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EFFECTS OF DROUGHT STRESS ON GROWTH PARAMETERS AND ANTIOXIDATIVE ACTIVITY OF CORIANDER (*CORIANDRUM SATIVUM* L.)

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ABSTRACT

Drought stress such as other environmental stress is one of the growing concerns in agriculture management worldwide. The effect of drought stress on the morphological characteristics and antioxidative enzymes activity such as catalase (*CAT*), ascorbate peroxidase (*APX*) and guaiacol peroxidase (*GPX*) in coriander were investigated in pot conditions as completely randomized design with three replications. The result showed that morphological traits and enzymes activity were significantly difference with increasing drought stress in coriander. Based on cluster analysis, group which had a total dry matter trait was the best group for determining the most effective traits on dry matter production. At low drought stress condition (70% FC), included traits were APX, plant height and shoot dry weight which selected as an important traits in dry matter production. The stepwise multiple linear regression results show that shoot dry weight and leaf dry weight (R^2) were recognized as most effective traits which determined total dry weight production at 50% FC drought stress level.

Keywords: Drought stress, Antioxidative enzymes activity, Cluster analysis

INTRODUCTION

Environmental stress is main limiting factor to crop production around the world. Study of environmental stress such as a drought stress and role of detect and evaluation of crop grows and performance is very obvious (Geertans and Raes, 2009). Drought stress is a common environmental stress factor seriously affecting crop production in different regions, particularly in arid and semi-arid regions. Droughts are attracted the attention of environmentalists, ecologists, and agricultural scientists as an environmental calamity. Timing and characteristics of rains, low relative humidity, temperatures, high winds, intensity and duration of rain, including distribution of rainy days during crop growing seasons and onset and termination, play an important role in the occurrence of droughts. Due to the increase of population and development of the agricultural, energy, and industrial sectors, the demand for water has increased widely, and water scarcity has been occurring almost every year in many parts of the world (Mishra and Singh, 2010) such as Iran (Golbashy et al., 2010). Reports showed that in semi arid regions of Iran, drought decrease season length (Magorokosho et al., 2003), disturb photosynthesis and assimilate remobilization which finally reduces grain

weight (Vaezi and Ahmadikhah, 2010). Multiple biochemical pathways determine the tolerance to drought stress. These pathways play the role in protection of protoplast functions, maintenance of ion homeostasis and control of water flux. drought stress, like other abiotic stresses, can also lead to oxidative stress through the increase in reactive oxygen species (ROS), such as superoxide (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•), which are highly reactive and highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acid, etc, causing lipid peroxidation, protein denaturing and DNA mutation, respectively (Abou Zeid and Hassan, 2011). The role of antioxidative defense systems in plant reactions to drought stress was comprehensively documented in *Gypsophila aucheri*, which is a xerophytic plant (Sekmen Esen et al., 2012). In another report, physiological and antioxidative responses of sunflower (*Helianthus annuus*) cultivars under drought stress were evaluated, and the efficiency of antioxidative systems in coping with drought effects was obvious (Baloğlu et al., 2012).drought stress decreases growth of some medicinal plants, including *Bupleurum chinense* DC. (Zhu et al.,

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2009)and *Hypericum brasiliense Choisy* (Nacif de Abreu and Mazzafera, 2005). *Coriandrum sativum* L. is a culinary and medicinal herb of the *Apiaceae* family commonly known as coriander. The roots and leaves o f C. *sativum* are rich with aromatic flavor (Sreelatha et al., 2009) and are popularly used in soups in Iran cooking. Previous studies on this herbs how their various medicinal properties, including antidiabetic, antioxidant, hypocholesterolemic, hepatoprotective, antihelmintic, antibac-terial, anticancer and anxiolytic activities (Padmaa, 2009; Asgarpanah and Kazemivash, 2012). Studies on *C. sativum* have always paying attention on the aerial divisions of the herb (Wangensteen et al., 2004; Hashim et al., 2005). Although the common effects of drought on plant development are fairly well known, the primary effects of water deficit at the molecular and biochemical levels are not well understood (Bhatnagar-Mathur et al., 2009). Furthermore, the metabolic and physiologic reactions of crops to dry environments have been well studied, but similar studies are lacking in aromatic and medicinal plants (Saeidnejadi et al., 2013) such as coriander. The objective of the present investigation was to study effects of

drought stress on growth parameters and activity of antioxidant enzymes in coriander (*corianderum sativum* L.) in order to evaluate the relative significance of these parameters in imparting tolerance.

MATERIALS AND METHODS

In order to study physiological and biochemical parameters of Coriander (*Coriandrum sativum* L.) under drought stress an experiment was designed based on completely randomized design with three replications under greenhouse conditions at Sari Agricultural Sciences and Natural Resources University. In each pot, 30 plants were maintained. Six weeks after sowing, the seedlings were treated by drought treatments at three levels of control, 40% and 70% FC. Coriander plants were harvested 10 days after stress treatments.

Measurements of chlorophyll fluorescence
Chlorophyll fluorescence parameters were recorded at room temperature with a portable PAM-2500 fluorometer (Walz, Effeltrich, Germany). The saturation pulse method was used for analysis of quenching components (Schreiber et al. 1986). Prior the measurements, the attached leaves were dark-adapted for 5 min in leaf-clips (Tarchoune *et al*., 2012). Minimum fluorescence (*Fo*) and maximum fluorescence (*F*m) were measured

upon excitation of leaves with the dark adapted leaves whereas (Fo), (Fm) and (F) was obtained upon excitation of leaves with the light adapted leaves. Maximal fluorescence in the dark-adapted state (Fm) and the light-adapted state (Fm) was measured following a 0.8 s saturating pulse at 5000 μmol m^{-2} s^{-1}. Measurements of Fm and Fm' were performed with the measuring beam automatically switching to 20 kHz during the saturating flash. The value of Fm was long enough for primary quinone electron acceptor of PSII (QA) to be fully oxidized. The steady-state fluorescence during exposure to natural illumination (F) was also measured. All measurements of initial chlorophyll fluorescence of PSII in darkness (Fo) and initial chlorophyll fluorescence of PSII during illumination (Fo) were performed with the measuring beam set to a frequency of 600 Hz. (Duanet al., 2005). Using both light and dark fluorescence parameters, we other fluorescence parameters were calculated as follows: (1) efficiency of excitation energy capture by open PSII reaction centers, $Fv/Fm = (Fm - Fo)/Fm$; (2) the photochemical quenching coefficient, $qP = (Fm - F)/(Fm - Fo)$, which measures the proportion of open PSII reaction centers (Van Kooten and Snel, 1990); (3) the non-photochemical quenching coefficient, $qN = 1 - (Fm - Fo)/(Fm - Fo)$; and (4)photochemical conversion; Y(II) ($(Fm - F)/Fm$), (5) primarily thermal losses, corresponding to the sum of non-regulated heat dissipation and fluorescence emission; Y(NO) (F/Fm) and (6) regulated thermal energy dissipation related to NPQ; Y(NPQ) ((F/Fm) − (F/Fm)) (Genty et al., 1996; Klughammer andSchreiber, 2008) and (7) non-photochemical quenching, NPQ = $(Fm/Fm') - 1$ (Bilger and Björkman, 1990; Essemine et al., 2012)

Deterination of antioxidative enzymes

For preparaing enzyme extracts, fresh leaf samples (0.5 g) were homogenized in 5 ml buffer (0.05 M tris HCl buffer, pH=7.5, 3 Mm MgCl2, 1 Mm EDTA) using a mortar and pestle followed by centrifugation. The enzyme extracts were used to determine GPX, APX and CAT activities (Garratt et al., 2002). For determining APX, 2 mM ascorbate was added in extraction buffer (Sandhya,2010).

The reaction mixture (3 ml) for GPX contained 50 mM phosphate buffer (pH = 7), 1 ml guaiacol 1% and 0.3 ml enzyme extracts. After equilibration at 30^C for 1 min, the reaction was started by addition of 1 ml H$_2$O$_2$ 1% (w/v) and GPX activity was measured by following the decrease in absorbance at 420 nm by using UV−visible spectrophotometer

(Upadhyaya et al., 1985). For measurement of APX, 0.1 ml enzyme extracts was added to the reaction mixture containing 50 mM phosphate buffer, 0.2 ml H_2O_2 1% (w/v), 0.5 mM ascorbate, 0.1 mM EDTA and decrease in absorbance of ascorbate (extinction coefficient 2.8 mM-1 cm-1) was read at 240 nm (Asada and Chen,1989). Activity of CAT was measured by adding 50 ll of enzyme extract to the reaction mixture containing phosphate buffer and H2O2 and following the decrease in absorbance of H2O2 (extinction coefficient 0.036 mM-1 cm-1) within 1 min at 240 nm (Maehly and Chance, 1959)(Sandhya, 2010). Protein concentration was estimated according to Bradford (1976) using bovine serum albumin as standard (Jebara et al., 2010).

Chlorophyll (Chl) contents andinorganic elements: Total Chl content was determined following the method described by Lichtenthaler & Buschmann (2001). Chlorophyll was extracted by pure methanol and its contents were determined using spectrophotometer (Spekol 1300, Japan). The absorbance of the extract was recorded at 652.4 and 665.2nm and Chl content was calculated by equation 1.

[Chls a+b] (µg/ml)= $24.93A_{652.4}+1.44A_{665.2}$ [Equation 1]

Where $A_{652.4}$ and $A_{665.2}$ are the absorbance at 652.4 and 665.2nm, respectively. Determination of Na and K was performed spectrophotometrically by flame photometry (Hajiboland et al., 2010). The data were analyzed using GLM procedures included in the SAS statistical package and mean separation was done according to LSD at the 0.05 probability level.

RESULTS AND DISCUSSION

The results of the ANOVA for estimated traits showed significant differences ($P<0.01$) for all traits (Table 1).Antioxidant activities can be affected by location and growth conditionsof the Coriander plant (Tang et al., 2013). Drought impacts on crop and medicinal plant yields have been reported by some researchers (Mohamed and Abdu, 2004; Renau-Morata et al., 2012).

Correlation coefficient analysis

Correlation among different traits is generally due to the presence of linkage of different genes. Environment play an important role in the development of phenotypic correlation (Jaffee and Price. 2007). In some cases, environment affects both the traits simultaneously in same direction or sometime in different direction. Phenotypic correlation is the net result of genetic and environmental correlation (Cattell, 1960).

Table 1: Analysis of variance for the studied traits under different levels of drought in coriander

S.O.V	df	CAT (x_1)	GPX (x_2)	APX (x_3)	Chl a (x_4)	Chl b (x_5)	Chl a + b (x_6)	Carotenoid(x_7)
Drought	2	0.0000308**	0.0000615**	0.0015747**	1.732**	0.794**	4.769**	0.035**
Error	6	0.0000005	0.0000011	0.0000045	0.039	0.005	0.037	0.000
CV (%)	-	13.78	19.75	12.48	9.76	16.25	9.55	4.37

**, significant at 0.01 probability level

Continue of Table 1. Analysis of variance for the studied traits under different levels of drought in coriander

S.O.V	df	Plant height (x_8)	Leaf fresh weight (x_9)	Shoot fresh weight (x_{10})	Total fresh weight (x_{11})	Leaf dry weight (x_{12})	Shoot dry weight (x_{13})	Total dry weight (Y)
Drought	2	2.088**	2664.441**	6856.445**	16677.583**	104.719**	165.631**	597.547**
Error	6	0.013	10.183	57.310	50.755	4.310	3.468	25.724
CV (%)	-	6.75	6.95	6.47	5.78	9.49	18.5	13.38

**, significant at 0.01 probability level

Table 2: Correlation coefficient between morphological and physiological traits with total dry weight at different drought stress levels

Traits	Total dry weight (Y)		
	FC	70% FC	40% FC
CAT (x_1)	0.110	-0.731*	-0.938**
GPX (x_2)	0.373	-0.619	-0.648
APX (x_3)	-0.187	0.576	0.770*
Chl a (x_4)	-0.007	0.413	0.084
Chl b (x_5)	0.467	0.907**	0.873**
Chl a + b (x_6)	0.355	0.580	0.371
Carotenoid (x_7)	-0.220	-0.663	-0.915**
Plant height (x_8)	0.661	0.970**	0.943**
Leaf fresh weight (x_9)	0.648	0.749*	0.937**
Shoot fresh weight (x_{10})	-0.017	0.751*	0.424
Total fresh weight (x_{11})	0.673*	0.767*	0.741*
Leaf dry weight (x_{12})	0.784*	0.871**	0.982**
Shoot dry weight (x_{13})	0.687*	0.963**	0.996**

Significant at * $P \leq 0.05$ and ** $P \leq 0.01$

Based on table 2, at FC drought stress condition, results revealed that total fresh weight (x_{11}), leaf dry weight (x_{12}) and shoot dry weight (x_{13}) traits had positive phenotypic correlation with total dry weight at 0.05 probability level. Total dry weight would increase if total fresh weight (x_{11}), leaf dry weight (x_{12}) and shoot dry weight (x_{13}) traits would be increased. Biomass production is usually considered a drought tolerance criterion, and these findings are similar to other study results to a great extent (Purcell et al., 2000). All other traits had no-significant phenotypic correlation with total dry weight.

Cluster analysis

Cluster analysis was used to arrange a set of variables into clusters. The aim of cluster analysis was to establish a set of clusters so that cases with in a cluster were more similar to each other than within other clusters

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(Anderberg, 1973). The cluster analysis was performed using a measure of similarity levels and Euclidean distance (Eisen et al., 1998). In this experiment, cluster analysis categorized measured traits into different groups (Figure 1). Based on cluster analysis, group which had a total dry matter trait was the best group for determining the most effective traits on dry matter production. Accordingly, cluster analysis among traits was done at all drought stress levels (FC, 70% FC and 40% FC). According to results of cluster analysis at FC drought stress level, included traits were CAT (x_1), GPX (x_2), total fresh weight (x_{11}) and leaf dry weight which categorized in one group and recognized as the most effective traits in dry matter production (Figure 2). At low drought stress condition (70% FC), included traits were APX (x_3), plant height (x_8) and shoot dry weight (x_{13}) which selected as an important traits in dry matter production. By increasing the amount of drought stress up to 40% FC, six traits were determined as effective traits in dry matter production. These traits included APX (x3), Chl b (x5), plant height (x8), leaf fresh weight (x9), leaf dry weight (x12) and shoot dry weight (x13) which categorized in separate group (Figure 3) and seems to appropriate for improving dry matter

production at high drought stress conditions. Effects of drought on the seed extract antioxidant activity of *Cuminum cyminum* L. by different methods were investigated, and it was shown that seeds under drought conditions had higher antioxidant activity (Rebey et al., 2012).

Stepwise multiple linear regression

Stepwise multiple linear regression were used to determine the variable accounting for the majority of total yield variability. Stepwise program computed a sequence of multiple linear regression in a stepwise manner. One variable was added to the regression equation at each step. The added variable was the one which induced the greatest reduction in the error sum of squares. It was also the variable which had the highest partial correlation with the dependent variable for fixed values of those variables already added. Moreover, it was the variable which had the highest F-value (Leila and Al-Khateeb, 2005). The stepwise multiple linear regression results were parallel to cluster analysis finding. The stepwise multiple linear regression procedure has been also used by Firoozi et al (2012). At FC drought stress level (control), leaf dry weight and Chl b (R^2) were important in the total dry weight production (Table 6). Furthermore, at 70% FC drought stress level,

the traits included shoot dry weight, leaf dry weight and carotenoid (R^2) had an important role in total dry weight production (Table 7). Also, shoot dry weight and leaf dry weight (R^2) were recognized as most effective traits which determined total dry weight production at 50% FC drought stress level (Table 8).
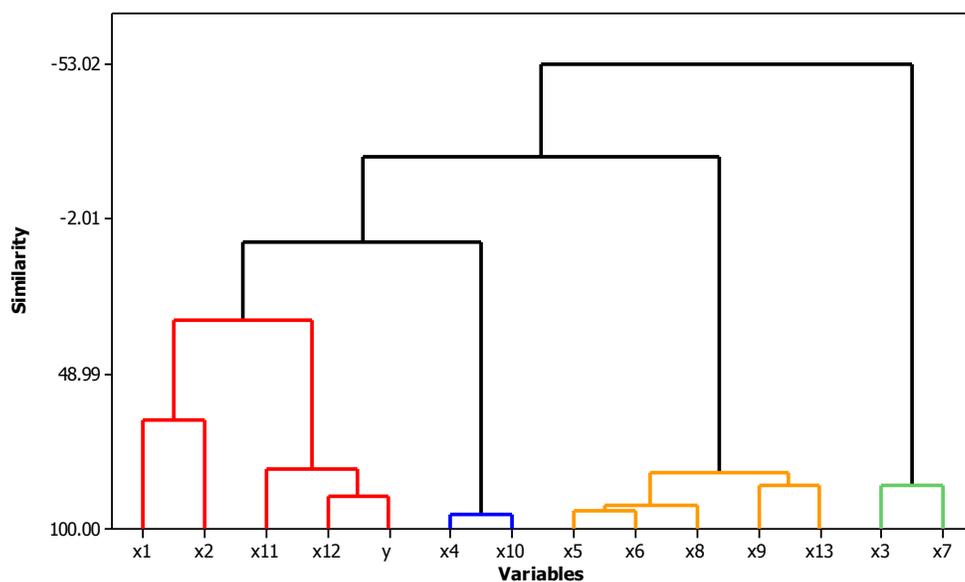

Figure 1: Segregation of traits at FC drought stress level using the hierarchial cluster analysis
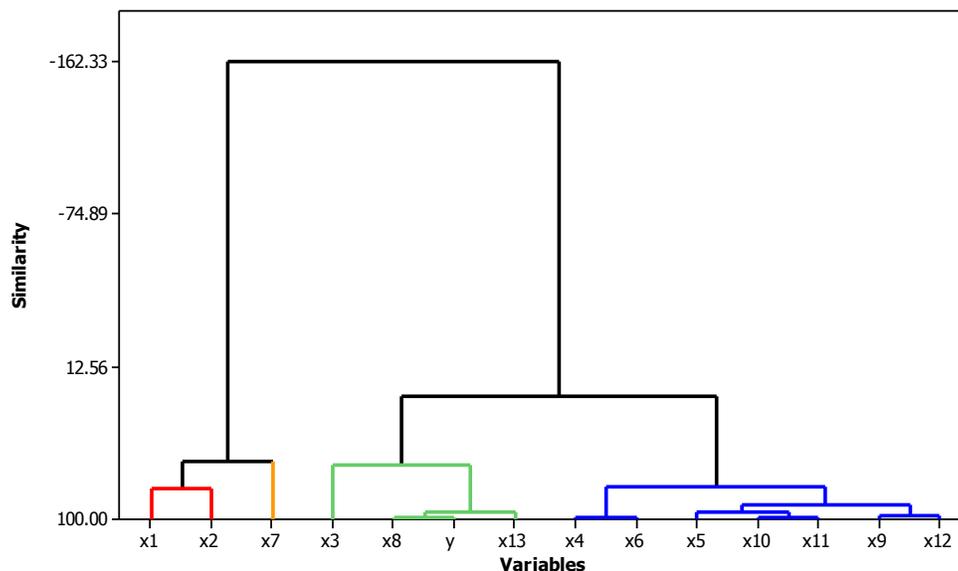
Figure 2: Segregation of traits at 70% FC drought stress level using the hierarchial cluster analysis
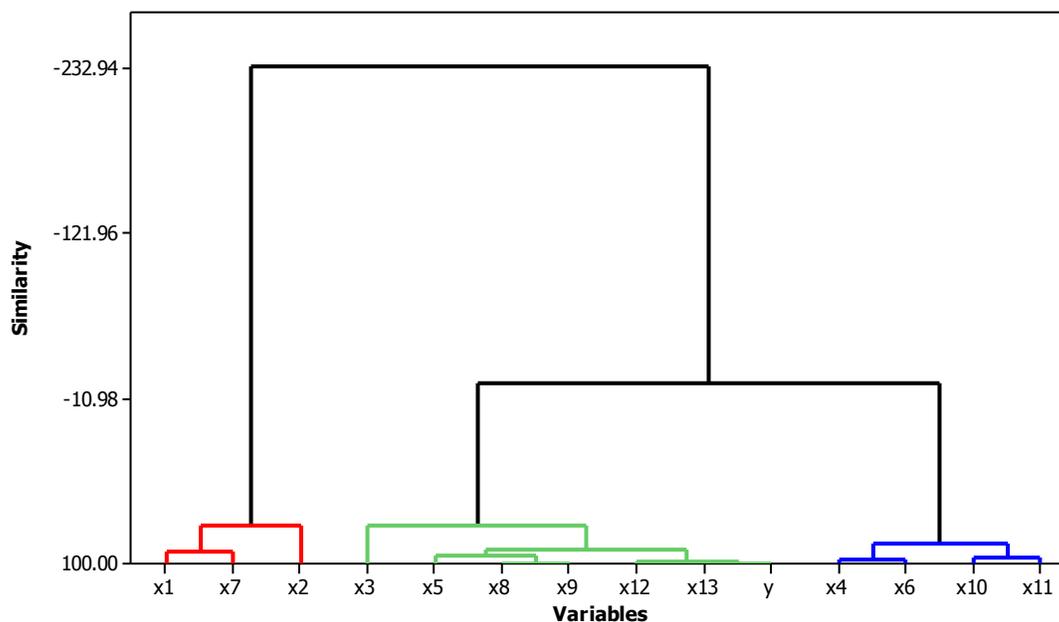

Figure 3: Segregation of traits at 40% FC drought stress level using the hierarchial cluster analysis

Table 3: Similarity and distance levels of the estimated characters (variables) using the hierarchical cluster analysis at FC drought stress level

Step	Number of clusters	Similarity level	Distance level
1	13	95.2101	0.09580
2	12	94.0183	0.11963
3	11	92.0514	0.15897
4	10	89.2209	0.21558
5	9	85.8141	0.28372

6	8	85.7433	0.28513
7	7	81.3725	0.37255
8	6	80.0090	0.39982
9	5	63.8665	0.72267
10	4	31.3408	1.37318
11	3	5.3649	1.89270
12	2	-22.5206	2.45041
13	1	-53.0218	3.06044

Table 4: Similarity and distance levels of the estimated characters (variables) using the hierarchial cluster analysis at 70% FC drought stress level

Step	Number of clusters	Similarity level	Distance level
1	13	99.439	0.01122
2	12	98.954	0.02093
3	11	98.501	0.02998
4	10	97.943	0.04113
5	9	96.348	0.07304
6	8	95.852	0.08297
7	7	92.033	0.15933
8	6	82.411	0.35178
9	5	81.292	0.37416
10	4	68.417	0.63167
11	3	66.840	0.66321
12	2	29.527	1.40945
13	1	-162.331	5.24662

Table 5: Similarity and distance levels of the estimated characters (variables) using the hierarchical cluster analysis at 40% FC drought stress level

Step	Number of clusters	Similarity level	Distance level
1	13	99.809	0.00382
2	12	99.438	0.01123
3	11	98.159	0.03681
4	10	97.278	0.05443
5	9	95.880	0.08239
6	8	94.433	0.11134
7	7	91.463	0.17075
8	6	90.059	0.19882
9	5	85.925	0.28150
10	4	75.092	0.49815
11	3	74.977	0.50047
12	2	-20.952	2.41903
13	1	-232.936	6.65873

Table 6: Relative contribution in predicting total dry weight, F-value and probability by the stepwise procedure analysis at FC drought stress level

Model	Traits	*B*	Std. Error	Beta	R Square	*T*	Sig.
	(Constant)	-23.408	24.022			-0.974	0.367
2	Leaf dry weight (x_{12})	2.073	0.402	0.799	0.61	5.153	0.002
	Chl b (x_5)	5.939	1.874	0.491	0.24	3.168	0.019

Table 7: Relative contribution in predicting total dry weight, F-value and probability by the stepwise procedure analysis at 70% FC drought stress level

Model	Traits	*B*	Std. Error	Beta	R Square	*T*	Sig.
3	(Constant)	0.251	0.086			2.904	0.034
	Shoot dry weight (x_{13})	1.000	0.000	0.693	0.94	8652.768	0.000

Model	Traits	B	Std. Error	Beta	R Square	T	Sig.
	Leaf dry weight (x_{12})	0.999	0.000	0.382	0.03	4714.546	0.000
	Carotenoid(x_7)	-0.180	0.063	0.000	0.02	-2.848	0.036

Table 8: Relative contribution in predicting total dry weight, F-value and probability by the stepwise procedure analysis at 40% FC drought stress level

Model	Traits	B	Std. Error	Beta	R Square	T	Sig.
	(Constant)	0.026	0.024			1.077	0.323
2	Shoot dry weight (x_{13})	1.000	0.000	0.692	0.99	2351.127	0.000
	Leaf dry weight (x_{12})	0.999	0.001	0.316	0.01	1075.353	0.000

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