

RESPONSE OF GRAPE GENOTYPES TO WATER DEFICIT: ROOT, SHOOT GROWTH AND ENDOGENOUS HORMONES

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SUMMARY

Grape varieties respond to decrease in soil water status by stomatal closure and reduced shoot growth with simultaneous increase in root growth. Most of the water deficit responses are known to be regulated by endogenous hormonal contents like abscissic acid (ABA) and cytokinins. To determine how these endogenous hormones are related to changes in shoot and root morphology, four grape genotypes were grown in pots and subjected to different levels of soil moisture stress for 14 days. None of the genotypes could survive beyond 4 days under 100 % stress conditions. There was an increased accumulation of ABA and corresponding decrease in cytokinins at 50 % stress compared to control (100 % irrigation). Among the genotypes tested Flame Seedless had highest ABA and lowest cytokinin levels and it also had highest root to shoot length ratio and root to shoot dry weight ratio. It is presumed that the reduction in shoot growth and stomatal conductance observed may be due to their ability to synthesize and accumulate ABA with the onset of soil moisture stress.

Key words: ABA, cytokinins, grape genotypes, root to shoot length ratio, soil moisture stress, stomatal conductance

INTRODUCTION

Even though grape originated in temperate regions, it is considered to be a water stress adapted species in India as it is well acclimatized to arid and semi arid regions of the country. Drought is considered to be a limiting factor for crop yield (Schultz 1986). Studies have indicated that when water is available grapevines maintain high stomatal conductance and can keep internal CO₂ concentration at higher levels and, when water becomes limiting, it closes the stomata in response to even small decrease in soil water potential (Schultz 2000). A regulatory mechanism that contributes to sensitivity of vines to such water deficits involves the stress hormone ABA. In addition to closing stomata, ABA promotes

characteristic developmental changes that could equip the plants better to face water deficits including restriction in shoot growth (Creelman *et al.* 1990) and leaf area expansion (Lacoeur *et al.* 1995) and stimulation of root extension (Sharp *et al.* 1994). When water availability is limited, root growth is generally less inhibited than shoot growth (Sharp and Davies 1989). The most well known manifestation of this differential sensitivity is the increase in root to shoot dry weight ratio as a basic adaptation to water stress. Information about ABA content in grape genotypes commonly grown in India are less investigated and reported. Hence, this investigation was taken up to see the extent of ABA and cytokinin accumulation in grape vines during soil moisture stress and their influence on root and shoot growth.

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MATERIALS AND METHODS

The experiment was conducted at the experimental farm of Indian Institute of Horticultural Research, Bangalore during 2002-03. Rooted cuttings of the grape genotypes namely Flame Seedless, Thompson Seedless, Sharad Seedless and Tas-A-Ganesh from nursery beds were transplanted into earthen pots containing growing media of garden soil, farm yard manure and sand (2:1:1). When the potted plants attained six months of age, three levels of moisture stress *viz.*, control (100% irrigation), 50 % stress (50 % irrigation) and 100 % stress (without irrigation) were imposed for 14 days. The experiment was conducted in three replications under factorial CRD design.

Observations on gas exchange parameters like photosynthetic rate, stomatal conductance and transpiration rate were recorded using portable photosynthesis system (Model 6200, Licor, USA). The leaf samples were collected at the beginning of the experiment under field capacity condition and at the end of stress cycle from all the treatments and were analyzed for Abscissic acid (ABA) and Cytokinins, zeatin riboside and dihydro zeatin riboside (t-ZR and DHZR).

Estimation of ABA and Cytokinins

The fully developed immature leaves were collected in icebox and were brought to laboratory. Leaves were washed in distilled water and 10 g leaf tissue was ground in 80% methanol and filtered using whatman filter paper. The residue was left overnight with 80 % methanol. It was filtered and filtrates were pooled. The filtrate was dried using rotary flash evaporator at 35°C under vacuum and the residue was dissolved in distilled water. The water extract was partitioned thrice against di-ethyl ether after adjusting pH to 3.0 with 0.1 N HCl.

The ether fraction was dried in the flash evaporator at 35°C and the residue was dissolved in 5ml tris buffer (20 mM, pH: 7.4) for ABA estimation.

Aqueous phase was partitioned with water saturated n-butanol at pH of 8.0. The butanol fraction was dried under reduced pressure at 35°C in the flash evaporator and the residue was dissolved in 5ml tris buffer (20 mM,

pH: 7.4) for estimation of cytokinins like zeatin riboside and dihydro zeatin riboside(ZR and DHZR).

ELISA technique was used to quantify ABA (WEILER, 1982) and Cytokinins (BARTHE and STEWART, 1985) in plant tissue employing laboratory raised polyclonal antibodies. The quantity of hormones was expressed as ng / g fresh weight of the tissue.

At the end of the stress cycle, plants were uprooted from the pots and total shoot length, total root length, total shoot dry weight and total root dry weight were measured. Root to shoot length ratio and root to shoot dry weight ratio were derived from respective values. Statistical analysis was performed as per the procedures of Gomez and Gomez (1984).

RESULTS AND DISCUSSION

A progressive reduction in stomatal conductance was observed in all the grape genotypes with increased soil moisture stress (Table 1). None of the genotypes could survive beyond four days without irrigation (100 % stress). Though there was reduction in stomatal conductance in all the genotypes with increased soil moisture stress Flame Seedless recorded highest stomatal conductance ($0.41 \text{ mol m}^{-2} \text{ sec}^{-1}$) followed by Thompson Seedless ($0.40 \text{ mol m}^{-2} \text{ sec}^{-1}$) on 14th day of stress cycle at 50 % stress. It has been demonstrated by Corriea *et al* (1990) that stomatal closure by plants is an early response to water stress and this reduction induces restriction in CO₂ assimilation rate. However, the reduction in assimilation (A) was less than the reduction in stomatal conductance (gs) thus increasing water use efficiency (A/gS){Table 2}. This is in accordance with results of Escalona *et al.* (1999) as observed in grape genotypes. This response was more significant in Flame Seedless indicating its high assimilation rate even at reduced soil moisture content.

Significant difference in plant growth substance content of grape genotypes was noticed at all the levels of moisture stress (Table 3). There was an increase in the ABA content of stressed vines compared to non-stressed vines. But the extent of such increment varied with the genotypes. It was about nine folds in Flame Seedless from control to 50 % stress, while it was about

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Table 1. Influence of moisture stress on stomatal conductance (mol/m²/sec) in grape genotypes

Genotypes (G)	Days after initiation of stress cycle								
	4 th day			9 th day			14 th day		
	S 1	S 2	S 3	S 1	S 2	S 3	S 1	S 2	S 3
Flame Seedless	0.59	0.37	0.19	0.54	0.34	*	0.57	0.41	*
Thompson Seedless	0.50	0.42	0.19	0.50	0.42	*	0.52	0.40	*
Sharad Seedless	0.62	0.46	*	0.53	0.47	*	0.42	0.39	*
Tas-A-Ganesh	0.55	0.41	*	0.43	0.39	*	0.43	0.36	*
	G	S	GxS	G	S	GxS	G	S	GxS
SEm ±	0.003	0.002	0.052	0.013	0.018	0.023	0.014	0.012	0.025
CD at 5%	NS	0.076	0.014	0.013	0.034	0.069	0.042	0.037	0.074

S 1: Control (100% irrigation) S 2: 50% stress (50% irrigation) S 3: 100% stress (0% irrigation)

*: Plants died and the observations were not recorded.

Table 2. Influence of moisture stress on water use efficiency (μ mol CO₂ / m mol H₂O) in grape genotypes

Genotypes (G)	Days after initiation of stress cycle								
	4 th day			9 th day			14 th day		
	S 1	S 2	S 3	S 1	S 2	S 3	S 1	S 2	S 3
Flame Seedless	0.96	1.01	0.13	0.84	0.98	*	0.96	1.33	*
Thompson Seedless	0.92	0.97	0.15	0.91	0.99	*	0.91	1.02	*
Sharad Seedless	0.77	0.75	*	0.77	0.52	*	0.72	0.78	*
Tas-A-Ganesh	0.61	0.59	*	0.70	0.69	*	0.72	0.60	*
	G	S	GxS	G	S	GxS	G	S	GxS
SEm ±	0.003	0.030	0.060	0.042	0.036	0.072	0.046	0.039	0.079
CD at 5%	0.108	0.008	NS	0.121	0.105	0.210	0.134	0.116	0.232

S 1: Control (100% irrigation) S 2: 50% stress (50% irrigation) S 3: 100% stress (0% irrigation)

*: Plants died and the observations were not recorded

1-3 folds in other genotypes. The increased ABA content must have contributed to reduced stomatal conductance and thus net photosynthesis. Ability to synthesize ABA under stress conditions is a varietal behaviour as suggested by Regina and Carbonneau (1997) where four varieties of grapevines grafted on Fercal rootstock and

subjected to 50 % moisture stress behaved significantly different in terms of stomatal conductance and ABA accumulation. ABA plays a modulatory role in drought tolerance. This is in confirmation with studies of Wang and Wong (1997) in kiwi fruits where they could observe low ABA content under normal conditions but increased

Table 3. Influence of soil moisture stress on endogenous hormone content in grape genotypes

Genotypes (G)	Abscisic acid (ng/g tissue)				Zeatin riboside (ng/g tissue)				Dihydro zeatin riboside (ng/g tissue)			
	0		14 th day		0		14 th day		0		14 th day	
	day	S1	S2	S3	day	S1	S2	S3	day	S1	S2	S3
Flame Seedless	12.23	14.62	133.8	*	61.16	64.26	47.35	*	108.4	78.98	59.67	*
Thompson Seedless	31.71	24.62	49.97	*	70.86	80.42	52.91	*	49.50	51.03	32.98	*
Sharad Seedless	20.29	20.32	31.76	*	43.32	28.31	27.09	*	35.01	37.99	25.95	*
Tas-A-Ganesh	17.01	15.43	32.31	*	41.45	35.19	24.85	*	37.71	40.80	24.10	*
		G	S	GxS		R	G	GxS		G	S	GxS
SEM±	0.558	4.243	3.00	6.00	1.586	1.143	0.810	1.623	9.264	0.897	0.634	1.269
CD at 5 %	1.822	12.73	9.002	18.00	5.172	3.435	2.429	4.859	30.203	2.691	1.903	3.806

S 1: Control (100% irrigation) S 2: 50% stress (50% irrigation) S 3: 100% stress (0% irrigation)

*: Plants died and the observations were not recorded.

ABA under drought. In the present study also significant negative correlation was observed between ABA content and stomatal conductance at 50 % moisture stress.

The increased ABA content was coupled with significant reduction in cytokinin (both t-ZR and DHZR) among all the genotypes. This reduction was more in Flame Seedless and Thompson Seedless than in Sharad Seedless and Tas-A-Ganesh. Evidences for cytokinin as negative signal was confirmed by Hosaki *et al.* (1987) where under water stress conditions ABA concentration rose more rapidly but cytokinin concentration fell rapidly in melons. Reynolds and Naylor (1994) could observe increased ABA : cytokinin ratio and decreased stomatal conductance when grape genotypes Pinot Noir and Riesling were subjected to soil moisture stress. In spite of high ABA content in Flame Seedless under moisture stress, it exhibited higher stomatal conductance resulting in higher levels of photosynthesis.

There was an increase in root to shoot length ratio when non-stressed vines were compared with 50 % stressed vines (Table 4). The magnitude of increase was more in Flame Seedless and Thompson Seedless and was least in Sharad Seedless and Tas-A-Ganesh. Similarly there was increases in root to shoot dry weight ratio from

non-stress to stress conditions in all the genotypes except in Tas-A-Ganesh. Sharp and Davies, (1989) opined that when water availability is limited, root growth is generally less inhibited than shoot growth. Continuous water stress reduced vine growth and more dry matter accumulated in roots instead of shoots of grape vine Chardonnay (Palliotti *et al.* 2001). The increase in ABA content of drying roots combined with the availability of water drawn from the wetter roots may have impact on more root growth in Flame Seedless and Thompson Seedless as suggested by Dry *et al.* (2000) that vines subjected to partial root drying treatment developed higher root length than fully irrigated vines. ABA is known to maintain higher root growth under conditions of soil moisture stress which results in inhibition of shoot growth. The reduction in shoot development of dried vines is due to reduction in cytokinin content, because cytokinin plays an important role in stomatal activity and thus increased photosynthesis. Though ABA content of Sharad Seedless and Tas-A-Ganesh did not appreciably increase under stress, but their stomatal conductance decreased. This might be due to other precursors of ABA biosynthesis pathway that could have accumulated in the leaves, which could not be detected by the assay system used for ABA. In this context, Parry *et al.* (1998) observed increased accumulation of t-xanthoxin in water stressed

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Table 4. Effect of soil moisture stress on plant morphological characters of grape varieties on 14th day of stress cycle

Genotypes (G)	Root shoot length ratio			Root shoot dry weight ratio		
	S 1	S 2	S 3	S 1	S 2	S 3
Flame Seedless	1.55	4.02	*	0.84	1.01	*
Thompson Seedless	2.79	4.25	*	0.90	0.94	*
Sharad Seedless	3.31	1.39	*	0.85	0.91	*
Tas-A-Ganesh	4.41	2.33	*	1.15	0.69	*
	G	S	G×S	G	S	G×S
SEM ±	0.252	0.218	0.436	0.070	0.060	0.123
CD at 5%	0.736	0.637	1.270	NS	0.180	NS

S 1: Control (100% irrigation) S 2: 50% stress (50% irrigation) S 3: 100% stress (0% irrigation)

*: Plants died and the observations were not recorded.

flacca mutants of tomato to a similar extent as ABA in wild tomato plants. Significant correlation was observed for ABA and root to shoot length ratio under moisture stress conditions.

The reduced stomatal conductance, higher root to shoot length ratio, higher root to shoot dry weight ratio in response to increased ABA accumulation and reduced cytokinin content under moisture stress conditions in grape genotypes Flame Seedless and Thompson Seedless suggests their better drought tolerance capacity than genotypes Sharad Seedless and Tas-A-Ganesh.

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