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ORIGINAL ARTICLE

Drought tolerance screening in some safflower (*Carthamus tinctorius*) genotypes using physiological traits

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ABSTRACT
Drought stress is one of the major limitations to plant productivity across the world. Identifying suitable screening tools and also quantifiable traits would facilitate the crop improvement. In order to identify drought tolerant genotypes in safflower, an experiment was conducted in a randomized complete block design. The 15 genotypes of safflowers were grown under normal and stress condition with three replications. There was high variability among genotypes in response to drought. PI-1989290 had the highest CAT activity while under stress condition LRV-51-51 had the highest value. Drought stress influence POD activity dramatically, more than 2 fold, in Dincer, KINO-76 and PI-537598 genotypes, whereas this differences isn't significant in the CW-4440 and Yenice. Maximum RWC (0.64) was belonged to PI-1989290 and PI-537636-S while the lowest RWC was 0.61 in KINO-76 genotypes. The average of RWC for genotypes was obtained as 0.62. These physiological characters can be transferred to high yield genotypes to improve drought adaptability.
Keywords*: catalase, peroxidase, relative water content (RWC)*
Abbreviations*: CAT: catalase, POD: peroxidase, RWC; relative water content*

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INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the plants which have a high adaptation to different abiotic stress such as drought and salinity (Khalili Mosavi et al., 2009).

Interest in safflower has been increased as a result of drought tolerance and the application of safflower oil in human nutrition and industrial purposes. Nonetheless, it appears that there has not been an increase in safflower farms because of a wide variety of problems, mainly due to unavailability of adapted varieties to stress condition.

In general 33.5% of agricultural lands are devoted to dry cultivation and about 1.2 million/ha of lands under dry cultivation, more than 400 (mm) rainfall will receive (Mohammadi *et al.,* 2006; Shahriari Ahmadi et al., 2013). Thus, loss of yield in these areas is the main concern of plant breeders. Water deficit or drought is defined as a situation which adequate moisture for normal growth does not exist. In this situation, plants cannot successfully complete their life cycle and subsequently grain yield will decline (Kusaka et al., 2005; Shahriari Ahmadi et al., 2013). Drought stress usually induces the oxidative stress. In normal condition the reactive oxygen species (ROSs) are present in cell and have essential roles in various processes such as a plant cell death and in this condition generation of ROSs is tightly regulated but in the drought stress condition the cellular homeostasis is disrupted and the level of ROSs increases by unregulated manners (Shahriari Ahmadi et al., 2013; Panahi et al., 2013). The ROSs affects membrane lipids; inhibit enzyme activity and causes DNA and RNA damages (Mittler, 2002; Panahi et al., 2012). Catalase and peroxidase are key enzymes in decreases the ROSs especially in stress conditions (Simova-Stoilova et al., 2008).

Some investigations are reported on the effect of drought stress on safflower (Pandey et al. 20081). Omidi (2009) reported that developmental stages response differently to drought stress and subsequently effects on grain yield in safflower. It was reported that the seed yield of safflower decreased

sharply when drought stress was severe (Lovelli et al., 2007; Panahi et al., 2013). Development of stress tolerant varieties is always a major objective of many breeding programs but success has been limited by adequate screening techniques and the lack of genotypes that show clear differences in response to various environmental stresses.
In this regards, the objective of present study was to screen drought tolerance in Iranian safflowers genotypes to discover tolerant varieties to be utilized as gene donor in breeding programs for improving physiologically drought tolerance in high yielding genotypes.

MATERIALS and Methods

Plant material and stress treatment

15 safflower genotypes were selected for screening drought tolerance (Table 1). In each pot 5 seeds were sown. Experiment had three replications in each site and conducted in a Random Complete Blocks Design (RCBD) experimental design. Plants were irrigated normally until the onset of flowering. Subsequently under normal condition pots were well watered every day while under stress condition water withholding prolonged until visual wilting was recorded. To ensure the application of drought stress plants were watered whenever the RWC for the most wilted genotype reached to 0.52±0.2. In average it took five days to detect severe wilting in plants.

Relative water content (RWC) and chlorophyll content

A sample of 10 leaves were taken randomly from the flag leaves of each genotype and fresh weight (FW) was measured. Then, samples were placed in distilled water for 24 h and reweighed to obtain turgid weight (TW). Leaf samples were oven dried and weight in 70°C for 72 h (DW). RWC was calculated using the following formula:

$$RWC\ (\%) = \left[\frac{Fw - Dw}{Tw - Dw} \right] \times 100$$

ured using the chlorophyll meter (SPAD-502).

For enzyme assay leaves were collected. 0.2 g of Leaves were ground in liquid nitrogen and transferred to micro tubes. Total soluble protein was measured by Bradford method (Bradford, 1976). CAT activity was determined by the method of Aebi (1984). POD activity was measured by the method of Chance and Machly (1995). The enzyme activity was measured as UmL^{-1} and reported in Umg^{-1} protein.

Statistical analysis of data

Analysis of variance and correlation were performed using SPSS version 15 and Microsoft Office Excel 2007, respectively. Means of treatments were compared using Duncan's multiple range tests at $p \leq 0.05$. Means of treatments are presented in graphs with standard error bars.

RESULTS AND DISCUSSION

Large scale screening is essential to detect novel genetic resource for drought tolerance (Shahriari Ahmadi et al., 2013). There were significant differences among genotypes grown under stress and normal conditions. Regarding antioxidant enzymes under normal condition PI-1989290 had the highest CAT activity while under stress condition LRV-51-51 had the highest value. In general the results of ANOVA revealed significant enhancement in CAT activity under stress condition i.e. the mean CAT activity of normal and stress conditions were 1.4 and 2.1 (Umg-1 protein), respectively. However the magnitude of increase varied among genotypes. Under stress condition CAT activity for PI-537530 increased significantly compared to normal condition (Figure 1).

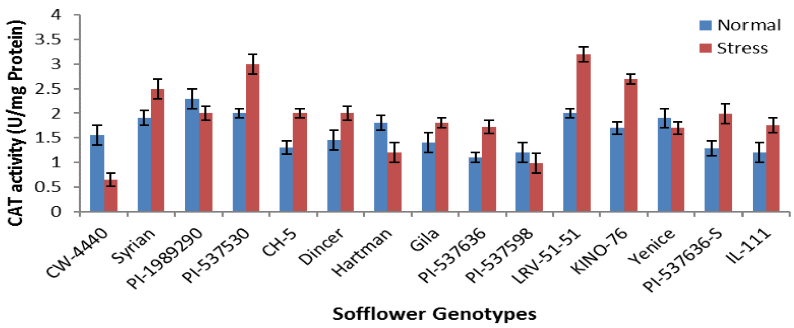


Figure 1: CAT activity in leaves of safflower genotypes under stress and normal conditions.

Results showed that POD activity also influenced significantly by drought stress ($p \leq 0.01$). As shown in the figure 2, drought stress influence POD activity dramatically, more than 2 fold, in Dincer, KINO-76 and PI-537598 genotypes, whereas this differences isn't significant in the CW-4440 and Yenice.
CAT and POD are key enzymes in scavenging and detoxification of hydrogen peroxide, a hazardous byproduct of photorespiration (Hameed et al., 2011). Induced activity of these enzymes under stress condition has been reported previously (Hameed et al., 2011; Khanna- Chopra and Selote, 2007; Shahriari Ahmadi et al., 2013).

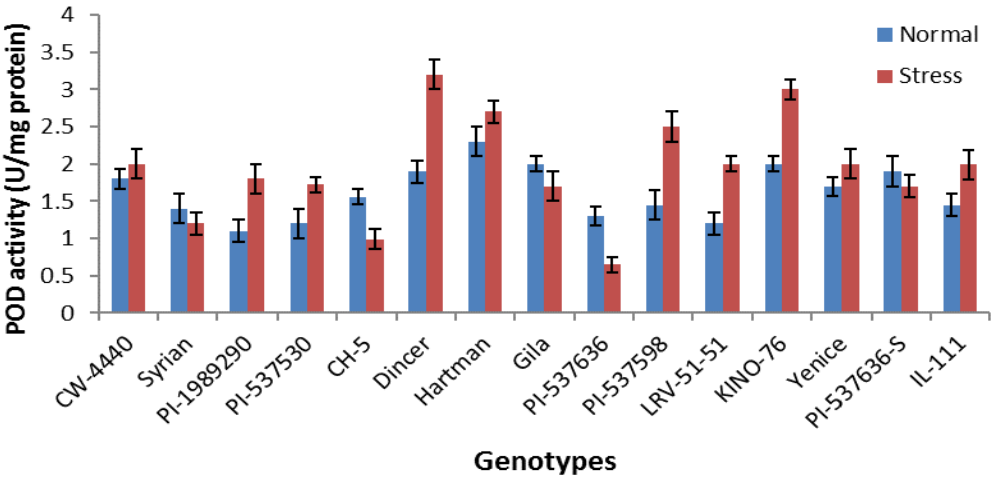


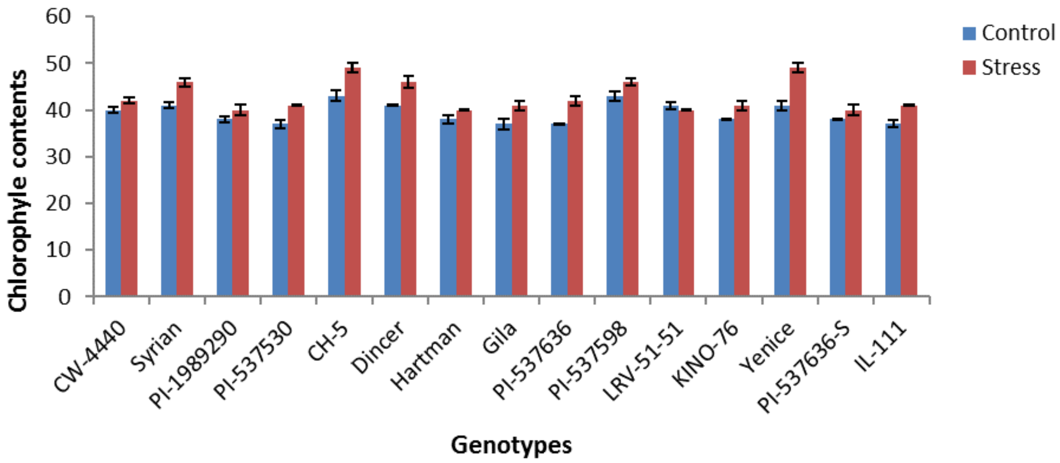
Figure 2: POD activity in leaves of safflower genotypes under stress and normal conditions.

Analysis of RWC in different genotypes in response to salt stress showed that different genotypes have different reaction to drought stress in the physiological levels (Table 1). Maximum RWC (0.64) was belonged to PI-1989290 and PI-537636-S while the lowest RWC was 0.61 in KINO-76 genotypes. The average of RWC for genotypes was obtained as 0.62. It means these genotypes experienced oxidative stress and their antioxidant enzymes triggered to detoxify cells. These results are in consistence with Loggini et al. (1999) and Simova-Stoilova et al. (2009) in which the antioxidant enzyme activity were reported higher in sensitive wheat cultivars. This variation in relative water content may be due to different ability of the genotypes in water absorption from the soil or different mechanisms in osmolyte accumulation in plant tissues to retain turgescence pressure (Shahriari Ahmadi et al., 2013).

Table 1: Mean value of relative water content (RWC) of 15 genotypes studied for drought tolerance screening along with standard error (±SE) under stress condition.

No.	Genotype	RWC
1	CW-4440	0.63±0.02
2	Syrian	0.62±0.05
3	PI-1989290	0.64±0.02
4	PI-537530	0.63±0.04
5	CH-5	0.62±0.02
6	Dincer	0.62±0.07
7	Hartman	0.62±0.04
8	Gila	0.63±0.02
9	PI-537636	0.61±0.06
10	PI-537598	0.62±0.03
11	LRV-51-51	0.62±0.05
12	KINO-76	0.61±0.05
13	Yenice	0.62±0.02
14	PI-537636-S	0.64±0.03
15	IL-111	0.63±0.01

Drought stresses effects dramatically in chlorophyll content in Syrian, PI-537530, CH-5, Dincer, KINO-76, Yenice, IL-111 genotypes (figure 3). CH-5 and Yenice had the maximum value (0.86 g/m2) for chlorophyll content under stress condition while, under stress condition CH-5 (0.76 g/m2) had the maximum content of chlorophyll.



According to Fernandez's finding (1992) genotypes are divided into four groups based on their yield under stress or non-stress conditions. A: genotypes which have similar desirable response under both stresses and non-stress conditions, B: genotypes which have desirable response just under none stress conditions, C: genotypes which have desirable response just under stressed conditions and D: genotypes which have not desirable response neither under stress or non-stress conditions. Considering to obtained results, it seems that PI-537598, Dincer, PI-250537and Gila genotypes were more capable to encounter the drought stress. Results from screening based on morphological traits were in agreement with findings of other researchers (Pourdad et al., 2006). Therefore, with respect to importance of physiological traits in crop production and its high hereditability, it is suggesting that antioxidant scavenger's enzymes should be used in breeding programs as reliable indexes for drought stress screening.

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