University of Birjand

تنشهای محیطی در علوم زراعی
Environmental Stresses In Crop Sciences
Env. Stresses Crop Sci.
Vol. 15, No. 2, pp. 361-373 (Summer 2022)

http://dx.doi.org/10.22077/escs.2020.3792.1916

Original article

Evaluation of physiological responses of cannabis (*Cannabis sativa* L.) ecotypes under different levels of irrigation

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Received 8 October 2020; Accepted 27 December 2020

Extended abstract

Introduction

Cannabis (*Cannabis sativa* L.) is an herbaceous annual plant belongs to Cannabacea Family. (Ahmad et al., 2008). The resistance to water shortage, the ability to grow in different climatic conditions, and great genetic diversity are features of this plant (Amaducci et al., 2008). Drought is one of the most important environmental stresses limiting crop production worldwide and has adverse effects on plant growth, development, which may result in decreased chlorophyll a and b and increased proline content of leaf (Lum et al., 2014; Karimi et al., 2016). Plants generally adapt to drought stress by inducing a variety of physiological, biochemical, and morphological responses, and each of these factors can be effective in introducing drought tolerant cultivars. Among the physiological properties, leaf water condition, membrane stability, photosynthetic changes and related factors are of great importance (Farooq et al., 2009). Considering the pharmaceutical and industrial importance of cannabis, this study was conducted to identify the drought tolerant and sensitive ecotypes of cannabis based on physiological responses.

Material and Method

This study was done in research greenhouse of University of Tehran, Iran, from February to July 2017 on the base of factorial experiment in as a completely randomized design (CRD) with three replications. The first factor consisted of three soil moisture levels [100% (normal irrigation), 75% (mild drought stress), and 50% (serve drought stress)] of field capacity (FC). Also, the 12 Iranian ecotypes of cannabis were the second factor where collected from different geographical regions of Iran including Urmia, Tabriz, Sanandaj, Dasht-e-Moghan, Rasht, Khomein, Daran, Qom, Shahrood, Kerman, Tabas, and Saravan. The seedlings thinning was done at 3-4 leaf pairs stage and four plants were maintained in each pot. At the time point of sex determination of plants, one female plant was kept for future study. The irrigation was done uniformly to all pots until the emergence of fifth pair of leaves and afterwards, irrigation treatments were applied. During applying irrigation treatments, the soil humidity of the pots was measured before each irrigation cycle. Relative water content, electrolyte leakage, chlorophyll a, chlorophyll b, total chlorophyll content and carotenoid pigments, proline content, catalase and guaiacol

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peroxidase enzymes were measured at full flowering period - early fruiting. The analyses of variance of obtained data were down using SAS software (v.9.2) and Duncan's multiple ranges test was used for comparing the averages at the significance level of $\alpha = 0.05$.

Results and Discussion

The results showed that the highest value of relative water content was obtained from the normal irrigation, which was 77.21% and was reduced to 16.70 and 31.13% under mild and serve drought stress, respectively. Interaction effect of Irrigation levels and ecotypes showed that Urmia ecotype had the highest value of relative water content in normal irrigation treatment, and Tabriz ecotype had lowest value of this parameter in severe drought stress. The electrolyte leakage Index was decreased by 10.54 and 24.11% at mild and severe drought stress, compared to normal irrigation, respectively. The highest value of electrolyte leakage was obtained from Tabriz ecotype in severe drought stress, and the lowest value of this parameter was obtained from Tabas and Saravan ecotypes in normal irrigation treatment. The highest values of chlorophyll b and total chlorophyll content were obtained for Tabas and Urmia ecotypes with 0.61 and 2.25 ($mg.g^{-1}$ fw), respectively, at normal irrigation treatment. The lowest values of this parameters were obtained for Tabriz and Dasht-e-Moghan ecotypes with 0.17 and 0.84 ($mg.g^{-1}$ fw), respectively, in severe drought stress. Water deficit decreased 28.12% of carotenoid pigments at severe drought stress compared to normal irrigation, and it increased values of proline, catalase and guaiacol peroxidase enzymes with 47.06, 29.18 and 22.78 (%) respectively, at severe drought stress compared to normal condition. The highest values of carotenoid pigments, proline, catalase and guaiacol peroxidase enzymes were observed in the ecotypes of Tabas, Urmia, Qom and Urmia [0.79 ($mg.g^{-1}$ fw), 1.27 ($mg.g^{-1}$fw), 0.0820 and 0.5800($Mc.min^{-1}$ mg^{-1} pro), respectively], and the lowest values of them were obtained for Tabriz, Dasht-e-Moghan, Khomein and Rasht ecotypes [0.34 ($mg.g^{-1}$fw),0.48 ($mg.g^{-1}$ fw), 0.0396 and 0.2744 ($Mc.min^{-1}$ mg^{-1} pro), respectively].

Conclusion

The results of this study showed that Tabas ecotype had a significant advantage in maintaining relative water content, maintaining chlorophyll content and maintaining membrane stability. The Tabriz Ecotype is the most sensitive ecotype for drought conditions. Because it lost the most values of the relative water content, chlorophyll content and membrane stability in stress condition compared to other ecotypes. Therefore, it can be concluded that the physiological parameters measured under drought stress conditions can be used as a criterion for the identification of tolerant and sensitive ecotypes.

***Keywords*:** Catalase, Chlorophyll, Drought Stress, Guaiacol Peroxidase, Prolin

Table 1. Coding, climatic and geographical characteristics of hemp ecotypes collection regions in Iran.

Ecotype	Ecotype Code*	Rainfall (mm)	Climate	Latitude, N	Longitude, E	Altitude (m)
Urmia (West Azerbaijan)	247 CS	341	Dry temperate	37° 52′	45° 4′	1345
Sanandaj (Kurdistan)	248 CS	458.4	Dry temperate	35° 30′	47° 03′	1538
Tabriz (East Azerbaijan)	249 CS	310	Dry temperate	38° 5′	46° 28′	1365
Dasht-e-Moghan (Ardabil)	250 CS	303.9	Dry temperate	39° 64′	47° 92′	388
Rasht (Gilan)	251 CS	1359	Humid temperate	37° 22′	49° 63′	3
Khomein (Arak)	252 CS	341.7	Semi-arid	33° 63′	50° 07′	1811
Daran (Isfahan)	253 CS	122.8	Semi-arid	32° 98′	50° 41′	2325
Qom (Qom)	254 CS	151.1	Semi-arid	34° 64′	50° 89′	934
Shahroud (Semnan)	255 CS	140.8	Semi-arid	36° 39′	54° 94′	1308
Kerman (Kerman)	256CS	135	Arid	30° 29′	57° 06′	1755
Tabas (South Khorasan)	257 CS	84.85	Arid	33° 86′	56° 93′	682
Saravan (Zahedan)	258 CS	90.6	Arid	27° 38′	62° 32′	1164

*: Ecotype codes were obtained from: Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran.

Table 2. Physical and chemical characteristics of the soil used for planting.

Soil Texture	Silt	Clay	Sand	OC	EC	pH	FC
	----------%----------				dSm^{-1}		%
Clay loam	38	32	30	0.82	1.62	7.9	22

Table 3. Analysis of variance on Physiological traits in different ecotypes of cannabis under deficit irrigation conditions.

S.O.V	df	Relative water content	Electrolyte leakage	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Irrigation levels (A)	2	5206.08 **	2536.95 **	0.4064 **	0.55 **	1.911 **
Ecotyps (B)	11	310.46 **	598.14 **	0.7253 **	0.050 **	1.033 **
A*B	22	26.77 **	14.26 *	0.0054 ns	0.0028 **	0.0135 **
Error	72	4.71	8.26	0.0034	0.0011	0.0073
C.V (%)	-	3.34	4.68	5.33	9.31	5.83

Table 3. Continued

S.O.V	df	Carotenoid	Proline	(CAT) Catalase	Gayacol Peroxidase (GPX)
Irrigation levels (A)	2	0.3049 **	2.87 **	0.0046 **	0.1195 **
Ecotyps (B)	11	0.1941 **	0.7266 **	0.0019 **	0.0832 **
A*B	22	0.0016 ns	0.0139 ns	0.0002 ns	0.0175 ns
Error	72	0.0090	0.0092	0.0001	0.0107
C.V (%)	-	17.06	10.81	18.36	23.05

*,** and ns: represent significant at of 5% and 1% probability level and not significant, respectively.

Table 4. Mean comparison of simple effects of irrigation levels and ecotype on physiological traits.

Treatment	Relative water content	Electrolyte leakage	Chlorophyll a	Chlorophyll b	Total Chlorophyll
	----------%----------		----------mg.g^{-1}Fw----------		
Irrigation					
Severe	53.18 ^{c}	69.49 ^{a}	0.99 ^{c}	0.23 ^{c}	1.22 ^{c}
Mild	64.32 ^{b}	62.16 ^{b}	1.11 ^{b}	0.37 ^{b}	1.49 ^{b}
Normal	77.21 ^{a}	52.74 ^{c}	1.21 ^{a}	0.48 ^{a}	1.69 ^{a}
Ecotype					
Urmia	71.52 ^{ab}	59.85 ^{de}	1.52 ^{a}	0.42 ^{c}	1.94 ^{a}
Sanandaj	59.30 ^{f}	71.53 ^{b}	0.82 ^{f}	0.30 ^{de}	1.12 ^{f}
Tabriz	60.17 ^{f}	74.41 ^{a}	1.09 ^{d}	0.32 ^{de}	1.41 ^{d}
Dasht-e-Moghan	56.05 ^{g}	68.72 ^{c}	0.55 ^{g}	0.29 ^{e}	0.84 ^{g}
Rasht	60.62 ^{f}	66.96 ^{c}	1.29 ^{c}	0.32 ^{de}	1.61 ^{c}
Khomein	63.01 ^{de}	61.34 ^{d}	0.94 ^{e}	0.33 ^{d}	1.27 ^{e}
Daran	63.46 ^{d}	57.91 ^{e}	1.05 ^{d}	0.33 ^{d}	1.38 ^{d}
Qom	67.39 ^{c}	54.39 ^{f}	1.28 ^{c}	0.33 ^{d}	1.61 ^{c}
Shahroud	61.07 ^{ef}	66.95 ^{c}	0.84 ^{f}	0.31 ^{de}	1.16 ^{f}
Kerman	70.51 ^{b}	53.73 ^{f}	1.06 ^{d}	0.40 ^{c}	1.46 ^{d}
Tabas	73.42 ^{a}	50.91 ^{g}	1.31 ^{c}	0.53 ^{a}	1.84 ^{b}
Saravan	72.29 ^{ab}	50.84 ^{g}	1.45 ^{b}	0.47 ^{b}	1.92 ^{a}

Table 4. Continued

Treatment	Carotenoid	Proline	Catalase (CAT)	Gayacol Peroxidase (GPX)
	---------mg.g^{-1}Fw---------		---------Mc.min^{-1} mg^{-1} pro--------	
Irrigation				
Severe	0.46 ^{c}	1.19 ^{a}	0.0778 ^{a}	0.5036 ^{a}
Mild	0.57 ^{b}	0.83 ^{b}	0.0669 ^{b}	0.4556 ^{a}
Normal	0.64 ^{a}	0.63 ^{c}	0.0551 ^{c}	0.3889 ^{b}
Ecotype				
Urmia	0.57 ^{bc}	1.27 ^{a}	0.0814 ^{ab}	0.5800 ^{a}
Sanandaj	0.47 ^{cd}	0.58 ^{e}	0.0556 ^{de}	0.4144 ^{cde}
Tabriz	0.34 ^{e}	0.55 ^{ef}	0.0696 ^{bc}	0.4278 ^{cde}
Dasht-e-Moghan	0.49 ^{cd}	0.48 ^{f}	0.0567 ^{de}	0.3878 ^{de}
Rasht	0.44^{de}	0.84 ^{c}	0.0494 ^{ef}	0.2744 ^{f}
Khomein	0.36 ^{e}	0.74 ^{d}	0.0396 ^{f}	0.3322 ^{ef}
Daran	0.62 ^{b}	0.97 ^{b}	0.0792 ^{ab}	0.5478 ^{ab}
Qom	0.64 ^{b}	1.04 ^{b}	0.0854 ^{a}	0.4800^{abcd}
Shahroud	0.52 ^{cd}	0.67 ^{d}	0.0591 ^{cde}	0.3900 ^{de}
Kerman	0.78 ^{a}	0.98 ^{b}	0.0643 ^{cd}	0.4467 ^{bcd}
Tabas	0.79 ^{a}	1.23 ^{a}	0.0820 ^{ab}	0.5611 ^{a}
Saravan	0.66 ^{b}	1.19 ^{a}	0.0769 ^{ab}	0.5300 ^{abc}

.In each column, averages with the same letters are not significantly different at 5% level of probability.

Table 5. The effect of interaction of irrigation levels in the ecotype on some physiological traits.

Irrigation	Ecotype	Relative water content	Electrolyte leakage	Chlorophyll b	Total Chlorophyll
		----------%	----------	----------mg.g^{-1}fw----------	
Severe	Urmia	57.97 nop	67.30ef	0.23 ij	1.60 efg
	Sanandaj	45.77 rs	79.77 ab	0.19 jk	0.93 rs
	Tabriz	42.11 t	84.43 a	0.17 jk	1.11 nop
	Dasht-e-Moghan	42.83 st	77.49 bc	0.20 jk	0.69 t
	Rasht	47.83 r	75.55 bc	0.20 jk	1.35 jkl
	Khomein	55.24 pq	70.57 de	0.21 jk	1.07 opq
	Daran	52.42 q	68.09 e	0.22 jk	1.16 no
	Qom	57.06 op	62.05 gh	0.21 jk	1.36 jkl
	Shahroud	48.21 r	75.16 bcd	0.20 jk	0.95 qrs
	Kerman	60.28 lmn	60.95 gh	0.27 hi	1.23 lmn
	Tabas	65.93 jk	56.81 ijk	0.38 ef	1.57 fgh
	Saravan	62.48 klm	56.81 ijk	0.32 gh	1.63 efg
Mild	Urmia	71.99 ghi	61.32 gh	0.46 bcd	1.97 bc
	Sanandaj	59.20 mn	74.53 cd	0.28 hi	1.10 npp
	Tabriz	60.89 klm	75.40 bc	0.31 gh	1.38 ijk
	Dasht-e-Moghan	55.17 pq	70.50 de	0.29 h	0.84 s
	Rasht	59.18 mn	68.78 e	0.33 fgh	1.60 efg
	Khomein	60.20 lmn	60.20 ghi	0.35 fg	1.30 klm
	Daran	63.07 jkl	54.40 jkl	0.32 gh	1.38 ijk
	Qom	66.11 j	53.78 jkl	0.31 gh	1.61 efg
	Shahroud	58.78 no	69.18 e	0.32 gh	1.17 mno
	Kerman	71.10 hi	53.43 kl	0.43 cde	1.51 ghi
	Tabas	73.90 fgh	51.81 klm	0.59 a	2.00 b
	Saravan	72.26 ghi	52.59 kl	0.50 b	1.99 b
Normal	Urmia	84.61 a	50.94 lmn	0.56 a	2.25 a
	Sanandaj	72.93 fgh	60.30 ghi	0.42 de	1.33 c
	Tabriz	77.50 cde	63.40 fg	0.48 bc	1.73 dc
	Dasht-e-Moghan	70.16 i	58.16 hij	0.38 ef	0.99 pqr
	Rasht	74.84 efg	56.55 ijk	0.43 cde	1.87 bc
	Khomein	73.59 fgh	53.25 kl	0.43 cde	1.44 hij
	Daran	74.90 efg	51.23 klm	0.45 bcd	1.61 efg
	Qom	79.00 bcd	47.34 mn	0.47 bcd	1.85 cd
	Shahroud	76.21 def	56.51 ijk	0.43 cde	1.36 jkl
	Kerman	80.18 bc	46.81 no	0.49 bc	1.65 ef
	Tabas	80.45 bc	45.26 o	0.61 a	1.95 bc
	Saravan	82.13 ab	43.13 o	0.59 a	2.14 a

In each column, averages with the same letters are not significantly different at 5% level of probability.