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Influence of Photosynthetic Pigments on Taxonomic Variations of Plant Species of *Zygophyllum, Tribulus* and *Fagonia* Genera of Zygophyllacea Family in Saudi Arabia

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The aim of the present study was to examine the influence of photosynthetic pigments composition in *Zygophyllum, Tribulus* and *Fagonia* plants in Saudi Arabia. We have selected *Zygophyllum, Tribulus* and *Fagonia* genera of Zygophyllacea family were collected from several geographical regions in Saudi Arabia. We have performed the pigment determination and total protein tests. It has been shown from our experiment that, leaves of studied plants were greatly varied in their photosynthetic pigments between different genera and within the same species from different location. The results showed a highly significant variations between the studied plants depending on plant species and their catchment location. The great differences of the climatic conditions between the different habitats of the studied plants resulted in the fluctuation of the estimated pigments and protein. We suppose that accumulation of protein under a certain stress suggests functional specialization for this protein and clarifies its role in the stress reaction.

Key words: *Zygophyllum, Tribulus, Fagonia,* Saudi Arabia.

Plants constantly have to adaptable to changing conditions of the environment, including to conditions of the lighting. These organisms receive the light signals through specialized photoreceptors, such as phytochromes, cryptochromes and fototropines. With their help, the plants have got the informations about the quantitative and qualitative changes in the composition of the spectral lights and about the time of the light exposure[1]. The vast majority of the biological processes is dependent upon the portion of the electromagnetic spectrum called visible light (or simply light). The denomination is due to fact that a short range of the light spectrum can be detected by the human eye, extending from about 380 nm (violet) to about 700 nm [red (R)]. However, many species are able to respond to wavelengths that fall outside the visible spectrum, such as shorter wavelengths (ultraviolet light-UV; \400 nm) and longer wavelengths [far red (FR); 700– 800 nm]. Therefore, as light is arguably a critical condition for life on earth, its interaction with organisms will always be an inexhaustible field of research. When we think of the association between light and plant development, photosynthesis is the main process that comes to mind[2].

Accordingly, if photomorphogenesis is the program by which light regulates many aspects the plant development, then photosynthesis is a photomorphogenic process, but since light is the

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source of energy for photosynthesis, and light quantity, quality, direction and diurnal and seasonal progression regulate photomorphogenesis, both process are separated in theory, as treated in the literature, but entirely dependent. To understand this question, from photosynthesis, which is the basis to plant structure and molecule production, light modulates several metabolic pathways affecting cell metabolism, including altered expression of up to several thousand genes which in turn generates a physiological response[3-4]. As is known, among the induced proteins, dehydrins (Group II late embryogenesis abundant (LEA) proteins) have been most commonly studied, yet we still have an incomplete knowledge of their fundamental role in the cell. They are evolutionarily conserved among photosynthetic organisms.

In particular, studies of stress-induced accumulation of five dehydrins in Arabidopsis revealed that two of them (LTI30 and COR47) accumulated primarily in response to low temperature. The level of another two proteins (ERD14 and LTI29) was up-regulated by ABA and low temperatures, whereas RAB18 was only found in ABA-treated plants[5]. The dehydrin-like protein P-80 (80-kD) from barley accumulated under cold acclimation though drought, ABA or heat stress did not increase the level of P-80[6]. The aim of the present study was to examine the influence of photosynthetic pigments composition in *Zygophyllum, Tribulus* and *Fagonia* plants in Saudi Arabia.

MATERIALS AND METHODS

In this study, we have selected *Zygophyllum, Tribulus* and *Fagonia* genera of Zygophyllacea family were collected from several geographical regions in Saudi Arabia including Riyadh, Quassium, Makkah, Aljubel and Afief. Also plant sheets were collected from different herbaria of King Saud University, Saudi Wildlife Commission and King Abdulaziz City for Science and Technology.

Pigments determination

Photosynthetic pigments, via, chlorophyll a, chlorophyll b and carotenoids were extracted and expanded young leaves according to Inskeep *et al*.[7]. Known weight (about 0.01 mg fresh weight) of leaves were immersed in 10 ml N, N-dimethylformamide (DMF) and kept overnight at 4°C. After incubation, chlorophyll (chl) content (Chl *a* and Chl *b*) and total carotenoid amounts were determined in the extract by UV-visible spectrophotometer (ultrospec 2100 pro- Amersham Biosciences, England). The absorbance of the solution was measured between 400 and 700 nm. Formula and extinction coefficients used for determination of photosynthetic pigments were:

Chl $a = 10.3\ E_{645} - E_{647}$

Chl $b = 19.7\ E_{647} - E_{645}$

Carotenoids = 4.2E 470 - (0.0264 Chl *a* + 0.426 Chl *b*)

Determination of Total protein

Proteins from leaves were extracted with an equal volume of 20 mM Tris-HCl buffer (pH 8.6) that contained 5 % β-mercaptoethanol (v/v), at 4°C. Each extract was filtered through the three layers of gauze and centrifuged at 10,000 *g* for 30 min (Germany). The supernatant was collected and assayed for total protein by (8).

Reagents

1) Reagents A (2 gm sodium potassium tartarate + 100 gm sodium carbonate dissolves in 500 ml 1N-NaOH complement to 1L distilled water).
2) Reagents B (2 gm sodium potassium tartarate + 1 $CuSo_4.5H_2O$ dissolves in 100 ml 1N-NaOH).
3) Reagents C (Folin – Ciocaiteau reagent solution dilute to ten volume of distilled water 1:10).

Add to 1 ml extract 0.9 ml Reagents A, then put it the water bath 50 for 10 min, after that add 0.1 ml Reagents B, then add 3 ml Reagents C, put it in the water bath 50 for 10 min, were determined in the extract by UV-visible spectrophotometer (ultrospec 2100 pro-Amersham Biosciences, England). The absorbance of the solution was 595 nm.

RESULTS

Variations in Photosynthetic Pigments of Zygophyllum, Tribulus and Fagonia genera collected from different regions of Saudi Arabia

For the evaluation of photosynthetic efficiency, the changes in photosynthetic pigments including chlorophyll a, b and carotenoids content were used in the present study. In this experiment,

plants were collected from different regions of Saudi Arabia. Photosynthetic pigments (chlorophyll a, b and total carotenoids) were determined as described earlier in materials and methods. It has been shown from our experiment that, leaves of studied plants were greatly varied in their photosynthetic pigments between different genera and within the same species from different location (Figs. 1-3).

The great variations in photosynthetic pigments content, depend on plant species as well as the catchment region. Comparing the different species within the same habitat, as well as, the same species within different habitats it was found that, photosynthetic pigments are depending on plant species and it's their habitat. Thereafter remarkable variations in leaf chlorophyll content of *Tribulus*, *Zygophyllum* and *Fagonia* plants

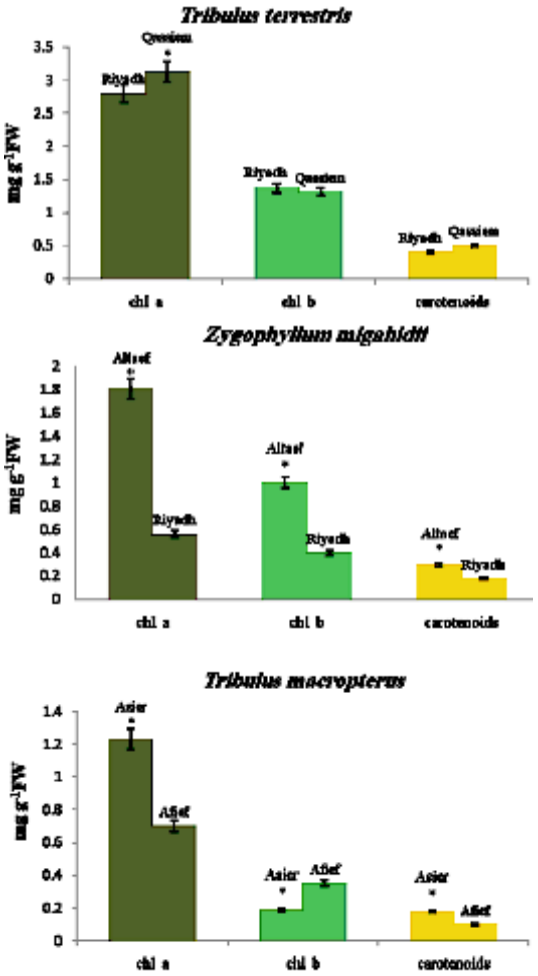

Fig. 1. Variation in chlorophyll a, b and carotenoids contents in *Zygophyllum simplex* and *Zygophyllum migahidii* collected from different locations in Saudi Arabia .Each value is an average of three replication. Significant differences between the two species (after correction of the significance level (p = 0.05) are indicated by asterisks

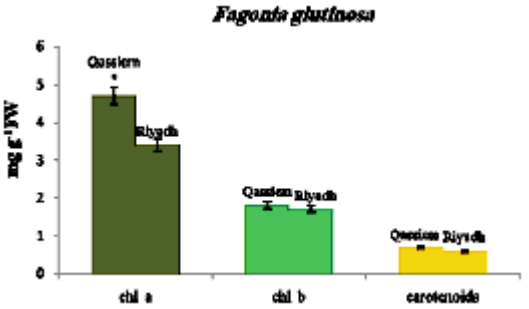

Fig. 2. Variation in chlorophyll a, b and carotenoids contents in *Tribulus terrestris* and *Tribulus macropterus* collected from different locations in Saudi Arabia .Each value is an average of three replication. Significant differences between the two species (after correction of the significance level (p = 0.05) are indicated by asterisks.

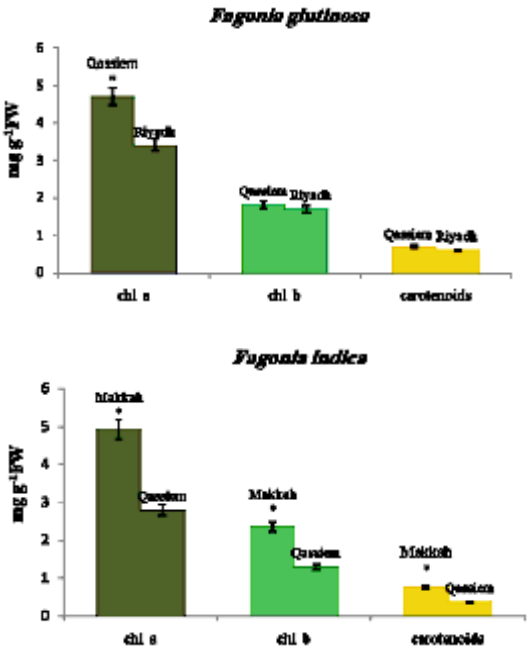

Fig. 3. Variation in chlorophyll a , b and carotenoids contents in *Fagonia glutinosa* and *Fagonia indica* collected from different locations in Saudi Arabia .Each value is an average of three replication .Significant differences between the two species (after correction of the significance level P = 0.05) are indicated by asterisks

collected from various locations were observed (Figs. 1-3).

Changes on protein content in Zygophyllum, Tribulus and Fagonia genera collected from different locations of Saudi Arabia

It is evident from Figure 4 that, total protein content was greatly varied between studied plant depending on plant species as well as the plant location. Thus, at *T.macropterus* collected from Afief, plant leaves contained 0.52 mg g^{-1} DW of protein, whereas, only 0.31 mg g^{-1} DW was present in *T.terrestris* collected from Qassim region. In other words, the less total protein content for *Z.simplex* species collected from Altaef was less than the same species in Riyadh region by 30%. Concerning the total protein in different plant genera within the same habitat, it was found that *Z.simplex* contain 0.3 mg g^{-1} DW compared with 0.4 mg g^{-1} DW in *T.terrestris* and 0.43 mg g^{-1} DW in *F.glutinosa* present in Riyadh, (Figure 4).

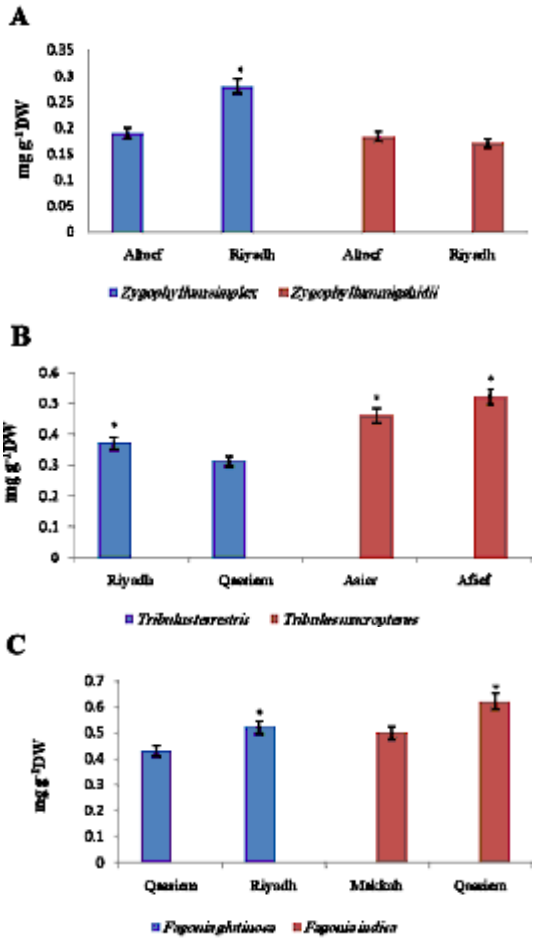

Fig. 4. Variation in protein content in *Zygophyllum* (A), *Tribulus* (B) and *Fagonia* (C) genera collected from different locations in Saudi Arabia .Each value is an average of three replicates .Significant differences between the two species(after correction of the significance level P = 0.05) are indicated by asterisks

DISCUSSION

In the present study, we determined the variations in photosynthetic pigmentation and as well as total proteins. For instance chlorophyll a and b contents for *T.terrestris* plants collected from Riyadh were equal to 2.8 and 1.36 mg g^{-1} FW, respectively. The corresponding values for the same species collected from Qassim were 3.13 and 1.31 mg g^{-1}FW, respectively. On the other hand the chlorophyll a and b contents for the other species *T.macropterus* were 1.23 and 0.19 mg g^{-1}FW, for the plants collected from Asier. Moreover the chlorophyll a and b contents for the plants collected from Afief were 0.7 and 0.35 mg g^{-1} FW, respectively. Comparing the carotenoids content in studied plants *F.indica* was founded to has the highest carotenoids content and reached to 0.77 mg g^{-1} FW, whereas the lowest carotenoids contents was founded in *T. macropterus* from Afief habitat. Carotenoids content for *T.terrestris* were 0.4 and 0.5 mg g^{-1} FW, for the plants collected from Riyadh and Qassim. The corresponding values for the other species *T.macropterus* collected from Asier and Afief were 0.18 and 0.1 mg g^{-1}FW, respectively. Similar pattern of differences was observed in other genera and species depending on the habitat from where the plant collected.

In *Z. simplex* and *Z. migahidii* plants collected from Riyadh, the chlorophyll a content were 3 and 0.56 mg g^{-1} FW, respectively. Whereas the corresponding values for chlorophyll b were 1.12, 0.4 mg g^{-1} FW and for carotenoids were 0.34 and 0.18, respectively.

Comparing the pigments content between different species of the same genus, it was found that chlorophyll a and b contents in *Z.simplex* were found to be 81.3 and 64.1 % , respectively more than in *Z.migahidii* middle region habitat (Riyadh). Great difference occurred for the same plants when collected from different habitats such as Altaef and Riyadh.

Data in Figure (3) shown that *F.glutinosa*

and *F.indica* were significantly differ according to species and different habitats. For instance the chlorophyll a for *F.glutinosa* collected from Qassim was higher than that collected from Riyadh by 27.4 %. Whereas the chlorophyll b for the same species were 1.81 and 1.70 mg g^{-1} FW and carotenoids were 0.7 and 0.6 mg g^{-1} FW for Qassim and Riyadh respectively. On the other species F.indica the plants collected from Makkah contained chlorophyll a higher than that collected from Qassim by 56.9 %. Carotenoids and chlorophyll b were also significantly differed, and reached to 44.7% and 50.6 % in Qassim less than Makkah.

As a consequence, the Chl *a/b* ratio differed significantly in all studied plants. A marked decrease in the ratio of chlorophyll *a/b,* from 2.7 to 0.7 occurred when the plant leaves were collected from Riyadh region in *Z. simplex* and *Z.migahidii* plants. Chl *a/b* ratio for *Z.simplex, T.terrestris* and *F.glutinosa* were 2.7, 2.1 and 2.1 mg g^{-1} FW, respectively for Riyadh habitat. *F.indica* collected from Makkah region showed a highly significant increment in photosynthetic pigments including chlorophyll a and b.

Also the same region (Riyadh) affect the total protein content in different species within the same genera, for example protein content *in Z.simplex* was 0.3 mg g^{-1} DW compared with 0.2 mg g^{-1} DW in *Z.migahidii*. On other word *F.glutinosa* collected Riyadh was found to contain nearly 2-fold highs protein than *Z.simplex* even though, the plants were collected from the same region and at the same time.

Our results indicated that the great variation of photosynthetic pigments including chl a, chlb and carotenoids could be resulted from the difference between the climatology of the collected area. Also the increment of carotenoids in certain species collected from specific habitat can protects the plants against some adverse climatic conditions such as drought, salinity, low temperature and high temperature.

Chlorophyll and carotenoid absorb radiant energy, which is used for photosynthesis. In many observed cases chlorophyll content declined significantly under different stress conditions[9-10]. The major role of carotenoid through direct quenching of triplet chlorophyll prevents the generation of singlet oxygen and protects from oxidative damage. Two carotenoid content classes, carotenoids show multifarious roles in drought tolerance including light harvesting and protection from oxidative damage caused by drought. Thus, increased contents specifically of carotenoids are important for stress tolerance[10]. Wang *et al.*,[11] reported that the carotenoid content in leaves of winter wheat increased under drought stress.

Confirmed results were obtained by a group of workers including[12-14]. High levels of proline enabled the plant to maintain low water potentials. By lowering water potentials, the accumulation of compatible osmolytes, involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortages within the organism[15].

However, the distribution of nitrogenous constituents was varied much in different plant species from location to another. Therefore, the total protein content in plants were consistently and significantly varied which means that the rate of metabolism, utilization and translocation of nitrogenous compounds was greatly retarded due to the effect of environmental conditions. Also, these results show that the effect of plant species on nitrogen metabolism was more exerted in the southern regions than in the west and northern regions. Other results suggest that atmospheric stress factors in highly polluted areas seem to affect accumulation of some flavonoids[16].

CONCLUSION

We suppose that accumulation of protein under a certain stress suggests functional specialization for this protein and clarifies its role in the stress reaction. Reaction to certain stress (drought, freezing, salt etc) seem to be valuable information for understanding the function of dehydrins in the cell. They are hypothesized to function, stabilizing large-scale hydrophobic interactions such as membranes structures or hydrophobic patches of proteins. Highly-conserved Polar Regions of dehydrins have been suggested to hydrogen-bond with Polar Regions of macromolecules, acting essentially as a surfactant, to prevent coagulation under conditions of cellular dehydration or low temperatures[17].

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REFERENCES

1. Mozdzen K, Saja D, Rys M, Skoczowski A., Impact of light spectral composition on the length and weight of the gametophyte of *polytrichastrum formosum* (hedw.) G.l. Smith, *plagiomnium cuspidatum* (hedw.) T.j. Kop. And *pleurozium schreberi* (brid.) Mitt. *Modern Phytomorphology* 2014; **5**: 73–78
2. Suetsugu N, Wada M., Cryptochrome B-light photoreceptors. *Curr Opin Plant Biol* 2003; **6**: 91–96.
3. Tepperman JM, Hudson ME, Khanna R, Zhu T, Chang SH, Wang X, Quail PH., Expression profiling of phy B mutant demonstrates substantial contribution of other phytochromes to red light- regulated gene expression during seedling de-etiolation. *Plant J* 2004; **38**: 725–739
4. Quail PH., Phytochrome-regulated gene expression. *J Integr Plant Biol* 2007; **49**: 11–20
5. Nylander M, Svensson J, Palva TE, Welin BV., Stress-induced accumulation and tissue-specific localization of dehydrins in *Arabidopsis thaliana*. *Plant Mol Biol* 2001; **45**: 263-279
6. Bravo LA, Close TJ, Corcuera LJ, Guy CL., Characterization of an 80-kDa dehydrin-like protein in barley responsive to cold acclimation. *Physiol Plant* 1999; **106**: 177-183
7. Inskeep WP, Bloom PR., Extinction coefficients of chlorophyll a and b in N, N-dimethylformamide and 80% acetone. *Plant Physiol* 1985; **77:** 483–485
8. Lowry OH, Rosbrough NJ, Farr AL, Randall RJ., Harttree-Lowry and Modified Lowry protein Assays. *J Biol Chem* 1951; **193**: 265
9. Nadler A, Bruvia H., Physiological responses of Potato plants to soil salinity and water deficit. *Plant Science* 1998; **137**: 43-51
10. Jaleel CA, Manivannan P, Wahid A, Farooq M, Somasundaram R, Panneerselvam R., Drought stress in plants: a review on morphological characteristics and pigments composition. *Int J Agric Biol* 2009; **11**: 100-105
11. Wang JR, Li SX, Li KI., Effect of water limited deficit stress during different growth stages on leaf enzymes of winter wheat. *Acta Bot Borel Occident Sin* 2001; **21**: 47-52
12. Zaifnejad M, Clarck RB, Sullivan CY., Aluminum and water stress effects on growth and proline of sorghum. *Journal of Plant Physiology* 1997; **150**: 338-244
13. Kundu PB, Paul NK., Effect of water stress on chlorophyll, praline and sugar accumulation in rape (*Brassica compestris* L). *Bangladesh Journal of Botany* 1997; **26**: 83-85
14. Sanchez FJ, Manzanares M, Andres EF, Ternorio JL, Ayerbe L., Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. *Field Crop Research* 1998; **59**: 225-235
15. Kumar A, Tamogami G, Iwahashi S, Prasad Agrawal H, Rakwal V, Transient R., Regulation of jasmonic acid inducible rice MAP kinase gene (OSBWMK1) by diverse biotic and abiotic stresses. *Plant Physiol Biochem* 2003; **41**: 355-361
16. Loponen J, Ossipov V, Lempa K, Haukioja E, Pilhlaja K., Concentrations and among-compound correlation of individual phenols in white birch leaves under air pollution stress. *Chemosphere* 1998; **37**: 1445-1456.
17. Ismail AM, Hall AE, Close TJ., Purification and partial characterization of a dehydrin involved in chilling tolerance during seedling emergence of cowpea. *Plant Physiol* 1999; **120**: 237-244.