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Original Research Paper

Response of selected scion and rootstock grape (*Vitis* spp.) genotypes to induced drought stress

Amulya S.[1], Prakash J.[1*], Singh S.K.[2], Verma M.K.[3], Patel V.B.[4], Thakre M.[1], Kumar C.[1] and Shankar K.[1]

[1]Division of Fruits and Horticultural Technology, ICAR- Indian Agricultural Research Institute, New Delhi - 110012, India
[2]ICAR- Indian Institute of Horticultural Research, Bengaluru - 560089, India
[3]ICAR- Central Institute of Temperate Horticulture, Srinagar - 191132, India
[4]Indian Council of Agricultural Research, New Delhi - 110012, India
[*]Corresponding author Email : singhjai2001@rediffmail.com

ABSTRACT

Climate change is expected to elevate drought frequency, straining agricultural freshwater resources. Developing drought-tolerant grapevine varieties is crucial. This study examined grape scion and rootstock genotypes under well-watered (WW) and induced-drought (ID) conditions. ID treatment reduced vine length by 11.34-35.15%, with *Vitis parviflora*, 110R, and Male Hybrid rootstocks showing superior growth. Root length increased under ID, indicating an adaptive moisture-seeking response. The ID treatment led to substantial reduction in leaf count and average leaf area, especially in Flame Seedless (27.71 and 19.07 cm^2, respectively). Drought stress elevated chlorophyll *a:b* ratio, affecting chlorophyll degradation in different genotypes. Significant variations were observed in leaf and root iron (Fe) and zinc (Zn) contents. Enzyme activities particularly peroxidase and polyphenol oxidase especially rose under drought, particularly in *V. parviflora* (3.39 µM guaiacol min^{-1} mg^{-1} protein and 1.33EU/ml/min respectively) likely to be contributing in drought tolerance mechanism. Principal component analysis (PCA) highlighted impact of traits on genotypes, emphasizing *V. parviflora*, Male Hybrid and Pusa Navrang as superior drought stress tolerant genotypes. Genotype clustering confirmed distinct groupings, while, correlation analysis unveiled intricate trait interactions.

Keywords: Clustering, drought-induced, principal component analysis, *V. parviflora*, well-watered

INTRODUCTION

Grape (*Vitis vinifera* L.) is a globally significant fruit crop, particularly valued for its role in wine production (Alston & Sambucci, 2019). Climate change projections indicate increased drought occurrences in traditional wine-growing regions, necessitating more irrigation (IPCC, 2014). Water deficits can hinder vine growth and yield but can enhance grape and wine quality unless severe (Chaves et al., 2010). Understanding grapevine responses to drought is crucial, especially with escalating water scarcity and irrigation constraints (Tsegay et al., 2014). Significant attention has focused on variations in drought tolerance among different grape genotypes, including scion and rootstock variability (Medrano et al., 2018). Rootstocks are essential for enhancing resistance to biotic and abiotic stresses, contributing to water absorption, nutrient uptake, and overall drought tolerance. Rootstocks can be categorized into those promoting greater vigour and drought resilience and those with lower vigor and reduced drought tolerance (Serra, 2013). A drought-tolerant scion is critical under stress, as it influences root development, stem and leaf dynamics, and water demand (Tandonnet et al., 2010). This study investigates the morphological and biochemical attributes of various scion and rootstock genotypes under induced drought stress conditions.

MATERIALS AND METHODS

Twelve grape genotypes, including seven scion cultivars (Pusa Navrang, Pusa Aditi, Pusa Trishar, Pusa Swarnika, Pusa Urvashi, Pusa Purple Seedless, Flame Seedless) and five rootstocks (110R, St. George, Dogridge, *Vitis parviflora*, Male Hybrid), were collected from the Grape Germplasm Block at ICAR-Indian Agricultural Research Institute, New Delhi. Dormant cuttings from 9 to10 year old vines, trained on the 'Kniffin' system, were potted (30×30 cm) in a soil peat mix (1:1:1) and maintained in a polyhouse for six months. The experiment spanned two seasons during 2021 to 2023; six-month-old self-rooted vines underwent a 21-day induced drought (ID) by withholding water, reducing soil VWC from 70% to 25% volume water content (VWC), while well-watered (WW) controls were maintained at 70% VWC. Moisture was monitored using a ProCheck moisture meter. The experiment followed a two-factor factorial completely randomized design (CRD) with three replications of five vine cuttings each. Vegetative growth parameters *viz.*, vine length (cm), stem girth (mm), length of the longest root (cm), number of leaves per plant and average leaf area (total leaf area/ number of leaves per plant) (cm^2) were recorded at the end of the 21 days, and biochemical parameters (Chlorophyll a ratio, peroxidase, and polyphenol oxidase activities) were assessed at 0, 7, 14, and 21 days. Chlorophyll was estimated using the DMSO method, peroxidase (POD) by Zhang et al. (2008) method, and polyphenol oxidase (PPO) by Halpin and Lee (1987) method, all measured with a UV-visible spectrophotometer. Iron and zinc in leaves and roots were determined using atomic absorption spectroscopy. Data were expressed as mean, standard deviation, and LSD ($d \leq 05\%$), followed by ANOVA. Correlation analyses, including principal component analysis (PCA), K-means clustering and heat maps were performed using R packages "FactoMineR", "corrplot", and "ggplot" in R 4.3.0 software.

RESULTS AND DISCUSSION

Analysis of variance for morphological and biochemical attributes of grapevines over two years showed significant differences between well-watered (WW) and drought-induced (ID) conditions. The vine length of all selected scion and rootstock genotypes significantly decreased with reduced water availability, highlighting the critical role of water in sustaining vine growth (Dry et al., 2015). A significant decrease in vine length from 70.33 cm to 41.01 cm was recorded in PT (41.68% reduction) followed by Flame Seedless (72.7 to 14 cm, 35.15% reduction) and PS (84.71 to 58.64 cm, 30.77% reduction). However, minimal decreases were found in Pusa Purple Seedless (43.69 to 37.51 cm) and Male Hybrid (86.64 to 76.81 cm). The highest overall plant height under ID condition was recorded in *V. parviflora* followed by 110R, Male Hybrid, Dog Ridge and Pusa Navrang, representing the vigorous growth of rootstock over scion varieties. A noticeable decline was recorded in the stem girth (mm) for genotypes subjected to ID compared to WW vines. Among the scion genotypes, Pusa Navrang and Pusa Purple Seedless (4.95 to 4.54 mm and 4.27 to 3.83 mm, respectively) showed a decrease in stem girth compared to WW conditions followed by Pusa Urvashi and Pusa Swarnika. Among the rootstocks, *V. parviflora* (5.80 and 5.81 mm) showed no difference between the treatments, followed by 110R (3.88 to 3.59 mm) and Male Hybrid (5.58 to 5.17 mm). This indicates the growth performance superiority of rootstock over scion. Under WW condition, the highest root length was observed in Pusa Purple Seedless (41.23 cm), followed by *V. parviflora* (36.44 cm) and110R (33.53 cm); however, under ID condition, the root length was significantly increased in all the genotypes (Table 1) as a plant's adaptive response towards moisture search. A similar trend was observed under controlled conditions. The highest number of leaves per plant was recorded in 110R (103.15), followed by Male Hybrid (84.52) and Pusa Navrang (78.04) under controlled conditions, while, it decreased significantly under water stress conditions but in a similar trend (Table 1). The sharp decline was recorded for the average leaf area under ID treatment, and the substantial detrimental effect of drought was detected on the leaf area. Flame Seedless was affected maximum with a decrease of 51.92%, followed by Pusa Trishar (26.50%). In contrary, 110R, *V. parviflora*, Male Hybrid and Pusa Navrang were able to manage the water stress with a 7.92, 11.67, 14.22 and 14.74% decrease in leaf area, respectively. The extent to which leaf growth is restricted may differ based on the type of tissue impacted (Wu & Cosgrove, 2000). For instance, the epidermal cells on the leaf surface might show reduced growth due to dehydration and stress. At the same time, the internal vascular tissues might maintain growth for a certain period to ensure water transportation within the plant. This differential response is an adaptation mechanism where certain tissues might prioritize water conservation overgrowth, while, others continue to support essential functions despite water scarcity.

Drought stress, due to reduced water availability, can decrease chlorophyll content, impacting photosynthesis (Flexas & Medrano, 2002). As shown in Fig. 1, the chlorophyll a:b ratio significantly increased ($p \leq 0.05$) in the ID plants compared to the WW control plants, irrespective of the varieties. This may be because chlorophyll degrades more rapidly than chlorophyll *a* (Shimoda et al., 2012). Considerable

Table 1 : Effect of induced drought stress on number of vine length, stem girth, longest root length, leaves per plant, average leaf area, on some grape genotypes

Genotype	Vine length (cm)		Stem girth (mm)		Length of longest root (cm)		No. of leaves/plant		Avg. leaf area (cm^2)	
	WW	ID	WW	ID	WW	ID	WW	ID	WW	ID
Pusa Navrang	89.08^{cd}	74.56^{de}	4.95^{bc}	4.54^{fg}	33.33^{e-h}	36.44^{c-f}	78.04^{cd}	62.66^{fg}	34.53^{def}	29.44^{gh}
Pusa Aditi	77.29^{bc}	55.52^{e-h}	5.14^{efg}	4.1^{j}	30.58^{g-j}	35.93^{bcd}	61.71^{fgh}	43.77^{jkl}	34.64^{def}	26.07^{hi}
Pusa Trishar	70.33^{ab}	41.01^{ef}	5.51^{gh}	4.31^{kl}	25.54^{kl}	35.06^{c-g}	55.72^{hi}	38.25^{lm}	36.3^{cd}	26.68^{hi}
Pusa Swarnika	84.71^{abc}	58.64^{ef}	5.37^{cde}	4.35^{ij}	24.35^{l}	30.62^{hij}	62.33^{fg}	50.27^{ij}	36.16^{cde}	27.25^{hi}
Pusa Urvashi	79.9^{ab}	65.45^{de}	5.57^{def}	4.55^{hi}	28.48^{jk}	34.42^{d-h}	68.06^{ef}	48.37^{jh}	39.21^{c}	29.44^{gh}
Pusa Purple Seedless	43.69^{ef}	37.51^{fgh}	4.27^{kl}	3.83^{l}	41.23^{b}	47.64^{a}	56.17^{ghi}	35.17^{mn}	27.32^{hi}	21.57^{jk}
Flame Seedless	72.7^{de}	47.14^{gh}	4.45^{fgh}	3.73^{k}	28.23^{jkl}	32.81^{f-i}	43.58^{kl}	31.5^{n}	39.67^{c}	19.07^{k}
110R	96.39^{fgh}	80.57^{h}	3.88^{b}	3.59^{def}	33.53^{e-h}	38.71^{bc}	103.15^{a}	89.86^{b}	33.3^{d-g}	30.66^{fg}
St. George	74de	57.68^{e-h}	4.56^{fg}	4.11^{ij}	28.34^{jk}	33.8^{d-h}	64.36^{ef}	40.47^{lm}	36.37^{cd}	25.05^{ij}
Dogridge	90.32^{cd}	76.24^{efg}	4.95^{bc}	4.23^{fg}	29.34^{ijk}	37.01^{cde}	77.55^{d}	62.69^{fg}	37.3^{cd}	29.9^{gh}
Vitis parviflora	106.02^{a}	89.8^{a}	5.8^{a}	5.81^{bc}	36.44^{c-f}	38.74^{bc}	65.61^{ef}	56.97^{gh}	61.49^{a}	54.31^{b}
Male Hybrid	86.64^{ab}	76.81^{bc}	5.58^{cd}	5.17^{efg}	31.25^{g-j}	36.86^{c-f}	84.52^{bc}	70.07^{e}	37.25^{cd}	31.95^{efg}
Mean	80.93 ±15	63.42 ±15.87	5.01 ±0.58	4.36 ±0.6	30.89 ±4.51	36.50 ±4.04	68.4 ±14.94	52.51 ±16.13	37.8 ±7.78	29.37 ±8.36
CV %	18.54	25.03	11.58	13.77	14.61	11.11	21.85	30.72	20.59	28.58
Genotype (G)	5.720		0.376		2.763		4.646		3.034	
Treatment (T)	2.33		0.153		1.128		1.897		1.238	
G×T	8.08		0.531		3.908		6.571		4.291	

LSD (d”0.05); WW: well watered; ID: induced drought

adverse effects on chlorophyll degradation were recorded in Pusa Aditi, Flame Seedless and St. George after 21 days of drought stress with the chlorophyll *a:b* ratio of 4.52, 4.47 and 4.3, respectively. Conversely, *V. parviflora* (3.07), Male Hybrid (3.4), Pusa Purple Seedless (3.34), and 110R (3.4) displayed a slight decrease in the chlorophyll *a:b* ratio under drought.

Under drought stress, the uptake and transport of essential micronutrients like iron (Fe) and zinc (Zn) can be affected, leading to reduced availability in leaves and increased accumulation in roots (Hanikenne & Bouche, 2023). After 21 days, leaf Fe content ranged from 92.84 µg g^{-1} in *V. parviflora* to 25.81 µg g^{-1} in Pusa Trisha under well-watered (WW) conditions, and from 84.25 µg g^{-1} in *V. parviflora* to

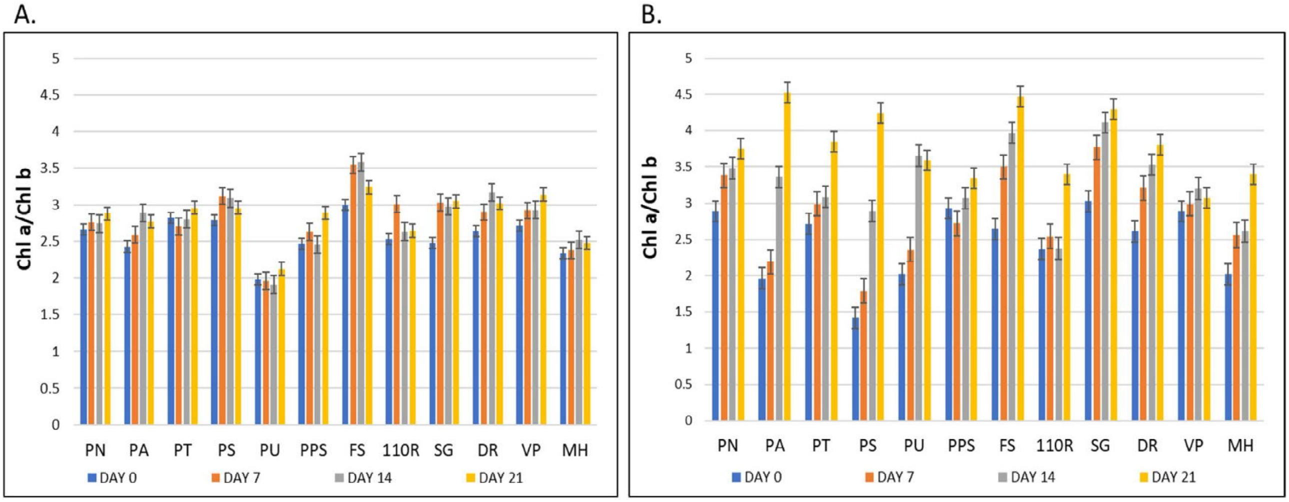

Fig. 1 : Responses of selected grape genotypes measured after different periods of stress (0, 7, 14 and 21 days) to chlorophyll *a:b* ratio under (A) well-watered-control and (B) induced drought conditions

Table 2 : Effect of induced drought stress on leaf and root Fe and Zn ($\mu g\ g^{-1}$) contents on some grape genotypes

Genotype	Fe ($\mu g\ g^{-1}$)				Zn ($\mu g\ g^{-1}$)			
	Leaf		Root		Leaf		Root	
	WW	ID	WW	ID	WW	ID	WW	ID
Pusa Navrang	78.09^{d}	55.11^{f}	84.53^{ij}	149.77^{b}	24.64^{eh}	20.84^{ij}	69.99^{c}	98.96^{a}
Pusa Aditi	35.89^{ij}	26.02^{lm}	62.36^{l}	85.76^{hij}	23.57^{hg}	14.64^{m}	28.93^{op}	35.46^{lmn}
Pusa Trishar	25.81^{lm}	24.32^{m}	48.92^{m}	80.17^{jk}	18.6^{k}	12.97^{n}	37.1^{klm}	40.16^{jk}
Pusa Swarnika	39.96^{hi}	27.22^{klm}	73.14^{k}	93.67^{ghi}	19.56^{jk}	14.34^{mn}	35.44^{lmn}	41.13^{jk}
Pusa Urvashi	47.02^{g}	32.18^{jk}	107.89^{e}	138.38^{c}	22.31^{gh}	16.41^{l}	39.39^{jkl}	46.74^{hi}
Pusa Purple Seedless	43.15^{gh}	30.99^{jkl}	81.68^{jk}	123.74^{d}	27.47^{bc}	17.01^{l}	42.35^{ij}	48.46^{gh}
Flame Seedless	27.41^{klm}	22.4^{m}	50.85^{m}	75.56^{jk}	16.49^{l}	10.6^{o}	31.08^{no}	46.56^{hi}
110R	85.59^{bc}	65.91^{e}	99.2^{efg}	170.87^{a}	28.37^{b}	23.55^{fg}	41.89^{j}	62.14^{d}
St. George	66.21^{d}	27.71^{klm}	82.05^{jk}	128.19^{cd}	24.14^{ef}	19.1^{k}	25.14^{p}	34.43^{mn}
Dogridge	80.42^{cd}	63.62^{e}	95.72^{fgh}	159.1^{b}	25.02^{e}	19.78^{ijk}	40.74^{jk}	56.11^{ef}
Vitis parviflora	92.84^{a}	84.25^{c}	130.41^{cd}	177.83^{a}	32.71^{a}	25.34^{de}	58.15^{de}	94.2^{b}
Male Hybrid	90.94^{ab}	61.78^{e}	105.07^{ef}	150.34^{b}	26.51^{cd}	21.11^{hi}	50.22^{gh}	52.51^{fg}
Mean	59.45 ±24.39	43.46 ±20.32	85.16 ±23.18	127.79 ±34.63	24.12 ±4.32	17.98 ±4.24	41.71 ±12.12	54.74 ±20.27
CV%	41.03	46.76	27.22	27.1	17.92	23.59	29.06	37.03
Genotype (G)	3.850		7.218		1.026		3.283	
Treatment (T)	1.572		2.947		0.419		1.340	
G×T	5.445		10.209		1.451		4.644	

LSD (d”0.05); WW: well watered; ID: induced drought

22.40 $\mu g\ g^{-1}$ in Flame Seedless under induced drought (ID) conditions (Table 2). In roots, Fe content ranged from 130.41 $\mu g\ g^{-1}$ in *V. parviflora* to 50.85 $\mu g\ g^{-1}$ in Flame Seedless under WW and increased to 177.83 $\mu g\ g^{-1}$ in *V. parviflora* and 75.56 $\mu g\ g^{-1}$ in Flame Seedless under ID. *V. parviflora* demonstrated the highest leaf Zn values (32.71 $\mu g\ g^{-1}$ WW and 25.34 $\mu g\ g^{-1}$ ID), while Flame Seedless had the lowest. Pusa Navrang roots showed high Zn levels (69.99 $\mu g\ g^{-1}$ WW and 98.96 $\mu g\ g^{-1}$ ID), whereas, St. George had lower levels. This increase in root minerals under drought mirrors findings by Yang et al. (2011).

The highest peroxidase activity following drought stress for 14 and 21 days was found in *V. parviflora* (1.70 and 3.39 µM guaiacol min-1mg-1protein, respectively), followed by Male Hybrid and 110R. Among the scion varieties, Pusa Navrang and Pusa Urvashi showed an exceptional increase. A similar trend was recorded in WW conditions; peroxidase activity of *V. parviflora*, 110R, Male Hybrid and Pusa Navrang were found to be higher than other genotypes (Fig. 2). A similar result was reported by Gurjar et al. (2015), where significant peroxidase activity was noted high in *V. parviflora*, Male Hybrid and Pusa Navrang in response to disease incidence indicating the resistance. During the 21-day drought period, *V. parviflora* displayed the highest polyphenol oxidase activity with values of 0.16 and 1.33 EUml-1min-1in WW and ID conditions, respectively. The lowest activity was exhibited in Flame Seedless (0.09, 0.28 EUml-1min-1) between the control and drought-induced conditions (Fig. 3). The uplifted levels of polyphenol oxidase enzyme activity under severe stress compared to normal moisture conditions suggest a potential link to enhanced plant tolerance to drought stress (Dastneshan et al., 2022).

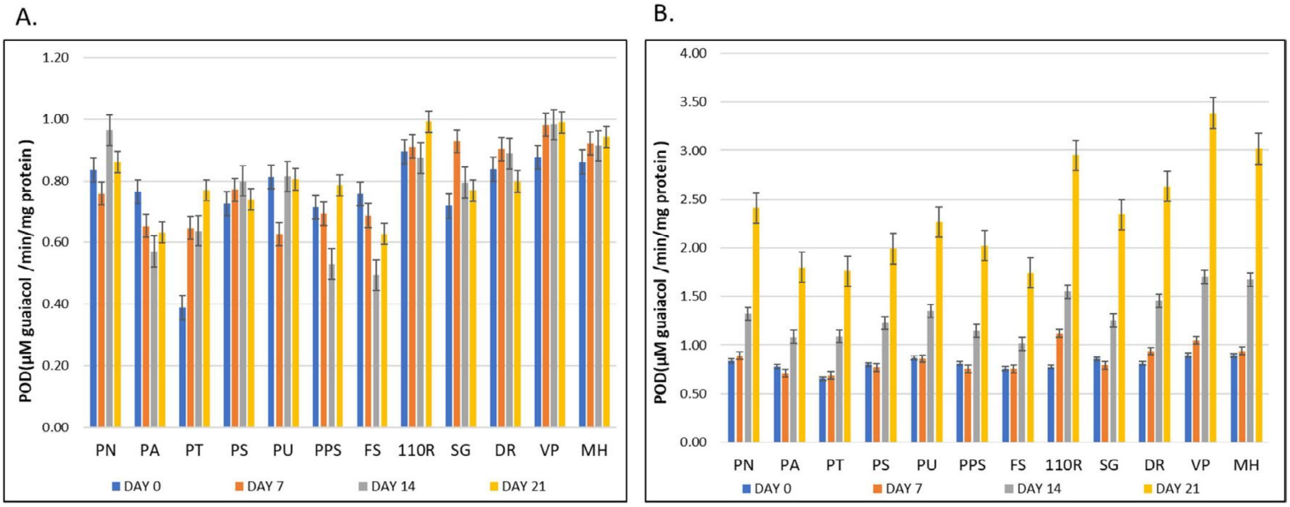

Fig. 2 : Responses of selected grape genotypes measured after different periods of stress (0, 7, 14 and 21 days) to Peroxidase (µM guaiacol min^{-1} mg^{-1}) under (A) well-watered-control and (B) induced drought conditions

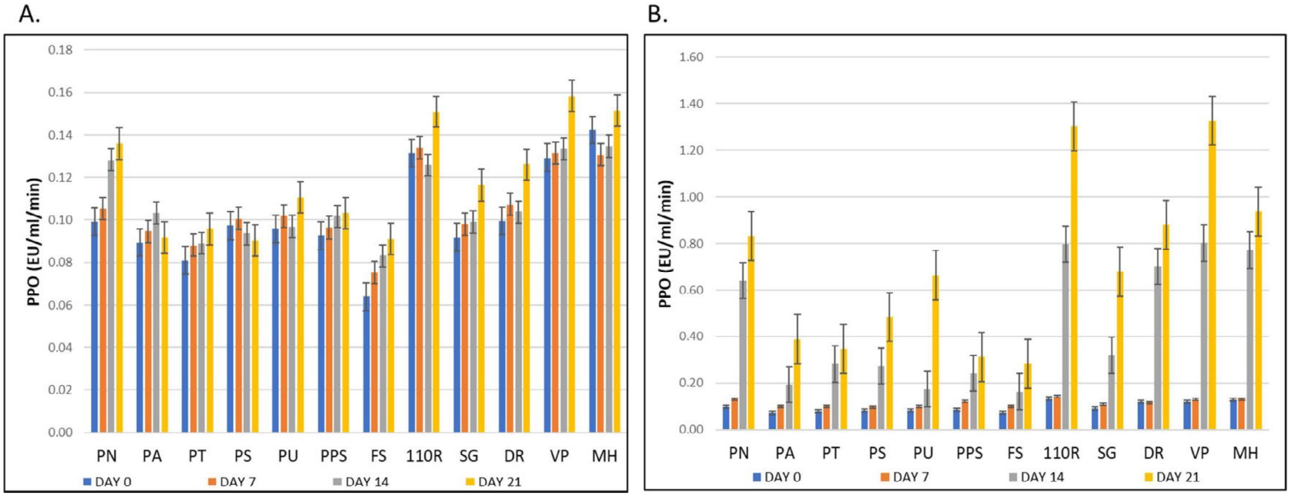

Fig. 3 : Responses of selected grape genotypes measured after different periods of stress (0, 7, 14 and 21 days) to Polyphenol oxidase activity (EU min^{-1} ml^{-1}) under (A) well-watered-control and (B) induced drought conditions

Principal component analysis (PCA) showed that the first four components accounted for 90.61% of the variance, with PC1, PC2, PC3, and PC4 contributing 60.90%, 13.70%, 10.10%, and 6.0%, respectively (Fig. 4c). The PCA, specifically focused on the first two axes, highlighted the substantial impact of observed traits on the genotypes (Fig. 4a). Principal component 1 highlighted positive associations with stem girth, leaf quantity, leaf Fe and Zn content, root Fe and Zn content, PPO and POD under both well-watered and limited water conditions; while, vine length, and average leaf area in the ID scenario. This primary component was influenced by various morphological and biochemical traits. PC2 was mainly influenced by vine length and average leaf area under WW conditions. Root length impacted PC3 (10.10%), while PC4 (6.0%) was influenced by chlorophyll *a:b* ratio (Fig. 4d). The first and second PCA values significantly contributed to the discrimination of diverse components, signifying the dominant representation of traits (Fahim et al., 2022). The PCA distinctly differentiated between genotypes and stress conditions. PC1 played a pivotal role in distinguishing data between control and drought-stressed scion/ rootstock combinations, as also reported in citrus scion/rootstock combination under NaCl stress (Shankar et al., 2023). Utilizing K-means, genotype clustering grouped 12 genotypes into six clusters on the factor map, based on similarities in morphological and biochemical traits (Fig. 4b). The genotypes

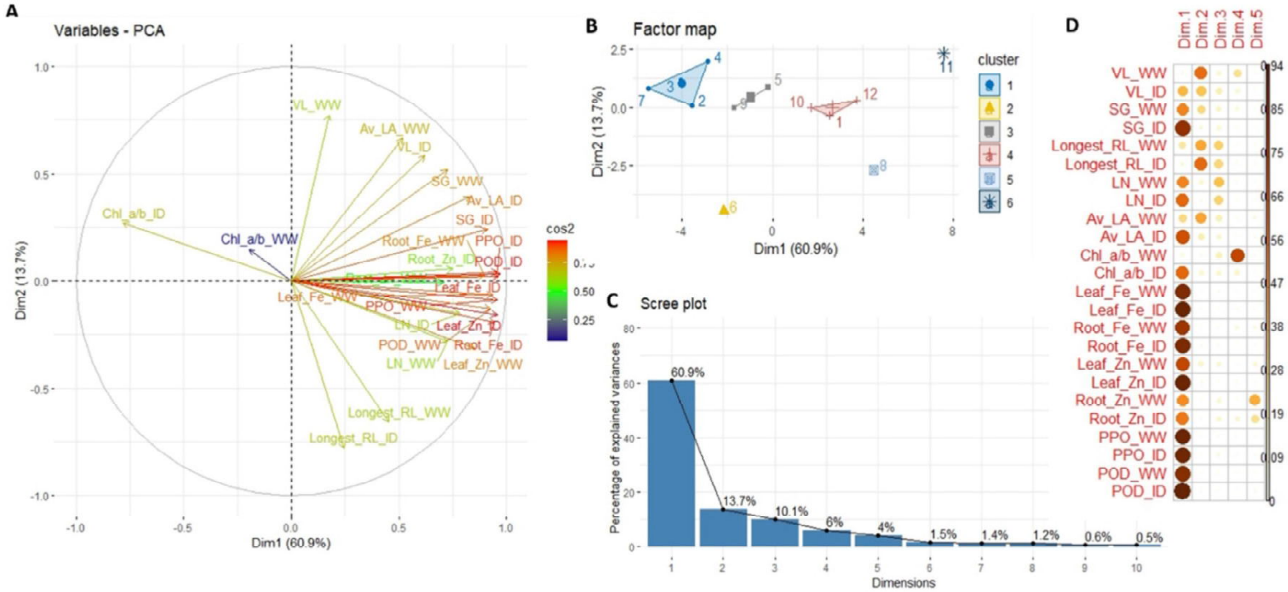

Fig. 4 : A. Principal component analysis (PCA) with morphological and biochemical parameters under well-watered-control and induced drought conditions and scree plots of PCA (B, C and D)

provide details of Cluster I (Flame Seedless, Pusa Trishar, Pusa Swarnika, Pusa Aditi), Cluster II (Pusa Purple Seedless), Cluster III (St. George, Pusa Urvashi), Cluster IV (Male Hybrid, Dog Ridge, Pusa Navrang), Cluster V (110R), and Cluster VI (*Vitis parviflora*).

Genotypes were categorized based on morpho-biochemical traits using cluster heat map analysis (Fig. 5), illustrating relationships among traits. The Euclidean squared distance metric showed correlations between genotypes and studied parameters. Previous studies utilized cluster analysis to classify genetic variations in plant matrices like wine and sweet cherries (Skendiet al., 2020; Ganopouloset al., 2015). Grouping the grape genotypes into five clusters was highlighted differences in their studied parameters, akin to the factor map in Fig. 4b. Notably, *V. parviflora* manifested superior drought stress tolerance, displaying strong correlations across morpho-biochemical parameters like vine length, stem girth, average leaf area, peroxidase, polyphenol oxidase and root iron and zinc content. Male Hybrid, Dogridge, 110R and Pusa Navrang formed a cluster exhibiting notable contribution compared to other genotypes. These findings underscore the significance of cluster analysis in discerning drought tolerance among grape genotypes based on peroxidase, polyphenol oxidase, leaf and root iron and zinc content, vine length, stem girth, leaf number, and average leaf area. Correlation between characters is indispensable parameter to evaluate whether the selection for one trait have influence on another (Majidi et al., 2009).

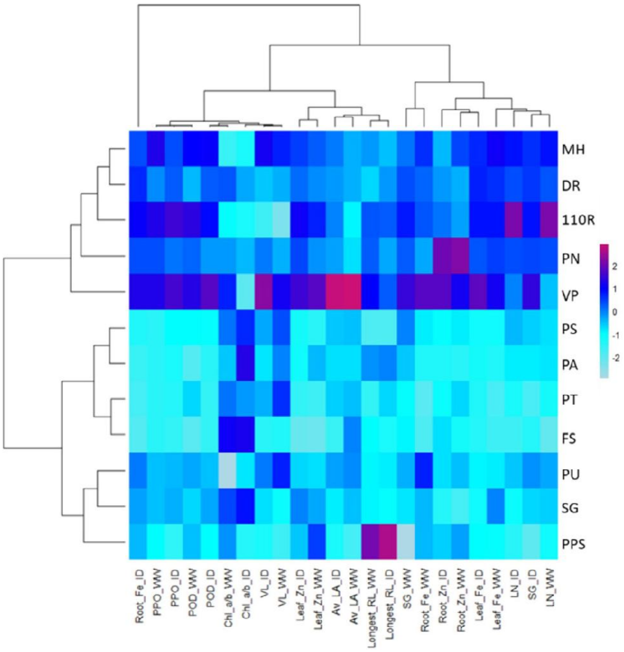

Fig. 5 : Cluster heat map analysis of 12 grape genotypes for drought tolerance

Correlation analysis (Fig. 6) revealed positive correlations ($p<0.01$ to $p<0.5$) among stem girth, leaf and root Fe and Zn contents, PPO, POD, and average leaf area. However, vine length, longest root length,

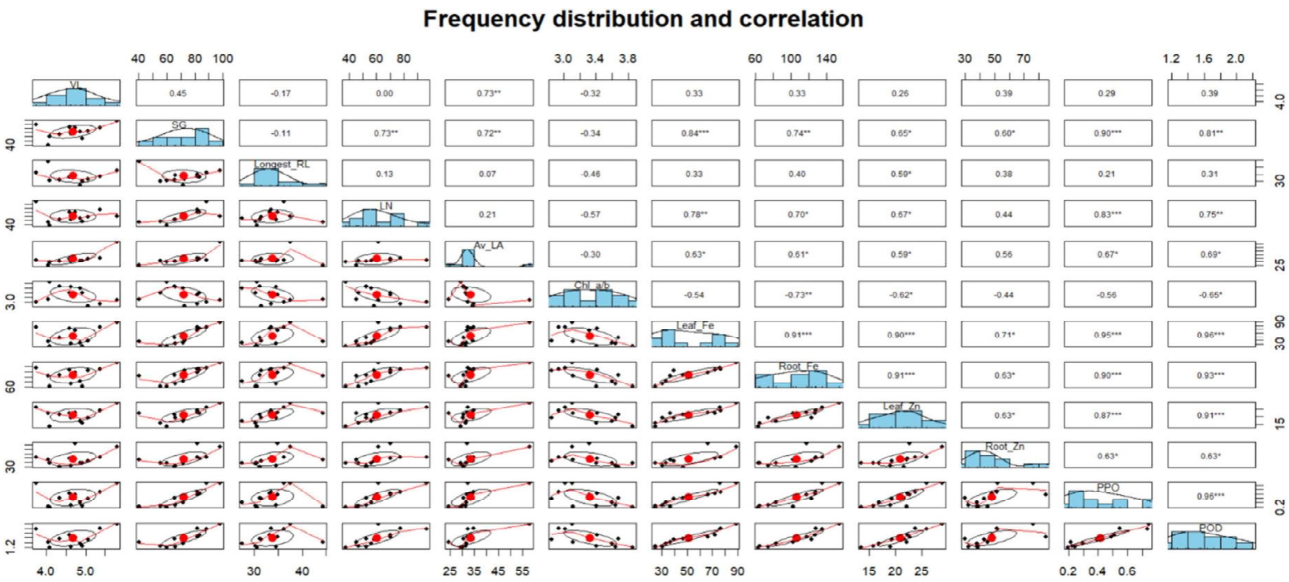

Fig. 6 : Correlation plot for 12 morpho-biochemical traits (vine length 'VL', stem girth 'SG', longest root length 'RL, number of leaves per plant 'LN', average leaf area 'LA', chlorophyll a:b ratio, 'chl a/b', leaf and root iron (Fe) and zinc (Zn) content, poly phenol oxidase and peroxidase based on 12 grape genotypes. The figure of diagonal line means the histogram of 12parameter's mean value. Linear regression relations (upper triangle) and phenotypic correlation coefficients (lower triangle) between traits. * and ** significantly at P <0.05 and 0.01, respectively

and chlorophyll *a*:*b* ratio showed non-significant ($p \geq 0.05$) or occasionally negative correlations with most parameters. This may be due to sample size limitations or genetic differences influencing growth and physiological traits. These insights are crucial for selecting drought-tolerant grape genotypes.

CONCLUSION

The present study revealed that drought-tolerant rootstocks like *V. parviflora exhibit* superior growth and resilience, highlighting their potential for sustaining grapevine growth under water-scarce conditions. Enzyme activity variations, mineral nutrient imbalances between leaves and roots, and PCA clustering emphasized the complex responses and the significance of genotype selection for drought resilience.

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