Indian Journal of Agricultural Sciences **85** (5): 628–33, May 2015/Article

Effect of water stress on growth and antioxidative defence system in *Cucumis* spp. at seedling stage

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Received : 6 August 2014; Accepted: 2 January 2015

ABSTRACT

Drought or moisture stress is one of the most significant environmental stresses causing huge loss to the agriculture worldwide, reducing average yield of major vegetables crop by more than 50%. In the current study, antioxidant responses under water stress were compared to detect water stress tolerant genotypes of *Cucumis* spp. Forty four genotypes of ten *Cucumis* spp. were evaluated for their respective drought tolerance and effect of water deficient on various morpho-physiological (root length, shoot length, root and shoot fresh and dry weight) and biochemical [Catalase, Ascorbate peroxidase (APOX) and Guiaicol peroxidase GPOX)] parameters. Of these 44 genotypes, 11.36% were tolerant, 29.54% were moderately tolerant and 38.63% were susceptible and 20.45% were highly susceptible. Decrease in root-shoot length, root-shoot fresh weight, root-shoot dry weight was observed in most of the genotypes. The activity of catalase and GPOX was increased in water deficit conditions while the activity of APOX decreased for most of the genotypes. Though none of the growth parameters and enzyme activity was correlated with tolerance level, various morpho-physiological and biochemical parameters, were associated under control and stress conditions.

Key words: Cucumis, Drought, Hydrogen peroxide, Reactive oxygen species

Cucurbits belonging to family *Cucurbitaceae*, which primarily comprise species consumed as food worldwide. Family consists of circa 118 genera and 825 species (Pandey *et al*. 2013). The genus *Cucumis,* comprises of a large amount of diverse varieties with a major native diversity centre in tropical and southern Africa, includes cucumber and melon. Cucurbits plants have a high water requirement and are considered to be drought stress sensitive. Prolonged exposure to low soil moisture, due to a lack of rainfall or irrigation, has been shown to reduce significantly fruit yield and quality (Elkner 1985). Transient water deficits are also observed in cucumber plants when transpiration rates exceed the rate of water uptake by the root system. Plant water deficits are evidenced by leaf wilting, closure of stomata, and, ultimately, a reduction in photosynthetic rate (Genty *et al*. 1987).

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Drought stress is a cause for reactive oxygen species (ROS) production and imbalance the energy intake and consumption by photosynthetic organ consequently denaturation of functional and structural proteins. However, ROS plays a dual effect under abiotic stress that depends on their overall cellular amount. At relatively low level, ROS triggers stress defense/acclimation responses as components of a stress-signaling pathway (Vranova *et al*. 2002). However, at phytotoxicity level, ROS become extremely deleterious, initiating uncontrolled oxidative cascades that damage cellular membranes and other cellular components resulting in oxidative stress and eventually cell death. Therefore, understanding plant responses to drought is of importance to develop stress tolerant crops.

To avoid negative effects of environmental stresses, plants have developed elaborate mechanism like activation of cell signalling pathways, production of stress proteins, up-regulation of anti-oxidants, accumulation of compatible solutes and activation of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase) (Xue *et al*. 2009). Thus, plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage.

In India several indigenous *Cucumis* spp. are found in arid environment and a few, viz *C. callosus, C. melo* var *momordica, C. melo, C. melo* var *chate* are cultivated under mixed cropping systems (Pandey *et al*. 2013). The aim of

this study is to investigate the response patterns of different well characterized *Cucumis* genotypes belonging to various species at seedling stage against drought stress, and to elucidate this response by determining activity of antioxidant enzymes like catalase (CAT), ascorbate peroxidase (APOX) and guaicol peroxidase (GPOX).

MATERIALS AND METHODS

The experimental material comprised a total of 62 genotypes of *Cucumis* spp was accessed for their drought tolerance level. The experiment was planted in two sets in plastic pots filled with vermiculite saturated with ¼ MS liquid media at 30°C in green house and were supplemented with 30 ml of water at an interval of every one day. After 7d, irrigation in one set was withdrawn in order to induce water deficit stress.

After 7 d of water stress induction, accessions were evaluated for shoot length, root length, root fresh weight and shoot fresh weight. In order to collect both root dry weight and shoot dry weight, each plant of both sets was dried in hot air oven at 65°C for 24 hr. Dry weight of the root and shoot was measured by using weighing balance.

For enzyme extracts and assays, leaf samples (0.2 g) from each treatment were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylene diamine tetra acetic acid (EDTA) and 1% polyvinyl polypyrrolidone (PVP). The homogenate was centrifuged at 15 000 rpm for 20 min at 4°C, and was used for activity measurements by considering protein amounts. The protein amount was determined by the Bradford method (Bradford 1976) using bovine serum albuminas a standard. For enzyme activity measurement, an appropriate aliquot dilution of crude extract was made.

CAT activity was measured by following the decomposition of H_2O_2 at 240 nm (coefficient of absorbance, ε = 39.4/ mM/ cm) in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 as described by Chance and Maehly (1955) for 1 minute. Enzyme activity was expressed as µmol of H_2O_2 decomposed/mg (protein)/ min.

The oxidation of guaiacol was measured by following the increase in absorbance at 470 nm (ε = 26.6/ mM/ cm) for 1 minute. The assay mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol and 10 mM H_2O_2 as described by Chance and Maehly (1955). GPOX activity was expressed as µmol (tetra guaiacol formation)/mg (protein)/min.

APOX activity was measured by following the decrease in absorbance at 290 nm due to ascorbate oxidation (ε = 2.8/ mM/cm) in a reaction mixture of 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 0.1 mM EDTA for 1 minute according to the method of Nakano and Asada (1981). APOX activity was expressed as mmol (ascorbate oxidised)/mg (protein)/min.

RESULTS AND DISCUSSION

Screening of genotypes

Water deficit drought is one of the most serious abiotic stresses in crop production as it causes significant decline in plant growth, development, and ultimately yield (Kusvuran *et al*. 2011). The present study has shown that species and genotypes differ for their tolerance level against water stress. Out of 62, 18 genotypes from different species of *Cucumis* failed to germinate in multiple replicates, probably because of loss of seed viability. Therefore, phenotypic and enzymatic evaluation was carried out on remaining 44 genotypes, of which five were tolerant (11.36%), 13 were moderately tolerant (29.54%), 17 were susceptible (38.63%) and 9 were considered as highly susceptible (20.45%) (Table 1;

Table 1 Effect of water stress on shoot–root mass, length and antioxidant enzymes activity in diverse *Cucumis* genotypes[@]

Species and genotype	Control plants									Water stressed plants								
	RL	SL	RFW	SFW	RDW	SDW	CAT	APX	GPX	RL	SL	RFW	SFW	RDW	SDW	CAT	APX	GPX
C. prophetarum																		
IC-258181*	15.5	4.01	0.06	0.31	0.01	0.02	0.004	0.415	0.140	10.5	3.8	0.03	0.16	0.02	0.01	0.006	0.455	0.221
IC-373402*	12.5	11.2	0.04	0.46	0.05	0.05	0.004	0.261	0.099	9.20	9.5	0.02	0.20	0.01	0.04	0.005	0.168	0.228
IC-467722*	12.0	8.03	0.04	0.30	0.02	0.01	0.004	0.317	0.109	10.0	9.5	0.02	0.10	0.01	0.03	0.004	0.187	0.200
C. utillosonas																		
IC-258163*	7.50	5.50	0.03	0.15	0.02	0.04	0.002	0.246	0.104	3.41	5.5	0.03	0.10	0.01	0.01	0.004	0.304	0.131
IC-276382#	22.0	13.0	0.16	0.88	0.02	0.06	0.004	0.186	0.089	11.4	4.5	0.07	0.13	0.05	0.03	0.004	0.146	0.111
IC-276541#	11.3	8.48	0.04	0.49	0.01	0.03	0.004	0.188	0.090	8.50	7.3	0.02	0.20	0.01	0.02	0.004	0.218	0.104
IC-276564#	28.5	24.0	0.39	0.87	0.03	0.06	0.005	0.124	0.074	21.2	9.0	0.15	0.56	0.05	0.02	0.006	0.097	0.188
IC-313031^	6.70	8.50	0.64	0.02	0.05	0.02	0.003	0.146	0.057	2.50	9.1	0.03	0.21	0.01	0.01	0.004	0.135	0.166
IC-321448$	7.50	9.52	0.36	0.50	0.03	0.02	0.004	0.119	0.174	5.20	4.8	0.12	0.19	0.03	0.02	0.005	0.085	0.241
IC-398779#	9.01	13.5	0.09	0.53	0.02	0.02	0.006	0.188	0.117	4.81	10.7	0.02	0.27	0.01	0.02	0.006	0.270	0.118
C. trigonous																		
IC-280785#	11.5	10.0	0.06	0.31	0.01	0.02	0.004	0.166	0.132	9.02	9.5	0.02	0.10	0.01	0.04	0.005	0.128	0.277

(*Contd.*)

Table 1 (*Concluded*)

Species and genotype	Control plants									Water stressed plants								
	RL	SL	RFW	SFW	RDW	SDW	CAT	APX	GPX	RL	SL	RFW	SFW	RDW	SDW	CAT	APX	GPX
C. agretis																		
IC-258167^	9.51	6.03	0.04	0.28	0.01	0.03	0.004	0.159	0.019	4.18	5.0	0.03	0.11	0.01	0.03	0.004	0.125	0.096
IC-276546$	11.5	15.5	0.19	1.68	0.02	0.05	0.005	0.168	0.075	5.89	7.1	0.05	0.26	0.02	0.04	0.006	0.220	0.110
C. memordica																		
IC-371709#	11.0	5.08	0.06	0.21	0.01	0.01	0.004	0.157	0.066	4.22	3.8	0.03	0.05	0.01	0.01	0.004	0.205	0.122
IC-415521$	8.02	10.0	0.15	0.79	0.01	0.03	0.005	0.221	0.178	4.50	6.2	0.02	0.06	0.01	0.02	0.007	0.150	0.237
IC-415531^	14.5	11.7	0.22	1.03	0.04	0.05	0.008	0.166	0.129	9.01	7.5	0.02	0.16	0.01	0.03	0.008	0.130	0.268
IC-415539^	10.0	14.5	0.23	1.30	0.02	0.04	0.005	0.093	0.074	6.51	12.5	0.02	0.14	0.01	0.02	0.004	0.245	0.073
IC-435555$	7.50	10.5	0.08	0.38	0.01	0.02	0.004	0.207	0.083	9.32	8.5	0.03	0.22	0.01	0.03	0.006	0.080	0.129
IC-433621$	5.50	5.80	0.06	0.17	0.02	0.01	0.006	0.185	0.081	3.50	4.0	0.02	0.08	0.01	0.03	0.006	0.112	0.131
C. melo var. *melo*																		
IC-297507^	8.5	11.2	0.72	0.87	0.03	0.04	0.004	0.156	0.116	13.5	6.1	0.06	0.29	0.01	0.05	0.005	0.167	0.124
C. callosus																		
IC-258113*	14.1	9.50	0.06	0.16	0.02	0.01	0.005	0.226	0.052	13.5	9.5	0.04	0.06	0.02	0.01	0.006	0.156	0.246
AHK-200$	19.5	9.80	0.15	0.56	0.01	0.03	0.006	0.125	0.089	14.0	10.2	0.03	0.18	0.01	0.03	0.006	0.085	0.150
SKY/DR/ RS-96#	7.02	8.20	0.03	0.25	0.01	0.01	0.006	0.126	0.134	8.49	10.2	0.06	0.22	0.02	0.04	0.007	0.153	0.180
SKY/DR/ RS-27#	9.08	11.5	0.07	0.41	0.01	0.02	0.003	0.145	0.100	7.51	9.5	0.02	0.06	0.01	0.01	0.004	0.116	0.178
SKY/DR/ RS-100^	9.50	11.5	0.09	0.40	0.01	0.03	0.007	0.105	0.098	7.03	9.5	0.05	0.21	0.01	0.02	0.006	0.125	0.104
SKY/DR/ RS-91#	11.0	9.45	0.11	0.35	0.03	0.02	0.005	0.166	0.070	11.0	8.5	0.04	0.21	0.02	0.02	0.007	0.082	0.138
SKY/DR/ RS-111^	17.7	12.0	0.22	1.13	0.02	0.11	0.004	0.124	0.037	16.0	9.5	0.03	0.12	0.01	0.02	0.005	0.085	0.130
SKY/DR/ RS-79^	19.5	11.5	0.28	0.87	0.03	0.04	0.004	0.153	0.073	15.5	9.8	0.06	0.45	0.02	0.03	0.004	0.135	0.109
IC-91205#	8.04	14	0.23	0.61	0.04	0.04	0.005	0.285	0.136	14.2	7.2	0.03	0.18	0.01	0.01	0.005	0.171	0.149
EC-428164^	20.5	25	0.41	1.44	0.02	0.06	0.005	0.128	0.104	12.5	12.5	0.04	0.40	0.01	0.04	0.005	0.081	0.174
C. melo																		
BS-25#	28.0	17.5	0.25	1.57	0.01	0.09	0.006	0.145	0.080	12.5	9.5	0.02	0.45	0.01	0.02	0.008	0.101	0.083
MM-1^	9.80	9.02	0.12	0.67	0.04	0.02	0.003	0.119	0.137	6.09	5.5	0.03	0.12	0.01	0.02	0.004	0.115	0.073
IIHR-3$	13.0	15.5	1.77	0.45	0.02	0.08	0.006	0.452	0.248	10.5	11.7	0.27	0.50	0.01	0.03	0.006	0.315	0.255
IIHR-18^	17.5	14.8	2.55	0.54	0.02	0.14	0.005	0.388	0.307	11.2	9.0	0.03	0.51	0.01	0.03	0.005	0.411	0.310
IIHR-81^	12.2	14.0	1.91	0.23	0.03	0.11	0.008	0.426	0.149	14.5	12.0	0.06	0.51	0.02	0.04	0.008	0.488	0.156
C. chate																		
Arya^	11.5	10.5	0.64	0.08	0.01	0.09	0.007	0.105	0.060	18.0	11.0	0.12	0.51	0.02	0.04	0.008	0.275	0.119
C. sativus																		
SPP-93#	14.6	11.0	1.32	0.35	0.03	0.07	0.006	0.163	0.126	12.5	10.0	0.06	0.47	0.02	0.03	0.009	0.220	0.179
SPP-56$	5.06	9.50	0.23	1.82	0.02	0.01	0.004	0.239	0.304	6.51	9.7	0.03	0.62	0.01	0.06	0.005	0.184	0.266
SPP-58^	17.5	14.5	1.79	0.02	0.01	0.01	0.007	0.466	0.169	9.50	10	0.09	0.56	0.01	0.04	0.007	0.132	0.170
SPP-63^	10.5	9.51	0.71	0.07	0.01	0.04	0.008	0.248	0.086	10.0	9.0	0.04	0.42	0.01	0.03	0.008	0.257	0.099
Super Vigour$	14.5	15.5	1.99	0.32	0.02	0.11	0.008	0.359	0.208	10.5	9.8	0.05	0.43	0.01	0.04	0.008	0.488	0.217
SwarnaAgeti$	10.0	14.5	1.65	0.31	0.03	0.05	0.004	0.379	0.141	8.02	9.8	0.09	0.10	0.01	0.02	0.004	0.461	0.213
VR-101^	8.48	7.05	0.50	1.32	0.05	0.01	0.006	0.083	0.215	6.80	6.2	0.07	0.26	0.01	0.03	0.005	0.115	0.343
C. hardwikki																		
IC-331626^	13.0	11.1	1.07	0.20	0.03	0.05	0.010	0.320	0.086	7.00	8.5	0.08	0.10	0.01	0.05	0.016	0.323	0.174

*Tolerant;#Moderately tolerant; ^Susceptible; $Highly susceptible;@RL- Root length; SL- Shoot length; RFW- Root fresh weight; SFW- shoot fresh weight; RDW- Root dry weight; SDW-Shoot dry weight; CAT-Catalase; APX- Ascorbate peroxidase;GPX- Glutathione peroxidase

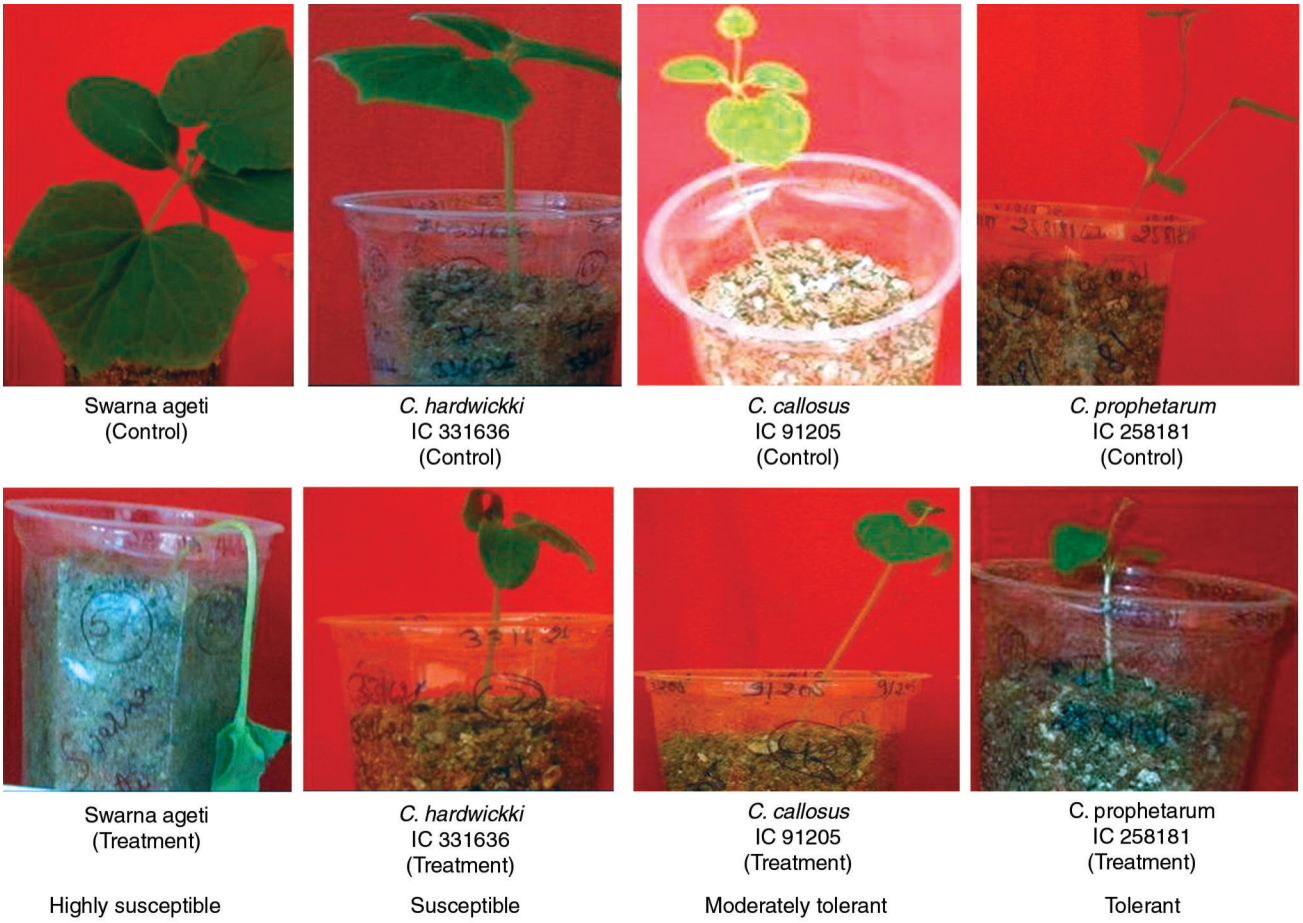

Fig 1 Differential phenotypic variations in *Cucumis* spp. under drought stress.

Fig 1). Among five tolerant genotypes three were belonging to *C. prophetarum* while in others species tolerant types were rare or nil in number. This classification is based on stress characteristics like desiccation of leaf and stem, drooping of leaves, curling of leaves at margins or tips, lodging and wilting of stem etc., developed by the plant under water deficit. This study sheds light on the relationship between drought stress and its effect on morpho-physiological (root length, shoot length, fresh and dry weights of shoots and roots) parameters of *Cucumis*.

Root length and shoot length

Root length is considered to be one of the most important characters for drought stress tolerance because roots are in direct contact with the soil and enhance the ability of the plant to capture water. The decline in various plant attributes in response to induced stress is a commonly observed phenomenon which is according to tolerance level in plant (George *et al*. 2013). Mean values of all the root/shoot traits are shown in Table 1. In control set, average root length was 12.5 cm with a range of 5 to 28.5 cm while in stress it ranged from 2.50 to 21.2 cm with a mean of 9.5 cm. Thus, drought stress caused a significant decrease in the root length of stressed plants as compared to corresponding control plants. Genotypes showed diverse responses in terms of root length under drought stress. However, maximum reduction in root length on account of stress was observed in BS 25, and was least in IC 258113. Exceptionally, in some genotypes (IC 435555, IC 91205, IIHR 81, SPP 56 and IC 29507) root length was increased under stress condition.

Similar to root length, there was a reduction in shoot length under water deficit condition with an exceptional increase in some genotypes was observed. Genotypes under control condition, showed shoot length variations from 4 to 25 cm with an average of 11.4 cm. Although under stress, mean shoot length was 8.45 cm with a range of 3.80 to 12.5 cm. On account of heat stress, maximum reduction in shoot length was observed in IC 276564 while it was east in IC 258181. However an increase in shoot length under water deficit condition was observed in four genotypes, viz. IC 467722, SKY/DR/RS 96, IC 313031, SPP 56 and Arya.

Many studies have shown that there are significant increases in root length and decreases in shoot length under drought stress and *vice-versa* (Tuna *et al*. 2010, Kavar *et al*. 2007). However, in our study a decrease in root length and shoot length was observed under water deficit condition. This decrease in root and shoot length under water stress may be due to reduction in mitotic cell division during water stress condition (Robertson *et al.* 1990). Decreased root length due to water stress was observed in wheat and maize (Nayar and Gupta 2006), moreover, root length was not associated with tolerance level and hence may not be

important for drought tolerance level *per se* in this species This could be because of evolutionary reasons. At the same time correlation studies further show that root length was significantly correlated with root dry weight and shoot length in treated plants supporting the earlier observations that root length is important for water and nutrient supply to plant for growth under stress.

Root and shoot weight

There was a decrease in average root fresh weight (RFW) under water deficit condition. RFW ranged between 0.03 to 2.55 mg under control condition while under stress it ranged from 0.02 to 0.27 mg. Overall,the effect of water stress was prominent on IIHR-3 and IIHR-81 and it was least in IC 258167. However, increased RFW was also observed in SY/DR/RS 96 genotype. Likewise, shoot fresh weight (SFW) ranged from 0.02 to 1.82 mg in control condition, whereas under water deficit condition it was lying between 0.05 to 0.62 mg. Under stress condition, genotype IC 276546 had very low SFWwhile and least shoot fresh weight reduction was observed in SKY/DR/RS-96 accession. Exceptionally, IC 313031, IIHR 3, IIHR 81, ARYA, SPP 93 and SPP 58 showed increment in SFW in water deficit condition.

Similarly to RFW, under control condition, root dry weight (RDW) ranged between 0.01 to 0.05 mg whereas in water deficit condition it ranged between 0.25 to 0.05mg. Abnormally, four (IC 258181, IC 276382, IC 276564 and SKY/DR/RS 96) genotypes showed increment in RDW during stress. Relatively lower influence of drought on dry biomass than on fresh mass indicates a presence of disturbance in water relations. Shoot dry weight (SDW) was ranged from 0.01 to 0.14 mg during control condition though in water deficit condition it was ranged from 0.01 to 0.06 mg. Strangely, similar to SDW, six genotypes (VR 101, SPP 58, SPP 56, SKY/DR/RS 96, IC 435555 and IC 280785) showed increment in SDW under stress.

In this study reduction in fresh as well as dry weight of both root and shoot was observed. However, the responses to water deficit conditions were genotype specific. This decrease in FW under stress may be a consequence of suppression of cell expansion and cell growth due to the low turgor pressure. Reduction in fresh weight under drought stress in wheat was also observed (Rane *et al*. 2001). Similar results were also observed in pearl millet (Kusaka *et al*. 2005) and *Abelmoschus esculentum* (Bhatt and Srinivasarao 2005).

Antioxidant enzyme activity

H_2O_2 is an important signaling molecule and its levels are kept under check by a sequence of H_2O_2 metabolizing enzymes. Any increase in H_2O_2 has severe consequences for the affected cell.An accumulation of H_2O_2 under stress cause extra functioning of H_2O_2 metabolizing enzymes. The balance between reactive oxygen species production and activities of antioxidant enzymes determines whether the oxidative signalling and/or damage will occur (Moller *et al*. 2007). Detoxification of reactive oxygen species in plants, induces enzymatic mechanisms such as superoxide dismutase, catalase, ascorbate peroxidase and peroxidase. Drought stress increased specific activity of CAT and GPOX in all the varieties tested.

There were significant variations in catalase activity among different genotypes under both stressed and non-stressed conditions. An increase in catalase activity was found in 40 genotypes while a decrease in same was observed for 4 genotypes under water deficit condition. Increase in catalase activity might be associated with high rate of H_2O_2 production under water stress. Average catalase activity in control was 0.051µmol/mg (protein)/min while under stress condition it was 0.0057 µmol/ mg (protein)/min (Table 1). Catalase eliminates H_2O_2 by decomposing it directly to water and oxygen. However, its activity was not found to be associated or correlated with genotype tolerance level; however increase in catalase activity is related with increase in stress tolerance (Kumutha *et al*. 2009). The observation of catalase activities may increase, decrease or remain unchanged under drought stress by Zhang and Kirkham (1996) supports our findings. This could probably happen due to accumulation of mutations in promoter or structural genes in the genotypes bred or maintained for higher yields under proper management.

The APOX activity was increased in 19 genotypes while decreased in 25 genotypes. APOX reduces H_2O_2 to water by ascorbate as a specific electron donor. Average APOX activity in *Cucumis* genotypes was decreased under water stress than control. Average APOX activity in control plants was 0.21 µmol/mg (protein)/min while under stress it was 0.19 µmol/mg (protein)/min. Increased APOX activity has been reported to play a role in resistance to oxidative stress (Sanchez-Rodriguez *et al*. 2010) during water deficiency. Average decrease in APOX activity in *Cucumis* spp. could be an indication that this mechanism might be controlled by metabolic conditions. However, its level is correlated with growth parameters in most of the spp. This difference could be attributed to evolutionary history of different species.

Average GPOX activity in control plants was 0.11 µmol/mg (protein)/min while under water stress it was 0.17 µmol/mg (protein)/min. The increase in enzyme activity was reported in most of the genotypes (40 out of total 44). Moreover, inter and intra species variations were also observed for GPOX activity.Increased in activity of GPOX may be due to high ROS formation due to drought stress. Higher peroxidase activity was also observed in groundnut (Reddy *et al*. 2003) and maize (Zhang *et al*. 1995) under drought stress.

Enzyme activity was not found to be correlated with the tolerance level of the genotypes; however significant correlations among a number of morpho-physiological traits were observed. Root length in control was found to be associated with SLC (Shoot length in control), whereas root length in treatment was associated with SLT (Shoot length in treatment), SFWT (Shoot fresh weight in treatment) and

SDWT (Shoot dry weight in treatment). Root fresh weight in control (RFWC) was associated with most growth parameters and enzymes but not the RDWC (Root dry weight in control). Root fresh weight in treatment (RFWT) had positive correlations only with RLT (Root length in treatment). It can be concluded from the present study that none of the morpho-physiological traits define drought tolerance in a species or genotype even. It is rather evolution of number of factors governing various traits provide tolerance to specific genotype.

ACKNOWLEDGEMENT

The paper presents the results from a project supported by NAIP (National Agricultural Innovation Project), entitled 'Bioprospecting of genes and allele mining for abiotic stress tolerance'. The authors would like to acknowledge Dr K V Bhatt, Principle Scientist, National Bureau of Plant Genetic Resources (NBPGR), New Delhi, for providing the germplasm.

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