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Impact of water stress on photosynthesis and secondary metabolites in snap melon and musk melon

Rakesh Bhargava*, Karun Gurjar, S. M. Haldhar, R. S. Singh and B. D. Sharma
Central Institute for Arid Horticulture, Beechwal, Bikaner – 334003, Rajasthan.
*Corresponding Author email : rbciah@yahoo.com
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Abstract

A study was conducted to assess the impact of water stress on physiological activities and metabolite constitution in musk melon and snap melon. The field grown plants were subjected to water stress for 5 and 10 days when the plants were 45 days old. Subsequently, the stressed plants were re-irrigated and allowed to recover up to 15 days after re-irrigation. The data on photosynthetic rate and associated parameters and secondary metabolites such as total sugar, phenols, alkaloids, tannins and flavonoid content were assessed at every stage. The data revealed that snap melon the photosynthetic rate recovered fast and nearly reached to the values in control plants, however in musk melon the recovery was low. The data on flavonoid constitution of snap melon reveals that the magnitude of this metabolite increases with imposition of stress as well as age of plants showing thereby that the plants have potential to scavenge the ROS moieties developed due to water stress.

Key words: Drought stress, secondary metabolites, Water use efficiency, Carboxylation efficiency

Introduction

Drought stress has profound effect on the physiological and biochemical process of plants. It has been documented growth and development, dry matter production and partitioning are all dependent on the water status of plant. Plant physiological process such as photosynthesis transpiration are dependent on the severity and duration of drought (Vadell and Medrano, 1992). It has been demonstrated that as soon as plants sense the drought stress, the stomata reduce the opening and hence restrict the transpiration rate (Flexas amd Medrano, 2002).

Apart from this, it also reduce the internal CO_2 concentration in mesophyll cells which affects the rate of photosynthesis (Wong *et al.*, 1985; Cormic, 1994). In some plants drought also reduce the rate of photosynthesis by non–stomatal factors also such as decreased carboxylation efficiency(Ramanjula *et al.*, 1998; Rouhi *et al.*, 2007) regeneration of RuBP (Vu *et al.*, 1999) loss of RUBISCO activity (Parry *et al.*, 2002), etc.

Drought also creates oxidative stress in plants (Weidner *et al.*, 2009). This leads to production of excess of reactive oxygen species and free radicals which can be neutralized either by scavenging systems, such as superoxide dismutase, catalase and peroxidase or by involving small molecules such as glutathione, ascorbate, carotenoids, flavonoid, phenols, etc. Among various compounds present in plant tissue, phenolics and flavonoid have anti oxidative properties (Rosicka-Kaczmarek, 2004). The antioxidative effect produced by phenols larely depends on their hydroxyl groups present in its structure.

In view of the above, the aim of the present study was to compare the physiological and biochemical activities in drought susceptible and drought tolerant species in order to assess mechanism adopted by them for drought endurance.

Material and methods

Plant Material

Snap melon (drought tolerant) and musk melon (drought susceptible) constituted the material for present study. The seeds were sown in field on 16th Feb., 2015 under recommended cultural practices. The plants were allowed to grow with irrigation up to 45 DAS. Thereafter, the plants were divided into two viz. (i) control- irrigated normally and (ii) stressed where irrigation was withheld upto 10 days. After completion of stress period, the stressed plants were re-irrigated and parameters under recovery were studied. The observation on photosynthetic rate and associated parameters and metabolite composition were recorded at an interfal of 5 days during stress and after re-irrigation as presented in table 1.

Photosynthesis and transpiration measurements

Net photosynthesis rate was measured by using Infra Red Gas Analyzer (CID 340, CID Inc., USA). The observations were recorded between 8.00 AM-10.00 AM in field. A minimum of three replications were taken for each

observations. The observations were recorded at fixed CO_2 (400 ppm) and light intensity between 900-1000 µE m^{-2} s^{-1}. Beside net photosynthesis, the data on transpiration and internal CO_2 concentration were also recorded. Using the above data, values of water use efficiency and carboxylation efficiency were also calculated for respective days of observation.

Estimation of metabolite composition

For estimation of metabolite composition, leaf samples from different treatments were collected and dried. The estimations were done using the dried powder.

Total sugars

For estimation of total sugars anthrone method as described by Thimmaiah (2016) with slight modification was adopted. 50 mg of sample was hydrolyzed with 2.5 ml of 2.5N HCL in boiling water for 1.5 hrs. The tubes were cooled and neutralized with $NaCO_3$. The final volume was made up to 50 ml. One ml of this solution was reacted with 4 ml of anthrone reagent and heated in water bath for 8 min. Subsequently the absorbance was read at 620 nm. The standard curve was made with different concentration of glucose.

Total phenols

The total phenols were estimated by using the method described by Malik and Singh (1980). For this 0.5g of powder sample was dissolved in 10 ml of methanol. The whole set up was left at room temperature for overnight. Subsequently, it was filtered and filtrate was allowed to dry at room temperature. The residue was dissolved in 5 ml of distilled water. From this 0.5 ml of sample was taken and volume made upto 3 ml. This was treated with 0.5 ml phenol reagent and kept at room temperature for 3 min. After this 2 ml of 20% sodium carbonate was added and solution was heated in boiling water for 1 min. The blue colour so developed was read on spectrophotometer at 650 nm. The standard curve was made with catechol.

Total Flavonoid

The flavonoid content in the samples were estimated by the method described by Ebrahimzadeh *et al.* (2008). For this 0.5 gm of powder sample was dissolved in 10 ml of methanol. After 24 hrs. The whole smash was filtered and filtrate was dried at room temperature. The final volume was made upto 10 ml with distilled water. To 1 ml of this test sample, 1.5 ml methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of potassium acetate and 1.8 ml of distilled water was added. The resulting yellow colour was read on spectrophotometer at 415nm.

Tannins

The tannin content in the sample was estimated by the method described by Schanderl (1970) with slight modifications. For this, 1 gm of powder sample was suspended in 30 ml of distilled water was boiled for 30 min. The whole solutions was filtered. One ml of filtrate was used as test sample by diluting with equal amount of water. To this 0.5 ml of Folin-Denis reagent and one ml of Na_2CO_3 was added and absorbance of blue colour thus produced was read at 700nm in spectrophotometer. The standard graph was made using tannic acid.

Alkaloid

For estimation of alkaloids, 2gm of sample was mixed with sufficient water so that it forms semi-solid paste. This was transferred in separating funnel and partitioned with chloroform. The pH of residues was adjusted to 8-9 by adding liquid ammonia in aqueous layer. This was again partitioned with 70 ml of chloroform and chloroform fraction was collected in pre-weights evaporating dish. From this the alkaloid content was estimated.

Results and Discussion

Photosynthesis and associated parameters

The data collected on effect of water stress on photosynthesis and associated parameters on musk melon are presented in table 1.

Table 1. Photosynthetic rate and associated parameters in musk melon under water stress and recovery

Treatment (Days after imposing stress)	Water stress				Control				
	Pn (µ mol CO_2 m^{-2} s^{-1})	Transpirat-ion (mmol H_2O m^{-2} s^{-1})	Leaf level WUE (µ mol CO_2 mmol H_2O^{-1})	Carboxylation efficiency (µ mol m^{-2} s^{-1}ppm^{-1})	Treatment (Days corresponding to stress treatent)	Pn (µ mol CO_2 m^{-2} s^{-1})	Transpirat-ion (mmol H_2O m^{-2} s^{-1})	Leaf level WUE (µ mol CO_2 mmol H_2O^{-1})	Carboxylation efficiency (µ mol m^{-2} s^{-1}ppm^{-1})
0	20.45	8.6	2.37	0.086	0	20.45	8.6	2.377	0.086
5	16.4	7.35	2.23	0.076	5	20.64	8.56	2.52	0.072
10	11.09	4.98	2.22	0.059	10	18.91	7.60	2.48	0.099
5 days recovery	16.1	8.25	1.95	0.084	15	19.01	8.22	2.31	0.114
10 days recovery	17.03	7.35	2.31	0.119	20	18.34	7.72	2.37	0.091
15 days recovery	17.52	7.46	2.34	0.08	25	17.85	7.65	2.32	0.864

Perusal of table 1 reveals that the photosynthesis rate at time of imposition of stress was 20.45 µ mol CO_2 m^{-2} s^{-1} which dropped to 16.4 µ mol CO_2 m^{-2} s^{-1} and 10.09 µ mol CO_2 m^{-2} s^{-1} after 5 and 10 days of imposition of water stress. Subsequently, on re-irrigation, the photosynthesis rate recovered and it reached to 16.1, 17.03 and 17.52 µ mol CO_2 m^{-2} s^{-1} at 5, 10 and 15 days after recovery, respectively. However, in controls, the photosynthetic rate was 20.45, 20.64, 18.91, 19.01, 18.34 and 17.85 µ mol CO_2 m^{-2} s^{-1} at 0, 5, 10, 15, 20 and 25 days after the start of experiment. Similarly, the transpiration rate in controls was 8.6, 8.56, 7.60, 8.22, 7.72 and 7.65 mmol H_2O m^{-2} s^{-1} at 0, 5, 10, 15, 20 and 25 days after start of experiment. In the treatments, the transpiration rate was 8.6 mmol H_2O m^{-2} s^{-1} at the start of experiment which dropped to 7.35 and 4.98 mmol H_2O m^{-2} s^{-1} at 5 and 10 days after imposition of stress. On recovery, the magnitude of transpiration rate goes to 8.25, 7.35 and 7.46 mmol H_2O m^{-2} s^{-1} at 5, 10 and 15 days after re-irrigation. Accordingly, the WUE of plants under treatment was 2.37, 2.23, 2.22 µ mol CO_2 mmol H_2O^{-1} at 0, 5 and 10 days after imposition of stress and was 1.95, 2.31 and 2.34 µ mol CO_2 mmol H_2O^{-1} at 5, 10 and 15 days after re-irrigation. In control plants the magnitude of WUE remained nearly same.

Table 2. Photosynthetic rate and associated parameters in snap melon under water stress and recovery

Treatment (Days after imposing stress)	Water stress				Control				
	Pn (µ mol CO_2 m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})	Leaf level WUE (µ mol CO_2 mmol H_2O^{-2})	Carboxylation efficiency (µ mol m^{-2} $s^{-1}ppm^{-1}$)	Treatment (Days corresponding to stress treatent)	Pn (µ mol CO_2 m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})	Leaf level WUE (µ mol CO_2 mmol H_2O^{-2})	Carboxylation efficiency (µ mol m^{-2} $s^{-1}ppm^{-1}$)
0	18.73	6.25	2.99	0.068	0	18.73	6.25	2.99	0.068
5	17.05	6.01	2.83	0.077	5	18.64	5.24	3.55	0.062
10	14.75	5.42	2.72	0.052	10	15.97	5.72	2.79	0.052
5 days recovery	19.88	7.87	2.52	0.080	5	15.92	6.83	2.33	0.059
10 days recovery	18.32	6.64	2.75	0.069	20	15.67	6.48	2.41	0.064
15 days recovery	18.64	7.09	2.62	0.070	25	14.97	6.01	2.49	0.064

Perusal of table 2 reveals that the photosynthesis rate at time of imposition of stress was 18.73 µ mol CO_2 m^{-2} s^{-1} which dropped to 17.05 µ mol CO_2 m^{-2} s^{-1} and 14.75 µ mol CO_2 m^{-2} s^{-1} after 5 and 10 days of imposition of water stress. Subsequently, on re-irrigation, the photosynthesis rate recovered and it reached to 19.88, 18.32 and 18.64 µ mol CO_2 m^{-2} s^{-1} at 5, 10 and 15 days after recovery, respectively. However, in controls, the photosynthetic rate was 18.73, 18.64, 15.97, 15.92, 15.67 and 14.97 µ mol CO_2 m^{-2} s^{-1} at 0, 5, 10, 15, 20 and 25 days after the start of experiment. Similarly, the transpiration rate in controls was 6.25, 5.24, 5.72, 6.83, 6.48 and 6.018 mmol H_2O m^{-2} s^{-1} at 0, 5, 10, 15, 20 and 25 days after start of experiment. In the treatments, the transpiration rate was 6.25 mmol H_2O m^{-2} s^{-1} at the start of experiment which dropped to 6.01 and 5.42 mmol H_2O m^{-2} s^{-1} at 5 and 10 days after imposition of stress. On recovery, the magnitude of transpiration rate goes to 7.87, 6.64 and 7.09 mmol H_2O m^{-2} s^{-1} at 5, 10 and 15 days after re-irrigation. Accordingly, the WUE of plants under treatment was 2.99, 2.83 and 2.72 µ mol CO_2 mmol H_2O^{-1} at 0, 5 and 10 days after imposition of stress and was 2.52, 2.75 and 2.62 µ mol CO_2 mmol H_2O^{-1} at 5, 10 and 15 days after re-irrigation. In control plants the magnitude of WUE remained nearly same.

Metabolite composition under water stress

The data on various metabolite compositions of snap melon and musk melon are presented in 3-7, under water stress and control. Perusal of data in table 3 reveals the changes in flavonoid content in musk melon and snap melon under control and water stress. The data on snap melon show that the magnitude of flavonoid was 3.16mg g^{-1} dry weight at the start of water stress. On imposition of water stress, its magnitude increased to 4.44 mg g^{-1} dry weight and reached upto 6.55 mg g^{-1} dry weight after 10 days of stress. On re-irrigation, its magnitude declined slightly and reached back to 3.46 mg g^{-1} dry weight after 15 days of re-irrigation. However, in control conditions the magnitude increased with increase in age of crop.

The initial value of flavonoid in musk melon was 2.18 mg g^{-1} dry weight which declined with imposition of water stress to the level of 0.81 mg g^{-1} dry wt. After 10 days

of imposition of stress. On re-irrigation, the magnitude increased with age of plant. However, under control conditions, the magnitude remained nearly constant.

Perusal of data in table 4 for reveals that changes in tannin content in musk melon and snap melon under control and water stressed. Data demonstrates that in snap melon the initial magnitude of tannin was 3.86 mg g^{-1} dry wt. which remained nearly constant with imposition of water stress. However, on re-irrigation the value increased significantly and reached to 5.01, 5.15 and 4.9 mg g^{-1} dry wt. on 5th, 10 th and 15 th days after re-irrigation. However, in control, the magnitude remained nearly constant.

Table 3. Total flavonoid content in plants under different treatment

Treatment	Total Flavonoid (mgg^{-1} dry wt.)					
	Day of initiation of stress	Days after stress		Days after re-irrigation		
		05	10	05	10	15
Musk melon (Control)	2.18± 0.021	1.92±0.011	2.11±0.055	2.75±0.130	1.71±0.010	1.966±0.055
Musk melon (Stressed)	2.18 ± 0.021	1.34±0.015	0.81±0.062	2.80±0.045	2.37±0.202	0.76±0.036
Snap melon (control)	3.16 ± 0.045	3.42±0.075	4.33±0.085	5.67±0.030	7.12±0.090	6.19±0.030
Snap melon (Stressed)	3.16 ± 0.045	4.44±0.087	6.55±0.133	5.186±0.106	4.36±0.096	3.46±0.140

Table 4. Tannin content in plants under different treatment

Treatment	Tannin (mgg^{-1} dry wt.)					
	Day of initiation of stress	Days after stress		Days after re-irrigation		
		05	10	05	10	15
Musk melon (Control)	3.24 ± 0.018	3.19±0.036	2.92±0.11	5.19±0.03	4.41±0.04	4.55±0.04
Musk melon (Stressed)	3.24 ± 0.018	2.34±0.061	3.30±0.04	5.11±0.21	5.033±0.045	4.05±0.015
Snap melon (control)	3.86 ± 0.044	3.79±0.065	3.5±0.062	5.44±0.04	5.38±0.036	5.41±0.025
Snap melon (Stressed)	3.86 ± 0.044	3.49±0.19	3.91±0.088	5.016±0.032	5.153±0.035	4.9±0.097

In musk melon under control condition, the magnitude declined slightly but remained nearly constant during stress period. On re-irrigation the magnitude increased and reached upto 5.11 mg g^{-1} dry wt. Similarly trend was observed under control condition also.

Perusal of data presented in table 5 demonstrates the changes in total alkaloid content in musk melon and snap melon under water stress. The data reveals that in snap melon plants maintained in control conditions, the magnitude of total alkaloid remained nearly same during the period of experiment. However, in plants under stress treatment, the magnitude increased to 2.18 mg g^{-1} dry wt. at 5th day of water stress, but it declined on 10th days of stress. On re-irrigation, the magnitude nearly remained same as at the initial stage. In musk melon, the magnitude of alkaloid content increased from 0.78 mg g^{-1} dry wt. at the start of experiment which increased to 1.46 mg g^{-1} dry wt. on 5th day of experiment but further the magnitude declined.

Table 5. Alkaloid content in plants under different treatment

Treatment	Alkaloid (%)					
	Day of initiation of stress	Days after stress		Days after re-irrigation		
		05	10	5	10	15
Musk melon (Control)	0.78 ± 0.08	0.8±0.1	1.56±0.15	1.65±0.05	1.0±0.1	1.24±0.045
Musk melon (Stressed)	0.78 ± 0.08	1.46±0.11	0.78±0.07	1.27±0.07	1.46±0.06	2.71±0.206
Snap melon (control)	1.44 ± 0.09	1.23±0.15	0.8±0.05	1.2±0.1	1.47±0.07	1.246±0.05
Snap melon (Stressed)	1.44 ± 0.09	2.28±0.10	0.8±0.1	1.43±0.057	1.2±0.05	1.48±0.076

The total sugar content in the leaves of snap melon and musk melon plants under water stress is presented in Table 6. Perusal of data on musk melon reveals that the initial value of total sugar was 34.52 mg g^{-1} dry wt. on

imposition of water stress and subsequent recovery, the values nearly remained constant. Similar trend was also observed under control conditions. In snap melon, under control conditions, the total sugar values remained nearly constant, but on imposition of water stress the total sugar level increased from 28.62 mg g^{-1} dry wt. To 41.63 mg g^{-1} dry wt. on 10^{th} days of stress. On re-irrigation the magnitude remained nearly same.

No specific trend was observed with respect to total phenolics in musk melon crop, but impositions of water stress increased the level of total phenolics in snap melon (Table 7)

Table 6. Total sugar content in plants under different treatment

Treatment	Total Sugar (mgg^{-1}dry wt.)					
	Day of initiation of stress	Days after stress		Days after re-irrigation		
		05	10	5	10	15
Musk melon (Control)	34.52 ± 0.48	36.12±0.13	39.0 ±1.89	32.29±0.51	43.52±1.37	37.69±1.95
Musk melon (Stressed)	34.52 ± 0.48	34.04±0.29	32.97±1.02	30.94±0.45	32.41±0.56	36.50±4.11
Snap melon (control)	28.62 ± 0.78	29.26±0.14	31.49±3.82	26.71±0.23	33.91±3.42	29.86±0.22
Snap melon (Stressed)	28.62 ± 0.78	28.88±1.17	41.63±1.32	37.27±0.08	40.39±0.44	36.89±0.25

Table 7. Total phenol content in plants under different treatment

Treatment	Total Phenol (mgg^{-1} dry wt.)					
	Day of initiation of stress	Days after stress		Days after re-irrigation		
		05	10	5	10	15
Musk melon (Control)	2.68± 0.04	2.9±0.09	2.25±0.011	2.94±0.015	2.73±0.105	3.06±0.238
Musk melon (Stressed)	2.68 ± 0.04	2.45±0.03	1.303±0.028	2.36±0.06	2.66±0.34	2.413±0.049
Snap melon (control)	3.84 ± 0.06	3.37±0.04	3.52±0.055	3.97±0.061	3.16±0.091	3.42±0.175
Snap melon (Stressed)	3.84 ± 0.09	4.48±0.15	4.94±0.261	5.12±0.191	5.21±0.211	3.58±0.108

Drought stress has profound effect on physiological activities of the plants such as growth and developments, photosynthesis, transpirations, etc. (Vadell and Medrano, 1992). It has been demonstrated that initially the impact is observed at stomatal level where the stomatal aperture is reduced (Flexal and Medrano, 2002) subsequently leading to decrease in photosynthesis rate, due to reduction in CO_2 availability (Wong *et al.*, 1985; Cormic, 1994).

During present study a comparison was made between water stress tolerant (Snap melon) and susceptible (musk melon) plants for their response towards induced water stress. The results highlights that in musk melon plants the photosynthetic rate decreased with imposition of stress. The pattern parallels with transpiration rate also which shows that this crop species goes for reduction in stomatal aperture leading to reduction in photosynthetic rate. The results revealed that in musk melon drought response is at stomatal level as is also shown in other mesophytic plants (Wong *et al*., 1995; Cornic, 1994; Reddy *et al.*, 2004 and Rouhi *et al.*, 2007). The data on carboxylation efficiency further demonstrate that a part of reduction in photosynthetic rate is also due to non-stomatal factor as has also been documented by Ramanjulu *et al.* (1998). The carboxylation efficiency also decreases substantially with imposition of water stress.

In case of snap melon, the photosynthetic rate decreases slightly upto 5^{th} day of water stress and to the tune of 21% by 10^{th} day of drought stress. Similar pattern is also observed with respect to transpiration rate. The results revealed that in snap melon the stomatal aperture remains open even under receding soil moisture level permitting the free flow of CO_2 and its subsequent fixation. That such phenomenon takes place is further supported by the fact that upto 5^{th} day of drought stress, the carboxylation efficiency is maintained.

Our results further demonstrates that recovery of photosynthetic rate is much faster in snap melon which is demonstrated by the fact that P_N rate was 19.88, 18.32 and 18.64 µmol CO_2 m^{-2} S^{-1} at 5^{th}, 10^{th} and 15^{th} days of after re-irrigation which was better than in control plants. However in musk melon, the recovery in rate of photosynthesis is slow and remain lower than that observed on day of imposition of water stress as well as in control plants such pattern has also been observed by Romero *et al.* (2004) who also demonstrated that in almond during subsequent

recovery the photosynthetic rate is same or ever better than that of control plants.

Yet another impact of drought on plants is that that it induces oxidative stress in plants for which the plants develop a system of antioxidative defense. One such mechanism in plants employ compounds such as flavonoid, phenols, proline, ascorbate, etc. for neutralization of reactive oxygen species (Qureshi *et al.*, 2007; Weinder *et al.* , 2007 and Weinder *et al.*, 2009).

The comparative studies made by us on snap melon and musk melon turned out to be a model to access the effect of drought stress in these crop species. The data on flavonoid content in snap melon reveals that both under control and stress conditions, that quantity of flavonoid increases with age of plants, but the magnitude of increase is more under drought stress as compared to control. This demonstrates that flavonoid is accumulated under drought stress which may be acting as an agent to neutralize reactive oxygen. On the contrary, in musk melon the magnitude of flavonoid decreases showing there by that they do not have potential to scavenge the additional reactive oxygen generated under drought stress. Similar results have also been shown with respect to phenols also where a marked increase in total phenols was recorded with imposition of drought stress (table 7). However, no change was observed in musk melon.

Thus, form the foregoing account it can be concluded that snap melon has stomatal as well as non-stomatal mechanism to maintain photosynthetic rate. They also have bimolecules to scavenge the additional reactive oxygen molecules developed under drought stress.

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