2014) studied 'Thompson Seedless' for the possible role of enzymes: polyphenol oxidase (PPO), peroxidase (POD), α-amylase and the rootstocks in delaying bud break under tropical conditions. They revealed that the stress relieving enzymatic activities of PPO and POD increase during initial stages of

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bud sprouting and further deplete at full bloom. Increased activity of α-amylase in bud acts as an indicator of translocation of carbohydrates from source (canes, cordons, stem and roots) to sink (bud) to fulfil the energy requirements during stressed conditions. However, the possible role of different enzymes and rootstocks on bud sprouting in coloured grape varieties is unknown under tropical conditions. The attempt was made to evaluate the performance of different rootstocks and their influence on stress relieving enzymatic activities during bud break of 'Red Globe' under tropical conditions.

Material and Methods

The experiment was conducted during the years 2016-17 and 2017-18 at the experimental block of ICAR-National Research Centre for Grapes, Pune (18.32°N, 73.51°E) in Midwest Maharashtra (India). The study was conducted on four year old 'Red Globe' vines grafted on '110R' (*Vitis berlandieri* x *Vitis rupestris*), 'Dogridge' (*Vitis champinii*), 'Salt Creek' (*Vitis champinii*) and '140Ru' (*Vitis berlandieri* x *Vitis rupestris*) as well as own rooted vines (*Vitis vinifera* L.) The vines were spaced at a distance of 3.04 m between rows and 1.83 m between vines within a row and trained on "Y" trellis system. Immediately after fruit pruning (during October), five vines/replication were selected and five canes on each vine were labelled randomly. The buds on canes were scooped by using a sharp razor blade at an interval of 0 (Stage I), 2 (Stage II), 4 (Stage III), 6 (Stage IV), and 8 (Stage V) days after pruning, respectively. The collected bud samples were then frozen in liquid nitrogen and preserved at -20 °C for further enzymatic assays. The observations on days taken for bud sprouts were recorded when 90 % of buds had sprouted on each rootstock.

Sample preparation, reagents and enzymatic assays: For all enzymatic assays, the samples were prepared by crushing 0.5 g buds to fine powder in liquid nitrogen using a pre-chilled mortar and pestle. The chemicals and reagents utilized for enzymatic assays were Na2HPO4, Na2CO3, K2HPO4, KH2PO4 (Thomas Baker Chem. Ltd. Mumbai, India); Acetic Acid, Sodium hydroxide, (Merck Ltd., Mumbai, India); Ethanol (Changshu Hongsheng Fine Chem. Ltd. Jiangshu Province, China); Catechol, Hydrogen peroxide (SD Fine chemicals Ltd, Mumbai, India).

Enzymatic assays: The catalase activity was assayed as per the method given by Sinha (1972). Addy and Goodman's (1972) method was followed for the peroxidase assay. The enzymatic activity of ascorbic acid oxidase assay (AAO) was done as suggested by Oberbacher and Vines (1963). The method given by Haplin and Lee (1987) was used for polyphenol oxidase assay (PPO).

Statistical analysis: The observations on days taken to bud sprout, bud sprout percent, and enzyme activity was recorded at different intervals after pruning. The experiment was conducted in Randomized Block Design (RBD) consisting of five treatments as rootstocks which were replicated four times and each replication comprised five vines. The generated data were processed and analysed by ANOVA using SAS and R statistical analysis tools.

Results

Bud break: The own rooted 'Red Globe' vines and vines grafted on '110R' rootstock sprouted after a shorter time of about 8 and 9 d, respectively (Tab. 1). Compared to these, the vines grafted on '140Ru', 'Salt Creek' and 'Dogridge' rootstocks took longer with 10, 12 and 14 d, respectively for bud break. The observations recorded for per cent bud sprouts on day 15 after pruning revealed that maximum per cent of bud break was noted on own rooted vines (80.68 %) followed by '110R' (73.55 %) and '140Ru' (68.22 %) during the years 2016-17 and own rooted plants (80.48 %) followed by '110R' (76.01 %) and '140Ru' (70.38 %) during 2017-18 while the least percentage of bud sprouting was recorded in 'Dogridge' grafted vines (50.96 % and 52.20 % during the years 2016-17 and 2017-18, respectively).

Table 1

Impact on days taken for bud sprouting and per cent bud sprouts in 'Red Globe' vines

Rootstock	Days taken for bud sprouting		% sprouted buds at day 15	
	2016-17	2017-18	2016-17	2017-18
140Ru	10.23	10.54	68.22	70.38
Dogridge	14.08	13.98	50.96	52.20
Salt Creek	12.13	11.97	66.13	65.57
110R	9.18	9.83	73.55	76.01
Own rooted vine	8.43	8.86	80.68	80.48
CD at 5 %	3.97	0.80	4.88	6.84

Catalase activity: The ANOVA results at different stages of bud growth showed significant difference in catalase activity among different rootstocks (Tab. 2). The data suggested that the catalase enzyme showed maximum activity during initial stage irrespective of the rootstocks and decreased marginally till IVth stage and further increased at Vth stage during the years 2016-17. During the initial stage of bud sprouting, the maximum catalase activity was recorded in vines grafted on '110R' followed by vines grafted on 'Salt Creek' and own rooted vines. The least activity was recorded in samples collected from vines grafted on 'Dogridge'. At stages II and III, the catalase activities were maximal in vines on '110R' and on '140Ru'. The vines grafted on 'Salt Creek' recorded highest catalase activity at stage IV followed by '140Ru'. Slight increase in catalase activities were observed at Vth stage in comparison to previous stages and activity was maximum on '110R'.

Whereas, during the year 2017-18, maximum catalase activities were recorded during initial stage of bud burst, irrespective of the rootstocks and then decreased gradually to the Vth stage. In the Ist and Vth stage, the highest catalase activity was shown in '110R' and '140Ru'. 'Salt Creek' grafted vines recorded least activity in both stages. During the IInd stage of bud sprouting, the highest catalase activity was recorded in '140Ru' grafted vines followed by own rooted vines. In the IIIrd and IVth stages, the highest catalase activity

Table 2

Variation in catalase enzyme activities in 'Red Globe' vines on different roots

Rootstock	Catalase (units (Kat f.)·mg^{-1} protein·g^{-1} FW)									
	2016-17					2017-18				
	Stage-I	Stage-II	Stage-III	Stage-IV	Stage-V	Stage-I	Stage-II	Stage-III	Stage-IV	Stage-V
140Ru	173.58	134.96	129.41	92.18	123.72	192.87	186.81	165.73	152.03	135.43
Dogridge	162.49	116.64	103.80	83.72	105.18	172.59	164.58	143.70	132.31	120.41
Salt Creek	211.89	104.70	104.88	92.25	92.35	157.16	160.51	162.35	141.81	96.09
110R	247.69	141.27	131.75	89.88	133.37	199.46	166.39	160.60	141.28	149.08
Own rooted vine	183.43	121.00	110.65	83.20	106.24	184.45	166.48	167.81	157.00	118.66
CD 5 %	33.10	18.99	14.60	4.98	14.80	15.51	NS	NS	12.80	26.46

was noted in own rooted vines and '140Ru'. The least activity was recorded in 'Dogridge' grafted vines.

Peroxidase activity (POD): The significant changes in POD activity among the different treatments were recorded at different stages of bud sprouting (Tab. 3). Significant differences were observed in POD activities among vines on different roots at all stages. However, the POD activities were decreased from initial stage to the last stage of observation during both years. In the initial stage in the first year of observation, maximum POD activity was expressed by the vines grafted on '110R' rootstock which was at par with own rooted vine while minimum value was noted in 'Dogridge'. Samples collected from vines on '110R' were found with maximum values of POD followed by own rooted vines at all stages except the IInd where POD activity was higher in own rooted vines followed by vines on '110R'. Minimum POD values were noted in 'Salt Creek' at IInd, IIIrd and Vth stages while it was minimum in 'Dogridge' in the Ist and IVth stages.

During all stages of the second year, the maximum POD activity was shown by the vines grafted on '110R' rootstock. At the initial stage, the vines grafted on '110R' rootstock showed maximum activity *i.e.* 8.074 units·mg^{-1} protein·g^{-1} FW and there was decreasing trend. It was only 0.813 units·mg^{-1} protein·g^{-1} FW at the last stage of study. The values of POD activities were followed by own rooted vines at Ist, IInd and IIIrd stages while at IVth and Vth stages '140Ru' performed better than own rooted vines. Minimum POD activities were recorded in 'Dogridge' at Ist and IIIrd stages while at IInd, IVth and Vth stages, 'Salt Creek' showed minimum POD activities.

Ascorbic acid oxidase activity (AAO): The noteworthy variations were recorded for AAO enzyme activities among vines on different types of roots (Tab. 4). At the bud sprouting stages, the AAO activities were increased from Ist to Vth stage of bud sprouting. At the initial stage of bud sprouts in the first year of experimentation, vines grafted on '140Ru' rootstock recorded maximum AAO activity (0.0039 units·mg^{-1} protein·g^{-1} FW) followed by vines on 'Dogridge' (0.0029 units·mg^{-1} protein·g^{-1} FW) and '110R' (0.0029 units·mg^{-1} protein·g^{-1} FW) while minimum activity was shown by own rooted vines which were found at par with activity in vines on 'Salt Creek' rootstock. In the second stage, vines on 'Dogridge' rootstock recorded maximum activity (0.0030 units·mg^{-1} protein·g^{-1} FW) while the samples collected from vines on '110R' and own rooted vines were recorded with minimum activity of AAO. From the third stage onwards, the AAO activity has increased to the maximum in all the rootstocks including the own rooted vines. Among the different rootstocks, vines grafted on '110R' rootstock showed progressive AAO activity after IInd stage of bud break. In the last stage of observations, vines grafted on '110R' were recorded with maximum activity of 0.0122 units·mg^{-1} protein·g^{-1} FW and followed by '140Ru' (0.0090 units·mg^{-1} protein·g^{-1} FW) and minimum activity was recorded in 'Salt Creek'.

Table 3

Variation in peroxidase enzyme activity in 'Red Globe' grape vines on different roots

Rootstock	Peroxidase (units·mg^{-1} protein·g^{-1} FW)									
	2016-17					2017-18				
	Stage-I	Stage-II	Stage-III	Stage-IV	Stage-V	Stage-I	Stage-II	Stage-III	Stage-IV	Stage-V
140Ru	6.170	2.820	1.385	0.471	0.470	7.477	4.479	1.902	0.951	0.670
Dogridge	4.458	3.392	1.193	0.410	0.414	4.934	4.471	1.495	0.811	0.544
Salt Creek	7.795	2.279	1.025	0.444	0.384	5.852	3.821	1.931	0.692	0.448
110R	9.520	4.029	1.924	0.576	0.797	8.074	5.447	2.815	0.964	0.813
Own rooted vine	8.124	4.117	1.867	0.571	0.483	6.750	4.914	2.680	0.792	0.660
CD 5 %	1.47	0.31	0.29	0.09	0.12	0.70	0.85	0.36	0.13	0.08

Table 4

Variation in ascorbic acid oxidase enzyme activity in 'Red Globe' vines

Rootstock	Ascorbic Acid Oxidase (units·mg^{-1} protein·g^{-1} FW)									
	2016-17					2017-18				
	Stage-I	Stage-II	Stage-III	Stage-IV	Stage-V	Stage-I	Stage-II	Stage-III	Stage-IV	Stage-V
140Ru	0.0039	0.0027	0.0066	0.0064	0.0090	0.0046	0.0044	0.0075	0.0097	0.0093
Dogridge	0.0029	0.0030	0.0056	0.0064	0.0070	0.0026	0.0045	0.0059	0.0085	0.0083
Salt Creek	0.0015	0.0028	0.0043	0.0053	0.0062	0.0030	0.0029	0.0054	0.0080	0.0064
110R	0.0029	0.0019	0.0077	0.0079	0.0122	0.0037	0.0032	0.0092	0.0113	0.0135
Own rooted vine	0.0013	0.0019	0.0062	0.0051	0.0079	0.0018	0.0021	0.0074	0.0090	0.0071
CD 5 %	0.0007	0.0005	0.0005	0.0007	0.0009	0.0006	0.0008	0.0012	0.0014	0.0015

In the second year, quite different trend was observed for AAO activities with significant differences. At the initial stage of bud burst, the vines on '140Ru' rootstock exhibited maximum AAO activity (0.0046 units·mg^{-1} protein·g^{-1} FW) followed by '110R' (0.0037 units·mg^{-1} protein·g^{-1} FW) and 'Salt Creek' (0.0030 units·mg^{-1} protein·g^{-1} FW) rootstocks. The least activity was measured in own rooted vines (0.0018 units·mg^{-1} protein·g^{-1} FW). At the IInd stage, AAO activities almost similar to I stage but an escalation was observed in in vines on 'Dogridge' where activity was increased from 0.0026 (stage I) to 0.0045 (stage II) units·mg^{-1} protein·g^{-1} FW which was found at par with vines grafted on '140Ru' (0.0044 units·mg^{-1} protein·g^{-1} FW) while it was decreased in vines on '110R'. At the IIIrd and IVth stages of bud burst, vines on '110R' were recorded with maximum AAO activity with values of 0.0092 and 0.0113 units·mg^{-1} protein·g^{-1} FW, respectively followed by '140Ru' (0.0075 and 0.0097 units·mg^{-1} protein·g^{-1} FW, respectively). While minimum activities were observed in vines grafted on 'Salt Creek' (0.0054 and 0.0080 units·mg^{-1} protein·g^{-1} FW at stage II and IV, respectively. In the last stage, the AAO activity was slightly decreased in all the rootstocks except in vines grafted on '110R'.

Polyphenol Oxidase activity (PPO): Data on PPO activities at different stages of bud burst are presented in Tab. 5. Significant differences were noted in PPO activities in vines on different type roots at all stages of both years. Decreasing trend was observed in PPO activities from the Ist to the IVth stage in all rootstocks. The trend was more prominent in the second year than the first year of the study. Data from the first year revealed that after the IIIrd or IVth stage, PPO activities were increased. In the first year of experimentation, vines grafted on '110R' rootstock recorded maximum PPO activity (0.0112 units·mg^{-1} protein·g^{-1} FW) followed by '140Ru' (0.0082 units·mg^{-1} protein·g^{-1} FW) while the rootstock 'Salt Creek' exhibited minimum PPO activity (0.0058 units·mg^{-1} protein·g^{-1} FW) at the Ist stage of sampling. At the IInd stage, the samples collected from vines grafted on '110R' rootstock again showed superiority and recorded with maximum PPO activity (0.0068 units·mg^{-1} protein·g^{-1} FW) followed by vines grafted on 'Dogridge' (0.0058 units·mg^{-1} protein·g^{-1} FW). But at the IIIrd stage, vines grafted on 'Dogridge' rootstock surpassed the samples from vines on '110R' in activity of PPO while minimum activity was recorded in own rooted vines (0.0010 units·mg^{-1} protein·g^{-1} FW). The maximum PPO activity was recorded in vines grafted on '110R' were 0.0040 and 0.0052 units·mg^{-1} protein·g^{-1} FW at IVth and Vth stages, respectively.

The vines grafted on '110R' rootstock recorded highest PPO activity except stage IV where maximum value was noted in own rooted vines. At the initial stage, highest PPO activity was recorded in vines grafted on '110R' rootstock (0.0188 units·mg^{-1} protein·g^{-1} FW) followed by '140Ru' (0.0156 units·mg^{-1} protein·g^{-1} FW) and minimum activity was estimated in vines on 'Salt Creek', during the second year of observations (Tab. 5).

During the second stage, PPO activity decreased in all rootstocks and it was maximum in the vines grafted on '110R' (0.0112 units·mg^{-1} protein·g^{-1} FW) which was at par with own rooted vines (0.0106 units·mg^{-1} protein·g^{-1} FW). At the IIIrd stage of bud burst, the maximum PPO activity found in the vines grafted on '110R' (0.0074 units·mg^{-1} pro-

Table 5

Variation in Polyphenol oxidase enzyme activity in 'Red Globe' vines

Rootstock	Polyphenol Oxidase (units·mg^{-1} protein·g^{-1} FW)									
	2016-17					2017-18				
	Stage-I	Stage-II	Stage-III	Stage-IV	Stage-V	Stage-I	Stage-II	Stage-III	Stage-IV	Stage-V
140Ru	0.0082	0.0047	0.0036	0.0017	0.0028	0.0156	0.0097	0.0053	0.0028	0.0025
Dogridge	0.0076	0.0058	0.0051	0.0016	0.0025	0.0112	0.0097	0.0062	0.0026	0.0020
Salt Creek	0.0058	0.0045	0.0021	0.0023	0.0036	0.0091	0.0077	0.0040	0.0031	0.0020
110R	0.0112	0.0068	0.0029	0.0040	0.0052	0.0188	0.0112	0.0074	0.0029	0.0026
Own rooted vine	0.0076	0.0052	0.0010	0.0033	0.0030	0.0152	0.0106	0.0061	0.0032	0.0022
CD 5%	0.0009	0.0014	0.0007	0.0006	0.0011	0.0018	0.0015	0.0010	0.0004	0.0004

tein·g^{-1} FW) followed by vines grafted on 'Dogridge' (0.0062 units·mg^{-1} protein·g^{-1} FW) rootstock and own rooted vines (0.0061 units·mg^{-1} protein·g^{-1} FW). The activity of PPO was minimum in vines grafted on 'Salt Creek' during initial three stages of bud burst whereas during IVth and Vth stages, it was found least in vines grafted on 'Dogridge'. From the IVth stage and onwards, there was not much difference found in PPO activity among all grafted rootstocks including own rooted vines.

Correlation study: The data in Tab. 6 revealed that the per cent bud break negatively correlated with activity of catalase (r = -96 and r = -99 resp. in the season 2016-17 and 2017-18, respectively) followed by PPO with the values of r = -83 (2016-17) and r = -82 (2017-18). POD also showed negative correlation with bud sprouting. AAO showed positive correlation with bud sprout during 2016-17 while it was found negatively correlated during 2017-18. Enzymatic activities in both the seasons were positively correlated with each other except activity of AAO with catalase during 2016-17 where negative correlation was recorded.

Discussion

In the present study, significant differences were observed for time taken for bud break and per cent bud break among the different rootstocks. The own rooted vines were early to sprout as compared to the grafted vines. The early bud sprouts in own rooted 'Red Globe' also supports the findings of Jogaiah *et al.* (2014) who reported early bud sprouts on own rooted 'Thomson Seedless' vines followed by those grafted on '110R' rootstock. Among the different rootstocks combinations, vines grafted on 'Dogridge' delayed the bud sprouts as compared to the other rootstocks under study. Jogaiah *et al.* (2014) also reported delayed bud sprouts in 'Thompson Seedless' grafted on 'Dogridge' while El-Morsi *et al.* (2006) reported that bud sprout of 'Superior Seedless' was higher on 'Freedom' rootstock than on 'Salt Creek'. Dieleman *et al.* (1998) evidenced that bud break and cytokinin concentration in bleeding sap of Rosa hybrid was affected by the genotype of the rootstock. The early bud sprout in own rooted 'Red Globe' and '110R' grafted vines in the present investigation were also correlated with higher per cent bud break. The lower percentage of bud break on 'Dogridge' rootstock might be due to the differences in genetic makeup of the rootstocks, the behaviour under given set of conditions will also be different which is reflected in the present study. The nutrient uptake efficiency differs with the stock-scion combinations. Until leaves become photosynthetically active, the bud acts as strong sink which draws carbohydrates stored during the last season. However, patterns of bud break appear to be more correlated with the capacity of buds to use soluble sugars than with sugar abundance in dormant structures (Leite *et al.* 2004, Bonhomme *et al.* 2005). The interaction of rootstock with scion has effects on bud break, vigor, nutrient uptake and other physiological activities.

Earlier, the adverse effect on human health using thiourea forced to use hydrogen cyanamide (a bud breaking chemical) in viticulture during the last two decades. Hydrogen cyanamide has been frequently used to break endo-dormancy of floral buds in grape and several studies were conducted to address its physiological and molecular basis (Or 2009 and Shulman *et al.* 1983). Boonyawat *et al.* (2016) and Perez *et al.* (2008) reported an increase in reactive oxygen species *i.e.* H_2O_2 level in hydrogen cyanamide treated grapevine buds due to the inhibition of catalase activity by reaction of nitrile groups with thiols and haematin groups of enzymes. In the present study it was observed that catalase activity was higher in the initial stage of observations and then a decreasing trend was noticed at the subsequent stages in all the rootstocks. However, there was no consistency in the decrease in catalase among the different rootstocks and stages. During the last stage, among all rootstocks in the present study, samples from vines on '110R' were recorded with slightly higher catalase activity. Pramanick *et al.* (2004) reported increased catalase activity in the early part of the dormancy cycle, then declined and was lowest at the bud break stage. At the time of dormancy, a lot of changes in buds and in chemical components mainly in phenolics, contents of endogenous hormones such as ABA, GA and IAA play an important role in regulating dormancy and bud break (Said *et al.* 2014, Khalil-Ur-Rehman *et al.* 2017).

Significant variations in POD and PPO activities recorded in the present study could be an indicator of endogenous

Table 6

Correlation coefficient among enzymatic activity, days to bud sprouting and % sprouted buds during season 2016-17 (A) and 2017-18 (B)

Parameters	% bud prout	Catalase	POD	PPO	AAO
(A)					
Bud sprout (%)	1.0000				
Catalase	-0.9640	1.0000			
POD	-0.5857	0.4581	1.0000		
PPO	-0.8343	0.8169	0.7116	1.0000	
AAO	0.0012	-0.1435	0.6321	0.4351	1.0000
(B)					
Bud sprout (%)	1.0000				
Catalase	-0.9943	1.0000			
POD	-0.7290	0.6697	1.0000		
PPO	-0.8281	0.8138	0.8549	1.0000	
AAO	-0.6093	0.5805	0.8058	0.9370	1.0000

changes and suggest their possible protective role in defensive mechanism against stress. Variations in the activity of these enzymes may also be attributed to the influence of rootstocks through uptake of water and nutrients. Perez and Lira (2005) found that the catalase activity increased during bud dormancy, reaching the highest level and thereafter decreased marginally. Xi *et al.* (2017) reported that the antioxidant enzymes like catalase and peroxidase increase when plants are subjected to stress conditions. As an outcome of the experiment it is observed that the maximum catalase activity occurred during initial stages and decreased marginally thereafter, which may lead to peak at bud break. The highest production of H_2O_2 during initial stages may be the reason for maximum bud sprout. Sudawan *et al.* (2016) concluded that the rise in H_2O_2 level leads to breaking of bud dormancy. These H_2O_2 molecules are broken by catalase into oxygen and water to avoid harmful effects on plants.

Catalase activity identified to play a driving role in inhibition of H_2O_2 production at bud break (Perez and Lira (2005) and Halaly *et al.* 2008). The simultaneous rise in H_2O_2 level due to inhibition of POD and catalase activities was recorded in grape buds subsequent to treatment with hydrogen cyanamide. Initiation of POD activities following hydrogen cyanamide treatment was observed due to the elevated H_2O_2 levels in bud cells because of low activity of catalase. The changes in enzyme activities were rapid and short lived and positively associated with higher and earlier bud break percentages. POD concentration was higher during the initial stage and thereby decreased sharply at the end of bud sprout. Among the different rootstocks, 'Red Globe' vines grafted on '110R' showed maximum POD activities followed by own rooted vines. However, the POD activity was negatively correlated with per cent bud sprouts. According to Passardi *et al.* (2005) peroxidase is involved in a wide range of physiological processes throughout the plant life cycle. Polyphenol oxidase and peroxidase are involved in plant defence mechanism (Thomma *et al.* 2001 and Xie *et al.* 2017). Peroxidase activity was lowest at the initial stages of dormancy then increased and was higher at dormancy breaking (Khalil-Ur-Rehman *et al.* 2019).

The increase in polyphenol activity in the initial stage and then decrease in the subsequent stages was found in the present study. However, the vines grafted on '110R' recorded higher PPO activity in all the stages thus confirming its superiority in bud sprouting among the different rootstocks. Li and Steffens (2002) noted that the activity is down-regulated with increasing age of leaf. Huystee and Cairns (1982) reported that the polyphenol oxidase play a major role during cell division, differentiation and primordial development in plants. It means highest PPO activity during initial stages of bud break is in correlation with increase in leaf mass and development of leaf primordia. The ascorbic acid oxidase (AAO) concentration in the present investigation was found increased with bud sprouting. In the initial stage, the concentration was lower; however, it was increased in the subsequent bud sprouting stages. The concentration was increased from stage III to V. AAO activity is related to cell extension and ultimately growth (Lin and Varner 1991, Pignocchi *et al.* (2003) and De Tullio *et al.* 2005). Increase in AAO activity is an indication of increase in production of photosynthetic oxygen and it could correlate with photosynthetic activities. So, the increasing trend in AAO activity observed during the experiment can be related with the maximum bud break.

To summarise the outcomes of present investigations, we deduced correlation using R among enzymatic activity and per cent bud sprouting. The parameter per cent bud sprouting was positively correlated with all the enzymatic studied. It was witnessed that the catalase and peroxidase activity might be directly taking part in breaking bud dormancy whereas AAO and PPO activity playing an apparent role in bud growth resumption.

Conclusion

The rootstocks used for 'Red Globe' scion have positive influence on enzymatic activities of stress relieving enzymes that lead to increased per cent bud sprouting and decrease in days to bud sprouting. The vines on '110R' and own roots performed equally well in release of bud dormancy under current experimental tropical conditions where double pruning and single cropping pattern is followed. Also, changes in enzymatic activities can be used as biological indicators to determine end of bud dormancy and recommencement of growth.

Acknowledgement

Authors are grateful to the Director, ICAR-National Research Centre for Grapes, Pune providing the facilities to carry out the present investigation. Authors also thankful to ICAR for financial support.

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Received January 14, 2020
Accepted March 9, 2021