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Adaptations of plant responses in *Agave sisalana* under drought stress conditions

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Key words: *Agave sisalana*, Drought, Physiological, biochemical, Water related attributes

Abstract

This study was aimed to characterize the drought tolerance at 10 and 2% field capacity in a desert plant *Agave sisalana* during a period of 2 months. Physiological, biochemical and water related attributes were investigated in order to explore agave plant's survival under water stressed conditions. Green house experiment was performed in CRD with four replications of drought stress imposed on six months old progeny of agave plants. Drought stress significantly decreased the photosynthetic rate (1.45 µmol m^{-2} s^{-1}), transpiration rate (0.15 mmol m^{-2} s^{-1}), stomatal conductance (0.9 µmol m^{-2} s^{-1}), leaf area (12.9 cm^2), relative water content (30.7%) and total chlorophyll content (0.24 mg g^{-1}). The concentration of biochemical indicators such as proline (4.26 µg g^{-1}) and Melondialdehyde (0.99 µmol g^{-1} FW) were increased by the increase in drought stress time from 10 to 2% field capacity. The correlation coefficients (r) between physiological, biochemical and water related traits were also found to be significant and showed positive and negative association among each other. This knowledge of stress inducible responses generated in the form of physiochemical attributes in water deficit crops can serve to be very useful in the future for better understanding of metabolic and gene regulatory pathways.

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Introduction

Drought is considered to be the premier limiting abiotic factor responsible for the crop water unavailability at the time of need. Major impacts of drought stress on plants include reduction in photosynthetic rate, leaf area and stomatal conductance to prevent water loss (Selote *et al.,* 2004). These generate several cellular responses in the form of solute and osmolyte concentration changes such as proline and MDA which helps the plants to survive in adverse conditions. These adaptive responses are controlled by specific sets of genes within the plants to avoid abiotic stresses in their agro ecosystem (Rodriguez-Uribe and O'Connell', 2006).

Agave sisalana are xerophytes of genus of the same name, belonging to the family Agavaceae and are well adapted in the desert. Their biological success in such difficult and dry climate is due to the long storage capacity of leaves and thick cuticle which minimizes non-stomatal water loss and ability of Crassulacean acid metabolism (CAM) to cope with long drought periods (Nobel, 1977). It has been cultivated mostly due to its importance as fiber production but its various traits related with drought tolerance are needed to be evaluated extensively for understanding its CAM ability (Wilhite *et al.,* 2007; Araus *et al.,* 2002). The magnitude of drought complexities produce by abiotic factors in cash crops can however be minimized by applying both genetic engineering and conventional breeding methods. In order to overcome the adverse effects of drought, it is necessary to develop transgenic crops transformed with up-regulated drought stressed genes. For this purpose, the knowledge of stress inducible responses generated in the form of morphological, physiochemical and biochemical attributes in water deficit crops can serve to be very useful (Vijayalakshmi *et al.,* 2012).

The objectives of this study was to determine the effects of drought stress tolerance on different physiological and biochemical processes in *Agave sisalana* plants by applying 10% and 2% Field capacity (FC) of water stress and comparing these attributes in control agave plants without drought stress treatment.

This is the first ever report on *Agave sisalana* under drought stress conditions. The physio-chemical and water related attributes have been found with variable responses under the 10 and 2% field capacity drought stress. This would lead to understand the genetic make-up of the genes involved in the tolerance of drought stress mechanism.

Material and methods

Plant material and treatment

Progeny of *Agave sisalana* plants was developed in the green house from the young saplings of already grown Agave plants taken from local nursery. Young saplings of agave plants were transplanted in composite soil (peat, sand, soil, 1:1:1) in mud pots placed in green house at temperature 25±2°C and relative humidity approximately 45-50%. The volume of water given to plants was calculated periodically to maintain the field capacity of the planted mud pots. After six months of progeny development, irrigation was withheld at 10 and 2% FC to impose the drought stress to the plants for 70 days while the control plants were irrigated normally. Completely randomized design (CRD) with four replications of each experimental unit was used.

Microscopic examination of leaf epidermal tissue

Microscopic glass slides were prepared for control, 10 and 2% FC drought stressed plants involoving peeling or scraping off the epidermal layer and mounting the tissue on microscope slide for further examination according to the method described previously (Prat, 1948). Distinct characteristics of the epidermis such as cell arrangement, size and shape of stoma were noted after examination under scanning electron microscope (SEM) at 10X.

Plants' physiological analysis under drought stress

Photosynthesis, transpiration, stomatal conductance and water-use efficiency: Physiological analysis of control, 10% and 2% FC drought stressed *Agave sisalana* plants were determined with infrared gas analyzer (IRGA) (model, LCA-4; Analytical Development Company, Hoddesdon, England) by putting IRGA leaf chamber on the top of intact leaves.

All these determinations were recorded at 14.30-15.30 h mid-day sunshine. Data was recorded in control, followed by 10 and 2% FC drought stressed plants.

Water related attributes and leaf surface area

The relative water contents (RWC) of control, 10% and 2% FC drought stressed plants were measured according to the method described by Barrs and Weatherley, (1962). Total leaf area of control, 10% and 2% FC drought stressed plants was measured with digital photographs with default parameters. Data was finally analyzed through Image J Software as reported by Igathinathane *et al.,* (2008).

Plant's biochemical analysis under drought stress

Proline content

Prolinecontens were estimated according to the method of Bates *et al.,* (1973). About 0.5 g sample of leaves of *Agave sisalana* were taken from control, 10% and 2% FC drought stressed plants. The sample was homogenized with 35% sulfosalicylic acid followed by filtration through Whatman filter paper. Homogenized filtrate (2mL) was reacted with 2 mL acid ninhydrin solution (1.25 g ninhydrin in 30 mL glacial acetic acid), 20 mL of 6 M orthophosphoric acid and 2 mL of glacial acetic acid for 1h at 100°C in a test tube. The ice bath was used to terminate the reaction. The mixture was extracted with 4 mL toluene, aspirated from the aqueous phase and placed at room temperature. Using toluene as blank, the absorbance of the mixture was measured at 520 nm. The proline concentration was determined from a standard curve using 0-100 µg L- proline calculated on fresh weight bases as follows.

µmolproline g^{-1} FW = (µg proline mL^{-1} x mL of toluene/115.5)/sample wt (g)

Lipid peroxidation assay

Malondialdehyde (MDA) level, an index for lipid peroxidation, was determined according to Quan, (2004). The leaves of control, 10 and 2% FC drought stressed agave plants were homogenized in 5 mL of 10% trichloroacetic acid (TCA) and centrifuged at 12,000g for 10 min. 2 mL of supernatant was added to 4 mL of 0.6% thiobarbutyric acid (in 10% TCA) and incubated at 100˚C in a water bath for 15 min.

The reaction mixture was placed at room temperature to terminate the reaction. After that, mixture was centrifuged at 12,000g for 10 min and absorbance of the supernatant was determined at 450, 532, and 600 nm on a spectrophotometer.

The concentration of MDA was determined by the following formula. C (µmol l^{-1})= 6.45(OD532-OD600)-0.56OD450.

Total chlorophyll content

Total chlorophyll content of agave plants was measured according to Arnon and Whatley, (1949). About 100 mg of control, 10 and 2% FC drought stressed leaves were grinded with 10 mL of 80% acetone to get the chlorophyll extract. The homogenate was placed at room temperature and left overnight. Absorbance of the final extract was read at 663 and 645 nm. The concentration of chlorophyll a, b and total chlorophyll (mg/g fresh weight) was calculated using Arnon's equations.

Statistical Analysis

Statistical analysis was done with STATISTIX V9.0 (Analytical software Tallahassee, USA) freely available online. The data obtained from CRD experiment was subjected to analysis of variance (ANOVA). LSD at (5% and 1%) was further used to compare the mean performance and significant differences for the parameters. Pearson's correlation coefficient was also determined to evaluate the association among physico-chemical attributes.

Results

Effect of drought stress on epidermal tissue of Agave plantleaves

Slides made with epidermis from leaves of control and drought stressed plants of *Agave sisalana* showed the difference in stomatal aperture. Plants under 10% drought stress showed stomata completely closed and 2% FC drought stressed plants showed the stomata partially closed, whereas, the control plants showed the stomata open on 10X magnification (Fig. 1 A-C).

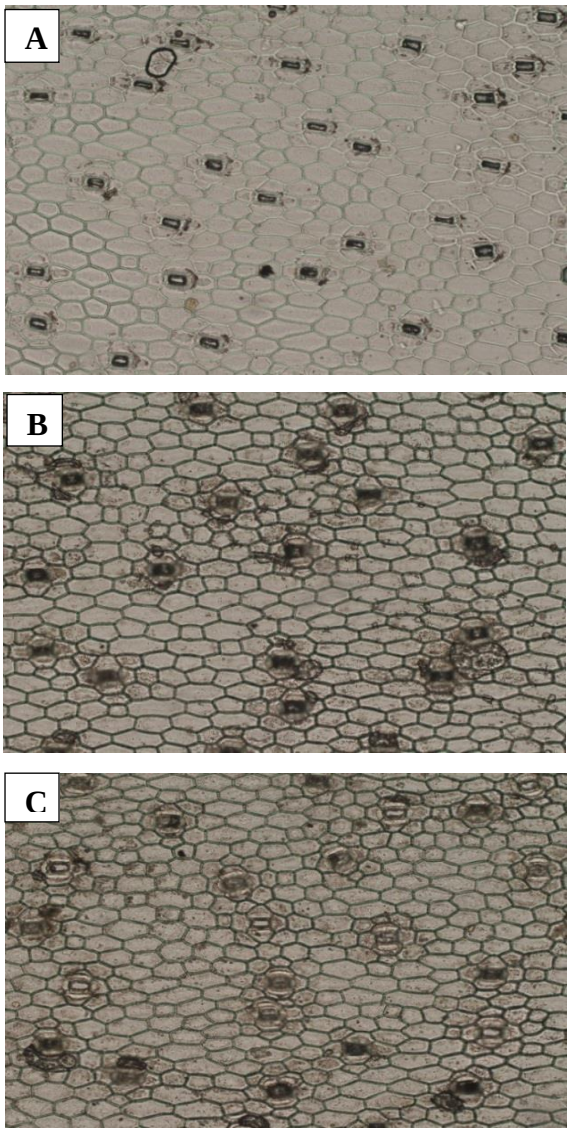

Fig. 1: Stomatal aperture of *Agave sisalana* leaf epidermis under microscope
A: Open stomata in control plants
B: Closed stomata in 2% FC drought stressed plants
C: Partially closed stomata in 10% FC drought stressed plants

Effect of drought stress on physiological behaviour of Agave plant

Photosynthesis rate

In control plants, 8.0 µmol m^{-2} s^{-1} photosynthetic rate was measured while it was 5.67 µmol m^{-2} s^{-1} and 1.45 µmol m^{-2} s^{-1} in 10% FC and 2% FC drought stressed treated plants respectively (Table 1). The photosynthetic rate of control plants was significantly higher followed by 10% FC drought stressed plants, whereas 2% FC treated drought stressed plants showed non significant photosynthetic activity (Table 2).

Transpiration rate

Transpiration rate measured for control, 10 and 2% FC drought stressed plants also revealed significant difference with the increasing drought stress intensity among the treatments. Highest transpiration rate of 3.71 mmol m^{-2} s^{-1} was measured in control plants followed by 0.40 mmol m^{-2} s^{-1} and 0.15 mmol m^{-2} s^{-1} in 10 and 2% FC drought stressed plants respectively (Table 1). The data is significantly variable at 5% level as mentioned in the Table 2.

Stomatal conductance

Highest stomatal conductance was maintained by the control plants (70 µmol m^{-2} s^{-1}) followed by 10% FC (44 µmol m^{-2} s^{-1}) and then at 2% FC (9.0 µmol m^{-2} s^{-1}) respectively (Table 1). Mean performance of the treatments showed that there is significant difference among control and drought stressed treated plants (10 and 2% FC) at $p \leq 0.05$ and $p \leq 0.01$ values. ANOVA states that the treatments differ significantly at 5% (Table 2)

Water use efficiency

Water use efficiency of control plants was maximum (0.5%) with gradual decrease in 10% FC (0.05%) and 2% FC (0.005%) plants. There was non-significant difference among the two drought stressed plants (10 and 2% FC) at $p \leq 0.05$ and $p \leq 0.01$ values (Table 1). ANOVA showed that there is significant difference in control and treated plants (Table 2).

Water related attributes and leaf surface area of Agave sisalana

The variables monitored related to water attributes showed a decline pattern. Non-significant difference of leaf relative water content was evaluated for control and 10% FC water stressed plants whereas 2% FC treated plants showed significant difference at $p \leq 0.05$ and $p \leq 0.01$.

Highest Leaf relative water content was exhibited by control plants (69.3%) followed by 10% FC (68.9%) and 2% FC (30.7%) drought stressed plants (Table 1). ANOVA showed the significant difference for the performance of leaf relative water content at ($p \leq 0.05$) (Table 2).

Reduction in leaf area was highest in 2% FC drought stressed plants because of the highest level of drought stress application. Mean performance? also proved highest leaf surface area in control plants (15.53 cm^2) followed by 10% FC (14.37cm^2) and 2% FC plants (12.9 cm^2) respectively (Table 1). ANOVA showed the significant variation for the treatments at ($p \leq 0.05$) (Table 2).

Effect of drought stress on plant's biochemical attributes

Proline content

Proline synthesis was increased as proline content in leaves of drought stressed (10% and 2% FC) plants. Highest proline content accumulation was 4.26 µg g^{-1} in 2% FC plants which is followed by 2.02 µg g^{-1} in 10% FC drought stressed plants. The control plants showed lowest proline content 1.23 µg g^{-1} (Table 1). ANOVA showed the significant difference among the variables at $p \leq 0.05$ (Table 2)

Malondialdehyde (MDA) level

The longer and more severe the drought stress is the lower is the activity of the protective enzymes and the higher the content of MDA.

In present study, highest MDA content was produced by 2% FC drought stressed plants followed by the quantity produced in 10% FC plants. Significant difference was recorded in control and drought stressed plants at $p \leq 0.05$ and $p \leq 0.01$ (Table 1).

Lowest MDA content was observed in control plants (0.068 µmol g^{-1} FW) and then drast-ically increase with increase drought intensity in 10% FC (0.209 µmol g^{-1} FW) and 2% FC (0.99 µmol g^{-1} FW) drought stressed plants**.** ANOVA suggeststhe significance of results at $p \leq 0.05$ (Table 2).

Total chlorophyll content

Under drought stressed conditions, plants started losing their chlorophyll content and photosynthetic machinery gets impaired.

Our results also showed lowest chlorophyll content under highest drought stress condition such as at 2% FC (0.24 mg g^{-1}) followed by 10% FC (0.34 mg g^{-1}) as compared to control plants which showed the chlorophyll content 0.59 mg g^{-1} respectively (Table 1). Analysis of variance also showed the significant results at $p \leq 0.05$ (Table 2)

Table 1. Mean Values for Physio-chemical and water related attributes under Control, 10 and 2% FC drought stress*Agave sisalana* plants.

Physio-chemical Parameters	P - value	Stress Levels		
		2 %	10 %	Control
Photosynthetic Rate	0.05	1.45a*	5.67b*	8.0c*
	0.01	1.45a^{NS}	5.67ab^{NS}	8.0bc^{NS}
Transpiration	0.05	0.15a*	0.40b*	3.71c*
	0.01	0.15a^{NS}	0.40ab^{NS}	3.71bc^{NS}
Stomatal Conductance	0.05	9.0a*	44.0b*	70.0c*
	0.01	9.0a**	44.0b**	70.0c**
Water Use Efficiency	0.05	0.005a^{NS}	0.05ab^{NS}	0.5*
	0.01	0.005a^{NS}	0.05ab^{NS}	0.5*
Total Chlorophyll	0.05	0.24a*	0.34b*	0.59c*
	0.01	0.24a**	0.34b**	0.59c**
Lipid Peroxidase	0.05	0.999a*	0.209b*	0.068c*
	0.01	0.999a**	0.209b**	0.068c**
Leaf Relative Water Content	0.05	30.7a*	68.9b^{NS}	69.3bc^{NS}
	0.01	30.7a**	68.9b^{NS}	69.3bc^{NS}
Proline	0.05	4.26a*	2.02b*	1.23c*
	0.01	4.26a^{NS}	2.02ab^{NS}	1.23c**
Leaf Surface Area	0.05	12.9a*	14.37b*	15.53c*
	0.01	12.9a**	14.37b**	15.53c**

*, denotes significant differences at 5% probability level ($P \leq 0.05$)

**, denotes significant differences at 1% probability level ($P \leq 0.05$)

^{NS}, denotes non-significant

Table 2. Analysis of variance (ANOVA) for Physio-chemical and water related attributes under Control, 10 and 2% FC drought stress *Agave sisalana* plants**.**

Physio-chemical parameters	Treatment MS	Error MS	F	P	C.V
Photosynthetic Rate µmol m^{-2} s^{-1}	33.06	1.33	24.80*	0.0013	22.91
Transpiration Rate mmol m^{-2} s^{-1}	11.84	0.0034	3484.15*	0.0000	4.11
Stomatal Conductance µmol m^{-2} s^{-1}	2811.0	1.00	2811*	0.0000	2.44
WUE µmol m^{-2} s^{-1}	0.2247	0.0033	66.74*	0.0001	31.37
Total Chl mg g^{-1}	0.0975	0.0004	243.75*	0.0000	5.13
LPO µmol g^{-1} FW	0.7553	0.000004	188842*	0.0000	0.47
Proline µg g^{-1}	7.4113	0.0004	18528.25*	0.0000	0.80
RWC (%)	1474.68	1.00	1474.68*	0.0000	1.78
LSA(cm^2)	5.2117	0.02	260.59*	0.0000	0.99

*, denotes significant differences at 5% probability level (P≤0.05)

Treatment MS= Mean square (estimate of variance between groups), Error MS= Average of square of error value, F= Significance probability (variance ratio between Treatment MS and Error MS), P=Probability value, CV (%)= Percent coefficient of variation, Total Chl (Total chlorophyll content), LPO (Lipid Peroxidation), RWC (Relative Water Content), LSA (Leaf surface area), WUE (water Use efficiency)

Correlation between physiological, biochemical and water related attributes

The correlation coefficients (r) among various physiological, biochemical and water related factors under drought stress conditions indicated significant decreasing pattern of physiological attributes and increasing trend of biochemical parameters except total chlorophyll content. Photosynthetic rate (A) was positively correlated with transpiration rate E (r=0.76), g (r=0.93), WUE (r=0.82), total Chlorophyll (r=0.88), LRWC (r=0.89) and LSA (r=0.94) whereas negatively correlated with proline (r=-0.93) and LPA(r=-0.92) at p≤0.05 (Table 3).

Table 3. Correlation coefficients (r) between physio-chemical and water related attributes of *Agave sisalana* plants under drought stress.

	PR	Trans.	SC	WUE	TC	LPA	Proline	LRWC	LSA
PR	*								
Trans.	0.7663	*							
SC	0.9349	0.8545	*						
WUE	0.8271	0.9789	0.8420	*					
TC	0.8831	0.9722	0.9418	0.9679	*				
LPA	-0.9215	-0.6646	-0.9555	-0.6640	-0.8055	*			
Proline	-0.9368	-0.7444	-0.9823	-0.7396	-0.8649	0.9936	*		
RWC	0.8952	0.5611	0.9082	0.5698	0.7266	-0.9901	-0.9685	*	
LSA	0.9494	0.8599	0.9953	0.8595	0.9521	-0.9451	-0.9728	0.9004	*

PR: Photosynthetic Rate; SC: Stomatal Conductivity; Trans: Transpiration; WUE: Water Use Efficiency, TC: Total Chlorophyll; LPA: Lipid Peroxidase; RWC: Related Water Content; LSA: Leaf Surface Area

Green: Positive Correlation among the parameters

Red: Negative Correlation among the parameters

Discussion

Today the world is meeting the challenges of low crop production due to continuous exposure of plants to biotic and abiotic stresses in their Agro ecosystem. *Agave sisalana* plants responds significantly to drought stressed conditions in the form of changes in various physiological, biochemical and water related attributes. Epidermis tissue of control and drought stressed leaves of *Agave sisalana* was observed on 10X magnification under microscope. Control plants showed opened stomata, whereas, the plants under drought stressed conditions (10% and 2% FC) showed closed stomata under examination.

Closed stomata under drought stress also correlates decreased photosynthetic and transpiration rate and stomatal conductance.

Drought stress has a major impact on the gas exchange characteristics of the plants and this is mainly due to the impaired photosynthetic machinery, stomatal closure to prevent the transpirational water loss, early leaf senescence, oxidation of chloroplast lipids and changes in structure of pigments and proteins (Vijayalakshmi *et al.,* 2012).

Agave sisalana plants treated with 10% and 2% FC drought stress, when compared with control or well watered plants showed a decreased pattern in all the physiological parameters. The significant decrease in photosynthetic activity of treated plants under drought stress is in accordance with the previous studies conducted on maize plants as reported by Anjum *et al.,* (2011a) and Jabeen *et al.,* (2008). Stomatal closure is another earlier response to drought which gives protection to plants from heavy water loss that progress to a noticeable decrease in stomatal and mesophyll conductance, increase intercellular CO_2 concentration and decrease photosynthetic rate (Chaves *et al.,* 2003). Plants respond differently in control and water deficit conditions (10% and 2% FC) for stomatal conductance. Our results are supported by Flexas and Medrano, (2002) as they reported that stomatal closure leads to decrease photosynthetic rate, inadequate CO_2 availability and Rubisco activity under water deficiency in plants. Our findings also revealed that all factors correlate one way or the other, which decrease CO_2/O_2 ratio in leaves and impaired photosynthesis process (Janson *et al.,* 2004; Moussa, 2006). Water use efficiency increases with the increase in drought stress, but in this study the results showed contradictory values with a markable difference among control and 10% and 2% FC water stressed *Agave sisalana* plants. These results are supported by the work of Jabeen *et al.,* (2008) as the water use efficiency differs significantly among various cultivars.

Relative water content is an indicator which reflects the plant inner strength to carry out the metabolic activities in tissues and an index for dehydration tolerance. Our findings in this experiment are in accordance with Moussa and Abdel-Aziz, (2008) who reported the decline in RWC in maize plants under drought stress. Another study by Yang and Miao, (2010) also reported that poplar species subjected to progressive water stress showed reduction of RWC in the drought stressed cuttings i.e., *Populuscathayana* 23.3% and *Populuskangdingensis* 16%. Nayyar and Gupta, (2006) revealed, the leaves progressed to drought, exhibit larger reductions in water potential, decrease the RWC, leaf water potential and transpiration rate with a considerable change in leaf temperature and over all abrupt plant metabolic activities. Leaf surface area was also reduced in 10 and 2% FC drought stressed plants when compared to the control. Our results are been supported by the findings of Sankar *et al.,*(2007) as they reported the decrease in leaf area after 50 to 70 days of drought stress.

Proline and other osmolytes' accumulation protect the cell membranes and proteins in plants subjected to abiotic traumas. It also regulates mitochondrial functions, influence cell division or death and regulates specific gene expression required immediate plants' recovery after stress (Szabados and Savoure, 2009). They maintain the quaternary structure of proteins and membrane integrity under water deficit condition and cause photo inhibition (Demiral and Turkan, 2004). Furthermore, it helps in stabilizing sub-cellular structures, scavenging free radicals, buffering cellular redox potential under drought stress conditions (Ashraf and Foolad, 2007). Increased concentration of proline reported in the present study has already been discussed in many researches related to drought-stressed wheat (Hamada, 2000), sorghum (Yadav *et al.,* 2005), bell pepper (Nath *et al.,* 2005), maize (Anjum *et al.,* 2011b). Generally, proline accumulation is higher in stress-tolerant plants as compared to sensitive ones due to variation in proline-oxidase production which may be an adaptability of the plants to combat with the stress environment (Sankar *et al.,* 2007).

Another biochemical marker for drought stress is malondialdehyde (MDA) produced by lipid peroxide-ation damage by free radicals or generation of ROS (Farooq *et al.,* 2009). These reactive oxygen species directly attacks membrane lipids and increase lipid peroxidation and the content of MDA (Mittler, 2002). Our results are in accordance with the findings of Meloni *et al.,* (2003) and Sakhanokho *et al.,* (2004) who reported that MDA content has been increased in the leaves under drought intensity which results in poor membrane stability, decreased chlorophyll content and finally results in ion leakage.

Chlorophyll content is another key indicator for determination of plant metabolic rate (Mohammed *et al.,* 2013). In the present study, decrease in chlorophyll content in drought stressed plants is in agreement with already reported findings in six *Triticumaestivum*cultivars (Nyachiro *et al.,* 2001), various sunflower varieties (Manivannan*et al.,* 2007b), two olive cultivars (Guer-fel *et al.,* 2009).

Conclusion

The *Agave sisalana* plants showed drought tolerance as the(evident by the) variation in physio-biochemical and water related attributes and the strong interac-tions in control, 10 and 2% FC drought stress. Decreased photosynthetic and transpiration rate and stomatal conductance and total chlorophyll content in drought stressed plants associates increased biochemical indicators (proline and MDA) production when compared with the controls.

This knowledge of stress inducible responses generated in the form of physiochemical attributes in water deficit crops can serve to be very useful in the future for better understanding of metabolic and gene regulatory pathway.

Novelty statement

This is the first ever report on *Agave sisalana* under drought stress conditions. The physio-chemical and water related attributes have been found with variable responses under the 10 and 2% field capacity drought stress. This would lead to understand the genetic make-up of the genes involved in the tolerance of drought stress mechanism.

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