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Studies on black leaf symptom development and its impact on nutrient profile and fruitfulness in Thompson Seedless grapevines grafted on Dogridge rootstock

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ABSTRACT

Salinity issues in the Indian vineyards have led to a number of nutritional imbalances leading to the decline in productivity of grapes. Black leaf is one such disorder which is adversely affecting the vineyards productivity. The vines having black leaf symptoms had significantly lesser number of fruitful canes than healthy ones. The symptom development reduced the bunch weight significantly. Chlorophyll content was significantly lower in the symptomatic leaves. The leaf analysis of symptomatic vines revealed significantly lower K, *i.e.* 0.35 % in petiole and 0.31% in leaf blade compared to 1.39% K in petioles and 0.59% in leaf blade in healthy vines. However, the leaf blades analysis of symptomatic vines revealed significantly higher Na (1.10%) compared to 0.46% in healthy vines. Lower K and higher Na content and narrow K:Na ratio was found to be associated with the symptom development. Phosphorus content was significantly reduced in the affected tissues. Chloride content in the tissue was not high enough to cause leaf blackening and necrosis. The micronutrients Zn, Fe, Cu and Mn were also not found associated with the development of symptom. High content of Na in vine tissues (>1.0%) grafted on Dogridge rootstock suggests that this rootstock could not exclude Na under saline irrigation.

Key words: Dogridge, Thompson Seedless, black leaf, salinity, sodium, potassium.

INTRODUCTION

Most of the vineyards in Maharashtra suffer from varying degree of salinity. Thus, rootstocks have become the mainstay in raising vineyards. Based on its ability to withstand salinity and drought, Dogridge has become the most commonly used rootstock. Deshmukh *et al.* (1) have reported that Dogridge could tolerate up to 6.5 dS/m NaCl salinity. However, ability of Dogridge rootstock to tolerate sodicity has become questionable as leaf blackening and necrosis have been observed by the authors in many Indian vineyards planted on calcareous soils and irrigated with saline water having EC as low as 1.30 dS/m. Depending upon soil, irrigation water and climatic conditions such symptoms may not appear during the initial two - three years. However, as the age of the vines advances, the symptoms start appearing. The symptomatic leaves initially show brownish/blackish brown discolouration on the margins in the form of an irregular shaped spot (blotch), which turns black, and later on the symptoms progress as marginal burning of the leaves (Fig. 1). The affected vines become woody and unproductive or produce small bunches depending upon the severity of the symptoms on a vine. The symptom development described here, however, differs from typical K deficiency, which included the location of leaves on a shoot where the symptoms were first noticed. The symptoms appear on both upper as well as lower surface of the leaves in contrast to the black leaf symptoms described earlier. The symptoms sometimes appear first on younger leaves and sometimes on older leaves on a shoot.

Fig. 1. Brownish/blackish brown discolouration on the margins in the form of an irregular shaped spot (blotch), which turns black followed by marginal necrosis.

There has not been general consensus regarding the causes of the disorder. Black leaf ("K deficiency") was most prominent at low K fertility and high yielding

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vineyards (Roberts and Ahmedullah, 12). According to Smithyman *et al.* (16), high crop levels and leaf K deficiency were not primary causes of black leaf in Concord grapes.

It is difficult to separate the effects of nutrient deficiency or toxicity particularly between Na and Cl by visual diagnosis. Marginal chlorosis and necrosis are also caused by the K deficiency as well as Na toxicity. The soil analysis carried after the appearance of the symptoms was not conclusive. Therefore, the present investigation was carried out to study the association of nutrients with the development of the black leaf nutrient disorder and the effect of disorder on fruitfulness of the canes and subsequently the yield of vines.

MATERIALS AND METHODS

The plant materials for this study were located in the vineyards of the National Research Centre for Grapes, Pune. The symptoms did not always develop on all the shoots on a vine in a season, variations in the tissue (petioles and leaf blade) nutrient contents in the healthy (visual No. symptoms) and affected shoots (symptomatic) from such vines were studied from different experimental blocks of the Research Farm. The healthy and affected vines located, randomly at 6 feet apart in the vineyards, received uniform fertilizer and irrigation. The soils of the vineyards were calcareous, alkaline in reaction exhibiting swelling and shrinkage behaviour. Vines were irrigated with water having EC = 2.0 dS/m; pH = 7.78; Ca^{2+} = 29.4 ppm; Mg^{2+} = 91ppm; Na^{2+} = 179.4 ppm; K = 0.95 ppm; Cl^- = 259.15 ppm; HCO_3^{2-} = 488 ppm, SO_4^- = 125.14 ppm and 37.0 ppm nitrate - N. Vines received uniform 160 kg N, 50 kg P_2O_5 and 160 K_2O on per hectare basis during the fruiting season. The soil samples were collected from the root zone up to 30 cm depth at the time of leaf sampling and at the time of tissue sampling. The exchangeable Na content in soil ranged from 1050 to 1175 ppm whereas exchangeable K content ranged from 375 to 475 ppm. The soil analysis carried after the appearance of the symptoms was not conclusive as the symptoms were observed on one arm of the vine and the shoots on other arms did not express the symptom. Hence, tissue analysis was carried out.

The tissues were sampled in February 2006 at the time of fruit ripening stage from Thompson Seedless vines grafted on Dogridge rootstock. Each set of sample consisted of three vines for both healthy as well as symptomatic vines. The data with respect to bunch weight and bunch numbers on healthy and affected canes were also recorded. In case of affected leaf samples, the dead portions of lamina were removed before analysis, it was difficult to. The nutrient content was studied after washing, oven drying at 70°C and grinding in cyclotec sample mill (Foss Tecator make). Nitrogen in the tissues was estimated by Kjeldahl method using Gerhardt semi-automatic distillation apparatus (VAPODEST 30). Another set of tissue samples was digested in block digestor in H_2SO_4: H_2O_2 mixture for estimation of P, K, Ca, Mg and Na. Phosphorus was estimated colorimerically by vanadomolybdate method. An atomic absorption spectrophotometer (Perkin Elmer AAnalyst 100) 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 San system). The data with respect to Ca, Zn, Fe, Cu and Mn were not found associated with the disorder hence not presented here.

The fruitfulness of the vines was compared by counting the number of canes per vine having well formed clusters/bunches after pruning. The chlorophyll was estimated by using DMSO data were statistically analyzed using Students 't' test and correlation coefficient worked out between chloride contents and nutrient analysed (Gomez and Gomez, 3).

RESULTS AND DISCUSSION

The vines suffering from black leaf symptom development showed significant reduction in bunch weight, berry weight, chlorophyll content in symptomatic vine parts, *i.e.* shoots and the fruitfulness in symptomatic vines was significantly reduced (Table 1). Black leaf symptom development was associated with a loss of chlorophyll and reduced photosynthetic ability suggesting damage to the photosynthetic system (Smithyman *et al.,* 16). In the vineyards under investigation, Na was found in higher quantity in the leaf and petiole.

Data on variations in the petiole and blade composition from the healthy and affected shoots are presented in Tables 2 and 3. Tissue (petioles and blades) sampled from affected shoots of the vines contained significantly lower K and P and significantly higher Na and chloride content compared to tissue samples from healthy shoots. Mean P, K, Mg, Na and chloride content in healthy petiole samples were 0.36, 1.39, 0.69 1.28 and 0.95 % respectively, whereas in affected petioles the mean P, K, Mg, Na and chloride were 0.26, 0.35, 1.46 and 1.12%, respectively. In leaf blades, mean P, K, Mg, Na and chloride content in healthy petiole were 0.27, 0.59, 0.29, 0.46 and 0.31 % respectively, whereas in affected petioles the mean P, K, Mg, Na and chloride were 0.18, 0.31,0.24, 1.10 and 0.49 %, respectively.

Table 1. Bunch weight, TSS and chlorophyll content in leaf blades of the healthy and affected shoots of Thompson Seedless vines.

Vineyard	Fruitful cane (%)		Average bunch weight (g)		TSS (°Brix)		Chlorophyll content on fresh leaf weight basis (mg/gfw)	
No.	H	A	H	A	H	A	H	A
1.	75	56	255	188	21.3	20.9	2.31	1.75
2.	80	52	236	201	21.5	21.4	2.13	1.77
3.	76	55	265	185	21.7	20.1	2.45	1.88
4.	82	60	235	170	21.3	21.2	2.67	1.79
5	78	46	242	205	21.2	20.8	2.19	1.59
6.	78	56	255	178	21.8	21.3	2.15	1.65
7.	76	58	258	195	21.3	21	2.35	1.89
8.	85	63	275	213	21.2	21.3	2.22	1.70
9	80	62	235	175	21.4	21.2	2.26	1.82
10	79	63	240	182	21.3	20.9	2.36	1.69
11	72	51	229	165	22.3	21.0	2.08	1.72
12	79	56	228	175	21.9	20.9	2.13	1.65
Mean	78.9	57.1	246.08	186.00	21.52	21.00	2.28	1.74
't' value	11.68**		9.37**	3.53*	9.21**			

H = Visually healthy leaf, A = Affected/symptomatic leaf; * significance at 5 % (p = 0.05);** Significance at 1% (p = 0.01)

Table 2. Nutrient content in petioles of healthy and affected shoots of Thompson Seedless vines.

Vineyard	N (%)		P (%)		Ka (%)		Mg (%)		Na (%)		Cl$^-$ (%)		K/Na	
No.	H	A	H	A	H	A	H	A	H	A	H	A	H	A
1.	0.56	0.46	0.39	0.25	1.75	0.38	0.67	0.75	1.23	1.60	0.82	1.17	1.42	0.24
2.	0.60	0.49	0.46	0.28	2.42	0.27	0.61	0.57	1.11	1.56	0.78	0.89	2.18	0.17
3.	0.42	0.49	0.33	c22	1.31	0.39	0.65	0.72	1.42	1.36	1.07	1.17	0.92	0.29
4.	0.49	0.46	0.32	0.28	1.19	0.32	0.61	0.54	1.39	1.44	0.82	0.92	0.86	0.22
5	0.57	0.53	0.43	0.24	1.35	0.43	0.71	0.75	1.44	1.52	1.17	1.31	0.94	0.28
6.	0.62	0.52	0.44	0.27	1.26	0.39	0.69	0.76	1.36	1.48	0.90	1.25	0.93	0.26
7.	0.58	0.51	0.32	0.30	1.38	0.40	0.65	0.60	1.40	1.56	0.82	1.17	0.99	0.26
8.	0.52	0.54	0.35	0.25	1.56	0.35	0.60	0.68	1.31	1.48	1.05	1.28	1.19	0.24
9	0.51	0.46	0.34	0.28	1.65	0.42	0.68	0.56	1.27	1.62	0.85	0.99	1.30	0.26
10	0.54	0.49	0.36	0.26	1.28	0.36	0.73	0.78	1.35	1.68	1.05	1.12	0.95	0.21
11	0.52	0.53	0.28	0.23	0.83	0.20	0.83	0.87	1.06	1.26	1.07	1.07	0.78	0.16
12	0.49	0.44	0.30	0.26	0.73	0.25	0.80	0.86	1.00	0.94	1.00	1.07	0.73	0.27
Mean	0.53	0.49	0.36	0.26	1.39	0.35	0.69	0.70	1.28	1.46	0.95	1.12	1.10	0.24
't' value	-2.10*		5.30**		7.78**		-0.43		-2.39*		-2.94*		7.11* *	

H = Visually healthy leaf, A = Affected/symptomatic leaf, ** Significant at (p = 0.01%)

High Na content in leaf as a result of saline irrigation was responsible for low K and leaf blackening in affected vines parts. Leaf blades in affected vine shoots contained toxic concentrations of sodium. According to Stevens (17), leaf sodium concentrations greater than 250 mmol/kg dry weight were associated with leaf necrosis. According to Nagarajah (10), values of 0.5% sodium would have toxic effect on Sultana vines. High soluble salt content of the irrigation water has been found associated with an accumulation of sodium, calcium and magnesium in the leaves and petioles, and a deficiency of nitrogen, phosphorus and potassium (Mataix *et al*., 9). Addition of NaCl in low doses in growth media decreases the K intake (Gracia and Charbaji, 5) and increasing levels of Na in growth media reduces the K concentration in different vine parts (Gracia and Charbaji, 4). The K: Na ratio was also significantly higher in both types of the tissues collected from healthy shoots compared to symptomatic shoots. The ratio of K: Na ranged from 0.73 to 2.18 in petioles of healthy leaves and 0.16 to 0.24 in affected ones. In healthy leaf blade samples, the K: Na ratio ranged from 1.04 to 1.84 whereas in affected samples it ranged from 0.17 to 0.46. Maintenance of high adequate levels of K is essential for plant survival in saline habitat (Grattan and Grieve, 6). High K: Na ratio in leaves, canes and roots was found to be associated with salinity tolerance in Thompson Seedless grape (Samra, 13).

As is evident from the Na concentrations in the vine tissues DogRidge (*Vitis champini*) rootstock couldn't prevent Na and chloride accumulation in the vine tissues under saline irrigation. Perennial plants unlike annual crops tend to accumulate salts in their organs leading to reduction in vegetative growth and yield over a time. Some of the elements like Na accumulate with time in the lamina and petiole (Hepaksoy *et al.*, 7). Further, salt exclusion capacity of rootstocks diminishes when exposed to high salinities (Walker *et al*., 18). Ramsey (*Vitis champini*) rootstock also could not prevent damaging accumulation of salt in Sultana vines where salinity was high and the petiole values being 1.786% Na and 2.02% chloride (Nagarajah,10). DRIS indices demonstrated a greater affinity for Na by Thompson Seedless vines raised on DogRidge rootstock (Sharma *et al.*, 15). Earlier studies on different stock scion combination also revealed that DogRidge could not prevent Na accumulation in Tas-A-Ganesh vines (Sharma and Upadhyay, 14).

The low P content in the affected tissues can not be associated with blackening of the blades as some of the healthy samples had lower P content than the affected ones (Table 3). Petiole chloride content exhibited significantly negative correlation with petiole P (r = -0.5376) and K (r = -0.5980). Blade chloride content was significantly and negatively correlated with petiole P (r = -0.6787) and K (r = -0.8182), and positively with Na (r = 0.7491). Higher tissue chloride content in the affected tissues could be responsible for lower P content in the affected tissues. However, the chloride content in leaf blade and petiole were not high enough to cause necrosis and leaf blackening. Chloride levels above 1.2% in leaf laminae are considered to be toxic (Ehlig, 2) whereas above 1.5% in petioles are considered to be excessive (Reuter and Robinson,11). Potassium and phosphorus contents in tissues were inversely related to chloride content. Garcia and Charbaji (5) reported a decrease in P concentrations

Table 3. Nutrient content in leaf lamina of healthy and affected shoots of Thompson Seedless vines at fruit ripening stage.

Vineyard number	N (%)		P (%)		K (%)		Mg (%)		Na (%)		Cl^- (%)		K/Na	
	H	A	H	A	H	A	H	A	H	A	H	A	H	A
1.	2.25	1.82	0.23	0.15	0.70	0.32	0.29	0.21	0.38	1.05	0.2	0.46	1.84	0.30
2.	1.69	1.58	0.29	0.15	0.61	0.21	0.28	0.21	0.43	1.23	0.2	0.49	1.42	0.17
3.	1.65	1.65	0.27	0.17	0.50	0.32	0.25	0.22	0.48	1.15	0.43	0.5	1.04	0.28
4.	1.87	1.89	0.29	0.24	0.73	0.38	0.28	0.25	0.50	0.83	0.36	0.48	1.46	0.46
5	1.74	1.61	0.30	0.19	0.55	0.32	0.27	0.23	0.45	1.22	0.39	0.46	1.22	0.26
6.	1.86	1.77	0.26	0.16	0.58	0.36	0.25	0.23	0.45	1.26	0.22	0.43	1.29	0.29
7.	2.06	1.74	0.29	0.15	0.52	0.34	0.26	0.22	0.48	1.15	0.22	0.54	1.08	0.30
8.	1.82	1.67	0.27	0.17	0.71	0.30	0.27	0.22	0.42	1.06	0.26	0.47	1.69	0.28
9	1.78	1.82	0.24	0.20	0.61	0.26	0.30	0.25	0.54	1.25	0.27	0.45	1.13	0.21
10	1.98	1.83	0.30	0.23	0.52	0.36	0.29	0.22	0.50	0.96	0.26	0.5	1.04	0.38
11	1.68	1.58	0.26	0.21	0.52	0.31	0.32	0.31	0.47	1.12	0.43	0.52	1.12	0.28
12	1.75	1.72	0.24	0.18	0.55	0.20	0.45	0.36	0.43	0.92	0.42	0.56	1.28	0.22
Mean	1.84	1.72	0.27	0.18	0.59	0.31	0.29	0.24	0.46	1.10	0.31	0.49	1.30	0.28
't' value	NS		7.15**		9.49**		2.25*		14.48**		-5.99**		12.47**	

H = Visually healthy leaf, A = Affected/symptomatic leaf; *significance at 5 % (p = 0.05); **Significance at 1% (p = 0.01).

with increased concentrations of NaCl in all plant parts. Low P content in the affected vine parts may also be due to reduced K content showing possible synergistic relationship between potassium and phosphorus.

The findings of the present study revealed that low potassium and high Na content, particularly in blades, caused leaf blackening and necrosis. Dogridge rootstock couldn't prevent accumulation of toxic concentrations of Na in Thompson Seedless vine tissues under saline irrigation although the rootstock has been introduced in India to cope up with salinity and drought stress. Chloride content was not associated with leaf blackening and necrosis in leaf blades as the chloride content was not high enough to cause leaf necrosis.

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