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Spatial and temporal responses of photosynthetic pigments, antioxidants, and ionic contents in a desert plant *Agave sisalana*

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As climate change is the main cause of environmental stresses which has become more severe in recent decades, affecting plant growth attributes to the major extent. In plants, disturbed ionic constituents induce oxidative stress which interrupts the cellular physio-chemical attributes leading to impairment of a variety of processes. A two-years (2018 and 2019) field study wa executed with the aim to estimate changes in photosynthetic pigments, ionic contents, and antioxidant potential in a desert plant species sisal (*Agave sisalana*) under spatial and temporal variations of five ecologically distinct zones, including Faisalabad, Layyah, Chakwal, Khushab, and Rawalpindi with three replications. Leaf traits such as nitrate (NO_3^-), phosphate (PO_4^{3-}), sodium (Na), potassium (K), calcium (Ca) and photosynthetic pigments (chlorophyll *a* and *b*, carotenoids), as well as antioxidants i.e. superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate (ASC) were determined in the collected samples to assess the requisite information. Results exposed higher accumulation of chlorophyll *a* and b contents in Chakwal region during the spring season while in the same season, the carotenoids content was higher in Khushab during both studied years. Moreover, autumn season was identified for having higher antioxidants activity in sisal. However, activities of superoxide dismutase, peroxidase, and ascorbate were noted significantly higher in Layyah, Rawalpindi, and Chakwal. Antioxidants were recorded in higher concentration during 2019 as compared to 2018 and more pronounced in spring and autumn with minor fluctuations. For spring season of the studied years, collected leaves of sisal exposed higher concentrations of ionic contents (K^+, Ca^{2+} Na^+, PO_4^{3-}, NO_3^-) as compared to other seasons. Temporal and spatial variations were proved changing and leaving significant impact on photosynthetic pigments, antioxidant potential and nutrient uptake hence, have antagonism with environmental factors of aridity and temperature.
Keywords: Nutrient, chakwal, ascorbate, catalase, sodium, aridity, temperature.

INTRODUCTION

Sisal is a CAM plant with significant tolerance to abiotic stresses, hence, responds to variations in temperature and environments to a greater extent (Shahzad *et al*., 2022; Sarwar *et al*., 2019). Reportedly, the sessile nature of such plants limits the choice to escape from their growing habitat (Zadnikova *et al*., 2015). Therefore, majority of these xerophytic species have evolved suitable mechanisms to cope with the stress situations through escape, avoidance, and tolerance either individually or in consonance with each other (Abobatta, 2019; Seleiman *et al.,* 2021). Endogenous crassulacean acid metabolism (CAM) also helps them to utilize the water more efficiently than the other plants (Winter *et al.,* 2015). Purposively, agave species have the innate capability to survive for more than one season without rainfall and tolerate extreme variations in the form of severe cold and hot temperatures (Nobel and Smith, 1983; Bergsten *et al.,* 2016; Sarwar *et al*., 2019). Climatic adversities become stress when hinders the growth and development of growing plants extensively and hampers the crop production and global food security (Khan *et al.*, 2021a). Stress primarily limits the photosynthetic activity of plants and this reduction in photosynthetic efficiency through production of ROS, the free-reactive oxygen radicals along with their derivatives (Sharma *et al.*, 2019; Pandey *et al.*, 2017). Moreover, ROS employs to transmit cellular signaling information in response to the changing environmental conditions in plants (Huang *et al.,* 2019). Abiotic stress creates the disturbance of the equilibrium between the generation of ROS and antioxidant defense systems. Unusually, the equilibrium between the toxic formation of ROS and their detoxification is maintained

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by both non-enzymatic and enzymatic antioxidants under severe environmental stresses (Hasanuzzaman *et al*., 2020). Various plant species have evolved adaptive mechanisms to compensate the effects of water deficit, salt and heat stresses (Parida and Das, 2005) through adjustments due to better plant adaptation to such adversities, with the most common namely osmotic adjustment (OA) (Chen and Jiang, 2010). Such OA is usually accompanied by the absorption of inorganic ions (Zeng *et al*., 2015) where the plants adapt to the prevailing conditions, to maintain the water potential gradient and suitable level of cell turgor (Woodruff *et al*., 2004). Similarly, Zheng *et al*., (2016) reported the spatio-temporal variation induced ion accumulations in tomato plants. Jin *et al*., (2020) found lower levels of ionic contents in the plant leaves exposed to drought stress. Although, the accumulation of certain antioxidants and ionic contents are stressed respondents yet, their accumulations lead to bound form of water in the plants leaves and stems in order to save them from their loss through transpiration (Rasul *et al*., 2012; Pan *et al*., 2013; Jahan *et al*; 2019) Moreover, production of super oxides and singlet as well as triplet oxygen radicals along with peroxides and hydroxyl radicals undergo scavenging mechanism due to CAT, POD, ASA and SOD based enzymatic antioxidants (Jahan *et al*., 2019) It is foremost speculated that the CAM plants including agave genus might have certain phenomena of accumulation of osmolytes and ions as well as enzymatic oxygen scavengers to mitigate the deleterious effects of ROS and resultantly have a durable growth and development due to intact photosynthetic apparatus and durable efficiency even under extreme environmental stressful conditions.

MATERIALS AND METHODS

Plant collection**:** The selection of the desert plant (*Agave sisalana*) was based on an ethnobotanical assessment. The desert plant was collected from five different ecological regions of Punjab, Pakistan i.e., Chakwal, Rawalpindi, Khushab, Faisalabad, Layyah (Table 1, Fig. 1). In each district, three natural growing *Agave sisalana* were identified and surveyed randomly to collect plant samples with three replications during peak seasons of spring, summer, winter, and autumn repeatedly for two years (2018 and 2019). Fresh plant material was transported by keeping in the coolers for analysis. Plant leaves samples were shade dried and stored at room temperature.

Table 1. Geographical indicators of eco-regions in Punjab, Pakistan.

Districts	Coordinates	Elevation (m)
Rawalpindi	33°36'02"N 73°04'04"E	493
Chakwal	32°55'49"N 72°51'20"E	498
Kliushab	32°17'55"N72°21'3"E	1,520
Faisalabad	31°25'0"N73°5'28"E	184
Layyah	30° 57'53"N 70°56' 23"E	147

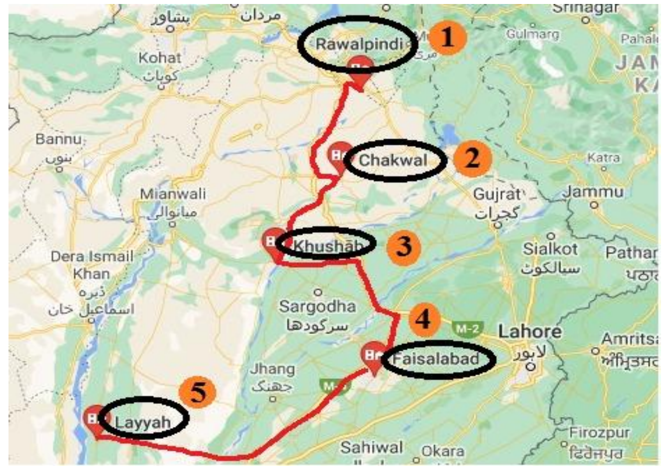

Figure 1. Map showing (a) Punjab and (b) five districts 1-Rawalpindi, 2- Chakwal, 3-Khushab, 4-Faisalabad, 5-Layyah.

Experimental analyses were executed at Agronomy and Botany Departments and at the Central High-Tech Lab of the University of Agriculture, Faisalabad, Pakistan.

Extraction of Photosynthetic pigments**:** The concentration of total chlorophyll and their *a*, *b* constituents and carotenoids were measured following the method of Arnon (1949) and Davies (1976), respectively. Leaf samples were extracted in ice-cold 80% acetone and filtered using a membrane filter. Pigments were separated and quantified by spectrophotometer (U19 2001, Hitachi, Tokyo, Japan).

Extraction of antioxidants**:** A 0.5 g sample of fresh leaves was ground in 10 mL phosphate buffer (pH 7.8), extract was further centrifuged at 15000Xg for 20 min. The supernatant was collected and further used for assessing the activity of superoxide dismutase, peroxidase, and catalase enzymes as per following protocols.

Superoxide dismutase**:** Superoxide dismutase activity in the *Agave sisalana* leaves was estimated using the method prescribed by Giannopolitis and Ries (1977). A 3 ml reaction solution was composed of enzyme extract (50 µL), NBT (1 mL), riboflavin (1 mL), methionine (500 µL), EDTA (500 µL) and phosphate buffer (950 µL). The reaction started with fluorescent light which produced blue formazone by photoreduction of NBT and was calculated by measuring absorbance at 560 nm using UV-vis Spectrophotometer (T60U, PG Instruments, Ltd., UK) every 30 sec intervals using quartz cuvette.

Catalase**:** Catalase (CAT) activity in sisal leaf samples was determined by following the protocol of Chance and Maehly (1955) with some modifications. A 3 mL reaction solution was composed of phosphate buffer (2 mL) and H_2O_2 (900 µL). The reaction was initiated via the addition of enzyme extract (100 µL). Absorbance of the reaction solution were recorded at 440 nm using a UV-visible spectrophotometer (T60U, PG Instruments, Ltd., UK).

***Peroxidase*:** Peroxidase (POD) activity in sisal leaf samples was estimated by the prescribed method of Chance and Maehly (1955) with some changes as described by Munir (2011). The Guaiacol oxidation reaction method (Pan *et al.*, 2013) was used to find out peroxidase activity. Final reaction solution (3 mL) was composed of sodium acetate buffer (2 ml, pH 7.0), guaiacol (400 µL) and H_2O_2 (500 µL) which was added with enzyme extract (100 µL). Changes in absorbance of reaction were measured at 470 nm spectrophotometrically.

***Ascorbate*:** Ascorbate activity was noted by the method of Dos Santos *et al.*, (2017). Leaf samples were extracted with 5% meta-phosphoric acid and reacted with 0.025% of 2,6-Dichlorophenolindophenol sodium reagent. The amount of ascorbate was calculated in mg/100 g fresh weight.

***Extraction of K^+, Na^+ and Ca^{2+} contents*:** The acid sulfuric digestion method was used to digest the dried leaf samples for ionic contents *and the samples were* assessed by using the method of Wolf (1982) on a flame-photometer (Jenway, PFP-7).

***Soluble Nitrate*:** Soluble Nitrate was measured by using the method proposed by Sah (1994). The leaf (0.5 g) was mixed with distilled water (5 mL) and autoclaved. Diluted the solution up to 50 mL. To 3 mL of the extract, 7 mL of chromotropic acid (CTA) was added and waited for 20 min. The absorbance was taken at 430 nm on a spectrophotometer.

***Soluble phosphate*:** For soluble PO_4^- ion, 1 mL extract and 2 mL of 2N HNO_3 were mixed in a test tube and volume was made up to 8 mL. Molybdate-vanadate reagent was added in 1 mL quantity and volume was made 10 mL (using distilled water). The absorbance was taken at 420 nm (Yoshida *et al.*, 1971).

Statistics: Two-way analysis of variance techniques was applied to summarize the experimental data (Steel *et al.*, 1997) (Statistix 8.1).

RESULTS

***Weather of growing season*:** Mean temperatures during both growing years (2018 and 2019) were cool from December to February (winter months) and mild to high in the spring months (March to April). However, a very high average temperature was noted in the summer (June to August) followed by a dry, warm autumn (October to November) (Fig. 2). In the experimented five ecological zones, the highest relative humidity was observed in KSB followed by CHK, FSD, LYH, and RWP while the minimum temperature was noted in RWP followed by CHK, KSB, FSD, and LYH in both growing years. The arid nature of the growing seasons was favorable for the investigation of any abiotic stressful impact on *Agave sisalana* species growing in these different ecological zones of Punjab Pakistan.

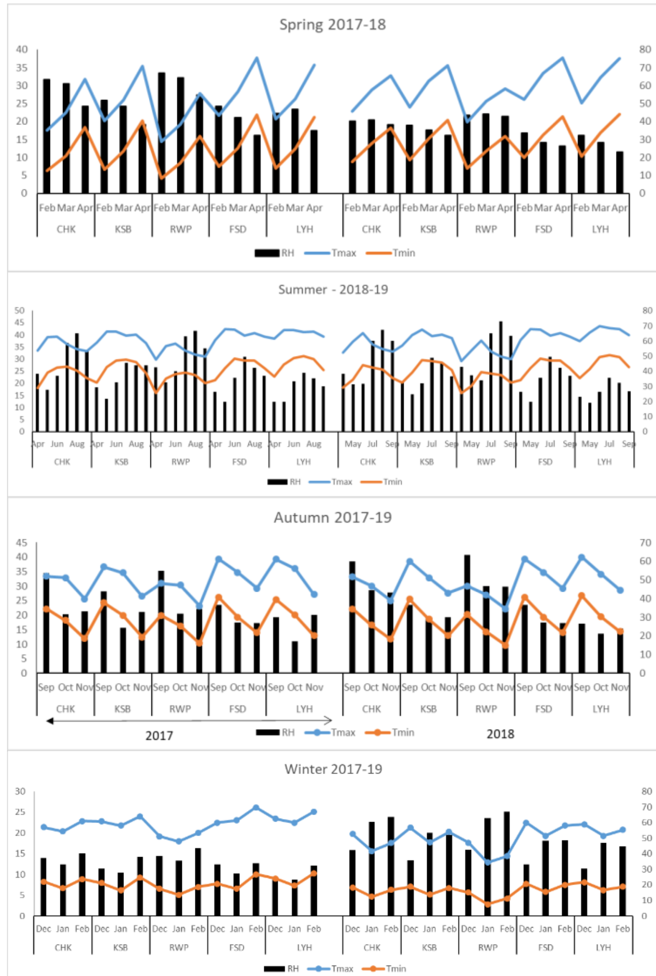

Figure 2. Weather data for two years and four seasons from 2017 to 2019

***Changes in photosynthetic pigments*:** A notable increase (P<0.05) in the concentration of chlorophyll *a* pigment was observed only during the spring months of both growing years (2018 & 2019), in the summer, winter, and autumn seasons, a significant (P<0.05) decrease in this pigment was recorded in respect of all five ecological zones (Table. 2 & 3). However, higher and increased chlorophyll content was recorded in the CHK region as compared to other regions during the spring months of both studied years whereas, this pigment content was the least at Layyah (Fig. 3). On the other hand, for chlorophyll *b* content, during 2018, the spring months induced the highest content with slight variation in seasons and a similar trend in 2019. RWP and L exhibited the highest and lowest contents of chlorophyll *b*, respectively without respecting yearly variations.

Table 2. Average (significant level, P<0.05) of photosynthetic pigments, antioxidants, and ionic contents of *Agave sisalana* during four seasons.

Seasons	Chl a	Chl b	Car.	SOD	POD	CAT	ASC.	K^+	Ca^{2+}	Na^+	PO_4^{3-}	NO_3^-
Summer	0.134*	0.189^{ns}	0.226*	0.144^{ns}	0.17*	0.14^{ns}	435.6^{ns}	4.15*	92.8*	22.7^{ns}	0.48*	0.63*
Autumn	0.129*	0.188^{ns}	0.188*	0.144^{ns}	0.12*	0.11^{ns}	479.8^{ns}	3.90^{ns}	85.1*	21.4^{ns}	0.52*	0.49*
Winter	0.128*	0.191*	0.190*	0.132*	0.17*	1.50^{ns}	420.6^{ns}	4.02*	92.6*	20.6^{ns}	0.43^{ns}	0.53*
Spring	0.184*	0.206*	0.193^{ns}	0.197*	0.16*	0.12^{ns}	429.1^{ns}	3.96^{ns}	94.4*	21.6^{ns}	0.57*	0.52*

Abbreviated as Chl: Chlorophyll, Car: Carotenoids, SOD: Superoxide dismutase, POD: Peroxidase, CAT: Catalase, ASC: Ascorbate, K+: Potassium, $Ca2^+$: Calcium, Na^+ : Sodium, PO_4^{3-} Phosphate, NO_3^-: Nitrate.

Table 3. Average (significant level, P<0.05) of photosynthetic pigments, antioxidants, and ionic contents of *Agave sisalana* at five different ecological zones.

Zone	Chl a	Chl b	Car.	SOD	POD	CAT	ASC.	K^+	Ca^{2+}	Na^+	PO_4^{3-}	NO_3^-
CHK	0.14^{ns}	0.22*	0.17*	0.15^{ns}	0.13*	0.13^{ns}	463.5*	4.22*	98.3*	24.5*	0.125*	0.520*
KSB	0.13^{ns}	0.17^{ns}	0.22*	0.12*	0.16^{ns}	0.14^{ns}	511.9*	3.40*	80.0*	18.9*	0.156*	0.186*
RWP	0.11*	0.17^{ns}	0.19*	0.13^{ns}	0.18*	0.15^{ns}	387.5*	3.50*	81.2*	20.6*	0.100*	0.427*
FSD	0.19*	0.17^{ns}	0.18^{ns}	0.15*	0.15^{ns}	0.12^{ns}	432.1*	3.93*	93.8*	21.1*	0.500*	0.470*
LYH	0.14^{ns}	0.21*	0.21^{ns}	0.21^{ns}	0.16^{ns}	1.84*	411.5*	4.97*	102.7*	22.7*	1.630*	1.110*

Abbreviated as Chl: Chlorophyll, Car: Carotenoids, SOD: Superoxide dismutase, POD: Peroxidase, CAT: Catalase, ASC: Ascorbate, K+: Potassium, Ca^{2+}: Calcium, Na^+: Sodium, PO_4^{3-} Phosphate, NO_3^-: Nitrate.

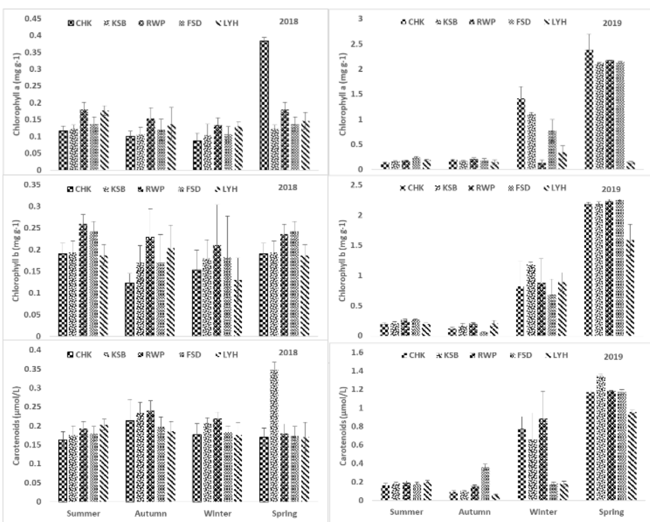

Figure 3. Photosynthetic attributes: chlorophyll a, chlorophyll b, and carotenoids in *Agave sisalana* collected from five different ecological regions during four seasons of both growing years (2018-2019).

Changes in antioxidant activity: During both years, superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) activities (P<0.05) gradual increased over the time and seasons in the experimented duration (four seasons of the year). Moreover, significant changes (P<0.05) in the enzymatic activities across the five ecological regions was also observed (Fig. 4, Table. 2, 3). In the first year (2018), spring season induced high superoxide dismutase activity in sisal leaves when compared with the rest of the months and seasons of the year.

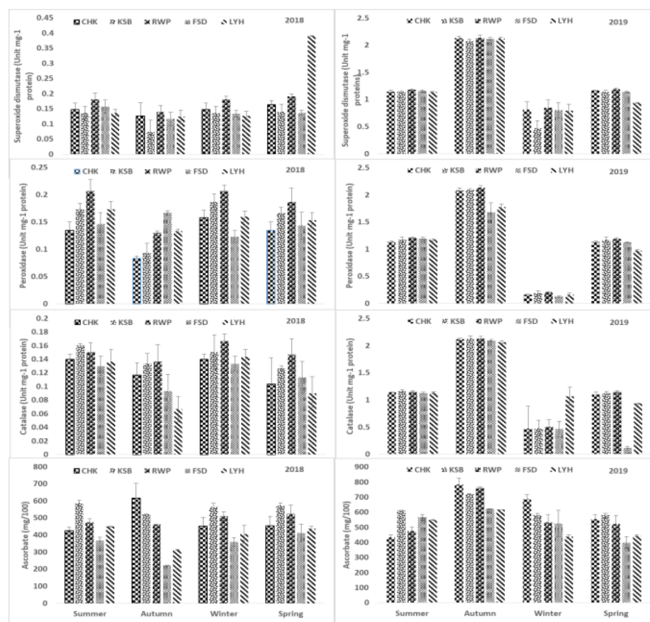

Figure 4. Antioxidants: superoxide dismutase, peroxidase, catalase, and ascorbate in *Agave sisalana* collected from five different ecological regions during four seasons of both growing years (2018-2019).

On the contrary during year 2019 (second experimental year), autumn season replaced spring in respect of SOD activities. For locations, SOD activity was significantly higher in dry region of Layyah when noted for both of hte years (Fig. 4). High POX activity was noted during summer than that of autumn and RWP emerged as location of high POX activity in the leaves of sisal during the year 2018. Whereas, for the second year (2019) POX activity was high in autumn in RWP and the least POX activity was recorded in sisal during winter months this year (Fig. 4). In leaves of desert plant species (sisal), catalase activity was significantly ($P<0.05$) increased with variations in seasons, but we found the highest activity during summer as compared to spring months (year-2018) (Table. 2) and (Fig. 4).

Spring season of 2018 was found favoring antioxidants and similarly for ascorbate contents for their significantly ($P<0.05$) high content however, such antioxidant potential lowers down appreciably at other periods of the year (Table. 2). Whereas, autumn season in the second year (2019) was recorded for higher ascorbate concentration. Chakwal region ($P<0.05$) notably found responsible for high ascorbate content in sisal leaves grown there during the studied years (Fig. 4, Table. 3).

Changes in ionic contents**:** Leaves of *Agave sisalana* collected from different regions exhibited significant variation ($P<0.05$) for ionic contents (K^+, Ca^{2+} Na^+, PO_4^{3-}, NO_3^-) where higher contents were recorded during spring months as compared to other seasons of the both studied years (Table. 2 & 3) and Rawalpindi was found leading region for accumulation of ionic contents during year I i.e., 2018. In 2019, potassium ion concentration in *Agave sisalana* was higher in spring and lowest in the Autumn season (Fig. 5).

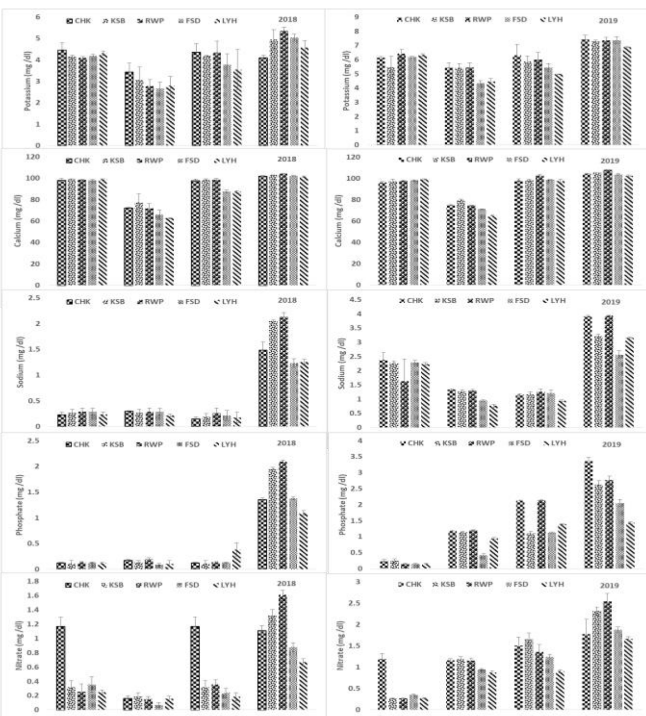

Figure 5. Ionic contents: Potassium, calcium, sodium, phosphate, and nitrate in Agave sisalana collected from five different ecological regions during four seasons of both growing years (2018-2019).

Similarly, spring months induced accumulation of higher calcium content in leaves with significantly higher Ca^{2+} in Chakwal. Again, for ionic contents, i.e., sodium, phosphate and nitrate, spring season and Chakwal conditions favored higher accumulations during both years.

DISCUSSION

During their growth period under field conditions, plant species experienced different types of environmental stresses and aridity/drought (water shortage) is one of them, which imposed threats to plant by stunting its growth. Under the climate change scenario, water shortage conditions are frequent and prevailing globally hence, influencing the biological systems and result frequent water shortage worldwide and results in adverse impact on the biological system. Photosynthesis, a ubiquitous mechanism for autotrophic plants, is highly sensitive to water dearth. Fathi and Tari (2016) reported photosynthesis as a first-line process that got changed due to water scarcity (Wang *et al.*, 2018). Ionic imbalances, lowering of transpiration and CO_2 and water inception as well as loss irregulated due to stomatal mal-functioning (Osakabe *et al.*, 2014; Pirasteh-Anosheh *et al.*, 2016; Xu *et al.*, 2016). Limiting water also lowered CO_2 absorption and transportation of non-structural carbon, a consequence due to stomatal closer (Parkash and Singh 2020) leading to carbon starvation which further affected various other mechanisms (Thompson *et al.*, 2017). In present study, the concentration of chlorophyll *a* pigment was increased only during the spring months of both growing years (2018 & 2019), however, in the summer, winter, and autumn seasons, a significant decrease in this pigment was recorded in respect of all five ecological zones (Table. 2 & 3). Mafakheri *et al.*, (2010) noted reduction in photosynthetic components i.e. total chlorophyll and *a*, *b* contents in chickpea cultivars under water deficit conditions and similar reduction was reported by Nasrin *et al.*, (2020), Khayatnezhad and Gholamin (2012) and Feng *et al.*, (2014) in other crops. According to present study outcomes, the higher and increased chlorophyll *a* content was recorded in the CHK region as compared to other regions during spring months of both studied years (Fig 3). Conformity of results were also reported by Zhang *et al*., (2021), who found spatial-temporal heterogeneity and its impact on chlorophyll contents of rice cultivars. Carotenoids are the lipophilic secondary metabolites, derived from the isoprenoid pathway and accumulated in different plant organs (Baranski and Cazzonelli 2017). Wu *et al*., (2020) reported drought responsive decreased accumulation of carotenoid contents in *Dunaliella salina* and similarly for egg-plants. This decreased accumulation of carotenoids proved sensitivity of carotenoids to drought as per finding of Mibei *et al.*, (2017). Furthermore, Norshazila *et al,*. (2017) also endorsed the finding of the present study regarding temporal variation in carotenoid concentration in pumpkin plant.

Stress induced production of reactive oxygen species (Zahra *et al.*, 2021) was identified for negative consequences at a cellular level as ROS species was causing damage to membrane system, lipids, DNA and amino acids and limited the enzymatic activities (Nizar *et al.*, 2022). As a result of stress upregulation, reactive antioxidant system in plants also got awaken and diluted the hazard to significant extent (Amari and Abdelly, 2021). In current study of both years, SOD, POX, and CAT activities gradual increased over the time and seasons in the experimented duration (four seasons of the year). Moreover, significant changes in the enzymatic activities across the five ecological regions were also noted (Fig. 4, Table. 2, 3). Khan *et al*., (2021b) recorded significant impact on antioxidant activity (SOD) under drought stress in rice plants. Soengas *et al.*, (2018) reported increased levels of antioxidants hence, confirmed suffering of plants with oxidative damage due to elevated temperatures in *Brassica oleracea*. Abedi and Pakniyat (2010) reported enhanced peroxidase and superoxidase activities in oilseed rape with decrease in catalase activity. Zhu *et al*., (2020) found increased levels of superoxide dismutase and catalase activities in cassava in the duration of water shortage. Nikalje *et al*., (2018 reported spatial and temporal variations in antioxidants levels of *Sesuvium portulacastrum*. Ascorbic acid (AsA), namely vitamin C, is one of the most abundant water-soluble antioxidants found in plant tissues (Seminario *et al.*, 2017). Ascorbate can scavenge reactive oxygen species and help for enhanced plant stress tolerance (Xiao *et al*., 2021). Zhu *et al*., (2020) found increased levels of ascorbate concentration in cassava under water shortage conditions. Seminario *et al*., (2017) noted significant relation between the ascorbate levels and drought situation in plants. Zhang *et al.*, (2018) found that higher ascorbate content could improve the rice resistance to high-temperature stress.

Water shortage reflects different responses in plants depending on the extent of soil dehydration (Forni *et al*., 2017). As water is the soul transporting medium hence, availability of soil nutrition entirely depends on extent of water scarcity primarily when considered for estimating growth and yield of plants (Cataldi *et al*., 2003). Ionic content Nikalje *et al*., (2018) also found differential spatial and temporal ionic contents levels in *Sesuvium portulacastrum.* Zheng *et al*., (2016) noted resembling response of sodium content to the spatio-temporal distribution in tomato plants as in desert plant in this study. In our study, the collected leaves from different regions displayed significant variation for ionic contents (K^+, Ca^{2+} Na^+, PO_4^{3-}, NO_3^-) where higher contents were recorded during spring months as compared to other seasons of the both studied years (Table. 2 & 3; Fig 5). Similarly, Jin *et al*., (2020) found lowered ionic contents in plant leaves exposed to drought as compared to salinity stress. The drought pattern remarkably enhanced the leaf water status and decreased toxic ion uptake (Rasul *et al*., 2016; Jin *et al*., 2020). However, results of this study suggests that the physiological responses to water limiting condition or temperature conspicuously depends on the temporal patterns and their combination with those stresses.

***Conclusions*:** Results confirmed major ionic accumulations in desert plant responded to spring season even under yearly fluctuations and in dry warm to hot regions whereas, the antioxidants were gathered aggressively under humid and subhumid hot conditions with seasonal and spatial fluctuations inducing drought tolerance in plants physiological and biochemical attributes in *Agave sisalana*.

***Author Contributions*:** MH and HM conceived and designed the research. SS, MH, and MA performed the experiment and wrote the initial draft of the manuscript. SS, HM and MH assisted with the analysis, revised subsequent versions of the manuscript, and provided technical guidance. SS and MH proofread and edited the final version. All authors have read and agreed to the published version of the manuscript.

***Conflict of Interest*:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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