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Anatomical Variability in Grape (*Vitis venifera*) Genotypes in Relation to Water Use Efficiency (WUE)

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Abstract: Grape (*Vitis venifera*), an important fruit crop having ability to give high monitory returns, but area under cultivation is limited due to lack of irrigation resources. Sixteen grape genotypes commercially grown in Western and Northern Maharashtra region were analyzed for anatomical polymorphism to characterize them in relation to Water Use Efficiency (WUE). No of xylem poles in roots, height of palisade mesophyll cells, no of stomata per 100 μm and conducting tissues in petiole were the key anatomical parameters, which will be helpful to select better genotypes for increased water use efficiency and higher yields. Palisade mesophyll height in high yielding stay green genotypes ranged between (103-117 μm) as compared to range of medium yielding stress susceptible genotypes (71-91 μm) indicating the increased photosynthetic rate and higher production due to this important trait. Fewer number and widely spaced stomata's in resistant plants reduces the chances of evapotranspiration. Genotype Trebbiano Toscano, Chenin Blanc and Syrah were identified as promising varieties for growing in tropical regions with irregular rainfall and water scarcity.

Key words: Xylem, anatomy, grape, conduit, water use efficiency (WUE)

Introduction

India has the distinction of achieving the highest productivity in grapes in the world, with an average yield of 30 t ha^{-1}. Maharashtra is a leading state with 34,000 hectares of area under grape cultivation and an annual production of 1,000,000 tones. Grape is a crop with high returns per unit area of land but not expanding fast due to: high initial and recurring cost, soil and water salinity, drought in the hot tropical areas and narrow variety base and lack of diversity in relation to water use efficiency.

Grape growers are facing great losses due to irregular rainfall, scarcity of water, low ground water level. Efficient use of water plays a key role in proper growth of plant and fruit development and the overall quality of grapes. Improved anatomical root and leaf traits can play crucial role in grape production viz; increase in number of xylem poles in roots, wide palisade mesophyll cells, less number of stomata in leaves, abundant conducting tissues in petiole. The present study is to focus the anatomical polymorphism of grape genotypes grown in Western and Northern Maharashtra regions.

Anatomical parameters are rather little explored in relation to drought resistance. These parameters are genetically governed and can be introgressed. Seventeen tomato genotypes were screened for petiole leaf anatomy and stomatal features to understand secret of drought resistance. Total petiole thickness, compact parenchyma and more number of xylem vessels were important characters of drought resistant genotypes. Leaf of resistant type had longer palisade mesophyll cells with compact arrangement. Thickness of leaves was invariably more in drought resistant genotypes (Kulkarni and Deshpande, 2006). Various stomatal characters such as sunken type and less number per unit area are associated with drought resistance (Davis, 1977). Guard cell size ranged between 78-92 μm in winter wheat genotypes and was important selection criteria for cold hardiness (Limin and Fowler, 1994).

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Parenchymas are principally storage tissues polyhedral in shape mainly related to storage, excretion like vegetative activities of plant. Apparently the potentiality to divide may be retained by parenchyma cells for many years (Barker, 1953). Many different food substances are synthesized and stored by parenchyma cells. These substances are stored in vacuolar sap or they may be discrete solid or fluid bodies in cytoplasm Amides, protein and sugar are dissolved in cell sap (Netolitzky, 1953). Importance of compactness of parenchyma cells was discussed by Liese and Grove (1961) in bamboo. Collenchyma walls may contain over 60% water based on fresh weight; over 200 per cent based on dry weight (Cohn, 1892). Deshpande and Kulkarni (2005) reported root morphological and anatomical parameters imparting drought resistance in tomato. Resistant genotype root length ranged between 60-70 cm whereas susceptible group had only 30-40 cm long roots. Number of xylem per cross section of root was 36.5 in resistant genotypes against 26.8 in susceptible groups. Cotton root anatomy was described and wide range of diversity was reported (Reinhardt and Rost, 1995).

Anatomical parameters in midrib cross-section having compact and long parenchyma in trispecies derivatives of cotton (Genus *Gossypium*) was reported (Ansingkar *et al*., 2004). Stability of anatomical parameters over seasons along with their genetics was discussed (Rajendran, 2004). In cotton, small and thick leaves with thick palisade tissue are associated with drought resistance (Bhatt and Andal, 1979; Singh *et al*., 1990, 1996). Additive as well as nonadditive effects for anatomical traits like number of stomata, number of epidermal cells, guard cell length and stomatal width were studied (Lakshminarayana *et al*., 1979). Thick leaves, high level of electrolyte, high chlorophyll content, few stomata and vigorous root growth are related to heat tolerance in Chinese Cabbage (Kuo *et al*., 1988).

Materials and Methods

Plant Material

Sixteen grape genotypes grown in Western and Northern Maharashtra region (India) were collected from growers field (Table 1). These genotypes were used for comparative morphological and anatomical analysis in relation to water use efficiency. Rootstock was used as control. All the experiments were performed in triplicates and the data obtained was statistically analyzed.

Anatomy and Micrometry

Leaf anatomy of all the genotypes was studied in detail using light microscopy by adopting standard methods of investigation (Jenson, 1962).

Table 1: Grape genotypes studied

			Fruit	
Serial	Name of variety	Origin	Color	Size
A	Syrah	California	Purple	Small
B	Cabernet Sauvignon	California	Black	Big
C	Shimphony	California	White	Big
D	Chenin Blanc	California	White	Small
E	Zinfandel	California	Black	Small
F	Baramati Purple	California	Black	Big
G	Tas-A-Ganesh	India	White	Small
H	Sanjeevese	Italy	Black	Big
I	Trebbiano Toscano	Italy	White	Small
J	Chardonny R-8	Italy	White	Small
K	Vermentino	Italy	White	Small
L	Root Stock	Wild	White	Small
M	Merlot	European	Purple	Medium
N	Chardonnay	European	White	Big
O	Vermianto	European	White	White
P	Gargamega	European	White	White

Leaf Sampling

The fully opened leaf was sampled to study the leaf anatomy.

Fixation

Leaf samples from similar stage of growth were fixed in the standard fixative FAA (90 mL of 90% alcohol+5 mL formalin (40% formaldehyde)+5 mL glacial acetic acid). The leaf samples were allowed to remain in fixative for 48 hours. Afterwards the samples were transformed to 70% alcohol to store them for further studies. Proper care was taken to maintain uniformity in leaf midrib size and portion.

Affixing the Sections

One per cent gelatin with little quantity of potassium dichromate was used as an adhesive. A few drops of gelatin were poured on the surface of a pre cleaned micro slides with the help of filter. The paraffin ribbons were cut into convenient lengths with the help of blade and placed on the adhesive surface. Gelatin could enable the sections to adhere to the slide, even at high temperature. The slides were then warmed over the warming plate maintained at nearly 45°C for a standard period of two hours to facilitate flattening stretching of ribbon. The excess adhesive was poured out and slides were later dried for 72 h under room temperature in dust free environment.

Micrometry

Magnification field of microscope with the help of calibrated ocular meter standardized with the help of stage micrometer. Observations on anatomical parameters of leaf (petiole and stomata) and root (xylem vessels) were recorded using ocular micrometer.

Number of Stomata (Per Microscopic Field)

The micrometric observations were recorded at 100X (10×10X) magnification field of microscope in area of one square millimeter.

Measurement of Chlorophyll Content

Total chlorophyll from leaves was measured as per procedure given by Arnon (1949) and Borse *et al.* (1998).

Results and Discussion

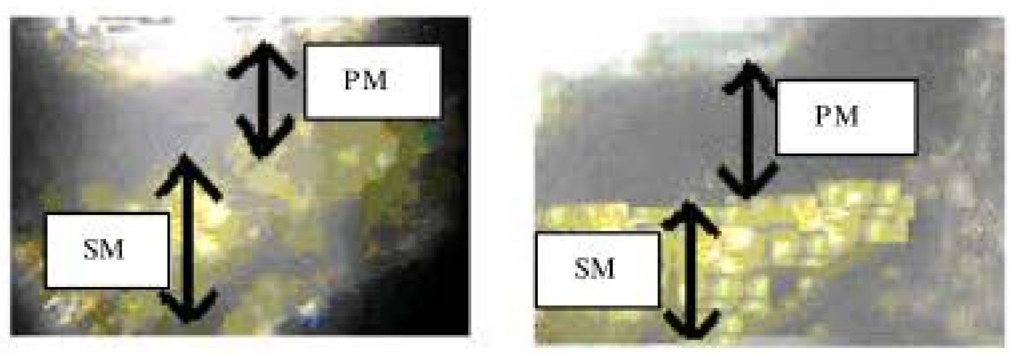
The grape genotypes collected for the present work were all of wine varieties originally of California and Italy origin, grown for last 15 years in India. These varieties showed variation in colour and size of the fruit as compared in Table 1. The leaf samples collected were morphologically differentiable and showed variation in size and weight of leaf ranging from 60-150 and 0.5-1.5 g, respectively. Table 2 shows that the genotype G (Tas-e-Ganesh), H (Sanjeevese)and C (Shimphony) shows more leaf size and genotype I (Trebbiano Toscano)and H (Sanjeevese) has more leaf weight than the genotype L (Root stock).

Anatomical Features of Grape Leaf

Pallaside mesophyll cells placed below the epidermis of leaf give strength and shape to the leaf. Palisade mesophyll compactness is responsible for holding the water content in the leaf and help to reduce evapotranspiration. Comparing the data depicted in Table 2, it is clear that genotype D (Chenin Blanc, 117 µm) with highest palisade mesophyll height followed by I (Trebbiano Toscano, 113 µm) and A (Syrah, 112 µm). This 40% increased height of palisade mesophyll cells provides an extra ability

Table 2: Variation in leaf anatomy of grape genotypes

		Leaf morphological and anatomical features						
Sr.	Name of variety	Leaf size (sq. cm)	Leaf weight (g)	Palisade mesophyll height (µm)	Spongy mesophyll height (µm)	No. of xylem poles in petiole	No. of stomata per 100 sq. µm	Distance between stomata (µm)
A	Syrah	81.0	0.519	112.00	99.00	33	4	81.00
B	Cabernet sauvignon	66.0	0.632	71.00	113.00	26	7	54.00
C	Shimphony	115.0	0.732	73.00	115.00	27	5	71.00
D	Chenin blanc	62.0	0.577	117.00	156.00	47	4	88.00
E	Zinfandel	79.0	0.822	79.00	98.00	32	6	69.00
F	Baramati purple	82.0	0.565	81.00	116.00	37	5	71.00
G	Tas-A-ganesh	142.0	0.683	103.00	119.00	40	5	70.00
H	Sanjeevese	132.0	1.357	91.00	145.00	42	4	88.00
I	Trebbiano toscano	93.0	1.658	113.00	112.00	50	4	101.00
J	Chardonny R-8	66.0	0.658	107.00	97.00	45	4	69.00
K	Vermentino	62.0	0.678	78.00	86.00	42	7	52.00
L	Root stock	39.0	0.264	83.00	95.00	43	6	72.00
M	Merlot	71.0	0.752	89.00	102.00	35	5	72.00
N	Chardonnay	76.0	0.623	76.00	110.00	28	6	76.00
O	Vermianto	86.0	0.726	86.00	108.00	35	5	64.00
P	Gargamega	81.0	0.658	92.00	97.00	32	5	70.00
	CD (at 5% level)	26.6	-	16.55	42.46	-	-	26.32



Shimphony Chenin Blank

Fig. 1: TS of leaf showing comparative tissue ratio variation in stress tolerant (chenin blank) and stress susceptible (Shimphony) genotype (PM-Palisade mesophyll, SM-Spongy mesophyii)

to leaves for increased utilization of available moisture resources leading to higher rate of photosynthesis. This difference is clearly visible as seen in Fig. 1, which shows thicker palisade mesophyll in stay green genotype (Chenin Blanc) as compared to thinner palisade mesophyll in water stress susceptible genotype (Shimphony).

Total biomass production of a genotype is water dependent, water in addition to its vital function is essential for generation of energy by photophosphorylation process required for the maintenance and normal growth of a plant. Chlorophyll content plays an important role in photosynthetic process by efficiently using the water. Figure 2 shows the relation between pallaside mesophyll height and total chlorophyll content of leaves. It is evident from graph that as height of palisade mesophyll increases, chlorophyll content of leaves also increases. This trend reflects secret of stay greenness of these genotypes.

The other anatomical feature of leaf for Water Use Efficiency is the number and size of the stomata. Water evaporates through intercellular spaces of parenchyma. From there water vapor escapes into atmosphere through stomata (90%) or cuticle (10%). Cuticle restricts transpiration and is chemically stable (Dicher, 1963). The density of stomata has been established as 100 to 300 mm^{-2} for leaves of many species (Stalfelt, 1956). The comparative data in Table 3 reflects, the number of stomata's in genotypes D (Chenin Blanc), I (Trebbiano Toscano) A (Syrah) have less (4 per 100 sq. µm) as compared to other genotypes. Also the distance between the stomata's is more as compared to other, which clearly indicates that it helps to decrease the rate of evapotranspiration.

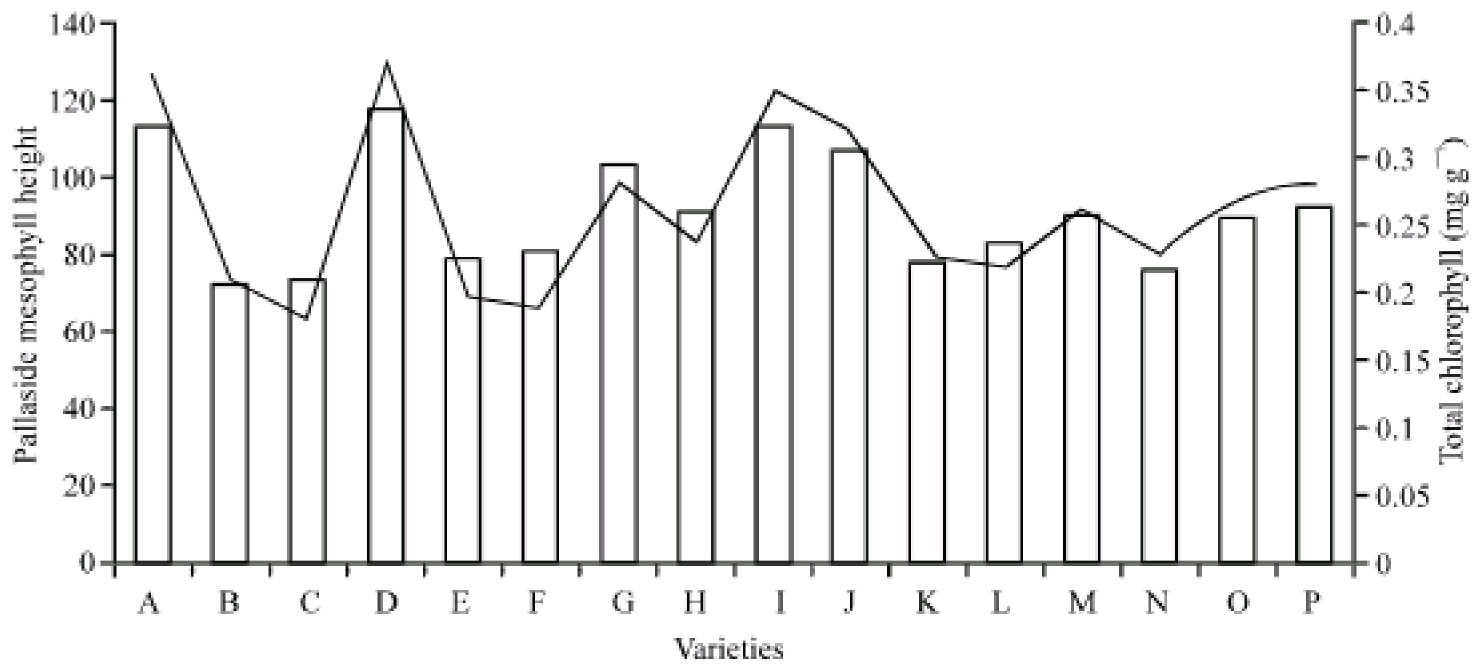


Fig. 2: Relation between pallaside mesophyll cell height and total chlorophyll content

Table 3: Parameters showing variation in root anatomy

		Root cross sectional data		
Serial	Name of variety	No. of xylem poles per root cross section	Distance between xylem poles (μm)	Diameter of mature xylem pole (μm)
A	Syrah	37.00	137.00	128.00
B	Cabernet sauvignon	22.00	89.00	132.00
C	Shimphony	40.00	114.00	78.00
D	Chenin blanc	39.00	142.00	124.00
E	Zinfandel	27.00	171.00	103.00
F	Baramati purple	49.00	109.00	87.00
G	Tas-A-ganesh	38.00	142.00	98.00
H	Sanjeevese	32.00	149.00	81.00
I	Trebbiano toscano	37.00	183.00	85.00
J	Chardonny R-8	38.00	151.00	89.00
K	Vermentino	35.00	95.00	98.00
L	Root stock	45.00	81.00	81.00
M	Merlot	36.00	126.00	92.00
N	Chardonnay	28.00	128.00	88.00
O	Vermianto	32.00	123.00	95.00
P	Gargamega	33.00	135.00	99.00
	CD (at 5% level)	5.95	19.87	27.36

Xylem poles in petiole play an important role in holding large amount of water. It acts as storage and conducting tissue providing water and essential vital biomolecules for the metabolic activities in a cell. The number and size of xylem poles is an important character of drought resistant plants. As seen in Table 2, it is clear that the genotypes D, have more (47-50) number of xylem poles than the genotype L (Root stock, 43). This difference is clearly visible in tolerant (Chenin Blanc) and susceptible cultivar (Shimphony) as shown in Fig. 4.

Anatomical Features of Grape Roots

Xylem poles in roots plays major role in feeder roots to affect rate of water absorption. Increased xylem diameter can increase water conducting ability of xylem poles provided strengths to pit membranes connecting them should be increased (Hack and Sprey, 2001). As compared in Table 2, genotype D (Chenin Blanc) exhibited highly desirable traits in relation to anatomical parameters ideal for water use efficiency. This is in accordance with the results obtained by Deshpande and Kulkarni (2005) where xylem diameter and number of xylem poles in drought tolerant mutant roots was observed to be more as compared to susceptible genotypes.

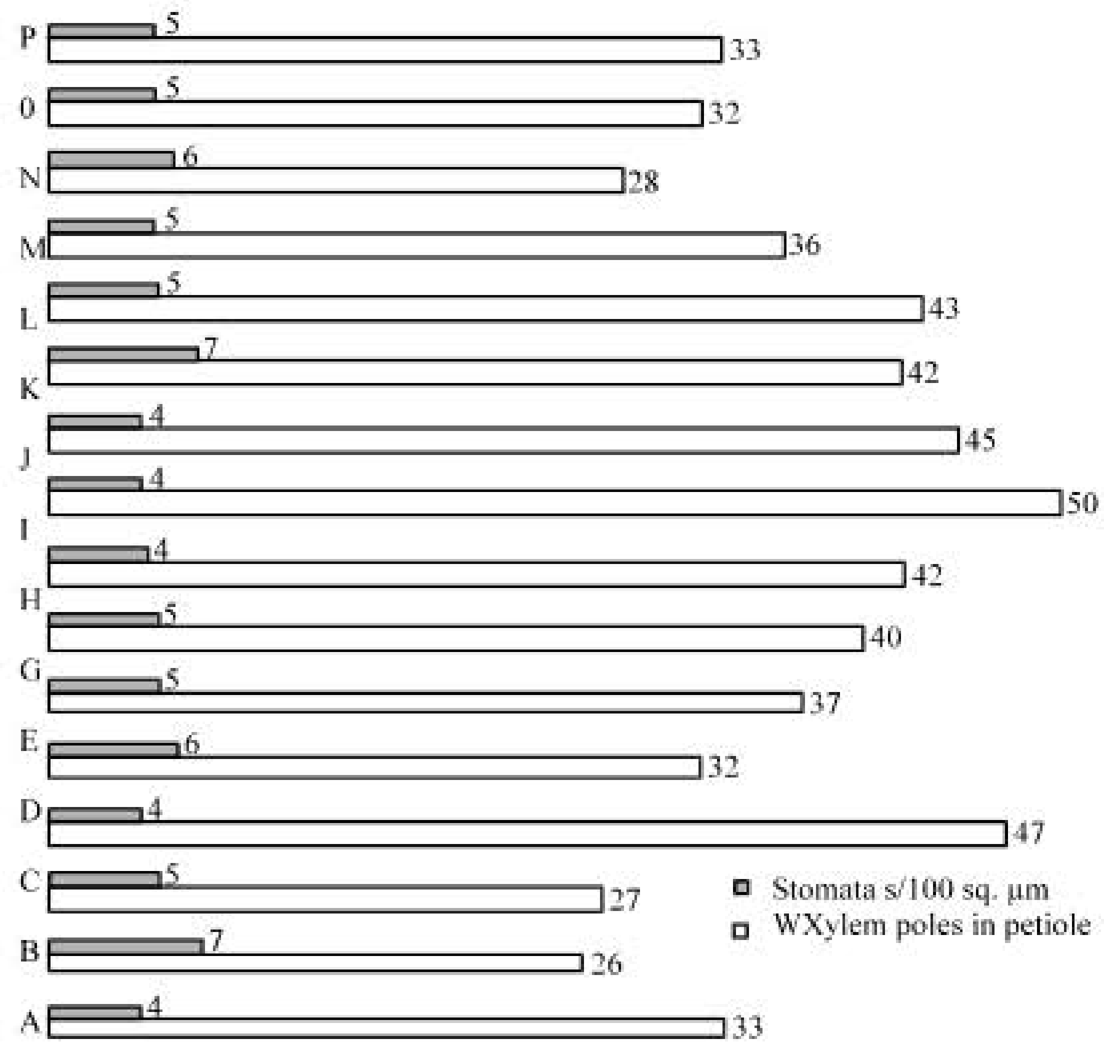


Fig. 3: Relation between root xylem poles and no of stomata on lower side of leaf

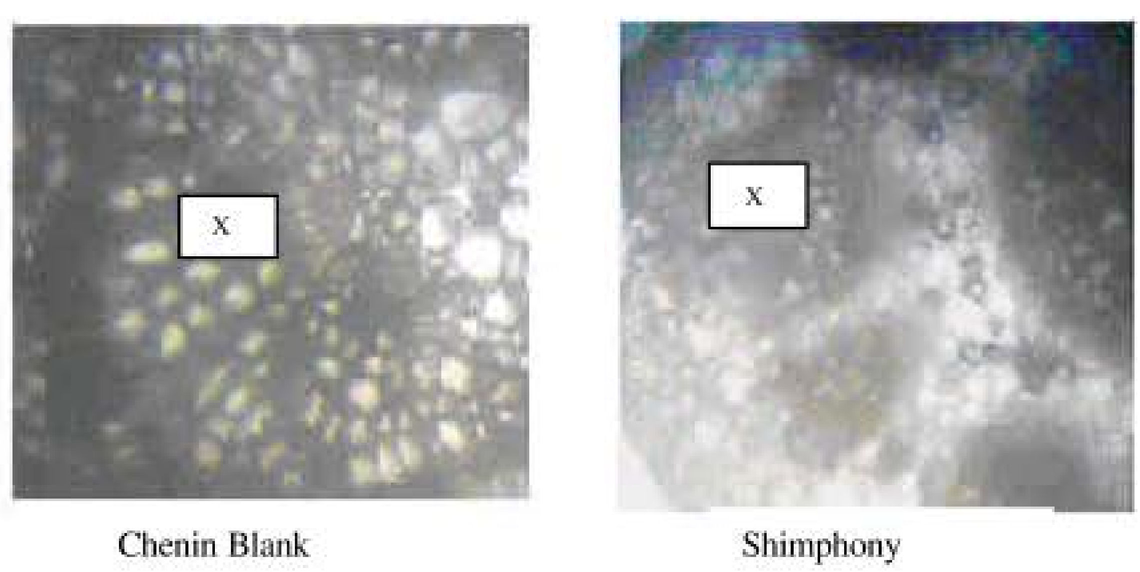


Fig. 4: TS of pstiole showing comparative xylem vessel diameter variation in stress tolerant (chenin blank) and stress susceptible (Shimphony) genotype (X-Xylem vessels)

Placement of xylem in roots also plays crucial role. The xylem poles placed at more distance cannot exchange water with each other through conduit pit membrane. Genotypes, which are closely placed, have that advantage. Table 3 shows that Genotypes Syrah (137 µm), Cabernet Sauvignon (89 µm) and root stock (81 µm) exhibited closely spaced xylem as compared to other genotypes under study. Similar findings were reported by Deshpande and Kulkarni (2005) while studying tomato root anatomy. McMichael *et al*. (1999) also reported higher number of xylem poles in cotton land races i.e., *Gossypium arboreum* and *herbaceum* as compared to *Gossypium hirsutum* which is more susceptible to water stress.

Wider the xylem poles more the ability to conduct water through conduit pit membrane which work as valve and are support strength to roots during stress situation (Hack and Sperry, 2001). Our results show similar trends. The xylem pole diameter was in range of (123-132 μm) in genotypes Chenin Blanc, Syrah, Chenin Blanc and Zinfandel as compared to lower range (81-98 μm) in susceptible genotypes. Under field condition, genotypes with diameter of higher magnitude were observed with advantage of luxurious vegetative growth and stay greenness.

Study of these anatomical parameters of leaf and roots helps in understanding the mechanism of water use efficiency in different genotypes. As compared to genotype K (Root Stock) which is having more feeder roots but thinner leaves and lower chlorophyll content, genotypes D (Chenin Blanc), I (Trebbiano Toscano) A (Syrah) exhibited desirable combination of leaf and root anatomical parameters with high chlorophyll content. These genotypes can prove to be ideal for grape growers in water scarcity area. Comparing the relation between numbers of xylem poles in roots with number of stomata per unit area of leaf on lower side (Fig. 3) reflects conclusion that more the number of xylem poles in roots lower the number of stomata in leaf per unit area. Genotype D (Chenin Blanc) exhibited superior anatomical features for water use efficiency amongst all genotypes studied. These results are parallel to the views and results obtained by Ansingkar *et al*. (2004).

Kariabasappa *et al*. (2006) reported highest average fruit yield and juice yield in genotypes I (Trebbiano) and D (Chenin Blanc) ranging from 20.75-22.63 t ha^{-1}; 1.411-1.448 hectoliter ha^{-1}. respectively (Average of 2 years on 4 and 5 year old wine yard). Genotypes B (Cabernet) and K (Vermentino) yields were in medium range from 13.65 to 16.28 t ha; 0.951-1.043 hectoliter ha^{-1}, respectively as compared with the remaining 14 grape genotypes. Interestingly the genotypes with higher yields are found to be with thicker leaves (225-273 μm) as compared to genotypes with medium yields (164- 184 μm). Genotypes with high yield magnitude are also having advantage of more number and wide xylem poles in roots, less number of stomata as desirable traits for improved water use efficiency. These positive traits were of lower magnitude in medium yielding genotypes.

Conclusion

Present investigation was an effort to study role of leaf and root anatomical parameters imparting increased water use efficiency and stay greenness to grape genotypes. Substantial importance of characters like long palisade mesophyll, lower number of stomata in leaves as well as more number of xylem poles in roots plays crucial role in increased water use efficiency resulting in higher photosyenthic ability and less evapotranspiration. Detailed genetic and molecular analysis of these traits will be helpful to further improve water use efficiency in grape genotypes for increased yield and fruit quality.

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