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Association of mineral imbalance with leaf chlorosis under saline irrigation in Sharad seedless grapes raised on Dog Ridge rootstock

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ABSTRACT

Under saline irrigation, development of leaf chlorosis/discoloration beginning from the upper leaves has been found to cause severe reduction in productivity and life span of the Sharad Seedless grape (*Vitis vinifera* L.) and its mutants grafted on Dog Ridge rootstock. In the present study, mineral accumulation in vine tissues over the years under saline irrigation and association of mineral imbalance with chlorosis/necrosis was investigated during the two pruning seasons in Sharad Seedless vines grafted on Dog Ridge rootstock. The contents of nitrogen, phosphorus, potassium, calcium and magnesium were significantly lower and sodium and chloride contents were found significantly higher in upper leaf lamina of symptomatic vines. Lamina N, P, K and Ca and Mg contents were positively correlated, whereas sodium and chloride contents were negatively correlated with chlorophyll content. Significant negative correlation was observed between chloride and phosphorus and nitrogen concentration in leaf tissues. Principal component analysis revealed a negative interaction between N, P, K and Na and Cl^- content in leaf tissues. Principal components (PCs) explained 91.7% of total variation in leaf lamina and were designated as PC1 (N+P+K-Na-Cl) and PC2 (N+P+K+Ca+Mg). Symptomatic vines accumulated higher sodium (>5.0 g^{-1}/kg) than potassium content in the leaf tissues and mean potassium content in symptomatic leaf lamina was <5.0 g^{-1}/kg. Increased accumulation of Na^+ and Cl^- in the chlorotic leaf tissues revealed that ability of Dog Ridge rootstock to exclude these salts diminished over time and long term studies are needed for identifying salt tolerant rootstocks.

Key words: *Vitis vinifera*, salinity, sodium toxicity, salt exclusion.

INTRODUCTION

In India, majority of the grape cultivation is confined to the semi-arid tracts of Maharashtra and Karnataka states. These areas often suffer from moisture and salinity stress and groundwater is major source of irrigation. Bhargava *et al.* (1) reported that 51% of the irrigation water samples taken from vineyards were not safe for the grape growing. Due to problems of salinity and moisture stress vines are raised on rootstocks. Dog Ridge is the preferred rootstock since vines grafted on it produce bigger size berries. Although the short term studies using the stock alone have shown that this rootstock is able to tolerate high salinity (Deshmukh *et al*., 3, Upreti and Murti, 17). Most of the salinity tolerance related studies in India have been conducted on Thompson Seedless and its mutants. However, scions also have an effect on salt accumulation irrespective of rootstock (Walker *et al*., 19) and level of salt induced chlorophyll degradation. Salt injury also depends on salt adaptation capacity which varies with scion genotype and the level of salinity (Siveretepe *et al*., 13; Grattan and Grieve, 5). Perennial plants including grapevines tend to accumulate salts in their organs and some of the elements like Na accumulate with time in lamina and petioles (Hepaksoy *et al.,* 6) and the results of short term studies without scion can prove misleading in long run. There is evidence that Salt exclusion capacity of rootstocks such as Ramsey (*Vitis champini*) diminishes when exposed to high salinity (Walker *et al*., 19).

Sharad Seedless grape is one of the important varieties of grapes in India. Under saline irrigation confusing leaf chlorosis/discoloration followed by necrosis is observed during the mid pruning season in many vineyards of this variety and its clones grafted on Dog Ridge rootstock. The severity of the symptoms is more during dry years. The symptoms differ from typical potassium deficiency that generally appears first on middle leaves and may appear first on basal or upper leaves depending upon the growth stage. Fruitfulness is reduced in vines which develop severe leaf chlorosis during the foundation pruning. The chlorosis (fading of green colour) begins from the upper (younger) leaves and progress towards basal leaves (Fig. 1A). Chlorosis is followed by formation of reddish/purple blotch near leaf margins and on upper as well as lower leaf surface. As the severity of the symptom increases, leaf margins dry up and either rolls up or down and entire leaf including veins develops purple/reddish discoloration (Fig. 1D).

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Fig. 1. Leaf chlorosis/discoloration development (A and B - initial chlorosis symptoms beginning from upper leaves at verasion stage.; C and D - leaf curling and sever chlorosis during cane maturity period)

Necrosis and premature leaf fall is also seen if a vine develop severe chlorosis during pruning season. Analysis of root zone soil was not conclusive and the present investigation was carried out to study the association of mineral deficiencies/toxicities with the development of varying severity of chlorosis under saline irrigation.

MATERIALS AND METHODS

The experimental vineyard was located at ICAR-National Research Centre for Grapes, Pune. The vines were drip irrigated and received 400-450 mm/ha irrigation in different years depending on the rainfall and as per availability of the irrigation water. The vines were raised on uniform package of practices during the last five years. The electrical conductivity of irrigation water ranged from 1.70 to 2.01 dS^{-1}/m, Ca from 29.4 to 32 mg/l; Mg from 91 to 100 mg/l and Na from 156.5 to 179.4 mg^{-1}/l; K from 0.88 to 0.95 mg^{-1}/l; Cl^- from 210.5 to 259.15 mg^{-1}/l. The soil samples were collected from the root zone up to 30 cm depth representing 20 cm radius from the point of dripper discharge at the time of leaf sampling (Table 1). The vineyard soil was clayey in texture (exhibited swelling and shrinkage behaviour), calcareous (free $CaCO_3$ ranging from 115-132 g^{-1}/kg), alkaline in reaction (pH varied from 7.90-8.12) organic carbon varied from 1.29 to 1.42 percent; EC (1:2) varied from 1.31 to 1.42 dS^{-1}/m. Amongst ammonium acetate extractable cations, calcium was most dominant (6740 to 7085 mg/kg) followed by magnesium (1815 to 2052 mg/kg), potassium (1140 to 1290 mg/kg) and Na (975 to 1050 mg/kg).

Leaf blade and petioles belonging to healthy and symptomatic leaves from 12 year old vines were used for diagnosis of the nutrient imbalance during two pruning seasons. Uniformity in sampling was maintained by selecting the leaves from the same node position. During foundation pruning season symptomatic and apparently healthy leaves were collected for analysis from different shoots present on a vine (90 days after foundation pruning). Leaf samples were also were collected from different vines with varying degree of chlorosis at veriason stage. On an average there were 15 fully expanded leaves on a shoot and leaves present from 3rd to 7th node position (lower leaves) and from 10th -14th node position (upper leaves) from the base were sampled separately (15 samples each) for diagnosis during foundation fruit pruning seasons since symptoms were more severe on upper leaves. Nitrogen in the tissues was estimated by Kjeldahl method and

Table 1. Nutrient content (g kg^{-1}) in leaf lamina and petioles of apparently healthy and symptomatic shoots during foundation pruning (n=15).

Class \ variable	Lamina				Petiole			
	Na	K	Ca	Mg	Na	K	Ca	Mg
Symptomatic	7.3	3.6	18.6	3.9	15.7	3.5	14.3	14.4
Healthy	2.8	6.4	28.6	6.8	14	11.8	14.6	15.1
Lambda	0.10	0.44	0.29	0.09	0.80	0.10	0.99	0.94
F value	241.3	36.1	70.2	292.6	7.2	242.5	0.2	1.8
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0122	< 0.0001	NS	NS

another portion of tissue samples was digested for estimation of P, K, Ca, Mg and Na (Kalra, 7). Phosphorus was estimated colorimetrically and an atomic absorption spectrophotometer (PerkinElmer ASS 400) was used to estimate K and Na in emission mode and Ca and Mg in absorption mode. The chloride in the tissue extract was determined by using flow injection system (Skalar make San system). The data with respect to Zn, Fe, Cu and Mn were not found associated with the disorder hence not presented here. Chlorophyll content was estimated by using DMSO in leaf discs from the margins of both healthy and affected vines. Significance of difference in mean nutrient concentration in non- affected (apparently healthy) and symptomatic leaves was tested using students' 't' test at a 0.05 probability level For correlation studies data from upper and lower leaves from fruit pruning studies was pooled together.

RESULTS AND DISCUSSION

Chlorosis was not observed by irrigating the vines with saline water the during the first three years. In the fourth year, slight chlorosis developed during late cane maturity in foundation pruning season and initiation of verasion stage in fruit pruning season During 5th year of irrigation with saline irrigation severe chlorosis followed by necrosis developed in both the pruning seasons. Vine nutritional status was monitored each year. Saline irrigation over the years resulted in significant increase in accumulation of Na and Cl^- in leaf tissues. In 2008-09 cropping season sodium and chloride concentrations in leaf lamina were 2.50 and 1.43 g/kg, respectively, and their concentration increased to 3.48 and 2.50 g/kg in 2011-12 and 4.18 and 3.6 g/kg, respectively, in 2011-13 cropping season at full bloom stage. The chlorophyll content (Table 3) in symptomatic leaves was significantly lower (1.53 mg/g) than apparently healthy leaves (2.83 mg/g). Development of chlorosis reduced the productivity of vines significantly. Number of fruitful canes reduced from 90 per cent in 2008-2009 cropping season to 62% in 2011-12 and reduced drastically to 15 per cent in vines which developed severe chlorosis during cane maturity period in 2012-13. Salt stress is known to reduce the life span of leaves and reduction in chlorophyll contents in the leaves by salinity has also been reported (Sivritepe *et al.*, 13). Salt stress causes accelerated senescence and, as a consequence, chlorophyll degradation. Sharma *et al*. (10) have reported reduced fruitfulness under saline irrigation in field grown 'Thompson Seedless' vines grafted on Dog Ridge rootstock exhibiting leaf necrosis and blackening.

Discriminant function analysis revealed significant differences for Ca, Mg and Na in leaf lamina and K concentration in petiole between apparently healthy and symptomatic tissue during foundation pruning season. Significantly higher concentration of Na (7.3 g kg^{-1}) and significantly lower concentrations of K (3.6 g kg^{-1}), Ca (18.6 g kg^{-1}) and Mg (3.9 g kg^{-1}) were observed in symptomatic leaf lamina (Table 3). Sodium concentration in petioles was also higher than potassium.

Discriminant function analysis during fruit pruning season showed the discrimination was highly significant for all the nutrients, however, K followed by Na discriminated the most between the two groups in the most in leaf lamina. Symptomatic leaf lamina contained significantly lower N (5.5 g kg^{-1}), P (2.2 kg^{-1}), K (4.1 g kg^{-1}), Ca (21.0 g kg^{-1}) and Mg (5.6 g kg^{-1}) concentration and significantly higher Na (8.1 g kg^{-1}) and Cl^- concentration (7.7 g kg^{-1}) than apparently healthy leaf (Table 3). Similar trend was observed in petioles with respect to nutrient content except for Mg which was higher in symptomatic petiole and Na followed by Cl^-, N and K discriminated the most between symptomatic and apparently healthy vines (Table 4).

Reduction in N, P, K, Ca and Mg in the present study is contradictory to short term study of Sivritepe *et al.* (13) conducted in pots using 110R rootstock. These authors have reported a significant elevation in N, P, K, Ca, and Mg in response to NaCl treatments. It may also be due to stock/ scion genotype differences in since scions also have an effect on salt accumulation irrespective of rootstocks (Walker *et al*., 19). Further, salinity effects in grapevines are accumulative and the results of short term studies may not be realistic. High soluble salt content of the irrigation water has been found associated with a deficiency of nitrogen, phosphorus and potassium in the leaves and petioles of vines (Mataix *et al*., 8). Although, chloride content was significantly higher in symptomatic leaves (lamina and petiole) and exhibited a negative correlation with chlorophyll content (r=0.712), but not high enough in the present to cause necrosis and leaf necrosis in the present study. Further, its concentration was less in upper leaf lamina (5.3 to 6.9 g kg^{-1}) than in lower leaf lamina where the symptoms were less severe (8.2 to 10.7 g kg^{-1}). Chloride content in petioles from symptomatic leaves was also below excessive level of 15.0 g kg^{-1} for grapevines. Chloride concentration more than 18.2 g kg^{-1} in leaf blades is considered excessive at veraison stage (Weir and Cresswell, 20). Although, Mg concentration was also reduced appreciably in symptomatic leaves it cannot be associated with the chlorosis symptoms reported in the present study since Mg content was higher than 2.5 g kg^{-1} in all the samples and less than 2.5 g kg^{-1} of Mg in petioles is associated with the development of leaf chlorosis (Wolf, 21).

Amongst the base cations, highest reduction was observed in potassium during both the sampling seasons. Sodium concentration in symptomatic leaf lamina was >5.0 g kg^{-1} and potassium concentration <5.0 g kg^{-1} in all the samples; whereas in apparently healthy leaf lamina K concentration was higher than Na during both seasons (Table 2 and 3). Higher accumulation of sodium in symptomatic leaf tissues resulted in narrow K/Na, Ca/Na and Mg/Na ratio. Increase in NaCl content of the nutrient solution decreased K, Ca and Mg contents of the various plant organs (Garcia and Charbaji, 4).

In order to identify the meaningful variables the data on N, P, K, Ca, Mg and Cl concentrations in leaf lamina and petiole were further subjected to a principal component analysis using ones as prior communality estimates. Only two components displayed eigenvalues greater than 1 and were retained. After varimax rotation two principal components (PC) explained 91.7% of total variation in leaf lamina and were designated as PC1 (N+P+K-Na-Cl) and PC2 (N+P+K+Ca+Mg) and the corresponding factor loadings are presented in

Table 2. Nutrient concentration (g kg^{-1}), nutrient ratios and chlorophyll content (mg^{-1}/g) in blades during fruiting season (n=30).

	Na	K	Ca	Mg	Cl	P	N	K/Na	Ca/Na	Mg/Na	CHL
Symptomatic lamina											
Mean	8.1	4.1	21.0	5.6	7.7	2.3	17.3	0.30	1.34	3.00	1.55
Min	7.0	3.5	16.4	3.6	5.3	1.9	15.7	0.22	0.82	2.15	1.23
Max	9.4	5.1	25.6	8.3	10.7	2.7	18.5	0.41	2.18	4.12	1.80
SDEV	0.7	0.5	3.5	1.6	1.8	0.2	0.8	0.05	0.45	0.62	0.15
Apparently healthy lamina											
Mean	3.1	9.1	24.0	6.7	3.3	3	19.9	1.86	4.39	9.47	2.83
Min	2.0	7.6	20.0	5.5	1.9	2.5	18.9	1.30	2.84	6.32	2.34
Max	4.8	11	27.1	8.7	4.6	3.6	21.2	2.65	5.95	12.51	3.34
SD	1.0	0.8	2.3	0.8	0.9	0.3	0.6	0.51	1.06	2.29	0.25
p-value	<0.0001	<0.0001	0.0003	0.002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Lambda	0.11	0.06	0.79	0.85	0.28	0.36	0.22				
F value	486.2	893.2	15.02	10.5	146.8	101.1	200.4				

Table 3. Nutrient content (g/kg), nutrient ratios in petiole during fruiting season (n=30).

	Na	K	Ca	Mg	Cl	P	N	K/Na	Ca/Na	Mg/Na
Symptomatic petiole										
Mean	18.3	5.4	14.4	11.0	11.5	2.2	5.5	0.17	1.16	0.91
Minimum	15.4	2.1	13.0	9.8	8.5	1.2	5.2	0.08	0.97	0.72
Maximum	21.3	10.9	16.8	12	13.4	3.5	6.0	0.33	1.46	1.1
SD	1.7	3.1	0.9	0.7	1.2	0.6	0.2	0.09	0.13	0.11
Apparently healthy petiole										
Mean	11.7	16.6	15.5	9.7	6.0	3.5	6.7	0.85	1.6	1.53
Minimum	9.6	10.5	13.7	7.6	4.3	2.3	6.0	0.49	1.08	1.27
Maximum	14.0	22.6	17.5	11.5	7.5	5.2	7.6	1.39	2.23	1.98
SD	1.1	4.6	1.0	1.3	1.0	0.9	0.4	0.3	0.3	0.2
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Lambda	0.16	0.32	0.75	0.69	0.14	0.57	0.26			
F value	315.6	121.5	19.6	25.9	367.6	43.6	162.9			

Table 4. Percentage of variance after varimax rotation in lamina.

	F1	F2	F3	F4	F5	F6	F7
Variability (%)	50.37	41.30	4.53	1.60	1.09	0.70	0.39
Cumulative %	50.37	91.67	96.21	97.81	98.90	99.61	100.000
Factor loadings after varimax rotation:							
	PC1	PC2					
Na	-0.929	-0.216					
K	0.875	0.450					
Ca	0.128	0.970					
Mg	0.084	0.951					
Cl	-0.971	0.167					
P	0.639	0.619					
N	0.723	0.621					

Table 5. Percentage of variance after varimax rotation in petiole.

	F1	F2	F3	F4	F5	F6	F7
Variability (%)	49.54	32.27	9.95	5.21	1.53	0.93	0.57
Cumulative (%)	49.54	81.81	91.76	96.97	98.50	99.43	100.00
Factor loadings after varimax rotation:							
	PC1	PC2					
Na	-0.598	0.690					
K	0.931	-0.197					
Ca	0.590	-0.250					
Mg	0.054	0.911					
Cl	-0.478	0.848					
P	0.939	0.036					
N	0.884	-0.363					

Table 5. In petioles after varimax rotation two principal components (PC) explained 81.8% of total variation and were designated as PC1 (K+N+P+Ca-Na-Cl) and PC2 (Na+Mg+Cl) and the corresponding factor loadings are presented in Table 6. In both the plant tissues Na and chloride exhibited negative interactions with N, P and K as is evident from negative factor loadings. The Na and K antagonism is quite strong in grapevines as increasing levels of Na in growth media reduces the K concentration in different vine parts (Gracia and Charbaji, 4).

Tissue Na and chloride contents exhibited a strong negative and significant correlation with chlorophyll content, whereas N, P, and K contents exhibited a strong positive correlation. Decrease in nitrogen and P concentration in symptomatic leaf tissues was due probably to NO_3^{-} and Cl^- antagonism and a significant inverse correlation between P and Cl^- content in the tissues in lamina (r= -0.503) and petioles (r= -0.403). A significant inverse correlation was also observed between N and Cl^- content in the tissues in lamina (r= -0.577) and petioles (r= -0.713). Inverse relationship of N and P with chloride content indicated the need for maintaining of adequate levels of these nutrients for proper vine growth under saline irrigation to reduce the toxic effects of specific ions like chloride. High soluble salt content of the irrigation water has been found associated with a deficiency of nitrogen, phosphorus and potassium in the leaves and petioles of vines (Mataix *et al.*, 8). Principal component analysis and significant negative correlation between the two elements in lamina (r= -0.886) as well petioles (r= -0.678) revealed a strong antagonism between K and Na. The potential for Na as a partial substitute for K in some, but not all plant species, is well documented. For cell extension, accumulation of K in vacuoles creates the necessary osmotic potential and high mobility of the osmoticum is needed, so only ions like Na can replace K. The Na and K antagonism is quite strong in grapevines as increasing levels of Na in growth media reduces the K concentration in different vine parts (Gracia and Charbaji, 4). Salt stress causes accelerated senescence and, as a consequence, chlorophyll degradation and maintenance of high adequate levels of K is essential for plant survival in saline habitat. Sodicity tolerance in grapevines is related to K/Na ratio in the leaf and not with absolute contents of sodium. Salt tolerant varieties maintained higher K levels than the sensitive ones (Troncoso *et al*., 15). Calcium and magnesium were also negatively correlated with sodium in the present study but the correlation was weak.

Sodium content in the lamina of symptomatic leaf samples ranged from 6.0 to 8.8 g kg^{-1} and according to Stevens (12) leaf Na concentrations greater than 250 mmol/kg (5.75 g kg^{-1}) are associated with leaf necrosis of grapevines. Visual symptoms of leaf margin discoloration (black or dark purple staining) and necrosis have been associated with blade levels above 5.0 g kg^{-1} Na (Christinsen, 2; Sharma *et al.*, 10; Sharma *et al.*, 11 and Upadhyay *et al.*, 16). Ramsey rootstock which also belongs to *Vitis champini* could not prevent damaging accumulation of salt in 'Sultana' vines, 17.90 g kg^{-1} Na and 20.2 g kg^{-1} chloride were found in petioles under Australian conditions where salinity was high (Nagarajah, 9). Toxic concentrations of Na in leaves were also observed in 'Cabernet Sauvignon' vines grafted on Salt Creek (*Vitis champini*) by Hepaksoy *et al.* (6) and in field grown 'Thompson Seedlees" grafted on Dog Ridge rootstock (Upadhyay *et al*., 16). There is evidence that salt exclusion capacity of rootstocks such as Ramsey diminishes when exposed to high salinities (Walker *et al*., 19). The toxic effects of salinity are cumulative, with tissue concentrations of NaCl generally increasing with duration of exposure. In our study apart from Na, chloride concentrations in some samples of symptomatic leaf lamina (>1.0 g kg^{-1}) and petioles (>13.0 g kg^{-1}) were near toxic range. These results of our study strengthens the view that ability of rootstocks to exclude salts diminishes when exposed to high salinities (Tregeagle *et al*., 14; Walker *et al*., 19; and Walker, 18)

It is evident that Dog Ridge rootstock could not restrict sodium uptake below toxic range under long-term exposure to saline irrigation and the leaf chlorosis and necrosis was associated with toxic accumulation of sodium in leaf lamina. Maintenance of adequate levels nutrients, particularly potassium, is essential to reduce the toxic effects of specific ions like sodium for vine survival under saline irrigation. High accumulation of Na^+ and Cl^- in the chlorotic leaf tissues revealed that the salinity effects are accumulative and the ability of Dog Ridge rootstock to exclude salts diminished over the time. Since salinity effects are accumulative, long-term research is needed to identify rootstocks with sustained capacity for both Cl^- and Na^+ exclusion.

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