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PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES OF *AGAVE SISALANA* RESILIENT ADAPTATION TO CLIMATIC AND SPATIO-TEMPORAL CONDITIONS

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Abstract

Plant's behavior varies physiologically and chemically in wake of its adaptation to environmental changes. Sisal (*Agave sisalana* Perrine) is less explored in climatic conditions of Pakistan. In the present work, physiological and biochemical responses of sisal plant under different natural environmental conditions in Punjab province of Pakistan's five districts having diverse climates from arid to semi-arid were selected. The selected districts included Chakwal, Khushab, Rawalpindi, Faisalabad and Layyah were studied through seasonal surveying during all four seasons and sampling of plant material were done for a period of two years 2017-2019. The data regarding total chlorophyll content, total soluble protein content, total soluble sugar content, total soluble phenolics, photosynthetic rate, transpiration rate, stomatal conductance, sub-stomatal CO_2 concentration and water use efficiency were investigated. The spring season reflected highest value of photosynthetic rate, transpiration rate, stomatal conductance, and sub stomatal CO_2 concentration at Rawalpindi district during 2018-2019 as compared to other seasons. The maximum total soluble sugars and total soluble phenolics content were also recorded in Rawalpindi district during spring of 2018-2019. Total soluble protein content increased in the Chakwal district during winter season of both years. However, total chlorophyll contents were maximum in spring season and were reduced during summer and autumn seasons in district Chakwal during 2018-2019. Overall spatial and temporal heterogeneity was clearly seen for the physiological and biochemical attributes of *Agave sisalana.* Based on hardy growth habit, sisal cultivation in problem soils affected by extreme dearth of water, frost and extensive salinity have been suggested as future thrust.

Key words: Gas exchange, Season, Environment, Heterogeneity, Punjab.

Introduction

Sisal (*Agave sisalana*) is known as a miracle plant for having multi-dimensional qualities as an important fiber-producing crop in the world having medicinal aspects with high capacity to withstand harsh environmental conditions (Yu, 2015). The plant is mostly cultivated for its high-quality natural fiber which is scraped from fresh leaves of plants in Eastern Africa, Brazil, India, and many countries in Asia (Yu, 2015; Hulle *et al.,* 2015). It is useful for manufacturing of handbags, floor mat, ropes, carpet etc. According to Fajrin (2016) 4.5 million tons of sisal leaves for fibers are produced annually around the world. Sisal represents 2% of the world's natural fiber production (Cruz-Magalhaes *et al.,* 2019). Agave plant species specially *Agave sisalana* is rich in Phyto-chemicals responsible for anti-inflammatory, analgesic, anthelmintic, antimycotic, antioxidant, antiviral, antituberculosis, antibacterial, gastro protective, bactericidal, and insecticidal activities (Debnath *et al*., 2010; Tewari *et al.,* 2014). The spatial heterogeneity varies with time (Van & Leeuwen, 2003) and adaptation of sisal to harsh climatic conditions can be attributed for its potential to conserve more water in their leaves (Corbin *et al*., 2015).

Agave sisalana grown successfully in regions where insufficient rainfall hinders the cultivation of other plant species (Debnath *et al.,* 2010). The thick cuticle on leaves helped to reduce non-stomatal water loss, helps agave plants to proceed crassulacean acid metabolism (CAM) photosynthesis by their reduced stomata (Davis *et al.*, 2019). The important role of the CAM photosynthetic mechanism modified by agaves i.e., stomata opening and uptake of CO_2 at night hours, thus allowing less water escape from transpiration mechanism (Arve *et al.,* 2011). The influence of abiotic stresses obstructs photosynthetic activity due to non-stomatal and stomatal reasons (Yadav *et al.,* 2020) and physiological aspects i.e., photosynthetic and transpiration rates, water use efficiency and stomatal conductance decreased with the increase of stress (Pan *et al.,* 2013). Riaz *et al.,* (2016) recorded decline of transpiration rate, photosynthetic rate, stomatal conductance, and total chlorophyll content in agave and Bouaziz *et al.,* (2014) found high amount of total soluble protein (35.33%), total sugars (45.83%), and lipid (2.03%). If plant face dry and harsh conditions, the cells may escape from water deficiency (Fathi & Tari, 2016) by tolerance and adaptation as a property whereby the plant works at tissue-specific and cellular level by producing proteins, sugars, enzymes and certain osmolytes that help it survive and work under harsh environmental situations (Arve *et al.,* 2011).

The effects of spatio-temporal variability on the physio-biochemical aspects of sisal (*Agave sisalana*) are widely unknown and demands insight research. This research work was performed to explore the Physio-biochemical analysis and to recognize the Phyto-chemicals variability in sisal plants collected in four distinct seasons from five ecologically diverse regions of the Punjab, Pakistan and its impact on planting environment and exploration of Phyto-chemicals diversity and concentration in sisal on basis of spatio-temporal variability, proving key role in the fiber and pharmaceutical industry.

Materials and Methods

The research experiment was performed to explore the spatial and temporal heterogeneity physiological and biochemical attributes of *Agave sisalana* Perrine renowned as sisal among succulent CAM plants was collected from ecologically diverse three sites in respect of all five selected districts and sub sites of Punjab, Pakistan as given in Table 1; (Fig. 1). In each location, surveyed randomly to collect plant leaves during the peak of all spring, summer, winter, and autumn seasons repeated for two years 2017-2019. Physiological and biochemical attributes of sisal plant leaves were determined by suitable analytical methods. Seasonal data on daily basis were collected from Pakistan Meteorological Department and presented in (Fig. 2) for all five locations and across two study years.

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

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

Gas exchange attributes and total chlorophyll content: Gas exchange attributes i.e., transpiration rate, photosynthetic rate, water use efficiency, stomatal conductance, and sub-stomatal CO_2 concentration, were measured in plant leaves using Infra-Red Gas Analyzer (IRGA- Model LCi, ADC Bio-Scientific Ltd. UK) without destructive sampling following the standard procedure. Three plant leaves were carefully chosen randomly from each site to represent canopy. The chlorophyll content was calculated by following the method given by Nagata & Yamashita (1992).

Total soluble sugars, phenolics and proteins: Total soluble sugar content was determined using the method of Gaines and Gascho (1985).

Total phenolics were determined as described by (Waterhouse, 2002) modified by (Munir, 2011).

Total protein content was observed from the solution as described by Bradford (1976).

Statistical analysis

The data were statistically analyzed by Fisher's Analysis of Variance (ANOVA) technique using Tukey's Honestly Significant Difference (HSD) test as described by Steel *et al*., (1997) using software Statistix version 8.1. Correlation 2-tailed test was also applied.

Fig. 1. Map showing the five Districts in Punjab Pakistan, 1-Rawalpindi, 2- Chakwal, 3-Khushab, 4-Faisalabad, 5-Layyah.

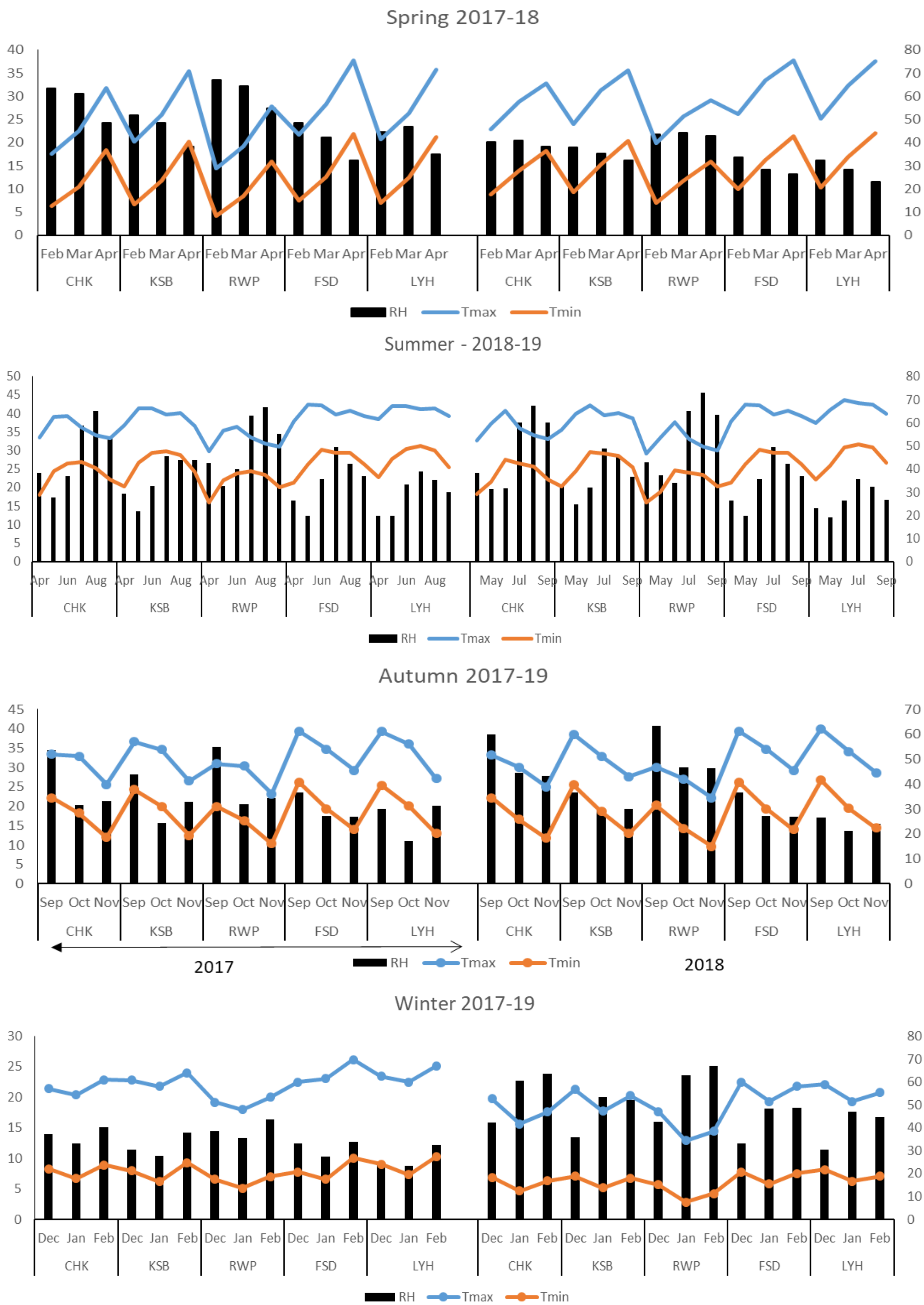

Fig. 2. Weather data of two years and four seasons during 2017-19.

Table 2. Two Way-Analysis of variance ANOVA (F-ratio) for physio-bochemical attributes in leaves, of *Agave sisalana* collected for five arid to semi-arid areas.

2-Factor ANOVA	Photosynthetic rate	Transpiration rate	Water use efficiency	Stomatal conductance	Sub stomatal CO_2 Conc.	Total Chlorophyll	Total soluble protein	Total soluble sugars	Total phenolics
Locations	8.81**	3.97*	2.42ns	8.86*	231.5***	1.06ns	5.43*	2.02ns	5.61*
Seasons	0.42ns	0.06ns	8.22*	369.3***	10.89**	1.28ns	0.62ns	0.83ns	0.63ns
Interaction	4.12*	3.10*	1.27ns	6.82*	22.19***	0.04ns	4.63*	0.94ns	0.76ns

Non-significant = ns, Significant * = p<0.05, Significant ** = p<0.01, Significant *** = p<0.001

Results

Physiological attributes variation in sisal by spatiotemporal variation: Photosynthetic rate (µmol. CO_2 $m^{-2}s^{-1}$) in sisal plant leaves were highly significant (p<0.01), among locations, while non-significant between seasons and the interaction among seasons and locations were insignificant (p>0.05), during 2018 and 2019 as in Table 2. Photosynthetic rate was higher (0.81 µmol. CO_2 $m^{-2}s^{-1}$) in sisal leaves collected from Rawalpindi location during spring while, the lowest value (0.09 µmol. CO_2 $m^{-2}s^{-1}$) was recorded at Layyah location during autumn 2018. The photosynthetic rate was significantly higher (2.85 µmol. CO_2 $m^{-2}s^{-1}$) at Rawalpindi location during spring followed by (1.31 µmol. CO_2 $m^{-2}s^{-1}$) winter 2019 as elucidated in Fig. 3.

The transpiration rate varied among locations (p<0.05) but not varied among seasons (p>0.05), and their interactions were observed significantly (p<0.05) during both years (2018-2019). The highest transpiration rate (0.50 mmol H_2O m^{-2} s^{-1}) was observed at Rawalpindi during spring followed by the autumn season 2018. Samples of sisal leaves collected from Rawalpindi location exhibited profoundly higher transpiration rate (2.56 mmol H_2O m^{-2} s^{-1}) during spring 2019 while the lowest value of transpiration rate was recorded during autumn as in Fig. 3.

The water use efficiency was elucidated non-significant (p>0.05) among locations but significant (p<0.01) among seasons and their interactions (location & seasons) were insignificant (p>0.05). During summer sisal plant leaves showed more water use efficiency (2.11 A/E) in samples collected from Rawalpindi location but lower stomatal conductance (0.28 A/E) were found in samples collected from Layyah location during autumn 2018. On the other hand, during winter 2019 we found maximum (4.66 A/E) water use efficiency in sisal leaves collected from Layyah location as in Fig. 3.

The stomatal conductance varied among locations (p<0.01) and seasons (p<0.001) and their interactions were noted significant (p<0.05) during 2018-2019 mention in Table 2. Stomatal conductance was observed the highest (3.16 mmol mol^{-1}) in sisal leaves collected from Rawalpindi location during spring 2018 and same trend was noted during spring 2019 as depicted in Fig. 3.

Sub stomatal CO_2 concentration in sisal leaves revealed significant (p<0.001) among locations, while seasonal effect was non-significant (p>0.05) and the interaction among the season and locations was also significant (p<0.001), during 2018 and 2019. Similarly, during spring 2018 showed maximum sub stomatal CO_2 concentration (2.13 µmol mol^{-1}) in sisal leaves collected from Rawalpindi location. While during spring 2019 maximum sub stomatal CO_2 concentration (3.93 µmol mol^{-1}) followed by summer autumn and spring season as in Fig. 3 was found.

Biochemical attributes variation in sisal by spatiotemporal variation: The total chlorophyll content was elucidated insignificant (p>0.05) among locations and seasons and their interactions were also insignificant (p>0.05) (Table 2). The present investigation revealed decreased level of total chlorophyll (0.24 mg g^{-1}) in *Agave sisalana* (sisal) leaves at Chakwal location during winter of the first season (2018) when compared to control samples, but increased level of total chlorophyll (0.57 mg g^{-1}) was noted during spring season of the second year (2019) at Chakwal location (Fig. 4). The highest value of total chlorophyll was recorded during the second year (2019) as compared to the first year (2018) of data recording.

The total soluble protein content varied among locations (p<0.05) but remained non-significant among seasons while their interaction was found significant (p<0.05) during both years (2018-2019) as given in Table 2. In case of total soluble protein content, the lowest concentration (0.02 mg g^{-1}) was noted during spring -2018 at Chakwal location while, the highest concentration (0.20 mg g^{-1}) was observed during winter season-2018 at Rawalpindi location as in Fig. 3. During the second year (2019) the lowest concentration of total soluble protein content (0.03 mg g^{-1}) was recorded in summer, but the highest values observed (1.44 mg g^{-1} and 1.12 mg g^{-1}) during winter followed by spring 2019. However, the highest value (1.44 mg g^{-1}) was noted at Faisalabad location during winter season-2019 as in Fig. 4.

The total soluble sugar content varied among the locations & seasons, and their interactions were found non-significant (p>0.05) during 2018-2019 as given in Table 2. On average, we found the highest level (2.89 mg g^{-1}) of total soluble sugar content in sisal leaves at Rawalpindi location during winter, but the lowest value (0.50 mg g^{-1}) was recorded at Faisalabad location during summer (2019). The highest concentration (0.59 mg g^{-1}) of total soluble sugar content was recorded at Khushab sites during autumn while, the minimum concentration (0.42 mg g^{-1}) was found at Faisalabad during autumn (2018) as given in Fig. 4.

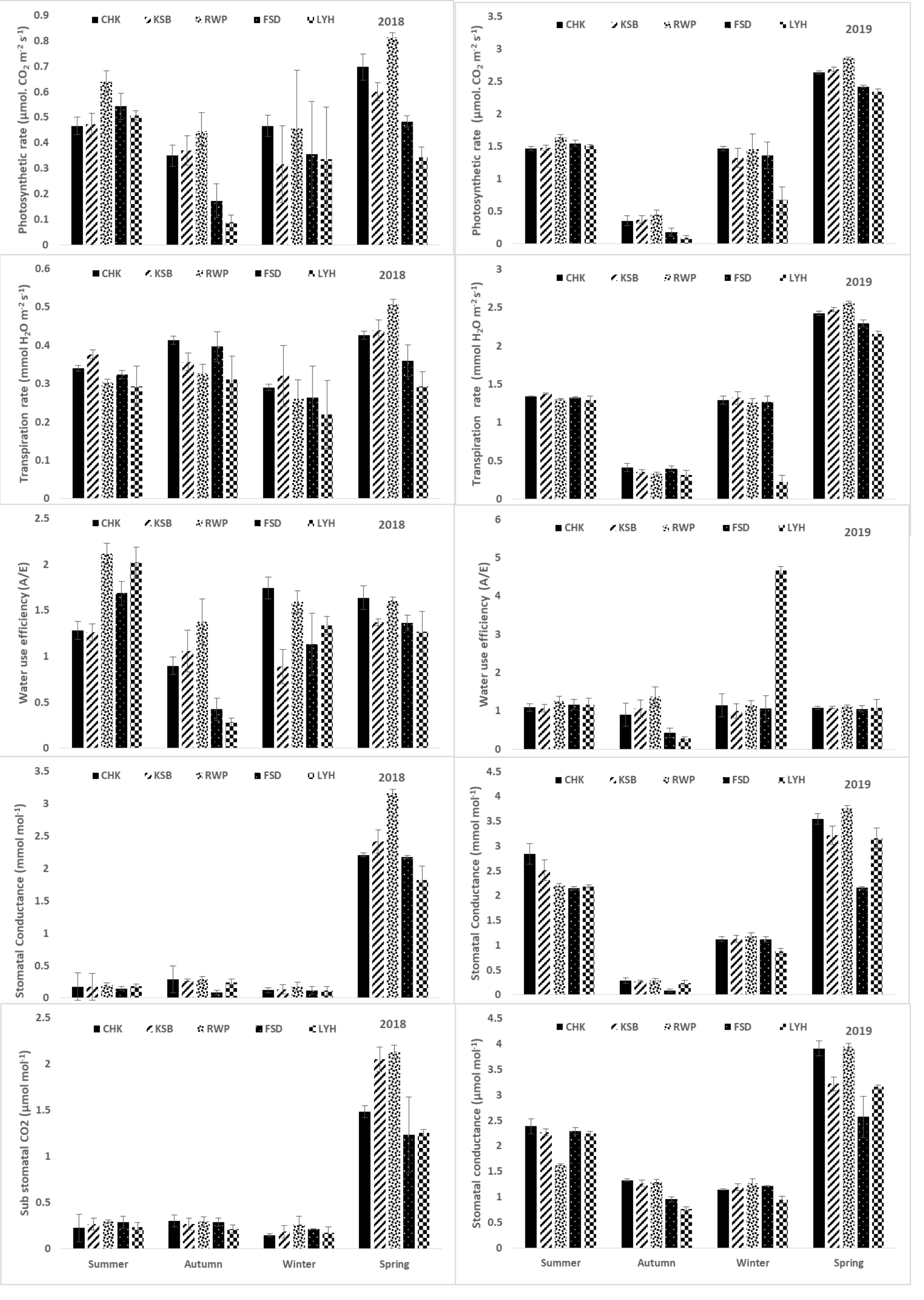

Fig. 3. Photosynthetic rate, Transpiration rate, Stomatal conductance, Water Use efficiency and Sub-stomatal CO_2 concentration *Agaves sisalana* leaves as affected by different seasons of years and locations.

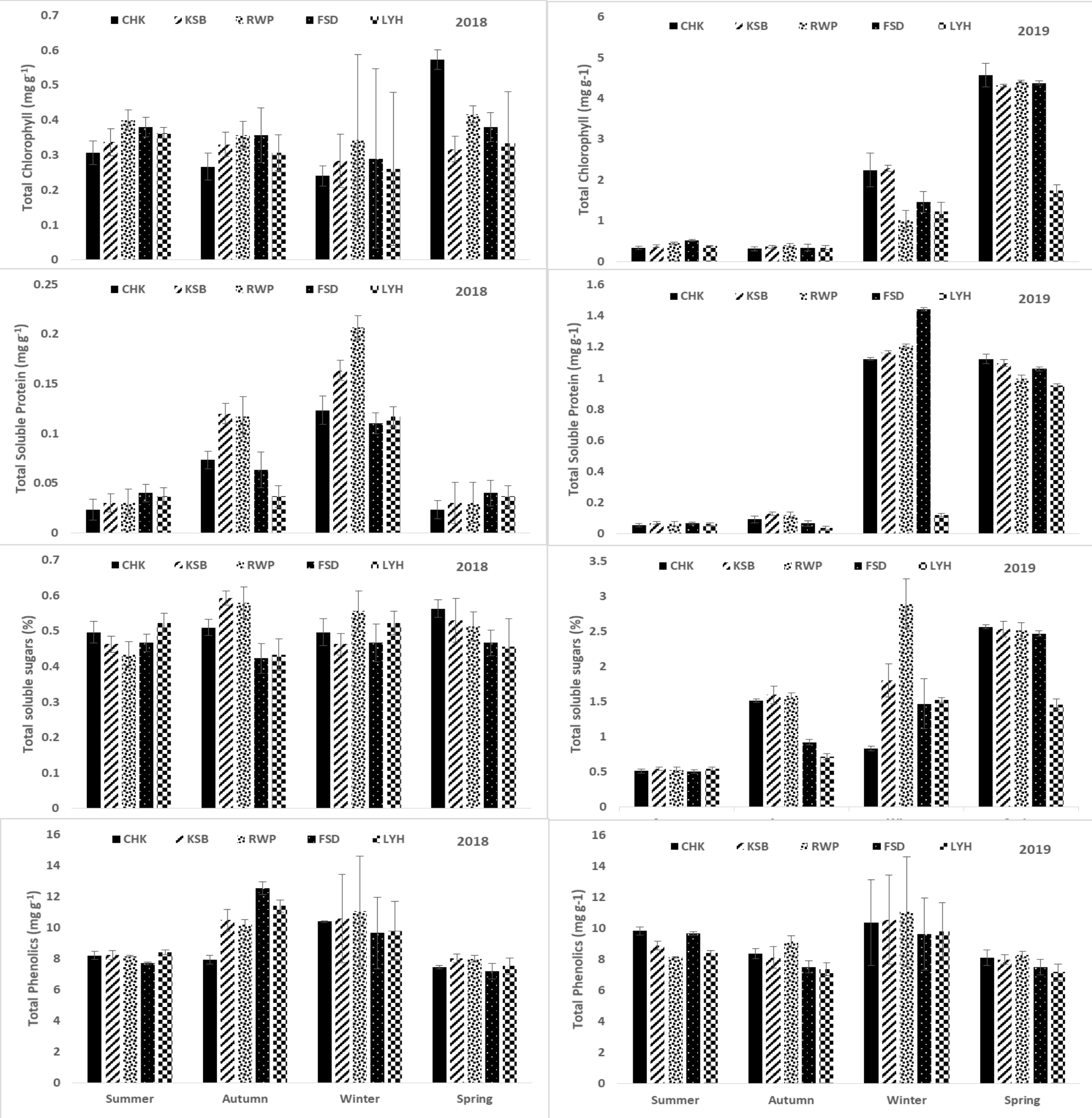

Fig. 4. Total Chlorophyll, Total soluble proteins, Total soluble sugar, and Total phenolics content of *Agave sisalana* leaves as affected by different seasons of years and locations.

The total phenolics were varied among locations ($p<0.05$), but their interaction across the seasons i.e. (locations × seasons) was insignificant ($p>0.05$) during 2018 and 2019 (Table 2). The tremendous increased levels of total phenolics concentration were found in sisal leaves obtained from five agro-ecological regions. The maximum total phenolics concentration (12.52 mg g^{-1}) was found at Faisalabad location in autumn while, the lowest concentration (7.44 mg g^{-1}) was noted at Chakwal location during spring of the year 2018 (Fig. 3). During the year 2019 the highest concentration (11.04 mg g^{-1}) of total phenolics was recorded at Rawalpindi location during winter but the lowest (7.18 mg g^{-1}) level was found at Layyah location during spring season of the year 2019 as demonstrated in Fig. 4.

Correlation: Pearson correlation exhibited that the total soluble sugars and total soluble phenolics had negative (-) correlation with photosynthetic rate while total chlorophyll positively (+) linked with photosynthetic rate of sisal during 2018 year (Table 3). Green or blue colors showed non-significant relation with parameters. However, we noted during the year 2019 total proteins showed positive relation with transpiration and photosynthetic rates but total chlorophyll had strong positive ($p<0.01$) correlation with transpiration rate, sub-stomatal conductance and photosynthetic rate in sisal leaves (Table 4). Different colors showed significant level in both tables (Tables 3 & 4).

Discussion

The world is facing limited crop productivity challenge due to the constant encounter of plants to environmental stresses (Riaz *et al.*, 2016) which are posing a severe threat to agro-ecosystem (Gull *et al.*, 2019). Plants being sessile confront stresses and develop potent adaptive tactics to avoid adverse effects to survive and thrive (Gull *et al.*, 2019). Sisal (*Agave sisalana*) responds to different environmental conditions in the form of changes in various physio-biochemical aspects. Physiological aspects of *Agave sisalana* plant leaves were measured by IRGA at five agro-ecological regions during four seasons of the years 2018 and 2019 (Fig. 3) in this investigation which is a modern, portable, recognized, and efficient tool. The photosynthetic rate in sisal leaves were computed higher at Rawalpindi during spring of both years 2018 and 2019 but observed lower at Layyah location during autumn of both years while, same trend was observed for transpiration rate and stomatal conductance (Fig. 3). These observations are characterized to thermal regimes variations in these localities where spring of Rawalpindi has moderate ascending trend of temperature to support higher photosynthetic rate while higher day temperatures and short nights at Layyah sites in autumn of both years, likewise transpiration rate and stomata conductance are supposed to be affected in similar pattern. Gas exchange attributes of plants were confirmed to be affected due to strenuously varying environmental conditions (Rasul *et al.*, 2012; Hafeez *et al.*, 2017; Gull *et al.*, 2019; Zhao *et al.*, 2020). Thus environmental stress influenced the gas exchange attributes including photosynthetic rate, transpiration rate etc. Ohashi *et al.*, 2006 found transpiration rate stomatal conductance and photosynthetic rate, were affected by water deficit environment in soybean plants. Zhao & Ji, (2016) observed spatial and temporal heterogeneity in maize transpiration response under arid climatic condition depicting a generalized response in different type of plants. As climate change led to drier and warmer conditions in semi-arid regions, the sustainable crop growth will become more challenging. Agaves being succulent can be productive plants of future that will respond favorably to dry and warm conditions through CAM system. *Agave sisalana* survives and is adapted in dry regions (Riaz *et al.*, 2016) and these adopted characters, including crassulacean acid metabolism (CAM), that allow them to survive under warm and the water deficit environment. Lawson & Blatt (2014) reported closure of stomata when examined under extreme water deficit condition which is another physiological character found in sisal for adaptation. Due to closure of stomata reduced transpiration rate, photosynthetic and stomatal conductance (Neales *et al.*, 1968; Reddy & Das, 2000; Arve *et al.*, 2011; Kluge & Ting, 2012; Pirasteh-Anosheh *et al.*, 2016; Henry *et al.*, 2019) was observed. The significant decline in photosynthetic activity of plants under drier and warmer seasons was also reported by (Anjum *et al.*, 2011). Another reason of stomatal closure was quick response to water stress which gave shelter to plants from severe water loss by a decline in stomatal conductance, increase in intercellular CO_2 concentration and decrease in the photosynthetic rate (Agurla *et al.*, 2018) and similar traits were also observed in sisal. This study elaborated that water use efficiency and sub stomatal CO_2 concentration of sisal leaves were more influenced in winter and autumn season compared to spring season during both years (Fig. 3). Mabapa *et al.*, 2018 observed decline in stomatal conductance, transpiration and increasing trend was noted for water use efficiency under water shortage and high temperature conditions. However, the gaseous exchange attributes were more affected i.e., the stomatal conductance, photosynthesis CO_2 intake and other gas exchange traits in response to locations and seasons (Fig. 3). Results of this study were in conformity with (Nayyar & Gupta, 2006; Jouany & Morgavi, 2007; Moussa & Abdel-Aziz, 2008; Flexas & Medrano, 2002; Jabeen *et al.*, 2008; Paul *et al*., 2017; Wang *et al*., 2018).

Table 3. Pearson correlation between physiological and biochemical attributes (2019 year) (Two-tailed test).

	Photosynthetic rate	Transpiration rate	Water use efficiency	Stomatal conductance	Sub-stomatal conductance
Total soluble sugars					
Total proteins					
Total chlorophyll					
Total phenolics					

Table 4. Pearson correlation between physiological and biochemical attributes (2018 year) (Two-tailed test).

	Photosynthetic rate	Transpiration rate	Water use efficiency	Stomatal conductance	Sub-stomatal conductance
Total soluble sugars					
Total proteins					
Total chlorophyll					
Total phenolics					

Negative (-)	ns	*	**
Positive (+)	ns	*	**

Significant level: ns= Non-significant, *, p<0.05, ** p<0.01

Total chlorophyll content is a vital indicator for the metabolic rate of plants (Riaz *et al.*, 2016). In present study outcome total chlorophyll content in sisal leaves were higher at Chakwal location during spring season compared to other locations and seasons Fig. 4. Zhang *et al.,* (2020) found spatial variation in the concentration of chlorophyll in grasses. Borsuk & Brodersen, 2019 noted spatial variation in concentration of total chlorophyll in leaves. Costa *et al.,* 2019 also found temporal and spatial influences on total chlorophyll content in plants. *Agave sisalana* leaf margins were serrated, and girth was succulent which resulted in limited occurrence of pigments and its density. Variable climatic features affecting seasonal and yearly data were meaningfully understandable when considered for sisal leaf composition as it raised a question on slow rate of sisal growth. Successful growth and survival of sisal in areas with moderate to dry environment was reported by Debnath *et al.*, (2010). Scientists have reported reduction in chlorophyll content of cereals, sunflower, and olives due to dry climatic conditions (Nyachiro *et al.,* 2001; Guerfel *et al.*, 2009). Some sugars, phenolics and proteins were subjected to abiotic trauma in plants (Riaz *et al.*, 2016). Higher levels of protein content of sisal leaves were found in winter seasons at Rawalpindi and Faisalabad locations during both years 2018 and 2019 respectively (Fig. 4). Merewitz *et al.,* 2011 found higher protein content in plants due to drought condition compared to control. These defense related proteins were involved in transcription, signaling, managing stressful situation and defense mechanism, protein synthesis, photosynthesis and photorespiration and energy metabolism, membrane and transport, cell structure and cell cycle, nitrogen assimilation and fatty acid metabolism as well as amino acid metabolism (Wang *et al.,* 2016). Annunziata *et al.,* (2019) found spatial and temporal impact on total soluble sugar content in wild plants under abiotic stress condition. The present results are in conformity with the previous researchers for other plant species (Ahmad *et al.,* 2018; Isah, 2019). We found higher levels of total soluble sugars in sisal leaves during autumn at Khushab location and at Rawalpindi during winter 2018 and 2019 respectively (Fig. 4). Carbohydrates, besides playing a vital function as Osmo protectants under water scarce condition have multiple roles as substrates for different cell processes and signaling molecules. Water deficit stimulated the accumulation of soluble sugars i.e., glucose and fructose in leaves of plants that contributed to starch biosynthesis and in sucrose transport to sink tissues While soluble sugar as glucose act as Osmo protectants and substrate components for cell respiration and fructose is related with secondary metabolite synthesis (Zivanovic *et al.*, 2020). Arabzadeh, (2012) found the accumulation of total soluble sugars in two plants species i.e., *Haloxylon persicum* and *Haloxylon aphyllum* under water stress. Zivanovic *et al.,* 2020 observed the increased level of total soluble sugar content in plants under saline and water scare condition. Annunziata *et al.,* (2019) found spatial and temporal impact on osmolyte accumulation due to abiotic stress in plants. In present results total phenolics content increased in *Agave sisalana* at Faisalabad during autumn and during winter at Rawalpindi location in year 2018 and 2019, respectively. Plant phenolics compounds has antioxidants properties, attractants (carotenoids and flavonoids), structural polymers, signal compounds, defense response chemicals and UV screens (flavonoids). Phenolics compounds are important in defense reactions, such as anti-inflammatory, anti-aging, and anti-proliferative activities (Lin *et al*., 2016). Clement *et al.,* (2007), found phenolics compound accumulation in plants that play role in growth of tobacco plants. Jaleel *et al*., (2009) observed agaves accumulate total phenolics content in autumn season and under water scarce condition. Samec *et al.,* 2021 also found increased level of phenolics content in plants under abiotic stress. Enzymes involved in phenolics biosynthesis and events relevant to it were highly influenced by extent of solar exposure and temporal features (Tolic *et al.,* 2017). Presented work is also agreed with the finding of Oszmianski & Wojdylo, 2005 thus suggesting sisal plants adaptation and leaves composition variability on the basis of spatio-temporal environmental uncertainties. We found significant correlation between physiological and biochemical aspects of sisal plant (Table 3 & 4). Chunthaburee *et al.,* (2016) found positive correlation of physio-biochemical attributes of plants when grown in saline soil. Toscano *et al.,* (2016) also found a positive correlation in water stress species but proline showed negative correlation with physiological attributes of plants. Hura *et al.,* (2007) also noted positive correlation between photosynthetic rate and transpiration rate with osmotic potential under water stress.

Conclusion

Climatic changes at regional and local levels are putting the food security at risk thus under variable spatial and temporal conditions of Punjab Pakistan, *Agave sisalana* was found flexibly existing with physiological and biochemical adjustments and adaptation to local climates tested across the seasons, locations, and years. The year 2018-19, with summer and spring seasons at locations of Rawalpindi, Khushab and Chakwal respectively were recorded as the most favorable for *Agave sisalana* physiology, gaseous exchange and biochemical attributes hence can be attributed as the best conditions for *Agave sisalana* with assured adaptability and propagation in these environmental conditions.

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