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Rootstock influenced metabolite changes during progressive salt stress conditions in Thompson Seedless grape

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ABSTRACT

Grape (*Vitis vinifera* L.) is a perennial fruit crop grown in the tropical and subtropical agro-climatic zones of India and suffers from moisture and salinity stress. In the present study, the effect of rootstock on metabolite changes in 'Thompson Seedless' under salt stress was studied. Grafted and own-rooted vines showed differential response to salt stress. Among 24 metabolites, proline, ornithine, and norleucine significantly accumulated in salt-treated own-rooted vines as compared to grafted vines whereas phenolics content of grafted vines was higher than the own-rooted vines. Accumulation of Na^+ and Cl^- were observed in the leaves of stressed own-rooted vines within three days of salt treatment as compared to 15 days in grafted vines. Principal component analysis revealed rootstock specific response to the stress

Key words: *Vitis vinifera*, amino acids, phenolics.

INTRODUCTION

Grape (*Vitis vinifera* L.) in India is grown in the tropical and subtropical agro-climatic zones over an area of 139 thousand ha with an annual production of 2.92 million MT (NHB database 2017-18). The grape grown under semi-arid tracts suffers from moisture and salinity stress. Salinity adversely affects the quality and yield of the grapes when grown on own-roots. Hence, use of salt-tolerant rootstocks gained impetus in major grape growing regions of India. The chief contributing factor towards salinity is ground water. In Maharashtra, a major grape growing region in India, more than 50% samples of irrigation water had EC more than 1.0 dS m^{-1}, where growth is restricted due to salinity (Bhargava *et al.*, 2). The Na^+ content also increased from 0.20 - 10.78 meq l^{-1} during 1980 - 1981 to 0.20 - 70.74 meq l^{-1} during 1999 - 2004. Consequences of salinity become more significant when grapes are grown in hot, dry climate compared to cool, humid climate (Walker *et al.*, 14).

Plants adapt to salinity by three mechanisms; osmotic stress tolerance, Na^+ exclusion and tissue tolerance (Munns and Tester, 8). Also, many processes are involved in modulation of biochemical activities and development based on stress sensing and salt-stress responsive signal transduction, such as various compatible solute/osmolytes, polyamines, reactive oxygen species (ROS) and antioxidant defense mechanism, ion transport etc. (Hasegawa *et al.*, 4). The role of amino acids, phenols and organic acids in the mitigation of adverse conditions of biotic and abiotic stress has been studied in different grape species (Cramer *et al.*, 3, Król *et al.*, 7, Taware *et al.*, 12).

Use of salt tolerant rootstock is recommended to overcome the problem associated with salinity stress. Rootstocks differ in their ability to withstand different abiotic stresses. Rootstock '110 Richter', a hybrid of *V. berlendieri* × *V. rupestris* has ion exclusion property and sustain grape productivity under saline condition (Upadhyay *et al.*, 13). In the present study, the effect of progressive salt stress was analyzed in 'Thompson Seedless' grapevines grafted on rootstock '110 R' and compared with own-rooted vines. Rootstock–scion combination induced metabolic changes and differential responses at morphological and physiological levels were observed.

MATERIALS AND METHODS

The experiment was conducted on grape (*Vitis vinifera* L.) cv. Thompson Seedless grown on its own-root and grafted on '110 R' rootstock, at ICAR-National Research Centre for Grapes, Pune (latitude 18.31° N, longitude 73.55° E). Sixteen months old potted vines were used for this experiment. The plants were raised in black soil with 40% clay and a soil moisture of 38% was maintained. The vines were regularly nourished with half-strength Hoagland's nutrient solution. Salt treatment was imposed by irrigating vines with 150 mM NaCl salt solution and control vines with water of EC<0.7 dS m^{-1}. Leaves from control and treated vines were sampled at 6 hr., 24 hr., 48 hr., 7th day, 15th and 30th day after treatment and stored at -20°C till use.

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The leaves were detached from petiole, washed with distilled water and oven dried at 70°C. Leaf samples were ground in Cyclotec sample mill (Foss Tecator, Hillerod, Denmark) followed by digestion in Block digester with H_2SO_4 : H_2O_2 mixture. The digested samples were analyzed for sodium on Analyst 100 Atomic Absorption Spectrophotometer (Perkin Elmer, Waltham, MA, USA) on emission mode and chloride was analyzed by using flow injection system (Skalar, San system). The leaves at 4th and 5th position of control and treated grapevines were collected from three biological replicates at each time point and processed separately for analysis of amino acids, phenols, and organic acids. For organic acid and phenol estimation, sap was collected from the sampled leaf as per the method described by earlier workers (Wallis and Chen, 15, Wallis *et al.*, 16). The sap was freeze-thawed, centrifuged at 8000 rpm at 4°C for 5 min and stored at -20°C until further use. Organic acids were estimated on ultra HPLC 1260 Series with Diode Array Detector (DAD) at wavelength 214 nm according to the method described earlier (Jogaiah *et al.*, 5).

For estimation of phenolic compounds, a measured amount of sap was diluted in mobile phase that consisted of 95% of solution A (0.2% acetic acid in 10% acetonitrile) and 5% of solution B (0.2% acetic acid in acetonitrile). Chromatographic analysis was performed using Zorbax Eclipse plus C18 column on ultra HPLC 1260 series with DAD (Agilent Technologies, USA). For amino acids estimation, leaf samples were extracted in 5 ml of 0.1% formic acid in 20% methanol, centrifuged and the supernatant was filtered. The filtrate was estimated on HPLC (Perkin Elmer 200 series) coupled to mass spectrometer (API 2000 Applied Biosystem, Canada) equipped with electrospray ionization (ESI+) probe and a chromatographic column, Zorbax SB C-18 (4.6mm × 50 mm × 1.8 µm, Agilent technologies).

The data were analyzed using SAS Version 9.3. The mean of three biological and two technical replicates was used to calculate fold change in salt stressed vines as compared to control vines. The principal component analysis (PCA) was performed to analyze grouping of different samples.

RESULTS AND DISCUSSION

The data on the effect of salt stress on the accumulation of different metabolites are given in

Table 1. Effect of salt stress on mean content (ppm) of major metabolites in grafted and own rooted grapevines.

Metabolites	TS/OR			TS/110R		
	Control	Stressed	Sig	Control	Stressed	Sig
Alanine	6.24	9.84	***	4.82	4.9	NS
Arginine	39.54	139.88	***	270.41	236.53	NS
Asparagine	25.24	25.85	NS	23.83	23.08	NS
Glutamic acid	31.17	33.19	NS	26.46	27.35	NS
Glycine	0.32	0.45	***	0.32	0.33	NS
Hydroxyl proline	0.31	0.35	NS	0.84	0.87	NS
Lysine	4.83	4.35	NS	3.53	3.16	NS
Norleucine	0.23	0.63	**	0.22	0.29	NS
Ornithine	9.6	15.06	***	23.66	21.63	NS
Proline	6.17	7.86	***	17.59	16.46	NS
Serine	12.24	11.16	NS	10.22	10	NS
Lactic acid	0.04	0.06	*	0.1	0.1	NS
Tartaric acid	2.35	2.41	NS	3.5	2.24	**
Malic acid	2.42	2.23	NS	5.25	3.12	**
Caftaric acid	30.14	130.36	***	112.23	181.31	*
Catechin hydrate	8.84	12.98	***	15.98	21.69	***
Vanillic acid	0.38	0.63	***	0.21	0.28	NS
Resveratrol	0.17	0.17	NS	0.38	0.44	NS
Qurcetin hydrate	0.43	0.61	NS	1.85	3.04	NS
Total phenols	49.2	141.89	***	134.36	202.52	**

* - significant at 5%, ** - significant at 1%, *** - significant at 0.1%, NS – non significant

Table 1. Among amino acids, only 11 amino acids were detected in both own-rooted and grafted vines. Amongst all, arginine was the most abundant amino acid followed by glutamic acid and aspartic acid. Individual amino acid profile showed variation in their concentrations between salinity levels and time points. In own-rooted vines, accumulation of six amino acids (alanine, arginine, glycine, proline, ornithine, norleucine) was recorded in salt-treated vines which were significantly different from control vines. The mean content (ppm) of these amino acids in treated own-root vines was 9.98 (alanine), 151.77 (arginine), 7.87 (proline), 0.45 (glycine), 0.63 (norleucine) and 15.06 (ornithine) as compared to 6.03, 40.58, 6.18, 0.32, 0.27 and 9.94 respectively in control vines. In own-rooted vines, the content of all the amino acids except serine also showed significant differences at various time point and significant salinity level x time interaction effect.

As the data in Table 2 indicated, after 15 days of stress, in own root grapevines the highest fold change was recorded for arginine (19.45 fold) followed by norleucine (15.20 fold), ornithine (2.53 fold) and proline (2.33 fold). At the early stage of stress i.e. 6h after stress, the content of arginine (8.48 fold), ornithine (2.51 fold), hydroxyl Proline (2.24 fold) was increased significantly in treated vines. However, in grafted vines, amino acid contents of control and treated vines were not statistically significant. However, as indicated in table 2, significant accumulation or degradation of some amino acids were observed at specific time points.

The increase in amino acids under salt stress could be due to amino acid production and/or from enhanced stress-induced protein breakdown. Cramer *et al.* (3) reported accumulation of several amino acids, sugars and organic acids under moisture and salinity stress in wine grape variety Cabernet Sauvignon. Generally, stress tolerant plants have higher levels of stress-related metabolites under normal growth conditions and/or accumulate larger amounts of protective metabolites, such as proline and soluble sugars, under unfavorable conditions, indicating that their metabolism is prepared for

Table 2. Fold change in metabolite contents in salt stressed vines at different time points.

	TS/110R					TS/OR				
	6h	24h	48h	7d	15d	6h	24h	48h	7d	15d
Alanine	1.12	1.08	2.19	0.58*	1.11	1.39	1.68*	2.07*	1.55*	1.27
Arginine	0.96	0.50*	1.86*	0.79*	0.37*	8.48*	0.59*	3.08*	1.88*	19.45*
Asparagine	1.32*	0.85	1.20	0.78*	0.87	0.68	0.78	1.05	0.92	1.67*
Glutamic acid	1.25	1.11	0.91	0.95	0.94	0.62*	1.04	1.27*	0.77	1.62*
Glycine	0.98	1.21	0.91	0.79	1.16	1.29	1.48*	1.28	1.07	2.02*
Hydroxy proline	0.76	1.57*	0.88	1.48*	0.88	2.24*	0.82	1.71	0.44*	1.29
Lysine	0.79	0.94	0.99	0.89*	0.88	0.87	1.03	0.87	0.93	0.79
Norleucine	0.26*	0.54*	0.86	1.13*	4.20*	0.91	0.24	1.11	16.50*	15.20*
Ornithine	0.49*	2.07*	0.45*	1.24*	1.03	2.51*	1.28	1.19	0.77	2.53*
Proline	0.86	1.25	0.52	1.23	0.87	1.33	0.74	1.37	0.83	2.33*
Serine	0.55*	1.28	1.36*	0.96	1.04	0.74	0.77	1.00	1.11	0.91
Lactic acid	0.57*	0.89	0.95	0.67	0.47	0.44	2.38	2.10	1.00	1.23
Tartaric acid	0.98	0.79*	0.37*	0.88	0.70*	0.80	1.90*	1.48*	0.88	0.88
Malic acid	0.93	0.87	0.58*	1.22	0.49*	0.64	2.05*	1.30	0.80	0.91
Caftaric acid	0.88	1.40*	1.28*	0.40*	0.90	10.44*	2.63*	1.89*	1.19	11.49*
Catechin hydrate	1.11	1.55	1.72*	0.93	0.99	3.80*	1.07	0.58	1.20	1.25
Vanillic acid	1.50	4.25*	ND	2.36	0.55	4.42*	2.90*	1.59*	1.74*	1.20
Resveratrol	1.07	1.26*	1.56	1.18	0.77	7.51*	1.55*	0.65	0.55	0.98
Quercetin hydrate	0.84	3.24*	1.27	0.30*	14.66*	0.09*	0.62	ND	ND	2.76*
Total phenol	0.90	1.43*	1.33*	0.48*	0.94	8.20*	2.00*	1.55	1.17	5.68*

*Indicates that the fold change was statistically significant. ND: not detected

adverse growth conditions (Krasensky and Jonak, 6). In the present study also, it was observed that control grafted vines accumulated the higher quantity of amino acids (arginine, glycine, norleucine and ornithine) than control own-rooted vines, which could have helped grafted vines in withstanding the salinity stress. The role of proline as an osmo-regulator is well documented. In our experiment, proline content was higher in grafted vines than own-rooted vines under control conditions. However, under salt treatment, the proline was significantly higher in salt-treated own-rooted vines as compared to control whereas in grafted vines, its content was comparable in control and salt treated vines. These results are in agreement with Cramer *et al.* (3), who reported two-fold increase in proline content after 16 days of salt stress in Cabernet Sauvignon on own root.

Among the nine detected phenolic compounds only three viz., catechin hydrate, caftaric acid, and resveratrol were commonly present in all the samples at all the time points. The total phenolic content varied between control and salt-treated vines and also among different time points. Significantly higher amount of total phenols was found in salt-treated grafted as well as own-rooted vines as compared to control vines (Table 1). In salt-treated own rooted vines, the content of catechin hydrate (12.98 ppm), caftaric acid (130.36 ppm) and vanillic acid (0.63 ppm) was found to be significantly higher than the control vines. The content of other phenols was not affected by salinity stress. At 6h and 15 days of stress, caftaric acid was increased 8.68 and 11.49 folds respectively in stressed own rooted vines. In grafted vines, the content of caftaric acid (181.31 ppm) and catechin hydrate (21.69 ppm) was significantly increased in salt-treated vines as compared to control (112.23 and 15.98 ppm respectively). The content of most of the phenolics compounds as well as total phenol in the control grafted vines was much higher than own-rooted control vines suggesting that rootstock 110R has the inbuilt potential of accumulating more phenolic compounds in scion variety and thus imparts it better ability to thrive under stress situations as compared to own-rooted vines. The potential of phenolics to act as an antioxidant is mainly due to their properties to act as hydrogen donors, reducing agents and quenchers of singlet O^{-2} (Rice-Evans *et al.*, 9). Accumulation of different phenols and change in phenol composition of grape leaf and roots in response to different stress have been reported earlier by Król *et al.* (7) and (Berli *et al.*, 1).

Among organic acids, lactic acid, tartaric acid, and malic acid were detected in all the samples. The content of lactic acid was significantly different in control (0.04 ppm) and salt-treated (0.06 ppm) own-rooted vines while the content of other organic acids did not change due to salt stress. In contrast, in grafted vines, salt stress resulted in significant decrease in the amount of tartaric acid (2.24 ppm and 3.5 ppm in treated and control respectively) and malic acid (3.12 ppm and 5.25 ppm in treated and control respectively). Though the mean content of lactic acid was not significantly different between control and treated grafted vines, however, its content was significantly decreased at 6h. Significant degradation of tartaric acid was also observed at all the time points. The reduced content of organic acids and their degradation under salt stress may be involved in compensating for ionic imbalance (Sanchez *et al.*, 10).

Imposition of salinity treatments led to significant increase in leaf Cl^- and Na^+ contents in vines as compared to control. The accumulation of leaf Na^+ and Cl^- was higher in own-rooted vines than grafted vines (Table 3). Significantly higher levels of leaf Na^+ and Cl^- levels were recorded at early as well as late time points in salt-treated own-rooted vines while in treated grafted vines significantly higher levels of Na^+ and Cl^- was recorded only at 30 days. This clearly indicated the immediate sensitive response of own-rooted vines to salt stress and their inability to restrict uptake of these two ions from soil. However, in grafted vines, the reduced concentration of these ions in earlier stages indicated the ability to restrict the uptake of these ions and their subsequent transport to leaves. Relatively lower accumulation of Na^+ and Cl^- in grapevines grafted on rootstocks 110R, B-2/56 (a clone of 110R) and 1103P as compared to own-rooted vines under field conditions has been demonstrated by several studies (Sharma *et al.*, 11, Upadhyay *et al.*, 13).

Two-dimensional plot of principal component analysis (Fig. 1) based on metabolite changes was performed to study the grouping pattern of the analysed samples. PCA1 (29.1%) and PCA2 (17.1%) together explained 46.2% of variability. In 2-D plot, control samples of own root vines at all the time points except 24h were placed closely. In salt treated vines, an increase in salt ions was recorded at an early stage of 6h and 24h, indicating the early response of the vines to salt stress. At 48h the accumulation of sodium and chloride increased which was accompanied with the increase in arginine, proline, alanine and catechin hydrate content. The contents of most of the bio constituents were comparable at 48h and 7 days resulting in co-grouping of these samples. Treated samples at 15 days was separated from all other samples, thus indicating the major differences in bio constituent

Table 3. Effect of salt stress on leaf Na^+ and Cl^- content (%).

Salt ion	Factor	Salinity level (T)				Time Point (TM)								I
	Sample	C	T	LSD	Sig	6h	24h	48h	7d	15d	30d	LSD	Sig	T × TM
Cl^-	TS/OR	0.37	1.08	0.11	***	0.47	0.46	0.60	0.62	0.98	1.24	0.2	***	***
	TS/110R	0.28	0.64	0.03	***	0.31	0.30	0.31	0.37	0.40	1.10	0.05	***	***
Na^+	TS/OR	0.22	0.58	0.09	***	0.26	0.31	0.43	0.37	0.51	0.49	0.15	**	**
	TS/110R	0.06	0.14	0.008	***	0.07	0.08	0.07	0.07	0.07	0.28	0.01	***	***

C: Control; T: Treated; I: Interaction, TS/OR: own-root grapevines, TS/110R: grafted grapevines * - significant at 5%, ** - significant at 1%, *** - significant at 0.1%.

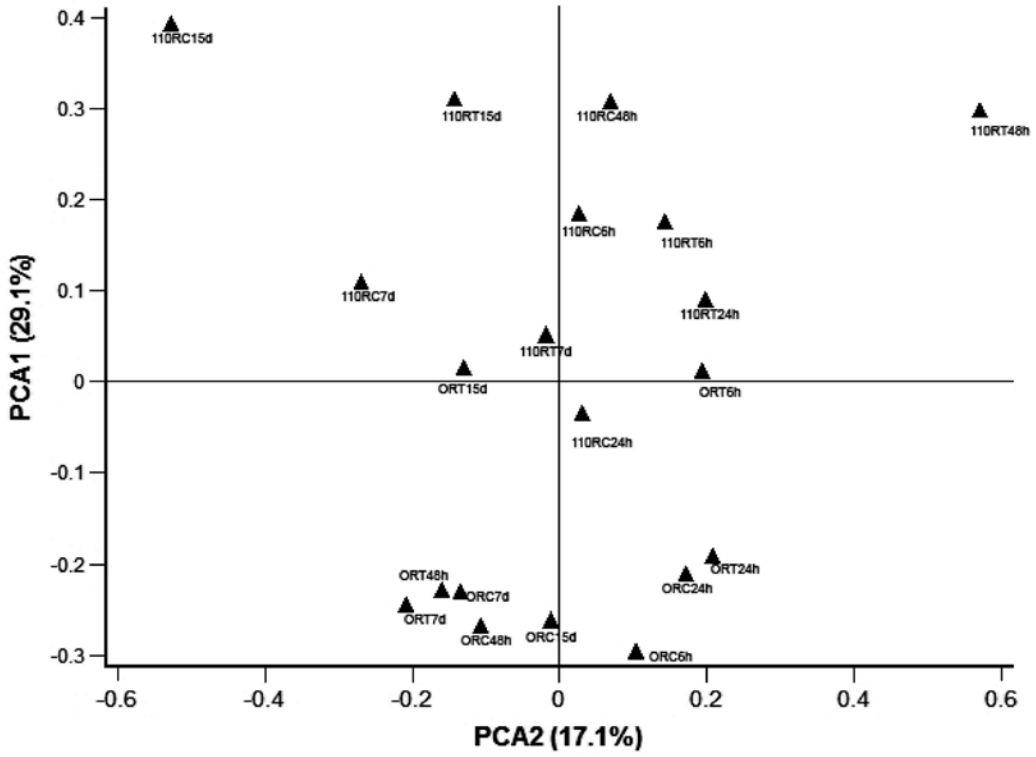

Fig. 1. 2-D plot of Principal component analysis of metabolite content in control and treated vines of own-root and grafted vines.

levels in salt-treated vines at the late stage. In grafted vines, first two coordinates did not show any specific pattern, however the control treated vines of 6h, 24h, and 7 days were placed close to each other. The leaf sodium and chloride content were not much varied among control as well as treated vines and levels of bio constituents remained comparable in both control and treated vines at these time points. As the salt treatment progressed, the 15 days treated vines showed a higher amount of leaf Cl^- and Na^+ along with increased levels of several metabolites and control and treated vines were placed afar.

Metabolic networks are highly dynamic and role of each metabolite in normal and stress condition changes according to the severity of the stress. This study identified metabolites at different stages of salt stress in own-root and grafted vines, which could be used as biomarkers for assessing the stress status of vines

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