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Original article

Evaluation of the effect of cold stress on physiological and biochemical traits of commerical sugarcane cultivar CP69-1062 with *Saccharum spontaneum* species in seedling stage

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Extended abstract

Introduction

Cold is one of the most important and primitive factors limiting plant growth, especially tropical and subtropical plants. Sugarcane is a tropical and subtropical plant that produces 70% of the total world's sugar. So its impact on the global economy is transparent and undeniable. The main problem with most sugarcane cultivars is their extreme sensitivity to cold, which reduces the yield, sugar content and quality of their syrup. The long-term meteorological statistics of Khuzestan province in the past years, show that temperatures below 0°C occur in the sugarcane cultivation and industries almost every year. This factor has a direct effect on reducing sugar production as well as reducing final income. Among the commercial varieties of sugarcane, "*Saccharum officinarum* var CP69-1062" cultivar has high yield and produces a lot of sugar, but its main problem is its sensitivity to cold and encountering with this stress, which may reduce the yield and content of sugar that can be extracted from it. In contrast, "*Saccharum spontaneum*" is a wild cultivar with high sucrose content and resistant to biotic and abiotic stresses such as cold stress. So, according to aforementioned, this study was conducted to investigate the effect of cold stress on morphological, physiological and biochemical traits of two sugarcane cultivars CP69-1062 (cold-sensitive) and *S. spontaneum* (cold-tolerant) under controlled conditions, to: (1) Reporting putative Cold-tolerance cultivars, Aiming to expand their production and use them in breeding programs such as backcross and producing new tolerant cultivars, (2) Reporting The most important traits affected under cold stress and (3) Reporting stable traits under normal and cold stress conditions.

Materials and methods

The experiment was performed as factorial experiment in a completely randomized design with three replications in the tissue Culture and biotechnology Laboratory and Research Greenhouse of Khuzestan Sugarcane Development Research and Training Institute, and cold chamber in the 2019-2020 crop year. To apply cold stress, sugarcane seedlings were placed in cold chamber and exposed to 0°C and -4°C for

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24 hours. After the end of the stress period, sampling and analysis were immediately performed. Finally, morphological, physiological and biochemical traits of stressed and control samples such as malondialdehyde content (MDA), electrolyte leakage (EL), leaf pigments, catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), supraxide Dismutase (SOD), proline and total water soluble carbohydrates (Wsc) of the leaf were measured.

Results and discussion

The first effects of cold stress were observed on photosynthesis pigments among all cultivars. However, the sensitive cultivar had a greater reduction compared to tolerant cultivar. Cold stress significantly increased electrolyte leakage (EL) and malondialdehyde (MDA) content from 25 to -4°C in all cultivars, particularly in sensitive cultivar. Moreover, proline content and total water-soluble carbohydrates (WSC) of leaf showed significant increased under cold stress, particularly in tolerant cultivar. Under cold stress, Catalase (CAT) activity significantly decreased compared to control temperature, which was accompanied with negative correlation with proline content. In addition, Superoxide dismutase (SOD), Peroxidase (POD) and Ascorbate peroxidase (APX) activity was higher than control temperature in all cultivars, particularly in tolerant ones. The intensity of these changes were less at -4°C due to intracellular freezing, ice crystals formation and the abolition of cellular metabolic activities. Phenotypicaly, at 0°C, the leaves of the tolerant cultivar showed little change in appearance in comparision with control temperature (25°C). On the other hand, at -4°C the effects of seedling damage were clearly observed.

Conclusions

The results showed that both sensitive (*S. officinarum* var CP69-1062) and tolerant (*S. spontaneum*) cultivars had different morphological, physiological and biochemical responses under cold stress compared to control temperature and to each other, that this case indicates different genomic structure, genetic capacity, and their other characteristics in cold tolerance. In addition, *S. spontaneum* cultivar had better and more efficient defense mechanism than commercial variety CP69-1062 and thus, was more tolerant to cold stress. Finally the set of these physiological and biochemical changes in both sensitive and tolerant cultivars, is to maintain survival and yield under cold stress.

***Keywords*:** Antioxidants, Cold tolerance, Electrolyte Leakage, Proline, Malondialdehyde, Water-Soluble Carbohydrates

Fig. 1. Effect of cold stress on sugarcane leaves at -4°C

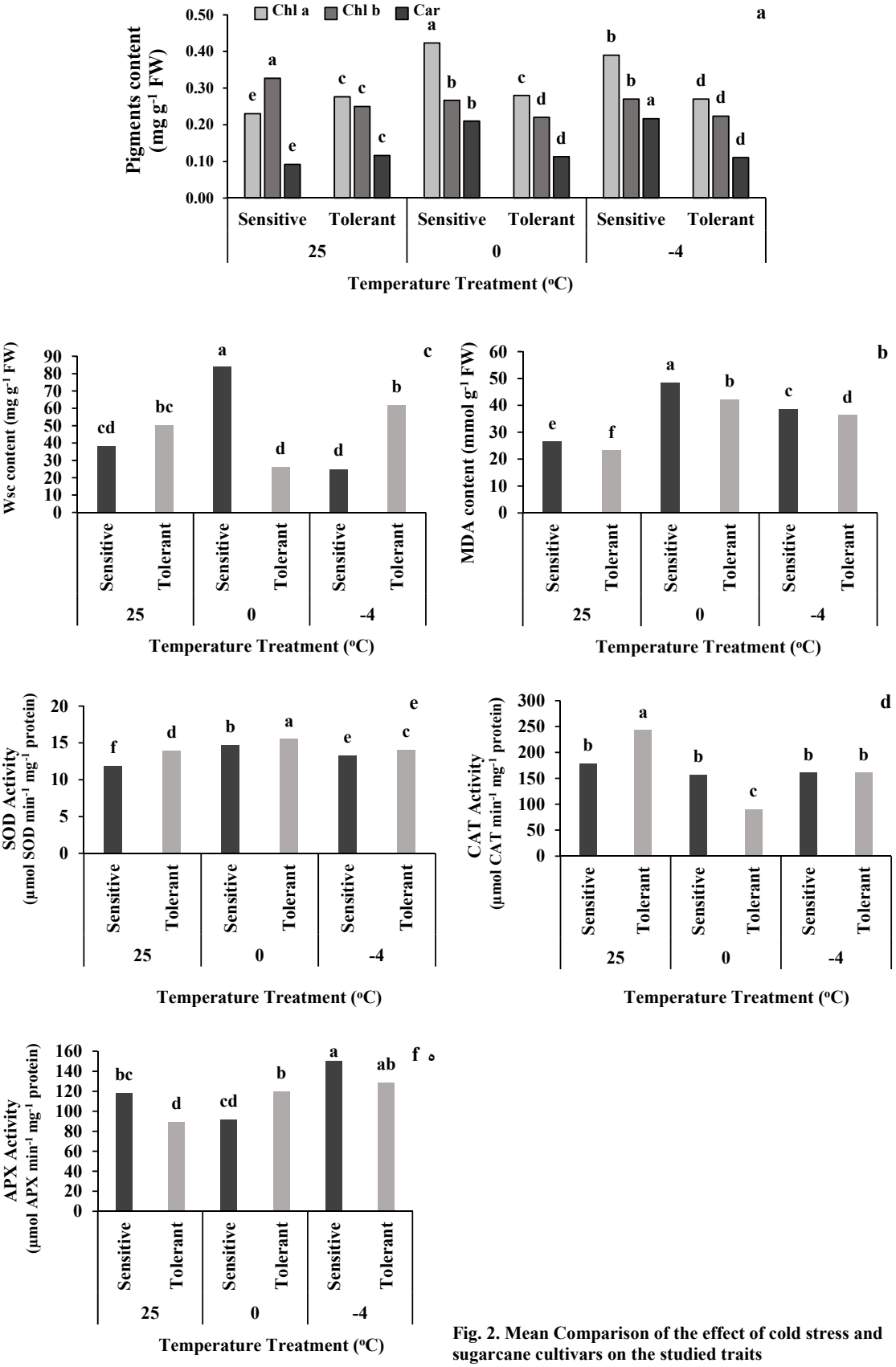

Fig. 2. Mean Comparison of the effect of cold stress and sugarcane cultivars on the studied traits

Table 1. Variation analysis of the effect of cold stress and sugarcane cultivars on the studied traits

Source	df	Chl a	Chl b	Carotenoide	Proline	Malondialdehyde	Electrolyte leakage
Replication	2	0.00002	0.00004	0.00000	0.00004	1.760	0.888
Treatment	2	0.01602**	0.00377**	0.00662**	0.00027*	636.592**	17.690*
Cultivar	1	0.02347**	0.01445**	0.01502**	0.0012**	68.864**	70.726**
Treatment * Cultivar	2	0.01610**	0.00045**	0.00871**	0.00002 ^{ns}	6.996*	0.778 ^{ns}
Error	10	0.00001	0.00003	0.00001	0.00004	1.016	2.421
CV (%)	-	*1.01463*	*2.18793*	*2.54755*	*1.22143*	*2.814*	*1.836*

Table 1. Continued

Source	df	Water soluble carbohydrates	Catalase	Superoxide dismutase	Peroxidase	Ascorbate peroxidase
Replication	2	89.055	3233.389	0.001	0.054	64.889
Treatment	2	250.888 ^{ns}	11596.222**	7.505**	0.895**	2447.722**
Cultivar	1	43.555 ^{ns}	8.000 ^{ns}	6.504**	1.100**	249.388 ^{ns}
Treatment * Cultivar	2	3617.555**	6536.000**	0.802**	0.019 ^{ns}	1435.388*
Error	10	94.389	438.055	0.003	0.061	207.689
CV (%)	-	20.430	12.693	0.386	1.650	12.382

The ns, * and ** symbols show non significant and significant different at the 5% and 1% probability level, respectively.

Table 2. Mean Comparison o the effect of cold stress and sugarcane cultivars on the studied traits

Stress	T(°C)	Cultivar	Chl a	Chl b	Carotenoide	Proline	Malondialdehyde	Electrolyte leakage
			----------mg g^{-1} FW----------				Mmol.g^{-1} FW	(%)
Cold stress	**0°C**	***S. officinarum* (CP69-1062)**	0.42 ^{a}	0.27 ^{b}	0.21 ^{b}	0.51 ^{cd}	48.40 ^{a}	89.01 ^{a}
		S. spontaneum	0.28 ^{a}	0.22 ^{d}	0.11 ^{d}	0.52 ^{ab}	42.08 ^{b}	84.28 ^{bcd}
	-4°C	***S. officinarum* (CP69-1062)**	0.39 ^{b}	0.27 ^{b}	0.22 ^{a}	0.51 ^{bc}	38.45 ^{c}	86.18 ^{ab}
		S. spontaneum	0.27 ^{d}	0.22 ^{d}	0.11 ^{d}	0.52 ^{a}	36.30 ^{d}	82.32 ^{cd}
Control	**25°C**	***S. officinarum* (CP69-1062)**	0.23 ^{e}	0.33 ^{a}	0.09 ^{e}	0.49 ^{d}	26.45 ^{e}	84.97 ^{bc}
		S. spontaneum	0.28 ^{c}	0.25 ^{c}	0.12 ^{c}	0.51 ^{abc}	23.18 ^{f}	81.67 ^{d}

Table 2. Continued

Stress	T(°C)	Cultivar	Water soluble carbohydrates	Catalase	Superoxide dismutase	Peroxidase	Ascorbate peroxidase
			(mg g^{-1} FW)	----------U mg-^{1} protein----------			
Cold stress	**0°C**	***S. officinarum* (CP69-1062)**	84.11 ^{a}	157.14 ^{b}	14.71 ^{b}	15.09 ^{ab}	91.84 ^{cd}
		S. spontaneum	26.09 ^{d}	89.29 ^{c}	15.50 ^{a}	15.47 ^{a}	119.90 ^{b}
	-4°C	***S. officinarum* (CP69-1062)**	25.00 ^{d}	160.71 ^{b}	13.30 ^{e}	14.87 ^{b}	150.00 ^{a}
		S. spontaneum	61.94 ^{b}	160.71 ^{b}	14.07 ^{c}	15.48 ^{a}	128.57 ^{ab}
Control	**25°C**	***S. officinarum* (CP69-1062)**	38.42 ^{cd}	178.57 ^{b}	11.88 ^{f}	14.32 ^{c}	118.20 ^{bc}
		S. spontaneum	50.45 ^{bc}	242.86 ^{a}	13.92 ^{d}	14.81 ^{b}	89.29 ^{d}

Each number is the average of three repetitions (SE ± Mean). The data in each column, which have at least one common letter, are not significantly different at the 5% probability level based on the LSD test.

Table 2. Correlation between studied traits in controlled conditions

	Traits	1	2	3	4	5	6	7	8	9	10	11
1	**Chl a(1)**	1										
2	**Chl b(2)**	-0.055	1									
3	**Car(3)**	0.979**	0.026	1								
4	**Pro(4)**	0.240	-0.86**	0.167	1							
5	**MDA(5)**	0.711**	-0.351	0.617**	0.157	1						
6	**EL(6)**	0.702**	0.325	0.650**	-0.580*	0.644**	1					
7	**WSC(7)**	0.328	-0.040	0.205	-0.101	0.263	0.236	1				
8	**CAT(8)**	-0.102	0.273	-0.058	-0.186	-0.63**	-0.228	0.218	1			
9	**SOD(9)**	0.316	-0.84**	0.186	0.605**	0.630**	0.049	0.202	-0.44	1		
10	**POD(10)**	0.142	-0.82**	0.056	0.684**	0.548*	-0.159	0.188	-0.43	0.770**	1	
11	**APX(11)**	0.012	-0.012	0.144	0.235	0.124	-0.078	-0.519*	-0.30	-0.219	0.031	1

The * and ** symbols significant different at the 5% and 1% probability level, respectively.