Original Research Article

Effect of seasonal changes on photosynthetic pigments and proline contents of some plants growing naturally in Tayma region, Saudi Arabia

Abstract

The present study was undertaken to understand adaptive behavior and the possibility of osmotic adjustment adopted by three plant species to tolerate harsh environmental conditions during the winter and summer seasons based on some soil and plant variables as well as the relationships among them. The plant species studied are *Hyoscyamus muticus* L. (*H. muticus*), *Anabasis setifera* Moq. (*A. setifera*) and *Teucrium polium* L. (*T. polium*) and were collected from Tayma Governorate, Tabuk region, Saudi Arabia. ANOVA results showed that the plant species, depths, and their interaction significantly affected most physicochemical properties of the soil supporting three plant species. Also, the plant species, seasons, and their interaction highly significantly affected photosynthetic pigments and proline contents. The highest values for pH and Ec in *A. setifera* soil, for Na^{+}, Mg^{++} and Cl^{-} in *H. muticus* soil, and for other studied chemical properties in *T. polium* soil were recorded. Significantly increased water content % was observed in A. setifera soil at 20-40 depth across the winter season. Chlorophyll a (Chl.a), chlorophyll b (Chl.b) and Chl.a+b contents in *H. muticus* plants and Chl.a/b, total carotenoids, total pigment and proline contents in *T. polium* plants were significantly increased in comparison to the other plant species. Compared to the winter season, the amount of proline and photosynthetic pigments increased significantly in the summer season. The principal component and biplot analysis displayed that the three plant species were quite separated based on the variables studied, and showed positive correlations among most soil and plant variables, but these associations varied in their degree and consistency. The positive correlations were observed for PC1 with K^{+}, Ca^{++}, HCO_3^{-} and SO_4^{--} in soil as well as Chl.a/b, total carotenoids, total pigment and proline in *T. polium* plants. While PC2 showed highly positively correlated to Na and Mg in soil as well as Chl.a, Chl.b and Chl.a+b in *H. muticus* plants. As a result, the above soil chemical properties are associated closely with the above plant variables in the two previous plant species. The statistical study shows that due to its adaptive behavior and potential for osmotic adjustment, *T. polium* type generally adapts better to the dry desert environment and seasonal changes.

Key words: *H. muticus, A. setifera, T. polium, soil properties, photosynthetic pigments, proline, PCA.*

1. Introduction

The productivity of ecosystems is decreased, along with the diversity and relative abundance of species, by factors such as soil degradation, drought, global warming, extinction of perennially palatable species, overgrazing, and human-caused activities [1,2]. Numerous researchers have noted seasonal variations in the chemical or biological components of a variety of plant species, for example Peters et al. [3], Ramírez-Briones et al. [4], Huang et al. [5], El-Absy [6]. Seasonal variations affect the different biochemical characteristics of the wild plant species, these variations significantly were noticed in the active components of these species, and these variations are caused by variations in environmental factors including rainfall, temperature, and other factors [7,8]. El-Absy [6] and Kamel and El-Absy [9] conducted a study on the seasonal variation in the chemical composition of soil and plants and found that significant differences were seen to represent real environmental variations in chemical composition throughout the seasons, species, and their interactions.

All soils contain a variety of soluble salts, the most prevalent cations being calcium (Ca^{++}), magnesium (Mg^{++}), and sodium (Na^{+}), which are associated with soil salinity [10]. Misra and Tyler [11] reported that although nutrient content may increase as the volume of soil water declines and results in enhanced uptake by plants for a brief period, low water availability in soils may have diverse impacts on it. Plants require rather high amounts of the so-called macroelements Ca^{++}, Mg^{++}, and K^{+} with values of >0.1% of dry mass, in addition to the metabolic mineral elements nitrogen, phosphorus, and sulfur [12].

Desertification is a severe issue in semi-arid climates, where there is a rapid increase in soil erosion and a gradual decline in vegetation cover [13]. Two important environmental conditions that affect plant productivity and spread are drought and salinity factors [14]. Plant species have developed a variety of techniques to cope with harsh environmental changes in all types of climates and terrains and to lessen negative impacts of ions from those areas of the plants where they may be damaging, in order to adapt and flourish to salt and drought conditions [15,16]. Plant species can regulate their osmotic pressure by accumulating osmolytes that are compatible with their cells' metabolic processes [17]. Aldamegh et al. [18], Pradhan et al. [19] and Salama et al. [20] mentioned that dry weather conditions may activate the production of secondary phytochemical compounds or accumulation of numerous reactive oxygen species and osmolytes with high concentrations such as proline, which will play a critical role during stress acclimation in plants. Proline has been suggested to have a variety of roles in osmotically challenged plant tissues, including osmotic adjustment, protection of plasma membrane integrity, a source of carbon and nitrogen, as well as a sink of energy or decreasing power [21-23]. The higher plant tissues that absorb light include important pigments called chlorophylls and carotenoids. Through the process of photosynthesis in the cell, they are able to convert the radiant energy of sunlight into the chemical energy of organic carbon compounds [24]. These compounds may have improved the plants' ability to withstand the effects of salinity and drought conditions [25]. Due to the plant species capacity to maintain turgidity and water absorption, desert plants can withstand drought conditions [26]. Additionally, to achieve this the plant species undergo morphological, physiological, and molecular modifications [27,28].

The physical, chemical, and biological characteristics of the soil as well as plant growth are all impacted by the pH of the soil [29]. Where understanding the relationship between geographically heterogeneous soil characteristics and fertility is necessary due to public concern over boosting soil productivity and crop input efficiency [30]. Because soil and plant species are interdependent, the amount of organic matter in the soil as well as the type and nature of vegetation affect its physical, chemical, and biological characteristics [31,32]. Numerous research, including those by Al-Ghamdi [25], Al-Mutairi [33], Salama et al. [34] and El-Ghani and Amer [35] investigated the effects of soil factors on different plant species. El-Ghani and Amer [35] stated that the correlations between the recorded soil characteristics and the vegetation are examined using multivariate statistical methods. The principal component analysis (PCA) has been used to study the relationship between soil parameters and plant variables by many researchers, for example El-

Absy [6], El-Ghani & Amer [35] Ferraz et al. [36], Metwally et al. [37], Abdel-Fattah et al. [38] and El-Absy [39].

The major aim of this research was to calculate soil physicochemical properties and study seasonal variations of photosynthetic pigments and proline contents in three plant species growing under natural conditions in Tayma Governorate, Tabuk region, Saudi Arabia, as well as, elucidate the relationship between soil and plant variables by PCA and Biplot analysis. These plant species are *Hyoscyamus muticus* L. (*H. muticus*), *Anabasis setifera* Moq. (*A. setifera*) and *Teucrium polium* L. (*T. polium*).

2. Materials and methods

2.1. Study area

The current study was conducted between January 2022 (winter) and August 2022 in Tayma Governorate, Tabuk Region, Saudi Arabia (summer). Tayma is a city located on the western edge of the great sand dune desert, the Nafoud Al-Kebir. The oasis of Tayma is located in the Province of Tabuk, 255 km southeast of the city of Tabuk and extends between latitude: 27°37′47″N and longitude: 38°32′38″E (Fig. 1). This area has an arid climate with year-round temperatures that range from 0 °C in the winter to approximately 50 °C in the summer. Less than 150 mm of precipitation per year on average is relatively little [40].

Fig. 1. Location map of Tayma Governorate, Tabuk Region, Saudi Arabia. (https://geanderson.files.wordpress.com/2014/11/maps_tayma.jpg).

The weather information for 2022 regarding temperature, relative humidity, and rainfall is shown in Fig. 2. Average high and low temperatures, relative humidity, and rainfall for the historical period of 2022 were reported with values of 28.41 °C, 16.05 °C, 29.44%, and 3.16 mm, respectively. The months of June, July, and August have the highest high and low temperatures, while January had the lowest temperature readings. The months with the highest relative humidity % were January and December, while the ones with the lowest percentages were May and June. The highest rainfall was observed between January and October months, but the lowest rainfall occurs in the months of June and July.

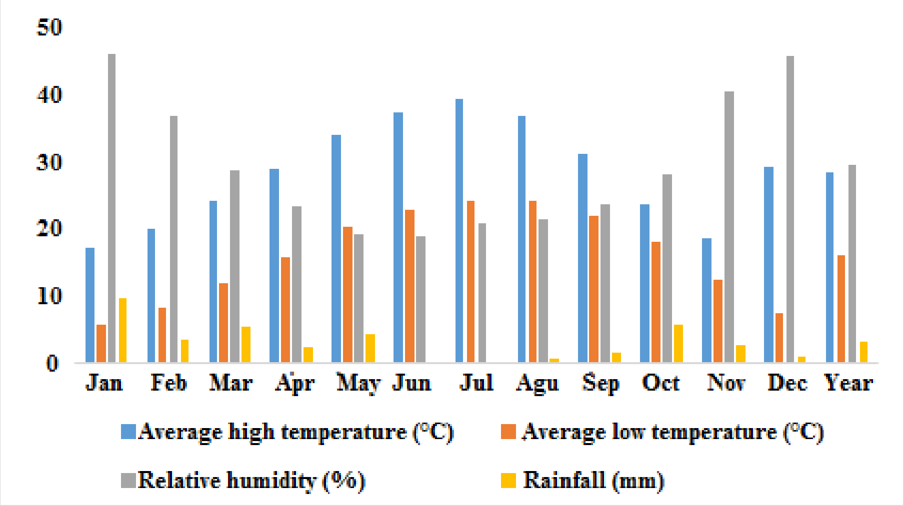

Fig. 2. Monthly average high and low temperatures (°C), relative humidity (%) and rainfall (mm) in the region studied (https://tcktcktck.org/saudi-arabia/tabuk/tayma).

2.3. Soil physical and chemical properties

In the study area, soil samples were carefully taken from the soil associated with *H. muticus*, *A. setifera*, and *T. polium* at two random depths of 0-20 cm and 20-40 cm. Each sample was divided into three replicates, which were then transported to the lab in sealed tins to be utilized for soil physical and chemical parameters. As recommended by Jackson [41] and Rowell [42] for soil texture, soil samples were air-dried, sieved, and utilized for the physical analysis of soil particles. The results of the physical analysis are reported as a percentage of the original weight. The Rowell [42] described approach was used to determine the soil moisture content. According to Jackson [43], the soil-water paste was used to measure electrical conductivity (EC; ds/m) and pH value for each sample. Using a saturation paste devised by Tuzuner [44], the soil chemical properties including sodium, potassium, calcium, magnesium (Cation; meq/L), chlorides, bicarbonate, and sulphate (Anion; meq/L), were determined.

2.4. Plant analysis

The samples of *H. muticus, A. setifera*, and *T. polium* were manually randomly collected in triplicates at the region under investigation in both the winter and summer seasons of 2022. Then they were put in plastic bags before being transported right away to the lab for preparing and processing. The contents of the photosynthetic pigments were measured spectrophotometrically, and using the wavelengths of 663, 645 and 470 nm, the chlorophyll a (Chl.a), chlorophyll b (Chl.b) and total carotenoids were determined by equations of Lichtenthaler [45], respectively, as follows:

$$Chl.\ a\ (\mathrm{mg/g^{-1}\ FW}) = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times V/(1000 \text{ x } W)$$

$$Chl.\ b\ (\mathrm{mg/g^{-1}\ FW}) = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V/(1000 \text{ x } W)$$

$$\text{Carotenoids}\ (\mathrm{mg/g^{-1}\ FW}) = [(1000\ A_{470}) - (2.27\ Chl.\ a) - (81.4\ Chl.\ b)\ /226] \text{ x } V/(1000 \text{ x } W)$$

Where, V: is the volume of the extracting liquid, W: is the weight in grams of the fresh leaf sample, and A_{663}, A_{645} and A_{670}: are the corresponding wavelengths of the light density value, respectively.

The proline content of samples in the three plant species was determined at 16 DAA according to the standard method of Bates et al. [46]. Following sample collection, preparation, and processing, a spectrophotometer (SPECTRO UV-VIS RS Spectrophotometer, Labo Med, Inc.) was used to determine the proline content on a fresh weight basis by utilizing the equation below:

$$\text{Proline } (\mu\text{moles/g of fresh plant materials}) = \{(\mu\text{g proline/mL} \times \text{mL toluene})/115.5\ \mu\text{g}/\mu\text{moles}\}/(\text{g sample}/5)$$

2.5. Statistical analysis:

The Komolgorov-Smirnov test was used to confirm the normality of the data distribution in this study. Then, using the Steel & Torrie [47] method, the measured data were subjected to two- and three-way ANOVA tests as well as the coefficient of variation (CV%) to identify any significant differences ($p \leq 0.05$ and $p \leq 0.01$) in the impact of the experimental factors and their interactions. The resulting data were presented as average ± standard error (SE), and multiple comparisons were decided using the least significant difference test (L.S.D.) at the 0.05 level of probability, according to Steel & Torrie [47]. For a better understanding of the correlation between the soil and plant variables in both seasons, principal component analysis (PCA) was used. All statistical analysis was performed using the computer software program OriginPro 2018 b9.5.0.193.

3. Results and Discussion

3.1. Soil analysis

The physical properties % of the soil supporting *H. muticus*, *A. setifera*, and *T. polium* in the two depths at the studied area are shown in Tables (1). The two factors of plant species ($P < 0.01$) and depths ($P < 0.05$) significantly influenced the percentages of sand, silt, and clay in their soil. While the species x depths interaction showed insignificant ($P > 0.05$) effects for these soil properties. These results were consistent with earlier research by Salama et al. [48], Moustafa et al. [49] and Al-Taisan [50]. The plant type of *T. polium* recorded a significantly higher sand % compared with the other two plant species investigated. However, the highest percentage of silt and clay was found in the soil associated with *A. setifera* type in the region studied. During the 0-20 and 20-40 depths, the sand % in the 20-40 depth as well as the silt and clay % in the 0-20 depth had recorded the highest values at plants soil. As for the interaction between the two factors, the highest values of sand% in *T. polium* soil at the 20-40 depth, and silt and clay % in *A. setifera* at the 0-20 depth were observed in this area studied. The soil of three plant species generally had higher sand percentages in the two soil depths than other soil particles, indicating a sandy texture in the soil at the study location. According to USDA [51], due to rapid water infiltration and percolation, as well as strong soil aeration, sandy soil, sometimes referred to as light-textured soil, has a low ability to hold water. El-Absy [39] and Moustafa et al. [49] mentioned that the proportions of sand, silt, and clay in soils collected from various environments differed significantly.

Table 1. Physical proprieties % of the soil supporting three plant species in two depths at Tayma Governorate, Tabuk Region, Saudi Arabia.

Factors	Sand	Silt	Clay	Soil Texture

Species				
H. muticus (H)	82.85±0.92b	11.17±0.61a	5.98±0.32b	Sandy
A. setifera (A)	81.37±0.45b	11.67±0.21a	6.97±0.31a	Sandy
T. polium (T)	91.17±0.55a	5.90±0.45b	2.93±0.15c	Sandy
P–Values	0.00^{**}	0.00^{**}	0.00^{**}	
Depths				
0 – 20	84.38±1.55b	10.06±0.88a	5.56±0.69a	Sandy
20-40	87.88±1.63a	8.10±1.06b	4.03±0.58b	Sandy
P–Values	0.07^{*}	0.08^{*}	0.08^{*}	
Species x Depths Interaction				
H x (0 – 20)	82.26±1.39a	11.71±0.88a	6.03±0.50a	Sandy
H x (20 – 40)	83.44±1.40a	10.63±0.89a	5.93±0.51a	Sandy
A x (0 – 20)	80.63±0.53a	11.76±0.34a	7.62±0.19a	Sandy
A x (20 – 40)	82.10±0.45a	11.58±0.30a	6.32±0.19a	Sandy
T x (0 – 20)	90.25±0.51a	6.72±0.27a	3.03±0.27a	Sandy
T x (20 – 40)	92.09±0.65a	5.09±0.50a	2.83±0.17a	Sandy
P–Values	0.95^{ns}	0.49^{ns}	0.20^{ns}	
CV%	1.87	10.74	11.11	

Statistically significant differences at $*p \leq 0.05$ and $**p \leq 0.01$; ns: indicate the non-significant difference. Different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test

The chemical proprieties of the soil supporting three plant species in two depths at the region studied are shown in Table 2. The two-way ANOVA demonstrated a significant effect (P < 0.05 or 0.01) of plant species, depths and their interaction on all soil chemical properties under study, with the exception of Ec, HCO_3^- and SO_4^{--} by depth factor, as well as pH, Ec and Ca^{++} by species x depths interaction. Our results were in line with El-Absy [6], El-Absy and Kamel [13], Han et al. [52] and Jamin et al. [53].

The highest values of Na^+, Mg^{++} and Cl^- in *H. muticus* soil, pH and Ec in *A. setifera* soil and other soil chemical proprieties in *T. polium* were recorded at the region under study. While the values of pH, Ec and Ca^{++} were higher in the second depth compared with the first dept. The opposite is true for other soil chemical proprieties evaluated from the two depths. During the two depths, the values of Na^+ and Mg^{2+} in *H. muticus* soil as well as K^+, Ca^{++}, HCO_3^- and SO_4^{--} in *T. polium* soil were higher than in the other soil chemical proprieties. On the other hand, the highest values of pH and Ec in the second depth and Cl^- in the first depth were observed in *A. setifera* soil. The study site tended to be alkaline because the pH values were higher than 7.7 in the two depths across the plant species soil. Marschner [54] mentioned that cation elements including magnesium, calcium, and potassium as well as other elements become less soluble at alkaline pH values higher than 7.5 due to chemical interactions with particular substances, such as HPO_4 and $CaCO_3$. Because soluble salts are more prone to accumulate in arid soils due to low precipitation and temperature rise, decreases in EC values may have a significant influence on plant health and nutrient availability [55,56]. The rise in total soluble salts in plant species soil could be the cause of alkalinity, as reported by Salama et al. [20].

Local variation in the soil properties surrounding single plants was what caused the diversity in plant species [57], and significant variations in environmental variables reflect the variability in the soil texture and properties [33]. The location with respect to the distance from the sea and the types of surrounding mountains may be the cause of the variance in values of soil chemical properties between various environments, thus both of which have a substantial impact on soil characteristics [49]. In general, soil properties like texture, pH, the presence of microaggregates, major cations, and organic matter can have an impact on the rhizospheric community either directly or indirectly [58]. The chemical and physical properties of the soil have a significant impact on plant growth patterns [59] due to their impact on water supplies [60], thus the plants are able to adjust and flourish in environments with various soil characteristics [61].

Table 2. Chemical proprieties of the soil supporting three plant species in two depths at Tayma Governorate, Tabuk Region, Saudi Arabia.

Factors	pH	Ec (ds/m)	Cation (milliequivalent/Liter)				Anion (milliequivalent/Liter)		
			Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	HCO_3^-	SO_4^{--}
				Species					
H. muticus (H)	8.00±0.06a	4.90±0.47c	11.76±0.33a	0.60±0.03b	5.06±0.29b	114.33±0.43a	9.45±0.38a	2.45±0.11c	2.55±0.12c
A. setifera (A)	8.01±0.01a	6.68±0.40a	5.92±0.56b	0.29±0.04c	3.70±0.46c	112.30±0.72b	9.42±0.75a	3.17±0.34b	3.24±0.03b
T. polium (T)	7.71±0.04b	5.08±0.29b	6.30±0.38b	1.30±0.06a	7.65±0.39a	109.35±0.32c	8.01±0.25b	7.07±0.27a	4.41±0.14a
P–Values	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.01^{*}	0.00^{**}	0.00^{**}
				Depths					
0 – 20	7.90±0.07a	4.76±0.30b	7.47±0.95a	0.81±0.16a	4.94±0.63b	112.39±0.73a	9.38±0.55a	4.24±0.65a	3.35±0.28a
20 – 40	7.91±0.06a	6.34±0.33a	8.52±0.99b	0.65±0.14b	6.00±0.65a	111.60±0.90b	8.53±0.29b	4.22±0.83a	3.45±0.29a
P–Values	0.94^{ns}	0.00^{**}	0.09^{*}	0.00^{**}	0.05^{*}	0.00^{**}	0.04^{*}	0.92^{ns}	0.41^{ns}
				Species x Depths Interaction					
H x (0-20)	8.02±0.10a	3.90±0.20a	11.24±0.38a	0.64±0.02c	4.55±0.27a	113.83±0.55b	9.70±0.58ab	2.37±0.19d	2.49±0.18c
H x (20-40)	7.98±0.10a	5.90±0.29a	12.29±0.36a	0.55±0.05c	5.57±0.29a	114.83±0.61a	9.20±0.59ab	2.53±0.12d	2.61±0.17c
A x (0-20)	8.00±0.01a	5.82±0.17a	5.40±0.80b	0.37±0.03d	3.15±0.63a	113.70±0.58b	10.90±0.52a	3.72±0.41c	3.19±0.03b
A x (20-40)	8.02±0.02a	7.53±0.18a	6.43±0.81b	0.21±0.01e	4.25±0.61a	110.90±0.59c	7.94±0.53c	2.63±0.32d	3.29±0.02b
T x (0-20)	7.69±0.07a	4.57±0.30a	5.78±0.46b	1.41±0.06a	7.12±0.49a	109.63±0.03d	7.55±0.33c	6.62±0.40b	4.36±0.22a
T x (20-40)	7.73±0.06a	5.60±0.29a	6.83±0.47b	1.18±0.04b	8.18±0.52a	109.07±0.67d	8.47±0.09bc	7.51±0.08a	4.46±0.21a
P–Values	0.80^{ns}	0.17^{ns}	0.04^{*}	0.08^{*}	1.00^{ns}	0.00^{**}	0.00^{**}	0.00^{**}	0.03^{*}
C.V.%	1.23	7.45	13.69	7.79	15.91	0.40		7.42	7.62

Statistically significant differences at $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$; ns: indicate the non-significant difference. Different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test.

The results in Fig. 3 revealed a highly significant effect (P < 0.01) of the plant species, seasons and depths on the water content % in soil associated with the three plant species. The same results were obtained by El-Absy [6], El-Absy and Kamel [13] and El-Lamey [62], who stated that the depths and seasons factors were significant effects on the soil water content% of plant species in various habitats studied. While the effects of the first and second interaction on the soil supporting three plant species in two depths across winter and summer seasons were insignificant. Significantly, the water content % increased with the soil of *A. setifera* type, followed by *T. polium* and *H. muticus* species. Additionally, the water content % was higher in the winter season and 20-40 depth compared with the other season and depth. Misra and Tyler [11] reported that seasonal effects may lead to beneficial changes in the water content of the soil, for example, an increase in the availability of phosphorus required for plant nutrition. In comparison to other interactions among the experimentally tested factors, the soil of *A. setifera* in the second depth throughout the winter season displayed increased water content%. However, it was discovered that *H. muticus* soil at the first depth throughout the summer season had the lowest water content%. According to Moustafa and Zayed [63], the moisture gradient is complex and related to a variety of environmental factors, including climatic drought, soil texture, the character of the soil surface, height and slope.

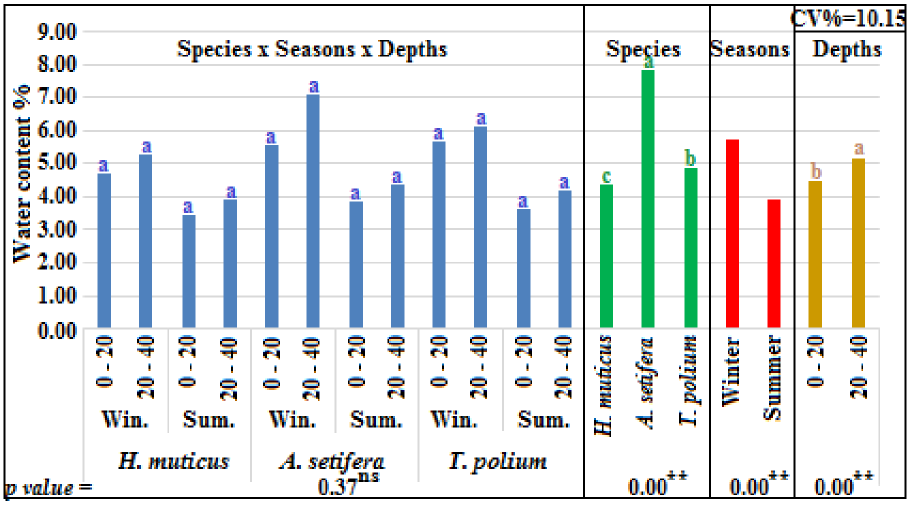

Fig. 3. Water content % of the soil supporting three plant species in two depths across winter and summer seasons at Tayma Governorate, Tabuk Region, Saudi Arabia. Statistically significant differences at $^{**}p \leq 0.01$; ns: indicate the non-significant difference. Different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test.

3.2. Plant analysis

Seasonal variations are the primary factor influencing the chemical composition of plants because they reflect seasonal variations in physiological needs and activities rather than availability in plant content [64,65]. Therefore, rich and diverse metabolic networks that enable the plant to produce a variety of chemicals support various strategies for adaptation [16]. The statistical analysis in Table 3 showed that plant species, seasons, and their interactions with the amounts of all photosynthetic pigments in the three plant species had highly significant effects ($P < 0.01$). Our findings were consistent with those of El-Absy [6], Uvalle Sauceda et al. [66], Devi et al. [67], Khater et al. [68], Malik et al. [69], Neamah and Jdayea [70], in which the findings likewise revealed seasonal variations had significant effects on all photosynthetic pigments in different plant species. The contents of chlorophyll a (Chl.a), chlorophyll b (Chl.b) and Chl.a+b showed a significant increase in *H. muticus* type, followed by *T. polium* and *A. setifera* species. While the contents of Chl.a/b, total carotenoids, and total pigment were significantly greater in *T. polium* than that in the other two plant species investigated. Moreover, all photosynthetic pigment contents displayed a significant increase in the summer season compared to the winter season, except Chl.a/b ratio (>1). In both of the studied seasons, the highest values were noticed for Chl.a, Chl.b and Chl.a+b contents in *H. muticus* plant and for total carotenoids and total pigment contents in *T. polium* plant. On the other hand, the Chl.a/b ratio had highest in *T. polium* and *A. setifera* across the winter season. The Chl.a/b ratios for the three plant species under investigation were higher than 1 because the Chl.a content was higher than the Chl.b content in those species across both seasons. El-Absy and Kamel [13], Salama et al. [34], El-Absy [39] and Huang et al. [71]) all reported similar findings to our study.

Morsy et al. [72] stated that desert plants were able to adapt to changes in light conditions and stressors thanks to the higher amounts of chlorophyll and carotenoids they attained in dry environments. While a decrease in Chl.a might be seen as a protective adaptation strategy that stops increasing photon absorption, based on a study by Ait Said et al. [73]. Also, chlorophyll can demonstrate a plants endurance for high pH environments [74]. The amount of chlorophyll in each leaf area shows how different plant species have adapted to the regional environmental circumstances [75].

Table 3. Photosynthetic pigments contents (g/100g fr. wt.) of the three plant species across winter and summer seasons at Tayma Governorate, Tabuk Region, Saudi Arabia.

Factors	Chlorophyll a (Chl.a)	Chlorophyll b (Chl.b)	Chl.a+b	Chl.a/b	Total Carotenoids	Total Pigment
Plant Species						
H. muticus (H)	2.58±0.05a	2.26±0.06a	4.84±0.11a	1.14±0.01c	408.15±3.37b	412.99±3.40b
A. setifera (A)	1.51±0.04c	1.23±0.01c	2.74±0.05c	1.23±0.02b	239.22±4.47c	241.96±4.50c
T. polium (T)	1.80±0.15b	1.33±0.26b	3.13±0.41b	1.53±0.19a	565.35±28.25a	568.47±28.66a
P–Values	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}
Seasons						
Winter	1.84±0.16b	1.38±0.20b	3.22±0.36b	1.46±0.12a	384.69±37.03b	387.91±36.99b
Summer	2.08±0.18a	1.83±0.17a	3.91±0.35a	1.14±0.01b	423.79±57.55a	427.70±57.74a
P–Values	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}
Plant Species x Season Interaction						
H x Winter	2.48±0.06b	2.14±0.02b	4.62±0.08b	1.16±0.01cd	403.62±1.73d	408.24±1.65d
H x Summer	2.68±0.02a	2.37±0.04a	5.05±0.06a	1.13±0.01ce	412.68±5.77c	417.73±5.71c
A x Winter	1.59±0.03d	1.24±0.02d	2.83±0.04d	1.28±0.01b	248.09±4.62e	250.92±4.58e
A x Summer	1.43±0.01e	1.21±0.01d	2.64±0.01d	1.18±0.01ce	230.35±0.20f	232.99±0.19f
T x Winter	1.46±0.04e	0.75±0.03e	2.21±0.06e	1.95±0.04a	502.35±1.15b	504.56±1.10b
T x Summer	2.13±0.07c	1.91±0.01c	4.04±0.07c	1.12±0.03de	628.34±4.62a	632.38±4.56a
P–Values	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}
C.V.%	3.60	2.86	3.03	2.70	1.70	1.66

Statistically significant differences at$^{**}p \leq 0.01$. Different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test.

Proline is an essential osmolyte produced at a high cost of energy, and it is increased in response to stress conditions [76]. As shown in Fig. 4, the results of the two-way ANOVA analysis revealed very significant effects ($P < 0.01$) of plant species, season, and their interaction on the proline content of the three plant species investigated as part of the region study. These findings agreed with those of Khater et al. [68] and El-Absy and Kamel [77]. The type of *T. polium* had a proline concentration that was significantly higher than that of *H. muticus* and *A. setifera* species. Significantly increased proline content of plants was observed in the summer season compared to the winter season. According to the interaction between the plant species and seasons, *T. polium* in the summer season demonstrated significantly higher proline content compared with other plant species in both seasons. In keeping with these findings, increased proline levels have also been observed under stressful conditions may increase *T. polium* plants tolerance to oxidative stress and aid in scavenging free radicals by Khater et al. [68] and El-Absy and Kamel [77]. Also, Halperin and Flores [78] reported *H. muticus* accumulated proline at a high rate when osmotically stressed. During *A. setifera* type, the proline accumulation and degeneration responded sensitively to climatic changes, as shown by Treichel et al. [79]. In most plant species, the proline can be utilized as a signal of altered physiological conditions including salt and drought stresses [6,13,80,81]. When it comes to osmotic adjustment and photoprotection, proline accumulation and chlorophyll content reduction in plant species appear to play a part, helping the plants resist salinity, hypoxia, and their combinations in their natural habitats [82].

Generally, it can be believed that weather conditions were the root of the notable variations in photosynthetic pigments and proline concentrations in the plant species under study because the variances across seasons were weather-related. Seasonal variation in defenses is frequent in plant species, and seasonal changes effects on plant metabolites have been well-documented [5].

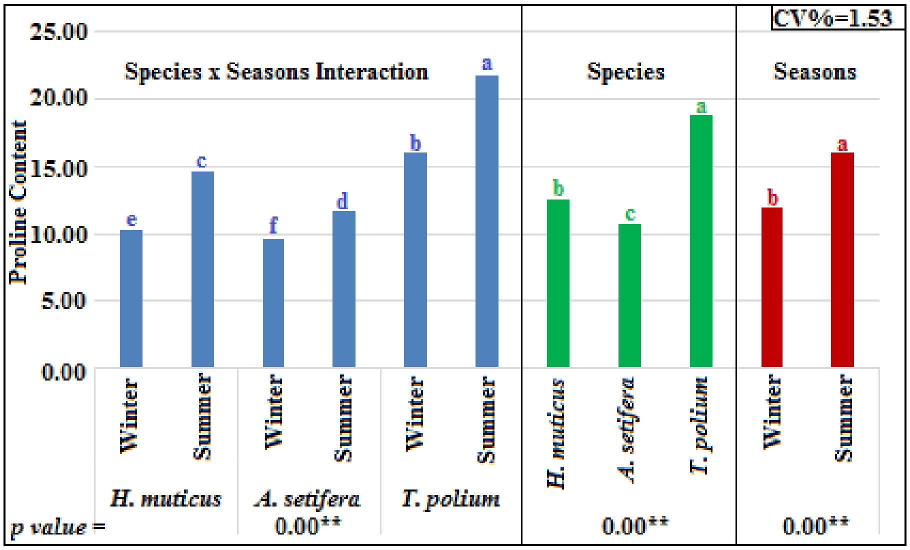

Fig. 4. Proline contents (mg/g) of the three plant species across winter and summer seasons at Tayma Governorate, Tabuk Region, Saudi Arabia. Statistically significant differences at $**p \leq 0.01$. Different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test.

3.3. Principal Component and Biplot Analyses:

For a clear understanding of correlations between soil and plant variables for plant species in both seasons, the principal component (PC) and Biplot analyses were performed (Fig. 5 and 6). The PC1 and PC2 displayed eigenvalues higher than one (>1) and they recorded 62.73% and 37.27% variability of the total variation of all evaluated variables, respectively. Therefore, the two first components express higher variability and support the choice of the variable with a positive loading factor because they better explain variability than a single variable. The PCA results obtained here are in line with El-Absy [6], Ferraz et al. [36], Metwally et al. [37], El-Absy [39] and Kooch et al. [83].

Positive loadings were noticed for K^+, Ca^{++}, carotenoids, total pigment and proline in both PCs, for water content, HCO_3^-, SO_4^{--} and Chl.a/b in PC1, as well as for Na^+, Mg^{++}, Chl.a, Chl.b and Chl.a+b in PC2. Generally, the PC1 with HCO_3^-, SO_4^{--}, and Chl.a/b contents as well as PC2 with Chl.a, Chl.b, and Chl.a+b contents showed the largest positive loadings (Fig. 5). These results harmony with findings by El-Absy [6,39] and Gil et al. [84], also who indicated these soil and plant variables are related to water stress and to salt stress. It is clear that these components represent a response to soil and plant variables evaluated, which has both positive and negative effects on both seasons at the study location. In order to increase the soil and plant variable examined under drought stress circumstances, the PC1 is therefore thought to be crucial. Similar to how PC1 and PC2 described various soil variables in this study, the PCA revealed distinct differences in soil characteristics across the study region [83]. In other studies, the soil variables EC, Ca^{++}, K^+, Na^+, Mg^{++} and Cl^- [85] and the soil properties EC, Na^+ and Cl^- [33] were the most important factors controlling the community structure of plants in the region under study.

The PC-Biplot for the soil and plant variables was made using PC1 and PC2, as shown in Fig. 6. Angles between variable vectors that are less than or equal to 90º (sharp angles) and greater than 90º (obtuse angles) represent a positive and negative correlation, respectively. The Biplot analysis showed strong positive correlations among soil variables [6,36,38,39,86] as well as among plant variables [87,88]. Hendry and Price [89] and Kaspary et al. [90] also reported a positive correlation between total chlorophyll and carotenoid contents which are crucial for assessing a plant's capacity to absorb light in the shade and defending it against stresses through photo-oxidation. Positive correlations were observed between water content% in soil and Chl.a/b, among pH, Mg^{++}, Cl^-, Chl.a, Chl.b, and Chl.a+b contents, as well as among K^+, Ca^{++}, HCO_3^-, SO_4^{--}, Chl.a/b, carotenoids, total pigment, proline contents. Na of soil was highly positively correlated with Chl.a, Chl.b, and Chl.a+b contents, but it had positively correlated with carotenoids and total pigment contents. Our results are as well in agreement with El-Absy [6,39] and Gil et al. [84]. In this regard, Gil et al. [91] found that major soil variables (Ca^{++} and Mg^{++}) and proline content in plants had positive associations using PCA, indicating that proline serves a functional purpose in the stress tolerance systems of the plant species under investigation.

The PC1 had higher soil and plant variables than the PC2. Furthermore, the first two PCs primarily distributed and distinguished these variables with the plant species into four groups. The first quarter (the highest for both PCs) comprised the K^+ and Ca^{++} soil variables as well as carotenoids, total pigment and proline contents, which showed related to *T. polium* type. As for the *H. muticus* type, which is found in the second quarter (the lowest PC1 and the highest PC2), has high amounts of the soil variables Na^+ and Mg^{++} as well as the plant variables Chl.a, Chl.b, and Chl.a+b. On the other hand, *A. setifera* type was linked with the soil variables Ec, pH, and Cl in the third quarter (the lowest PC1 and PC2). The fourth quarter (the highest PC1 and lowest PC2) included the water content %, HCO_3^-, and SO_4^{--} soil variables, which related to the Chl.a/b ratio. El-Absy [39] after using PCA and Biplot analysis, reported a strong positive association between the soil and plant variables suggesting that the chemical properties of the soil have an impact on the plant variables and that elevating the soil variables will elevate the plant variables. According to Salama et al., [92], there were clear negative associations between the soil variables including EC, Na^+, K^+, Ca^{++}, Mg^{++}, and Cl^- and the richness of plant species. Generally, PC1 was positively correlated to soil variables (K^+, Ca^{++}, water content %, HCO_3^-, and SO_4^{--}) and plant variables (Chl.a/b ratio, carotenoids, total pigment and proline contents), which related to the *T. polium* plants. These findings suggest that *T. polium* type may survive and sustain a variety of environmental conditions throughout the growing year thanks to its adaptive behavior and potential for osmotic adjustment.

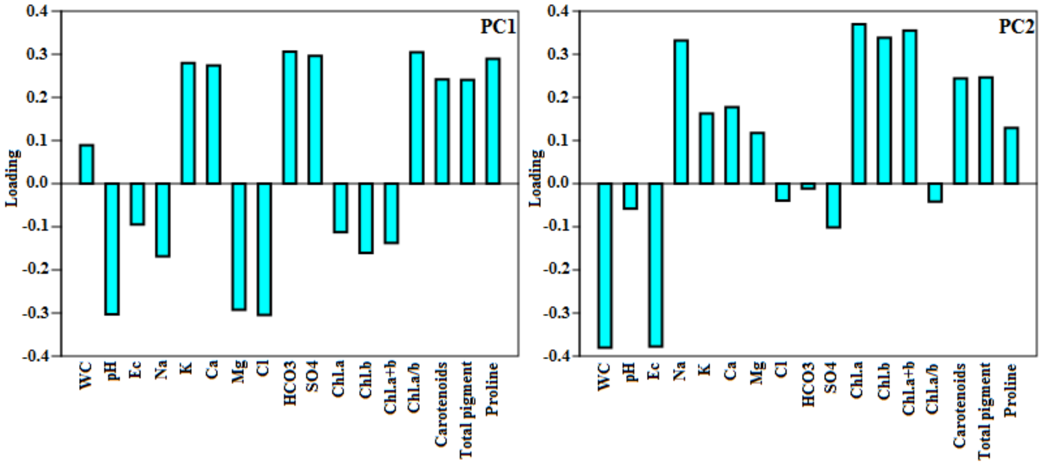

Fig. 5. The loading of the first two components with soil and plant variables of the three plant species. WC: water content %.

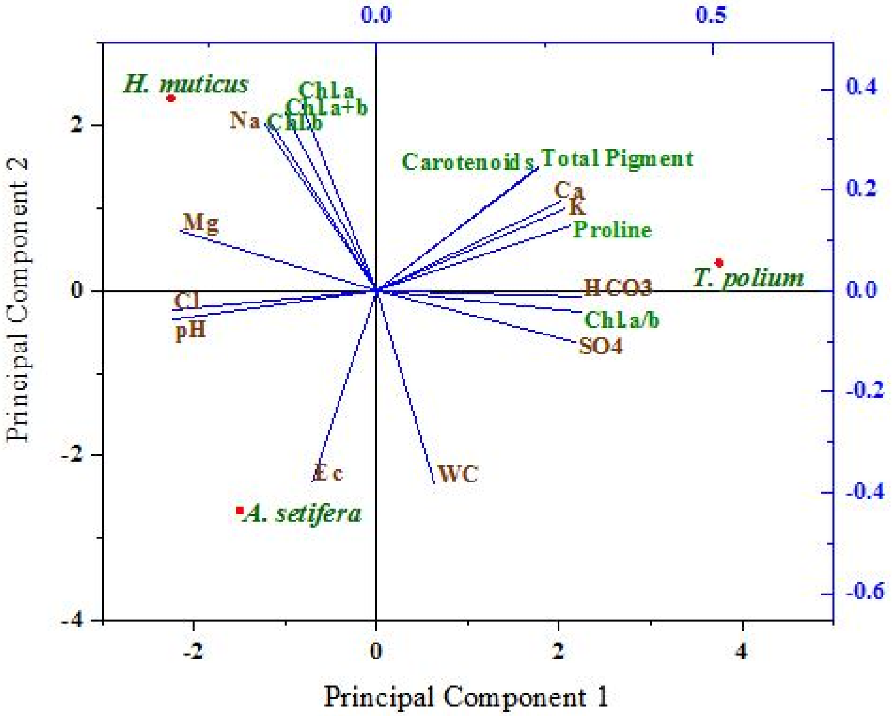

Fig. 6. Biplot diagram between PC1 and PC2 shows the correlation between soil (brown color) and plant (green color) variables with the three plant species (point red color) in both seasons. WC: Water content %.

5. Conclusions

In this study, significant effects ($P < 0.05$ or 0.01) by the plant species, depths and their interaction on most soil physicochemical properties, and by the plant species, seasons and their interaction on photosynthetic pigments and proline contents were observed. Most soil and plant variables examined in both seasons had a high positive association, according to PCA and Biplot analysis. These findings show how the three plant species varied in their responses to seasonal changes in the area under investigation. In comparison to *H. muticus* and *A. setifera* species, *T. polium* type generally adapts better to the arid desert environment and seasonal fluctuations because of its adaptive behavior and potential for osmotic adjustment.

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