AMINO ACID CONTENTS OF SOME LEGUME PLANTS GROWING IN WADI SUDR, SOUTH-WEST SINAI, EGYPT

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Abstract. The identification of soil properties and phytochemical compounds including the amino acid profile is at the base of this study, which investigates the adaptive behavior of three legumes plants *Acacia tortilis* (Forssk.), *Alhagi graecorum* Boiss. and *Retama raetam* (Forsk.) collected from Wadi Sudr, South-West Sinai, Egypt. The ANOVA displayed that most mechanical and chemical properties of the soil associated with the three plant species were significantly affected by plants and depths and their interaction ($p<0.01$). Also, water content was significantly affected by plants, seasons, depths and their interaction ($p<0.01$). As for plant analysis, the plants, seasons and their interaction was significant ($p<0.05$ or $p<0.01$) for most amino acid profiles, photosynthetic pigment contents and chemical compositions of three legumes species in Wadi Sudr region. The amount of most chemical properties was higher in the soil associated with *R. raetam* compared to the soil of the other plants. Most amino acids, photosynthetic pigments, Na^+, K^+ and SO_4^{2-} concentrations of the three studied plants in the dry season were higher than in the wet season. According to PCA, the PCA1 and PCA2 extracted had eigenvalue >1 and mainly distinguished the soil and plant variables in different groups across the three studied plants. According to biplot and based on the plants studied, PCA1 and PCA2 with the highest variability showed positive or negative correlation to soil and plant variables, but, they differed in their degree of significance/insignificance and consistency in quantity. The PCA revealed high positive correlations among some soil variables as well as among some amino acid profile and other plant variables under the plants studied. Some soil variables were positively correlated with some amino acids such as leucine, phenylalanine, valine, lysine, aspartic, arginine, serine and isoleucine as well as some photosynthetic pigments and other plant chemical concentrations.
Keywords: *amino acids, chemical composition, legume plants, PCA*

Introduction

The Sinai Peninsula occupies a portion of the foreland shelf of the Arabo-Nubian massif that dips gradually northward toward the Mediterranean Sea (Said, 1962). South Sinai is characterized by arid climatological conditions and a diversity of plant species. Wadi Sudr region is located in the southern section of the western coast of Sinai, which represents one of the largest and most developed wadis (Morsy et al., 2015; Mohamed et al., 2018). Wadi Sudr is characterized by diverse communities and species as well as the wide areas covered by well-developed plant communities, due to the abundance of extensive water resources, the channel broadness, the fragile nature of the sediments, the variation in the thickness of the surface sediments and the presence of local stone silos (Girgis and Ahmed, 1985; Morsy et al., 2015). Wadi Sudr is exposed to several environmental variables, which have impacted the ecosystem particularly the vegetation (Mohamed et al., 2018).

Climate change is the central issue of our time, with it is posing a major and increasing threat to global food security and affecting every country on every habitable continent (El-Hashash and El-Absy, 2019). Given the physical parameters (temperature, rainfall patterns, carbon dioxide fertilization) and changes in agro-ecosystems as well as the adaptive responses of human systems it is extremely difficult to predict the exact future

effects of climate change on plant productivity (FAO, 2016). Salinity, drought and environmental stresses are major constraints to plant growth worldwide, especially Egypt. Stress conditions induce the accumulation of numerous reactive oxygen species and osmolytes such as proline, soluble proteins, soluble sugars, and betaine, that will play a critical role during stress acclimation in plants (Pradhan et al., 2020; Salama et al., 2021).

The Legumes family (also called Leguminosae or Fabaceae) constitutes the third-largest family of flowering plants in the world after Asteraceae and Orchidaceae families (Pua and Davey, 2007). Legumes plants comprise of more than 19,000 different described species (Bennetau-Pelissero, 2019). Some legume species are annual herbs, some are vines, and some are bushes and trees distributed around the world in many different environments. In this study, three species of the legumes family (*Acacia tortilis* Forssk, *Alhagi graecorum* Bioss and *Retama raetam* Forssk) growing in Wadi Sudr region, Southwest Sinai, Egypt were chosen.

Acacia tortilis (Forssk.) Hayne ssp. raddiana (*A. tortilis*) plants are distinguished for growing in the desert due to their ability to tolerate the drought, salinity and alkalinity, drifting sand, grazing and repeated cutting as well as climatic changes in temperature (Derbel et al., 2007; Malakootian et al., 2018). It is native to arid and semi-arid areas of Africa and the Middle-East (Orwa et al., 2009). It is grown in the Sinai region, Egypt and *A. tortilis* seeds are used for animal fodder (Embaby and Rayan, 2016). It has also been used commercially and medicinally as it is considered beneficial for treating many different diseases such as skin allergy, cough and inflammatory reaction (Yadav et al., 2013). *A. tortilis* eventually has proved to be the most promising species for desert rehabilitation and greening (Gill and Al-Shankiti, 2015).

Alhagi graecorum Boiss. (*A. graecorum*) is grown naturally in xeric, halic and mesic habitats (Hassanein and Mazen, 2001) as well as found in deep moist, dry, rocky, or saline soils, and occasionally in cultivated fields (Boulos, 2009). It is native to North Africa, the Middle East, southeastern Europe and Russia (Awmack and Lock, 2002). It is widely distributed in Egypt and seems to have wide ecological amplitude, it has been recorded in various bioclimatic regions like Nile region, oasis, Mediterranean region, Eastern and Western Deserts, Red Sea coast and Sinai (Boulos, 2009). It is palatable and grazed by camels and camels in the deserts (Boulos, 2009), also the dried plants are used to treat bilharziasis, rheumatic pains, constipation, and worms (Boulos and El-Hadidi, 1984).

Retama raetam (Forsk.) Webb & Berthel (*R. raetam*) is usually found in the Egyptian Sinai Peninsula, Tunisia, Libya, Saudi Arabia and Northeastern Mediterranean region (Nasser et al., 2013). *R. raetam* used as a major source of fodder for livestock species such as sheep, goats and camels as well as in traditional medicine for the treatment of hypertension, diabetes, rheumatism, fever, inflammation, eczema, and microbial infections (Al-Onazi et al., 2021).

The growth of legumes plants and nitrogen fixation are affected by chemical compounds in the soil. Soil pH also affects the physical, chemical and biological properties of the soil as well as plant growth (Al-Mujahidy et al., 2013). Chemical properties in soil have been correlated with legumes plants, therefore, the details of soil property can be easily interpreted and allow rapid improvement of chemical properties in the soil during nitrogen fixation and root biomass (Yuvaraj et al., 2020).

In order for a plant to complete its life cycle, a plant needs relatively large amounts (>0.1% of dry mass) of essential macronutrients such as Ca^{2+}, Mg^{2+}, N, P, K^+ and S (Maathuis et al., 2009). Pyankov et al. (2001) stated that the concentrations of main chemical components as nitrogen, organic acids, and mineral substances recorded lower

values in stress-tolerant and higher values in ruderal species. Kamel and El-Absy (2020) explained that most of the chemical compounds in the plant were significantly affected by seasonal changes and different locations. This may reflect seasonal changes in physiological needs and effort, rather than availability in plant content (Estevez et al., 2010).

Proteins are a large set of organic molecules that are essential to the structure and functioning of cells in all living things (Aremu et al., 2017). Amino acids are the building blocks of proteins and are categorized into essential and non-essential amino acids based on their building in humans. Essential amino acids are synthesized only by plant species, whilst non-essential amino acids are synthesized by both plant species and people (Kumar et al., 2019). Plants are a rich source of amino acids, and glutamic and aspartic acids are the most abundant amino acids in plants (Kumar et al., 2017).

It was found that the interrelationships between different plant communities and environmental factors are very complex, which reflects simultaneous changes in factors like depth of groundwater, soil moisture and stability as well as salinity content (Zhang et al., 2005). The principal component analysis (PCA) method was extensively used to study the relationship between soil properties and vegetation have been conducted by several researchers for example Juhos et al. (2015); Ferraz et al. (2019) and Metwally et al. (2019). Also, the PCA has also been used to identify the differences between amino acids in different plant species (Zhu et al., 2017; Kaur et al., 2018; Kumar et al., 2019).

The aim of the current work is to study the adaptive behavior of some wild plants of the legumes family collected from Wadi Sudr through 1) determining and analysing the content of soil properties and phytochemical compounds including the amino acid profile 2) assessing the correlation between the environmental factors and plant properties using principal component analysis under dry and wet seasons.

Materials and methods

Study site

This study was carried out at Wadi Sudr in Ras Sudr, South Sinai, Egypt between August 2019 (dry) and January 2020 (wet). It was located at latitudes: 29°36′54″-29°51′54″ N, longitudes: 32°41′30″-33°09′ 07″ E (*Fig. 1*). From the North, Wadi Sudr borders Gebel El Raha (about 600 m), and from the South, Sinn Bishr (about 618 m). The Wadi Sudr originates from the hill slope of the EL-Tih plateau. The main trunk of the Wadi Sudr extends roughly in a northeast-southwest direction for about 55 km and flows into the Gulf of Suez at Ras Sudr town (about 55 km south of the El Shatt) (Girgis and Ahmed, 1985; Mohamed et al., 2018).

Collection and preparation of plant material

Three plants were used in this study, namely, *A. tortilis*, *A. graecorum* and *R. raetam* (*Fig. 2*). Three samples from the aerial parts in each species were collected at random from Wadi Sudr during the dry and wet seasons of 2019 and 2020, respectively. Drying of collected plant materials was done in the oven at 70°C to a constant weight after which dried samples were milled to a fine powder and stored in brown bags at room temperature until chemical analyses.
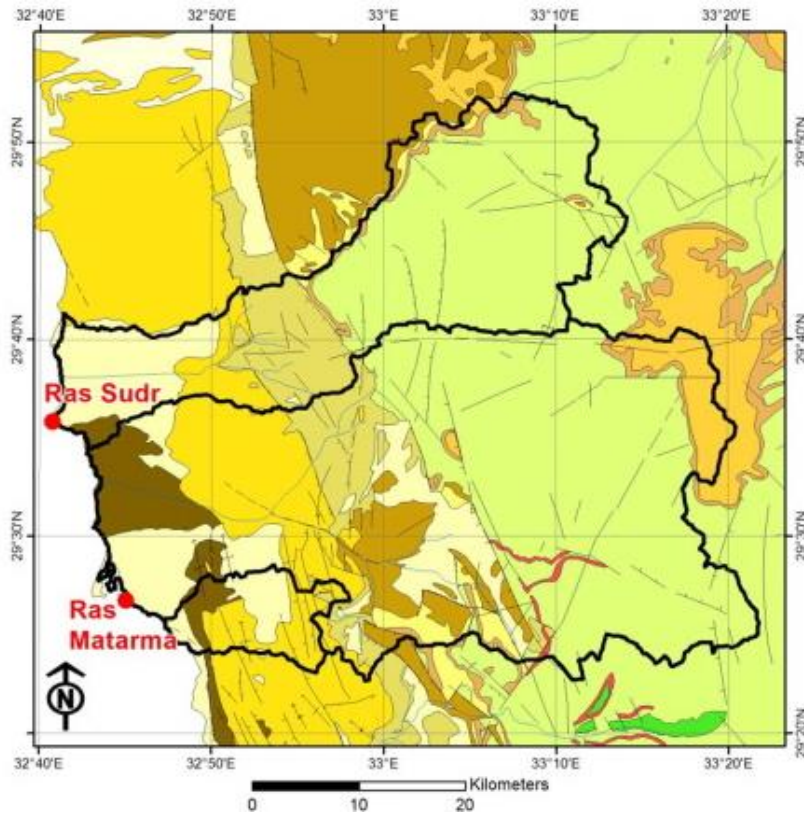
Figure 1. *Location map of Wadi Sudr, South-West Sinai, Egypt (Gabr and El Bastawesy, 2015)*
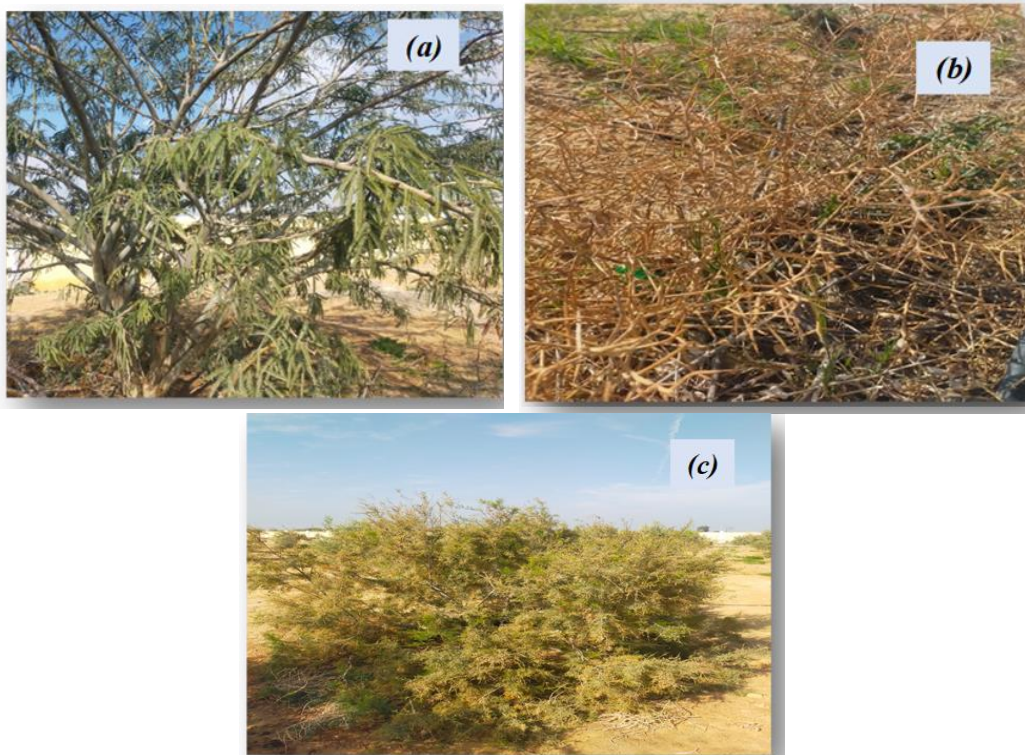

Figure 2. *The species studied from Wadi Sudr, (a) A. tortilis, (b) A. graecorum and (c) R. raetam*

Soil analysis

Soil samples were collected from the soil associated with the three studied plants carefully made from three random points at two depths (0-20 cm and 20-40 cm) at the Wadi Sudr. Three replicates were taken from each sample and carried to the laboratory in closed tins to be used for soil analyses. Soil samples were air-dried, sieved and used for mechanical analysis of soil particles as suggested by Jackson (1967) and Rowell (1994) for soil texture. The soil moisture content and sulphate concentration were calculated according to the method described by Rowell (1994). Electrical conductivity (EC) and pH value for each sample were carried out using soil-water paste, according to Jackson (1962), EC was expressed as mohms/cm. The mineral contents of soil including Cl^-, Ca^{2+}, Mg^{2+}, Na^+ and K^+ were determined using a saturation paste that described by Tuzuner (1990).

Plant analysis

The concentrations of Sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}), magnesium (Mg^{2+}), and sulphate (SO_4^{2-}) were determined by atomic absorption spectrophotometry (GBC Avanta E, Victoria, Australia) (Chapman, 1965). Total nitrogen (N) content was determined using the micro-Kjeldahl method (Bremner, 1965). Photosynthetic pigment parameters were quantified spectrophotometrically, using the wavelengths of 663, 645 and 470 nm, chlorophyll a, chlorophyll b and total carotenoids were calculated by equations of Lichtenthaler (1987), respectively. Crude protein % was determined by multiplying the total nitrogen by 6.25 according to Allen (1989). The plant water content was obtained following the equation described by Jin et al. (2017). All amino acid contents were analyzed at the Central Laboratories, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, using the Clait Amino Acid Analyzer SW (Pellet and Young, 1980)

Statistical analysis

Data values are expressed as Mean±standard deviation (SD). Analysis of Variance (two-way ANOVA) was performed to determine the effect of plant species (P), season (S) and P × S interaction using SPSS software package (version 20). The test of significance of the means was determined by the Least Significance Difference (LSD) when the ANOVA suggested a significant difference at $P \leq 0.05$ and $P \leq 0.01$. The Principal Components Analysis (PCA) was used to correlate the plant analysis with the soil variables studied using the computer program STATGRAPHICS Centurion 19.

Results and discussions

Soil analysis

The texture is one of the most important physical properties of soil. *Table 1* lists the means and ANOVA of mechanical properties for the soil adjoined of three species from the two depths across Wadi Sudr region. All mechanical properties (%) were highly significant (p<0.01) affected by the three plant species, the two depths and their interaction, except the two studied factors for very coarse sand had insignificant difference. Similarly, many authors, for example Mohammed et al. (2016), Abdedaiem et al. (2020) and Kamel and El-Absy (2020) have also reported that soil mechanical properties were significantly different between plants studied under different habitat

conditions. In contrast to these results, Desta et al. (2018) mentioned that soil physical properties measured were not statistically different ($p < 0.05$) under *A. tortilis*. The highest percentage of fine sand was found in the root-associated soil of the three plant species in two depths, followed by medium sand and coarse sand. The fine sand % was significantly higher in the soil associated of *A. tortilis* than other species at the two depths, also in the 20-40 depth than in the 0-20 depth across the three plant species. Thus, the adjoining soil of the studied plants and collected from 0-20 and 20-40 depths in the Wadi Sudr area are sandy in texture. This result is in line with earlier studies reported by El-Lamey (2020). Lal (2012) reported that, the leguminous plants enhance the soil physical properties by being a soil conditioner and enhancing physical residences.

*Table 1. Mechanical properties of the adjoining soil samples of the studied plants from different depths at Wadi Sudr*

Plants (P)	Depths (D)	Very Coarse Sand	Coarse Sand	Medium Sand	Fine Sand	Very Fine Sand	Clay and Silt	Soil Texture Class
R. raetam	0 – 20	0.78±0.01	9.33±0.01	26.41±0.18	58.38±0.23	2.64±0.02	2.46±0.13	Sandy
	20 – 40	1.32±0.00	3.96±0.08	5.46±0.00	86.7±0.31	1.70±0.09	0.86±0.01	Sandy
A. graecorum	0 – 20	0.73±0.03	6.21±0.11	12.35±0.04	76.13±0.28	3.85±0.03	0.73±0.11	Sandy
	20 – 40	1.61±0.06	2.92±0.09	5.04±0.09	86.81±0.15	2.82±0.04	0.8±0.09	Sandy
A. tortilis	0 – 20	2.51±0.03	6.68±0.10	4.38±0.11	84.02±0.21	1.46±0.07	0.95±0.12	Sandy
	20 – 40	0.73±0.01	5.34±0.07	2.11±0.08	90.17±0.19	0.92±0.10	0.73±0.08	Sandy
LSD for	P	NS	**	**	**	**	**	
	D	NS	**	**	**	**	**	
	P x D	**	**	**	**	**	**	

The results represent the values of mean ± standard deviation. Statistically significant differences at *$p < 0.05$ and **$p < 0.01$

The water content % showed significant differences ($P < 0.01$) between the plant species, seasons and depths as well as first and second-order interactions (*Fig. 3*). Likewise, significant differences of water content % in soil associated with the plants have been reported by Desta et al. (2018), El-Lamey (2020) and Liu et al. (2020). Higher values of soil water content were recorded in the case of *R. raetam* at the two seasons and depths than other plant species. Also, significant increases were found in the wet season and at 20-40 depth than in others season and depth for the soil water content of the three studied plants, due to rainfall, which leads to normal plant growth under Wadi Sudr drought conditions. The results of this study were in accordance with those of Singh (2004), Do et al. (2008) and El-Lamey (2020) who also observed higher value of water content % in the wet season than in the dry season.

The means and ANOVA of soil chemical properties associated with different plants at 0-20 and 20-40 depths were summarized in *Table 2*. According to the two-way ANOVA, all soil chemical analyses supporting the three plant species exhibited highly significant differences ($P < 0.01$) between the species, the depths and their interaction, except pH (between the studied factors) and SO_4^{2-} (interaction) which showed no significant differences. This is consistent with the previous studies by Desta et al. (2018) in *A. tortilis*, El-Lamey (2020) in *R. raetam* and Salama et al. (2021) in *A. graecorum*, Chemical properties of soil, such as electrical conductivity (EC), Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2-} and Cl^-

were significantly higher across the two depths in *R. raetam* than in the two plants *A. graecorum* and *A. tortilis*. While, the values of K^+ have increased significantly during the two depths in *A. tortilis* compared with their values in *R. raetam* and *A. graecorum*. In soil of plant species, the maximum amount of Na^+, K^+, Mg^{2+}, SO_4^{2-} and Cl^- were found in 0-20 depth and less in 20-40 depth, whilst Ec and Ca^{2+} were higher in the 20-40 depth than in the 0-20 depth. Soil pH associated with the three studied plants in Wadi Sudr tended to be somewhat alkaline, and there was no significant difference between the plant species and depths. The result of pH corroborates the result obtained by Salama et al. (2021), who also mentioned that alkalinity may be due to the increase in total soluble salts in soil of *A. graecorum*. Comole et al. (2021) mentioned that plants can adapt and thrive in locations with different soil properties.
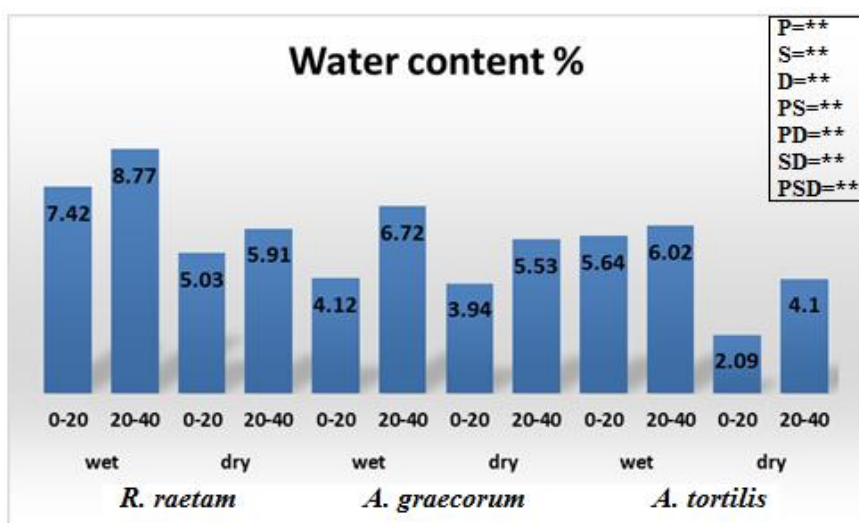
Figure 3. Water content % at the soil associated with three plant species at two depths and the two seasons during Wadi Sudr. According to three-way ANOVA test followed by LSD, water content % between the species (P), seasons (S), depths(D) and their interactions are significantly different (P < 0.01)

Table 2. Soil chemical properties associated with the studied plants from different depths at Wadi Sudr

Plants (P)	Depths (D)	Soil Chemical Properties							
		Ec	pH	Na^+	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Cl^-
R. raetam	0 – 20	27.87±0.09	7.41±0.09	40.40±0.14	10.50±0.07	19.80±0.08	49.85±0.08	11.72±0.13	37.70±0.12
	20 – 40	125.80±0.12	7.74±0.07	4.78±0.09	2.10±0.02	148.02±0.13	10.80±0.02	3.70±0.00	9.23±0.10
A. graecorum	0 – 20	21.97±0.10	7.09±0.01	21.32±0.12	11.20±0.08	9.49±0.03	31.71±0.10	8.57±0.07	12.90±0.08
	20 – 40	80.07±0.07	7.30±0.04	2.10±0.08	4.52±0.01	16.53±0.09	8.90±0.04	2.40±0.08	4.14±0.07
A. tortilis	0 – 20	17.77±0.02	7.27±0.08	10.49±0.11	20.20±0.09	5.94±0.09	15.11±0.05	7.43±0.11	7.20±0.05
	20 – 40	39.87±0.13	7.59±0.02	1.12±0.01	8.33±0.11	11.10±0.07	7.40±0.01	2.10±0.08	1.24±0.01
LSD for	P	**	NS	**	**	**	**	**	**
	D	**	NS	**	**	**	**	**	**
	P x D	**	NS	**	**	**	**	NS	**

The results represent the values of mean ± standard deviation. EC: Electrical Conductivity; Na^+: Sodium; K^+: Potassium; Ca^{2+}: Calcium; Mg^{2+}: Magnesium; SO_4^{2-}: Sulfate; Cl-: Chloride. Statistically significant differences at *p < 0.05 and **p < 0.01

Plant analysis

Photosynthetic pigment contents

Table 3 shows the photosynthetic pigment contents in the three plants under wet and dry seasons at Wadi Sudr area. Statistically significant differences ($p<0.01$) in chlorophyll a (Chl a), chlorophyll b (Chl b), total carotenoids, Chl a+b and total pigment were noticed among the three plants, seasons and their interaction, using two-way ANOVA. Chl a/b between the two seasons was significantly different ($p<0.01$), however, was not found to be statistically significant among the three plant species and plants x seasons interaction. These results similar to those described by Kebbas et al. (2015) in *A. tortilis*, Nasir Khan et al. (2016) in *R. raetam* and Salama et al. (2021) in *A. graecorum*.

Table 3. *Photosynthetic pigment contents of three legumes species during wet and dry seasons at Wadi Sudr*

Plants (P)	Seasons (S)	Chlorophyll a (Chl a)	Chlorophyll b (Chl b)	Total Carotenoids	Chl a+b	Chl a/b	Total pigment
R. raetam	Wet	15.54±0.28	9.63±0.08	159.79±0.38	25.17±0.09	1.63±0.01	184.96±0.31
	Dry	21.95±0.15	14.46±0.07	273.01±0.53	36.41±0.05	1.53±0.05	309.42±0.25
A. graecorum	Wet	5.27±0.09	3.36±0.11	895.31±0.62	8.63±0.12	1.73±0.12	903.94±0.19
	Dry	21.01±0.21	15.28±0.00	516.76±0.41	36.29±0.00	1.38±0.10	553.05±0.41
A. tortilis	Wet	16.79±0.09	9.74±0.03	649.73±0.75	26.53±0.07	1.74±0.09	676.26±0.22
	Dry	19.65±0.13	31.25±0.10	119.87±0.21	50.9±0.09	0.63±0.00	170.77±0.57
LSD for	P	**	**	**	**	NS	**
	S	**	**	**	**	*	**
	P x S	**	**	**	**	NS	**

The results represent the values of mean ± standard deviation. Statistically significant differences at $*p < 0.05$ and $**p < 0.01$

During the dry season, the maximum values were found for Chl a in *R. raetam* and for Chl b and Chl a+b in *A. tortilis*. As for the wet season, total carotenoids and total pigment in *A. graecorum* as well as Chl a/b in *A. tortilis* recorded the highest values. Generally, the concentrations of Chl a, Chl b and Chl a+b in the three plants as well as total carotenoids and total pigment in *R. raetam* were higher values in the dry season than in the wet season. While the maximum values of Chl a/b in the studied plants as well as total carotenoids and total pigment at *A. graecorum* and *A. tortilis* were registered in the wet season compared with their values in the dry season. Salama et al. (2021) reported the highest concentration of chlorophyll a and b in the summer season while their ratio in *A. graecorum* in the winter season were found to modify enabling the plants to adapt to light condition changes. Furthermore, for *A. tortilis*, Singh and Khajuria (1991) and Kebbas et al. (2015) found higher contents of total chlorophyll and carotenoids in the stressed plants, however, the Chl a/b ratio was not affected by the drought. Ait Said et al. (2013) hypothesized that a decrease in Chl a can be considered as a protective adaptive mechanism that prevents increased photon absorption. The Chl a/b ratio was higher due to higher Chl a content than Chl b content (Huang et al., 2021).

Mineral contents

Mineral contents of the plant species at the wet and dry seasons in Wadi Sudr area are presented in *Table 4*. Statistically significant differences ($p<0.01$) were noted in studied chemical compositions (Na^+, K^+, Ca^{2+}, Mg^{2+} and SO_4^{2-}) among the different studied plants, seasons, and their interactions. As for nitrogen content (N), significant differences showed among the three studied plants ($p<0.01$) and seasons ($p<0.05$), but insignificant for plants x seasons interaction. These outcomes are generally consistent with El-Lamey (2020), Al-Onazi et al. (2021) and Salama et al. (2021).

Table 4. *Chemical compositions of three legumes species during the wet and dry seasons at Wadi Sudr*

Plants (P)	Seasons (S)	Na^+	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	N
R. raetam	Wet	40.11±0.35	99.41±0.21	135.00±0.37	100.21±0.42	1.91±0.18	3.94±0.19
	Dry	54.31±0.22	150.00±0.64	133.10±0.83	94.50±0.34	2.12±0.21	3.84±0.20
A. graecorum	Wet	39.14±0.34	61.10±0.71	195.00±0.62	129.90±0.81	2.11±0.15	5.81±0.16
	Dry	47.21±0.43	62.50±0.62	82.21±0.41	124.50±0.51	3.41±0.12	3.61±0.15
A. tortilis	Wet	9.42±0.19	40.21±0.51	75.00±0.47	37.90±0.24	0.91±0.09	2.88±0.09
	Dry	10.94±0.25	82.51±0.34	69.20±0.29	35.50±0.31	1.49±0.20	1.99±0.08
LSD for	P	**	**	**	**	**	**
	S	**	**	**	**	**	*
	P x S	**	**	**	**	**	NS

The results represent the values of mean ± standard deviation. Na^+: Sodium; K^+: Potassium; Ca^{2+}: Calcium; Mg^{2+}: Magnesium; SO_4^{2-}: Sulfate; N: Nitrogen, Statistically significant differences at *$p < 0.05$ and **$p < 0.01$

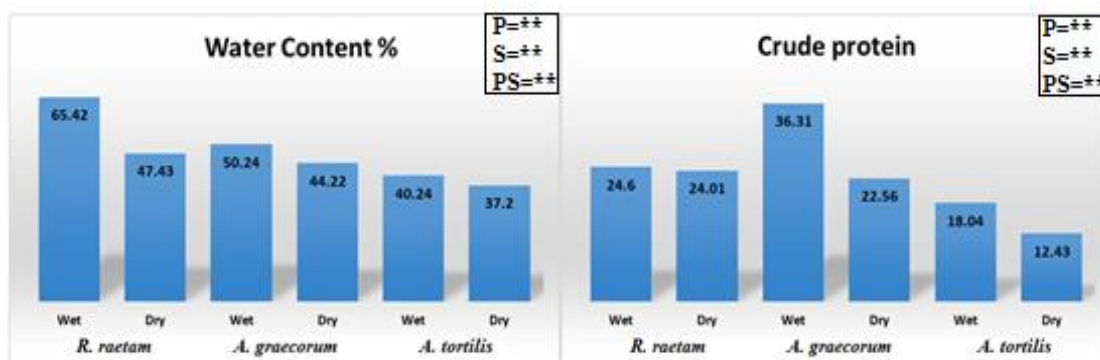During the three plant species, the dry season had higher values of Na^+, K^+ and SO_4^{2-} than those reported in the wet season. On the other hand, the values of Ca^{2+}, Mg^{2+} and N were higher in the wet season than in the dry season in the studied plants. Na^+ and K^+ contents at the two seasons recorded the highest values of *R. raetam*, followed by *A. graecorum* and *A. tortilis*. Further, the maximum Mg^{2+}, SO_4^{2-} and N contents were observed in *A. graecorum*, followed by *R. raetam* and *A. tortilis* during the two seasons. A higher Ca^{2+} content was found in *A. graecorum* at the wet season as compared to other species in the seasons, but lower in *A. tortilis* at the dry season. The anions and cations accumulation in *A. graecorum* were significantly increased during winter season (Salama et al., 2021), while total K^+ in *A. graecorum* (Salama et al., 2021) as well as potassium, Sodium, calcium and magnesium contents in *R. raetam* at Wadi Sudr (El-Lamey, 2020) showed the opposite response at the summer season. Rubanza et al. (2007) reported that *A. tortilis* had moderate to high levels of mineral contents.

Osuagwu et al. (2012) reported that plant species differ in content of the minerals as well as the reactions under adverse conditions in the same region. Na^+ is often stored in the vacuoles resulting in increased osmotic pressure (Wyn Jones, 1981) and avoid Na^+ toxicity (Salama et al., 2021), K^+ ions are essential for reducing the uptake of Na^+. Concentrations of Na^+ and K^+ as well as ion balance play important roles in plant salt tolerance (Zheng et al., 2015). To drought tolerance mechanisms, accumulated K^+ in *A. graecorum* may be employed during the dry seasons (Prajapati and Modi, 2012). Salama et al. (2015) stated that the absorption and removal of inorganic osmoregulatory

ions like K^+, Na^+, Ca^{+2} and Mg^{+2} are useful means of osmotic gradient re-adjustment in stressed plants. An increased concentration of Ca^{2+} and Mg^{2+} without reaching toxic levels counteracts the inhibitory effect of Na^+ and may contribute to its physiological salt tolerance mechanisms (Gul and Khan, 2006; Grigore et al., 2012).

Water content % and crude protein

Based on two-way ANOVA, statistically significant differences ($P < 0.01$) determined with respect to water content % and crude protein between the plants, seasons and their interaction (*Fig. 4*). Similar results were recorded in the study of Mabeza et al. (2014), Al-Qahtani et al. (2020), El-Lamey (2020) and Kamel and El-Absy (2020). Water content % and crude protein were significantly higher at the wet season than at the dry season in the three plant species. *R. raetam* recorded the greater value of water content % at the wet season, followed by *A. graecorum* and *A. tortilis*. Crude protein at the wet season had the highest value in *A. graecorum*, followed by *R. raetam* and *A. tortilis*. A similar result in *R. raetam* at Wadi Sudr was reported by El-Lamey (2020). As for *A. tortilis* it is well adapted to dry environments (Kebabs et al., 2015).

Figure 4. Water content % and crude protein of three plant species from Wadi Sudr during the wet and dry seasons. According to two-way ANOVA test followed by LSD, the parameters between the species, seasons and their interaction are significantly different (P < 0.01)

Amino acid composition

Table 5 outlines a detailed amino acid profile performed on three legumes plant species under the wet and dry seasons in Wadi Sudr area. Two-way ANOVA exhibited statistically significant differences between the three studied plants for all amino acids except glutamic acid, histidine, glycine and threonine. While, serine, glycine, arginine, alanine, phenylalanine, tryptophan and proline were statistically significant differences between seasons. As for plants x seasons interaction, the observed differences were not statistically significant for all amino acids, except serine and tyrosine. Similar results were obtained in the investigations conducted by Grela et al. (2017), El-Lamey (2020) and Salama et al. (2021).

The highest concentrations of aspartic acid, proline, leucine and arginine, and the lowest concentrations of cysteine and methionine were recorded in the three studied plants under the wet and dry seasons. Based on data of the two season, all amino acid concentrations of the three studied plants were higher in the dry season than in the wet season, except for isoleucine and lysine in *R. raetam*, cysteine in *A. graecorum* and

histidine in *A. tortilis*. The concentrations of aspartic acid, arginine, valine, phenylalanine, leucine and lysine were higher in *R. raetam* than in other plants. On the other hand, the maximum values of serine, glycine, tyrosine, cysteine and isoleucine were registered in *A. graecorum*. As for the other amino acids, they were greater in *A. tortilis* than in *R. raetam* and *A. graecorum* plants. The highest accumulation of proline had in *A. tortilis*, followed by *R. raetam* and *A. graecorum* during the wet and dry seasons at Wadi Sudr region. The levels of some amino acids were significantly reduced in *R. raetam* during summer season at Wadi Sudr (El-Lamey, 2020), while total free amino acids were increased in *A. graecorum* during winter season (Salama et al., 2021). As for *A. tortilis*, lower levels of isoleucine, lysine, and threonine were found (Embaby and Rayan, 2016). Osmotic modification may be accompanied by protein accumulation to improve drought tolerance of plant species (Salama et al., 2021). Also, the amino acids can play the same role during some mechanisms: acting as compatible osmolytes, regulating pH, acting as nitrogen or carbon reservoir (Ali et al., 2020), precursors for energy-associated metabolites, ROS scavengers, and potential regulatory and signaling molecules (Hildebrandt et al., 2015). Therefore, the synthesis of protein types rich in certain amino acids could be the key to survival for the species (Kasim et al., 2008). Proline accumulates under stress conditions and have more intimate association with survival adaptability of plants, considered to act as a compatible osmolyte (Verslues and Sharma, 2010) and stimulate root elongation at low water potentials (Yamada et al., 2005). Leucine, isoleucine and valine also play an important role in plant drought tolerance as an alternative source of respiratory substrates (Pires et al., 2016).

Table 5. *The amino acids profile (mg/g) of three different species from Wadi Sudr during wet and dry seasons*

Plants (P)	*R. raetam*		*A. graecorum*		*A. tortilis*		LSD for		
Seasons (S) Amino acids	Wet	Dry	Wet	Dry	Wet	Dry	P	S	PxS
Aspartic acid	16.33±0.01	17.53±0.09	13.66±0.10	14.35±0.08	12.23±0.11	12.91±0.10	**	NS	NS
Glutamic acid	5.94±0.06	6.81±0.10	6.86±0.08	8.01±0.11	7.72±0.00	7.04±0.09	NS	NS	NS
Serine	2.20±0.03	3.31±0.07	2.43±0.05	3.45±0.02	2.44±0.09	1.98±0.03	*	*	*
Histidine	1.44±0.05	1.32±0.08	1.22±0.09	1.13±0.07	1.62±0.08	1.54±0.01	NS	NS	NS
Glycine	3.26±0.00	4.32±0.03	3.85±0.05	4.98±0.06	3.88±0.10	4.65±0.07	NS	**	NS
Threonine	2.11±0.05	3.32±0.06	2.50±0.01	2.98±0.00	2.97±0.11	3.09±0.04	NS	NS	NS
Arginine	10.09±0.04	9.16±0.07	8.77±0.09	8.24±0.04	7.88±0.04	6.97±0.01	**	*	NS
Alanine	2.88±0.06	3.15±0.07	3.10±0.03	3.65±0.10	4.65±06	4.89±0.04	**	*	NS
Tyrosine	3.66±0.00	2.97±0.03	4.19±0.05	5.31±0.07	3.11±0.03	3.67±0.07	**	NS	*
Cysteine	0.52±0.06	0.50±0.08	0.70±0.09	0.670.01	0.56±0.07	0.54±0.02	*	NS	NS
Valine	6.43±0.03	6.23±0.04	4.26±0.04	3.96±0.00	3.33±0.04	3.84±0.07	**	NS	NS
Methionine	0.40±0.01	0.53±0.07	0.61±0.06	0.690.02	0.80±0.01	0.89±0.01	**	NS	NS
Phenylalanine	5.54±0.04	6.32±0.05	4.12±0.11	4.89±0.01	4.92±0.00	5.61±0.05	**	*	NS
Isoleucine	7.90±0.08	7.65±0.00	8.44±0.08	8.06±0.08	7.12±0.04	6.98±0.08	**	NS	NS
Leucine	9.44±0.07	9.89±0.04	7.10±0.06	7.27±0.10	8.70±0.08	8.98±0.06	**	NS	NS
Lysine	4.60±0.05	4.52±0.11	4.12±0.09	4.01±0.05	3.66±0.10	3.99±0.03	*	NS	NS
Tryptophan	1.230.00	1.65±0.01	1.88±0.00	2.08±0.11	2.44±0.00	2.97±0.01	**	*	NS
Proline	13.70±0.08	14.87±0.10	10.76±0.07	11.64±0.07	16.40±0.11	17.82±0.09	**	*	NS

The results represent the values of mean ± standard deviation. Statistically significant differences at *$p < 0.05$ and **$p < 0.01$

Principal component analysis

Principal component analysis (PCA) is a multivariate statistical technique. The PCA has been used to estimate the similarities and dissimilarities among the soil and plant chemical variables in the three studied plant species. The results are graphically displayed in a biplot of the first two PCAs (PCA1 and PCA2). Out of the PCAs, the PCA1 and PCA2 extracted had eigenvalues larger than one (Eigenvalue >1) (*Fig. 5*). While the rest PCAs had eigenvalues less than one (Eigenvalue < 1). Therefore, the PCA1 was kept for the final analysis, in which, the PCA1 and PCA2 explains variance more than an individual attribute (Sharma, 1996) and it expresses more variability and support to select the variable with a positive loading factor. The contributions of PCA1 to the total variance were higher than that of the other components, with PCA1 describing only about <60% of the measured data total variability in the original variables at the three studied plants. This result indicates that the first two PCAs may be used to summarize the original variables in any further analysis of the data, as well as to explain the total variation and the grouping of the PCAs. The PCA1 and PCA2 had mainly distinguished the soil and plant analysis across the three studied species in different groups. Thus, the PCA1 and PCA2 were employed to draw a biplot, and to explain relationships of soil and plant analysis across the plant species studied. The eigenvalue of the first four PCAs (Kumar et al., 2019; Liu et al., 2020) and the two first PCA components (Gil et al., 2014; Abdedaiem et al., 2020) are greater than 1, and they also explained the largest part of the total variance in soil properties, amino acids and environmental conditions at different plant species.
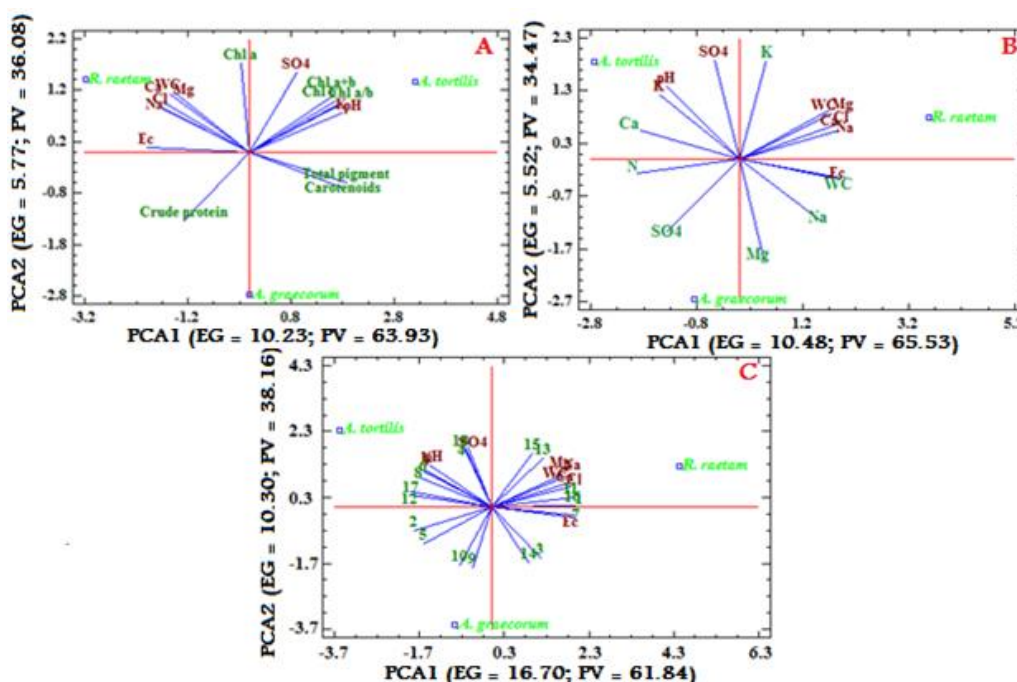
Figure 5. Biplot diagram based on first two PCs axes of soil chemical variables (brown points) with (A) Photosynthetic pigments and crude protein, (B) Mineral compositions and water content as well as with (C) amino acids contents in plants studied (green points). Symbols: EG: Eigenvalue; EV: Explained variance; EC: Electrical Conductivity; Cl-: Chloride; Na⁺: Sodium; K⁺: Potassium; Ca²⁺: Calcium; Mg²⁺: Magnesium; SO₄²⁻: Sulfate; WC: Water content; 1: Aspartic; 2: Glutamic; 3: Serine; 4: Histidine; 5: Glycine; 6: Threonine; 7: Arginine; 8: Alanine; 9: Tyrosine; 10: Cysteine; 11: Valine; 12: Methionine; 13: Phenylalanine; 14: Isoleucine; 15: Leucine; 16: Lysine; 17: Tryptophan; 18: Proline

During the three plant species, the soil variables were divided into two groups. The first group consisted of pH, K^+ and SO_4^{2-}. While, the second group comprised of other soil variables (*Fig. 5*). The soil variables inside each group were significantly positively or negatively associated with each other. Abdedaiem et al. (2020) showed that correlation between pH and EC of *R. raetam* was significant. Significant correlations among several soil chemical properties were observed by Liu et al. (2020). As for the plant chemical variables, positive or negative correlations were noticed among Chl b, Chl a+b, Chl a/b, between carotenoids and total pigment (*Fig. 5A*), among WC, Na^+ and Mg^{2+}, between N and SO_4^{2-} (*Fig. 5B*), among aspartic, arginine, valine, lysine, among threonine, alanine, tryptophan and methionine, among tyrosine, cysteine, glutamic and glycine, between serine and isoleucine, between phenylalanine and leucine as well as between histidine and proline (*Fig. 5C*) in the studied plants. Kaspary et al. (2020) mentioned that the importance of the relationship between Chl a and Chl b is due to assessing the ability of plants to capture light during shade. Positive correlations were found among mineral contents (Yinping et al., 2021). The most amino acids were significantly positively associated with each other (Kumara et al., 2019). No negative correlations were observed among the amino acids in plants studied by Kumar et al. (2017).

In the *Fig. 5A*, the soil variables i.e., pH, K^+ and SO_4^{2-} had a positive correlation with plant variables i.e., Chl b, carotenoids, Chl a+b, Chl a/b, total pigment, and that occupied the first and fourth quadrants of the diagram of *A. tortilis*, and also strongly correlated with PCA1. While, the rest soil and plant variables were positively associated with PCA2 and occurred in the second and third quarters with *R. raetam*. The EC, Na^+, Ca^{2+}, Mg^{2+}, Cl^- and WC variables in soil were positively correlated with K^+, WC, Na^+ and Mg^{2+} (*Fig. 5B*), leucine, phenylalanine, valine, lysine, aspartic, arginine, serine, and isoleucine (*Fig. 5C*) in plants. These soil and plant variables were strongly correlated with PCA1, which were located in first and fourth quarters of the diagram of *R. raetam*. On the other hand, the other soil and plant variables were located in second (*A. tortilis*) and third (*A. graecorum*) quarters, and that were strongly correlated with PCA2. These results indicated that the first two PCAs were affected by most soil and plant chemical variables across the three plant species studied. Also, the chemical variables of the soil associated with *R. raetam*, *A. graecorum* and *A. tortilis* during the winter and summer seasons show better soil chemical characteristics, which influence the distribution of legumes plants growing in Wadi Sudr, South-West Sinai.

The PCA1 had a high positive correlation with soil variables (Cl^-, Na^+, Ca^{2+}, Mg^{2+} and K^+/Na^+ ratios) and plant variables (water content, proline, and glycine betaine) and related to water stress and to salt stress (Gil et al., 2014). PCA1 included aspartic acid, glutamine, glycine, histidine, isoleucine, and serine, while, PCA2 consisted of cysteine and methionine, phenylalanine, and tyrosine (Kumara et al., 2019). Gil et al. (2014) reported that proline content had correlated with soil variables as Cl^-, Na^+ (positively) and soil moisture (negatively). Concerning chlorophyll, the contributions of soil and climate variables to the total variance were very low, while interspecific variation was the main factor affecting chlorophyll content (Li et al., 2018). Proline levels in the plants exhibited significant correlation with the variables associated with environmental stress (Gil et al., 2014), which indicate a functional role of proline in the stress tolerance mechanisms of plant species (Grigore et al., 2011).

Conclusions

Significant divergences for most soil mechanical and chemical properties between plants, depths and their interaction as well as for most amino acids, photosynthetic pigments contents and chemical compositions between plants, seasons and their interaction were observed by ANOVA. The highest contents of most soil chemical properties were recorded at *R. raetam*. The concentrations of most amino acids, photosynthetic pigments, Na^+, K^+ and SO_4^{2-} were higher in the dry season than in the wet season across the three studied plants. Based on the variation in the plants studied, the first two principal component analysis (PCA1 and PCA2) were strong enough to separate soil and plant variables. PCA1 and PCA2 formed different groups from soil and plant variables, which are closely related with each other, especially with some amino acids. The results of PCA from our study could be useful in future studies and may lead to the maintenance of cellular osmotic balance to protect the plant during different stress conditions.

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