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Physiological analysis of drought tolerance of cucumber (*Cucumis sativus*) genotypes

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ABSTRACT

Drought is one of the major factors limiting the growth and productivity of cucumber (*Cucumis sativus* L.) that adversely affects the desirable physiological and biochemical parameters. Thus, a field experiment was conducted during 2013–14 with 25 diverse genotypes of cucumber under four levels of irrigations, viz. 100% (control), 75%, 50% and 25% of the recommended irrigation. The yield reduction was as high as 51.97% under 25% of recommended irrigation. The physiological parameters such as proline, reducing sugars and phenol content increased significantly (P=0.05) as the drought stress increased from 100% irrigation to 25% irrigation level. In contrast, the relative water content (RWC), chlorophyll stability, membrane stability index (MSI) and fruit yield decreased significantly (P=0.05) with the increase in the intensity of drought stress in all genotypes. Among 25 genotypes DGC-1, DGC-19 and WBC-13 recorded better RWC, MSI, and lower yield reduction, while DGC-8, GS-3 and Barsati were highly sensitive to drought under all deficit irrigation levels (75%, 50% and 25%). These contrasting genotypes identified will be useful for mapping quantitative trait loci (QTLs) or genes for drought tolerance, and the best performing genotypes will be useful directly or as donors for genetic improvement in yield stability and water use efficiency in cucumber.

Key words: Chlorophyll, Cucumber, Drought tolerance, Proline, Relative water content

Drought is the major environmental constraint to crop productivity in the cucurbitaceous vegetables that are grown mostly as summer (March-June) crops in tropical parts of the world. Drought stress leads to inhibition of reduction in photosynthesis, respiration, protein synthesis and nucleic acid metabolism and thus growth and development (Bray *et al.* 2000, Zhu 2002).The reduction in growth is a consequence of drought induced modification in several physiological processes including modifications of water status, ion balance, mineral nutrition, stomatal behaviour, photosynthetic efficiency, carbon allocation, and utilization, etc. The rate of photosynthetic CO_2 assimilation is generally reduced by drought due to a reduced stomatal conductance and consequent restriction of the availability of $CO_2$for carboxylation and reactive oxygen species generation (Osakabe *et al.* 2014).

Better understanding on biochemical and physiological basis of drought tolerance mechanism in cucumber (*Cucumis sativus* L.) will not only help identify donors for component traits and devise effective breeding programs for genetic improvement of drought tolerance, but also help clone genes involved in drought tolerance and development of transgenic cucumber genotypes. Plant growth and fruit yield of cucumber are significantly affected by exposure to soil water deficits. Water-deficit stresses can also diminish cucumber fruit quality at harvest and during postharvest storage. Grafting cucumber onto *Luffa* was suggested to improve drought tolerance of cucumber (Liu *et al.* 2016). Despite the fact that cucumber is native to India and availability of vast genetic variability in the country, limited effort has been made to unravel the physiological basis of drought tolerance in cucumber and employ these mechanisms to develop drought tolerant cucumber genotypes. Hence, the present study was carried out to elucidate the physiological basis of drought tolerance in 25 diverse cucumber genotypes under three levels of drought stress under field conditions.

MATERIALS AND METHODS

The present experiment was carried out during 2014–15 at Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi. The materials for present investigation comprised of 25 germplasms of cucumber collected from various parts of India. Plants were exposed to four levels of irrigation treatments, viz. 100% of the recommended irrigation (control), and three levels of drought

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stress (75, 50 and 25% of the recommended irrigation).Two irrigations were given in the beginning for all treatments to have uniform germination.The drought stress was imposed 2 weeks after sowing. The recommended irrigation water amount (100% treatment) was calculated based on crop evapotranspiration calculated using formula given below (Allen 2006).

$$ET_c = ET_0 \times K_c$$

where, ET_c is amount of water required for the crop irrigation depth/area measured in mm, ET_0 is the monthly evapotranspiration and K_c is crop factor calculated by FAO Crop website.

Relative Water Content (RWC) of fresh leaves was estimated at 45 days after sowing (Barr and Weatherley 1962). RWC was calculated using the formula given below:

$$\text{Relative Water Content (\%)} = [(FW\text{-}DW) / (TW\text{-}DW)] \times 100$$

where, FW is Sample fresh weight, TW is Sample turgid weight, DW is Sample dry weight.

Membrane stability index (MSI) of fresh leaves was calculated as per the method suggested (Bailly *et al.* 1996). MSI was calculated as given below:

$$\text{Membrane stability index} = 1 - \frac{C1}{C2}$$

where, C_1=Conductivity of sample after exposure to 40°C, C_2= Conductivity of sample after exposure to 100°C.

Proline content of fresh leaves was determined using rapid colorimetric method.

$$\text{Proline } (\mu g/g \text{ Dry weight}) = \frac{\text{Proline concentration } (\mu g/ml) \times \text{Volume of toluene (ml)} \times 5}{115.5 \times \text{Fwt of sample}} \times \frac{\text{Fwt}}{\text{Dry weight}}$$

where, 115.5 is the molecular weight of proline.

Total chlorophyll content of fresh leaves was estimated at 45 days after sowing (Barnes *et al.* 1992).Total chlorophyll was calculated according to following formulae:

$$\text{Total chlorophyll (mg/g Dry weight)} = (20.2 \times A_{645}) + (8.02 \times A_{663}) \times \frac{V}{1000 \times Fw} \times \frac{Fwt}{\text{Dry weight}}$$

where, A=Absorbance at specific wavelength, V = Final volume of chlorophyll extract, Fwt = Fresh weight of sample, Dry weight = Dry weight of sample.

Chlorophyll stability index (%) = (Chlorophyll in stressed sample/ Chlorophyll in non-stressed sample) × 100

Reducing sugars of fresh leaves were estimated at 45 days after sowing (Somogyi 1952). From the standard curve, amount of reducing sugars present in sample was calculated according to following formulae:

$$\text{Reducing sugar in sample (mg/g Dry weight)} = \frac{\text{Sugar value from graph (mg)}}{\text{Aliquat sample used (0.2 ml)}} \times \frac{\text{Total volume of alchol free extract (10 ml)}}{\text{Weight of sample (100 mg)}} \times \frac{1}{1000} \times \frac{\text{Fwt}}{\text{Dry weight}}$$

Total phenol content was estimated following the suggested method (Bray and Thorpe 1954).

The performances of genotypes were compared by calculating a drought tolerance indexusing SAS Cluster procedures(SAS Institute 2000). We chose DGC-8 as the susceptible standard as it had lowest mean for yield under drought stress.

$$\text{Dought tolerance index} = \frac{\text{Observations of a character of a genotype on means of drought treatment}}{\text{Observations of same character on means of a check of drought treatment}}$$

For this purpose, six class intervals of index were defined based on the range of index for each character.

The experimental data were analyzed in randomized block design (RBD) and standard error of each mean was calculated to represent them on the bar diagram. The CD values were computed by multiplying the standard error of difference (SE_d) with table *t* value at error degrees of freedom (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Relative Water Content: The RWC decreased as the drought stress increased. Maximum RWC was recorded in genotype DGC-1 with 86.59% at 25% of recommended irrigation (Table 1). The minimum RWC in 25% of the recommended irrigation was recorded in DGC-8 with 40.74% (Table 1). The maximum drought index was recorded in genotype DGC-1 (index 2.20) with top score (1), whereas minimum drought index (1) for genotype DGC-8 with the lowest drought tolerance score (6) (Table 2).

The results in our experiment also showed that with the increase of stress in drought treatment, the RWC of genotypes DGC-1, DGC-19 and WBC-13 was the higher and minimum relative water content was recorded in DGC-8 and GS-3. These results indicate that a major adaptation system to drought exists for these resistant cucumber lines those are able to maintain low leaf transpiration rates and maintain more RWC which resulted into an osmotic adjustment through a proline accumulation (Patane *et al.* 2016).

*Membrane Stability Index:*The MSI decreased with increase in drought stress. At highest level of drought stress (25% of recommended irrigation), genotypes DGC-1, WBC-13 and DCG-19 showed MSI of about 50%, while DGC-8, GS-3 and Barsati had a MSI of about 28-32%.The maximum drought index (1.42) was estimated in DGC-1 with top score (1), whereas the lowest drought tolerance index (1.00) in DGC-8 (Table 2).

In the present experiment, MSI showed decreasing trend with the increase in drought stress. Our results are in agreement with the findings of previous researchers (Baroowa *et al.* 2016).

Proline content: The proline content of leaves increased as the drought stress level increased and reached the highest value at 25% of recommended irrigation in all the genotypes. The proline content of leaves increased significantly at higher (P=0.05) drought level 25% (133.28 µg/g Dry weight) over

Table 1 Influence of drought levels on relative water contentandproline content in different genotypes of cucumber

Genotype	Relative water content (%)					Proline content(µg/g Dry weight)				
	Irrigation levels					Irrigation levels				
	100%	75%	50%	25%	Means	100%	75%	50%	25%	Means
WBC-37	79.250	61.27	48.21	46.21	55.42	41.08	57.78	69.06	92.35	65.07
WBC-35	95.30	76.86	69.11	58.89	75.04	78.86	104.22	109.06	158.74	112.72
WBC-17	89.32	71.47	66.85	53.02	66.65	50.59	71.11	80.97	100.64	75.83
WBC-14	84.25	62.00	51.59	48.87	57.44	45.96	61.98	75.80	98.20	70.49
WBC-13	97.35	82.16	83.71	80.37	88.81	121.40	152.30	180.00	311.78	191.37
WBC-10	85.40	74.86	67.86	55.37	70.87	60.58	90.28	88.80	112.25	87.97
WBC1	87.43	75.69	67.93	56.33	71.85	69.03	94.66	91.01	112.99	91.92
RK-40	68.16	61.61	49.46	48.68	56.48	42.17	58.57	69.74	96.07	66.64
Pusa Uday	89.91	75.77	68.63	58.71	73.26	69.54	94.75	91.33	114.77	92.60
Pahari Barsati	79.37	73.63	67.49	52.99	68.37	54.06	77.60	86.90	110.86	82.36
HS-5	86.83	66.14	66.21	52.61	63.95	47.53	66.18	79.49	99.34	73.13
HS-1	92.21	77.77	74.37	69.39	81.19	104.49	131.00	140.84	199.56	143.97
GS-3	84.35	56.69	43.86	42.23	50.04	28.93	37.94	54.82	59.90	45.39
DGC-9	85.21	72.83	67.10	52.01	67.14	53.44	72.90	84.89	103.69	78.73
DGC-8	47.36	43.12	43.53	40.74	43.69	25.42	35.13	47.65	50.85	39.76
DGC-7	93.37	76.82	69.07	58.81	74.52	77.83	97.64	96.38	133.53	101.35
DGC-6	89.32	77.19	69.69	63.11	77.08	80.54	111.20	122.46	163.00	119.30
DGC-505	91.29	75.8	68.79	58.76	73.66	74.19	95.28	94.35	128.57	98.10
DGC-29	91.24	59.94	48.19	43.62	53.79	38.56	53.94	67.10	83.16	60.69
DGC-19	97.21	78.82	78.46	75.57	85.14	106.45	135.89	158.92	233.00	158.56
DGC-11	82.24	74.25	67.73	53.06	69.32	58.11	86.36	87.55	111.39	85.85
DGC-1	98.75	88.87	82.35	86.59	95.95	117.20	168.11	197.30	314.50	199.28
Barsati	84.24	57.65	44.42	42.35	50.69	33.52	43.29	60.14	69.07	51.51
7026-C	82.14	61.75	50.53	48.76	57.04	45.32	61.27	70.44	97.06	68.52
7026-B-76	81.24	59.65	46.79	43.49	53.07	35.12	48.00	63.97	74.38	55.37
	82.24	68.37	52.37	54.85	57.85	81.41	95.88	133.28		
SEm.±		*CD (P=0.05)*			*SEm.*±		*CD (P=0.05)*			
0.114		0.319			0.071		0.197			
0.046		0.128			0.028		0.079			
0.229		0.638			0.141		0.394			

control (57.85µg/g Dry weight) (Table 1). Drought score index showed that maximum index for genotype DGC-1 (5.01) and top score (1) followed by WBC-13 and DGC-19 (index 4.81, and 3.99, respectively) (Table 2).

Similar trend of increase in proline and soluble sugars with the increase in drought stress was reported in melons (Botia *et al.* 2005). Our results are supported by the reports, who indicated that higher proline contents involve a greater water-stress tolerance (Molinari *et al.* 2004).

Total chlorophyll: Total chlorophyll content of leaves as well as chlorophyll stability index decreased significantly with the increase in drought levels (Table 3). Maximum chlorophyll content was recorded under control condition with average chlorophyll content of 8.98 mg/g Dry weight. Significant genotypic differences in chlorophyll content were observed. At the highest stress levels, maximum total chlorophyll content was recorded in genotypes DGC-1 and WBC-13 with 13.88 and 12.11 mg/g Dry weight, respectively, while at the same stress level, DGC-8 and GS-3 recorded only about 2.2 mg/g Dry weight) (Table 3). At 25% of the recommended irrigation, the chlorophyll stability index was highest in DGC-1 and WBC-13, whereas minimum chlorophyll stability index was estimated for genotype GS-3 and Barasati (Table 3).

Higher total chlorophyll content was recorded in genotype DGC-1, WBC-14 and DGC-19 and lowest in genotype DGC-8 both under control and drought stress (25% irrigation level). However, the decrease in total chlorophyll content with increased drought stress was observed in all the genotypes. Decreased Chlorophyll level during drought

Table 2 Drought tolerance index and score amongst genotypes at mean value of drought stress treatments

Genotype	Total phenol content (mg/100g)		Proline content (µg/g dry weight)		Reducing sugar content (µg/g dry weight)		Membrane stability index (%)		Relative water content (%)		Yield per vine (kg)	
	Index	Score	Index	Score	Index	Score	Index	Score	Index	Score	Index	Score
WBC-37	1.51	6.00	1.64	6	1.56	5	1.13	5	1.27	5	1.22	6
WBC-35	2.39	4.00	2.84	4	2.36	4	1.30	2	1.72	3	1.92	4
WBC-17	1.70	5.00	1.91	5	1.76	5	1.20	4	1.53	4	1.38	5
WBC-14	1.60	5.00	1.77	5	1.65	5	1.17	4	1.31	5	1.27	6
WBC-13	3.90	1.00	4.81	2	3.70	1	1.39	1	2.03	1	2.74	1
WBC-10	1.87	5.00	2.21	5	1.93	5	1.25	3	1.62	3	1.65	5
WBC1	1.95	5.00	2.31	5	2.04	5	1.25	3	1.64	3	1.79	4
RK-40	1.53	6.00	1.68	5	1.58	5	1.14	5	1.29	5	1.21	6
Pusa Uday	1.98	5.00	2.33	5	2.06	4	1.26	3	1.68	3	1.76	4
Pahari Barsati	1.78	5.00	2.07	5	1.83	5	1.23	3	1.56	4	1.48	5
HS-5	1.65	5.00	1.84	5	1.70	5	1.19	4	1.46	4	1.38	5
HS-1	3.02	3.00	3.62	3	2.96	3	1.34	2	1.86	2	2.10	3
GS-3	1.06	6.00	1.14	6	1.15	6	1.01	6	1.15	6	1.04	6
DGC-9	1.74	5.00	1.98	5	1.78	5	1.23	3	1.54	4	1.40	5
DGC-8	1.00	6.00	1.00	6	1.00	6	1.00	6	1.00	6	1.00	6
DGC-7	2.23	4.00	2.55	4	2.19	4	1.29	2	1.71	3	1.89	4
DGC-6	2.55	4.00	3.00	4	2.54	4	1.32	2	1.76	3	2.03	3
DGC-505	2.08	5.00	2.47	4	2.13	4	1.28	3	1.69	3	1.83	4
DGC-29	1.42	6.00	1.53	6	1.48	6	1.11	5	1.23	5	1.20	6
DGC-19	3.32	2.00	3.99	3	3.31	2	1.35	2	1.95	2	2.41	2
DGC-11	1.83	5.00	2.16	5	1.88	5	1.24	3	1.59	4	1.54	5
DGC-1	4.30	1.00	5.01	1	4.16	1	1.42	1	2.20	1	3.03	1
Barsati	1.19	6.00	1.30	6	1.28	6	1.05	6	1.16	6	1.06	6
7026-C	1.56	5.00	1.72	5	1.61	5	1.15	4	1.31	5	1.23	6
7026-B-76	1.26	6.00	1.39	6	1.37	6	1.09	5	1.21	5	1.15	6
Range	1 –4.30		1 – 5.02		1 – 4.16		1 – 1.42		1 – 2.19		1-3.032	
Score												
1	3.76 - 4.30		4.36 -5.02		3.62 - 4.16		1.35 –1.42		1.99 – 2.19		2.69 – 3.03	
2	3.21 - 3.75		3.69 - 4.35		3.10 - 3.61		1.28 –1.35		1.79 – 1.99		2.35 – 2.69	
3	2.66 - 3.2		3.01 - 3.68		2.58 - 3.09		1.21 –1.28		1.59 – 1.79		2.01 – 2.35	
4	2.11 - 2.65		2.34 – 3.00		2.06 - 2.57		1.14 –1.21		1.39 – 1.59		1.67 – 2.014	
5	1.56 - 2.1		1.68 - 2.33		1.53 - 2.05		1.07 –1.14		1.19 – 1.39		1.338 – 1.67	
6	1.00 - 1.55		1.00 - 1.67		1.00- 1.52		1.00 –1.07		1.00 – 1.19		1.00 – 1.338	

stress has been reported in other species, depending on the duration and severity of drought (Kyparissis *et al.* 1995).

Reducing sugar: The reducing sugar content increased as the drought stress increased. Maximum reducing sugar content was observed in genotype DGC-1 (27.88mg/g Dry weight) at 25%, (22.10 mg/g Dry weight) at 50% and (24mg/gDry weight) at 75% of recommended irrigation.The maximum drought index was recorded in genotype DGC-1 (index 4.16) with top score (1) (Table 2).

The higher osmolyte concentration (proline and sugars) in DGC-1, WBC-13 and DGC-19 under drought stress might have helped to maintain structure and function of cellular macromolecules. However, proline accumulation cannot be used as a sole criterion for drought tolerance, as it also accumulates under other stresses such as high temperature, salt and starvation (Hong *et al.* 2000).

Total phenol: Maximum phenol content was observedin genotype DGC1 (26.16 mg/100g Dry weight) at 25%, (21.00 mg/100g Dry weight) at 50% and (21.11 mg/100g Dry weight) at 75% of recommended irrigation. DGC-1 with top score (1) followed by WBC-13 (index 3.90) with top score (1), whereas minimum index (1) was recorded in genotype DGC-8 followed by GS-3 (1.06) with the lowest drought tolerance score (6 each) (Table 2).

Table 3 Chlorophyll content and stability index of cucumber genotypes under different levels of drought stress

Genotype	Total chlorophyll (mg/g dry weight)					Chlorophyll stability index			
	Irrigation levels					Irrigation levels			
	100 %	75%	50%	25%	Means	75%	50%	25%	Means
WBC-37	6.37	4.35	4.04	3.01	4.44	68.2	63.4	47.2	59.6
WBC-35	10.72	9.49	6.58	8.15	8.74	88.5	61.4	76.0	75.3
WBC-17	7.39	6.20	4.67	3.65	5.48	83.9	63.2	49.3	65.5
WBC-14	7.02	4.65	4.15	3.13	4.74	66.2	59.2	44.6	56.7
WBC-13	16.32	14.90	11.82	12.11	13.79	91.3	72.4	74.2	79.3
WBC-10	8.06	7.37	5.06	4.75	6.31	91.4	62.7	58.9	71.0
WBC1	8.93	7.82	5.78	5.21	6.93	87.6	64.8	58.4	70.2
RK-40	6.63	4.39	4.05	3.02	4.52	66.1	61.1	45.5	57.6
Pusa Uday	9.03	8.05	5.93	5.44	7.11	89.1	65.7	60.2	71.7
Pahari Barasati	7.62	6.60	4.90	3.94	5.76	86.7	64.3	51.7	67.5
HS-5	7.11	5.53	4.44	3.55	5.16	77.8	62.5	49.9	63.4
HS-1	13.35	11.18	7.37	9.10	10.25	83.8	55.2	68.1	69.0
GS-3	5.22	3.04	2.89	2.20	3.34	58.1	55.3	42.2	51.9
DGC-9	7.52	6.45	4.85	3.68	5.63	85.7	64.4	48.9	66.3
DGC-8	5.00	2.56	2.25	2.15	2.99	51.1	45.0	43.0	46.4
DGC-7	10.63	9.28	6.40	6.74	8.26	87.3	60.2	63.4	70.3
DGC-6	11.25	9.84	7.00	8.18	9.07	87.5	62.2	72.7	74.1
DGC-505	10.18	8.84	6.31	6.52	7.96	86.8	62.0	64.0	70.9
DGC-29	6.14	4.21	3.97	2.76	4.27	68.5	64.6	44.9	59.3
DGC-19	15.57	12.95	10.08	9.30	11.98	83.2	64.8	59.7	69.2
DGC-11	7.77	7.18	4.92	4.51	6.10	92.4	63.4	58.1	71.3
DGC-1	18.10	17.22	13.60	13.88	15.70	95.2	75.1	76.7	82.3
Barsati	5.79	3.47	3.16	2.41	3.71	60.0	54.6	41.6	52.1
7026-C	6.94	4.56	4.13	3.10	4.68	65.6	59.5	44.6	56.6
7026-B-76	5.84	3.85	3.41	2.43	3.88	66.0	58.4	41.7	55.4
Means	8.89	7.26	5.84	5.27	6.81	75.84	62.20	52.54	63.54
	SEm.±	*CD (P=0.05)*							
Drought (D)	0.006	0.016							
Genotype (G)	0.002	0.006							
D × G (G)	0.012	0.032							

Our results are in conformity with the earlier findings (Martinez *et al.* 1994). The accumulation of phenolic compounds in stressed plants is negatively correlated with the accumulation of plant biomass (Abreu and Mazzafera 2005).

Consequences of drought stress on fruit yield: The fruit yield per vine decreased significantly with drought stress level increase (0.49kg at 25%) compared to control (0.98 kg). The genotypes also differed significantly to each other. Maximum yield per vine was recorded in DGC-1 (0.99 kg) at 25%, (1.14 kg) at 50% and (1.30 kg) at 75% of recommended irrigation. The minimum yield was observed in DGC-8 (0.24 kg) at 25%, (0.24 kg) at 50% and (0.78 kg) at 75% of recommended irrigation. The maximum drought tolerance index (3.03) and score (1) for genotype DGC-1, whereas the lowest drought tolerance index (1) and the lowest drought tolerance score (6) for DGC-8 followed by GS-3 (index 1.04 and 6score) (Table 2).

Yield reduction over control: The percentage of fruit yield reduction increased significantly with the increase in drought stress level. Maximum reduction of yield was recorded in GS-3(69.84%)at 25%, (167.76%) at 50% and (56.22%) at 75% of recommended irrigation. The minimum reduction was found in WBC-13 (33.67%) at 25%, (22.75%) at 50% and (15.83%) at 75% of recommended irrigation.

The average fruit yield reduction under drought stress was 36.16% at 75% irrigation and 51.97% at 25% irrigation. Thus, drought stress levels used in the study could be used to compare the drought tolerance of cucumber genotypes. Considering the fruit yield per vine and fruit yield reduction

under drought stress DGC-1, WBC-13 and DGC-19 were able to tolerate drought stress better.

The genotypes DGC-1, WBC-13 and DGC-9 were consistently at the top on the basis of drought tolerance score for all the physiological and biochemical traits, and, hence, these genotypes could be categorized as drought tolerant. But DGC-8, GS-3 and Barsati were at the bottom for majority of the traits including fruit yield, hence, weredrought susceptible. Thus, it may not be logical to suggest a single parameter as sole factor responsible for drought stress tolerance of cucumber genotypes.

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