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The impact of different habitats on some C3 and C4 species of poaceae growing in Nile delta

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Abstract

Seven sites were surveyed for collecting samples of C4 and C3 plants of Poaceae family from aquatic and terrestrial habitats. C4 plants include *Saccharum sp.*, *Saccharum spontaneum*, *Echinochloa stagnina*, *Echinochloa colona and Echinochloa crusgalli*. C3 plants include *Phragmite australis* and *Leersia hexandra*. Morphological description and anatomical characteristics of culm and leaf were studied in the two habitat. Growth rate and anatomical features of studied species affected by altering habitat from aquatic to terrestrial habitat. Morphological and anatomical characters were recorded and the data were analyzed using Past Software V. Cluster analysis was done depending on morphological characters and anatomical characters separately. The relationship between the studied species depending on morphological characters different from that based on the anatomical characters. Also, the relationship between studied C3 and C4 species affected by altering the habitat from aquatic to terrestrial one.

***Keywords**:* Poaceae, morphological characters, anatomical characters, Past Software V, Cluster analysis

Introduction

The subsequent floristic treatments of grasses of Egypt have been done by Täckholm & Täckholm (1941), Täckholm (1956, 1974), Cope (2005) and Ibrahim et al. (2016). The first key for the grasses of Egypt utilizing vegetative characters was completed by Hosni and Ibrahim (2004). The grasses are considered as important sources of raw material for the biomass and bioenergy industry such as *Arundo, Miscanthus*, and *Saccharum* (Hodkinson et al., 2015; Jones et al., 2015). They were dominated in many habitats such as grassland, reed swamp, salt-marshes and steppes.

The grasses were divided into two groups depending on the photosynthetic pathways: C3 and C4 pathways. C3 and C4 plants possess different biochemical and anatomical features that facilitate their adaptation to cool- temperate and warm-tropical environments, respectively (Edwards and Smith, 2010). Forty-six percent of grasses are C4 plants and together represent 61% of C4 species (https://www.quora.com/profile/Rajiv-Angrish). About 5000 species of grasses use C4 photosynthetic pathway.

The aim of this investigation is to study the effect of changing habitat on the morphological traits and the anatomical features as well as the relationship between studied C3 and C4 species by altering habitats from aquatic and terrestrial.

Materials and methods

Target species: The study targets belong to family Poaceae, subfamily Panicoideae, Arundinoideae and Ehrhartoideae. The species of C4 are; *Saccharum sp., Saccharum spontaneum L, Echinochloa stagnina* (Retz.) P.Beauv., *Echonochloa colona* (L.) Link, *Echinochloa crus-galli* (L.) P.Beauv., and species of C3 are *Phragmites australis* (Cav.) Trin. ex Steud. and *Leersia hexandra* Sw. (Table 1).

Target sites: The studied species were collected from seven sites (table 1) and the field observation were done at their natural habitats in Egypt during the summer season from 2017 to 2019. Identification and nomenclature of plant species was done according to Boulos (2005 & 2009), Ibrahim *et al.* (2016) and according to the catalog of life (http://www.catalogueoflife.org/col/search/scientific?22b700e5f4f3c91c3d6e6aa7e955a24b). Herbarium specimens were deposited at the Department of botany and microbiology, Faculty of Science, Herbarium, Damietta University.

Table 1: The surveyed sites of the studied plants.

Sites	Location	Plants
Damietta Port road	31°26'40.9"N, 31°45'22.9"E.	*E. colona* from aquatic habitat.
El-Salam canal station	31°23'44.8"N, 31°46'13.9"E.	*S. spontaneum, E. stagnina, P. australis* from terrestrial habitat.
Gas station Salam canal	31°23'45.8"N, 31°46'15.5"E.	*E. crusgalli* and *L. hexandra* from aquatic habitat.
EL-Salam canal bridge	31°23'45.7"N, 31°46'15.3"E.	S. *spontaneum, E. stagnina, P. australis* from aquatic habitat.
Kafr El-Arab	31°19'06.2"N, 31°18'07.6"E.	*E. colona* and *E. crusgalli* from terrestrial habitat.
Ezbet Awlad Hamam	31°22'30.9"N, 31°46'33.9"E.	*Saccharum sp.* from aquatic and terrestrial habitat.
Cherbas	31°16'49.2"N, 31°41'33.1"E.	*E. colona* and *L. hexandra* from terrestrial habitat.

Morphological characters:

Plant samples from three replicates of all studied species were collected for measuring their morphological characters.

Anatomical characters:

The sections of the third leaf and the third internode were fixed in FAA (formalin: acetic acid: 70% ethanol [1:1:18, v/v]). The samples were dehydrated by using ethanol series; starting with concentration of 70% and ended by 99.9 %. As this occurred using successive concentrations of ethanol in 10% increment. The fixed sections were stained with Fast-green double stain and Safranin O solution. Sections were mounted in Canada balsam after staining (Sass, 1958). Then examined using photomicrographs.

Statistical analysis:

Morphological, anatomical characters investigated were recorded and the data were analyzed using Past Software V.

Results

Morphological and anatomical characters

The observations of the morphological characters and the anatomical structure of the culm and leaf summarize in (table 2).

The studied species of Poaceae family from aquatic and terrestrial habitats classified into C4 and C3 plants. These C4 plants are *Saccharum sp., Saccharum spontaneum, Echinochloa stagnina, Echinochloa colona and Echinochloa crusgalli*. The C3 plants include *Phragmite australis* and *Leersia hexandra*.

Regarding the life span of the studied species in both aquatic and terrestrial habitats, it was perennial for all studied species except *E. colona* and *E. crusgalli* were annual.

From the present study, the morphological features of the plants did not change of the plants collected from the aquatic habitat to those collected from the terrestrial one. Vegetative characters can be used for plant identification until a flowering specimen is obtained for positive verification. It was complex to identify characteristics of the Poaceae.

Culm anatomical characters

The anatomical studies of species culm in the aquatic and terrestrial habitat show that all studied species have common culm anatomical characters where all culms have lignified epidermis. Cortex of all studied species consists of ground tissues containing group of collenchymatous cells except for *E. crusgalli*. Aerenchyma of the culm is present in all studied species except for *E. colona* and *E. crusgalli*. The culm primary ground tissue has cylindrical fibers except for *Saccharum sp.* in terrestrial habitat. The culm vascular bundles are scattered throughout the central ground tissue. The number of culm vascular bundle layers are the same for the same plant in the two habitat for all studied species except for *E. crusgalli* differ in the aquatic and terrestrial habitat (Fig. 1) (Fig. 2) (Table 2).

Leaves anatomical characters

All the studied species have common leaf anatomical characters where all studied species leaves with guard cells, silica and bulliform cells along the upper or lower or both epidermal cells. Their vascular bundles are surrounded by a bundle sheath which extends to the smallest veins.

A ring of mesophyll cells radiate from the vascular bundles (Kranz anatomy) is present in all studied C4 plants. The studied C4 species have two inner and outer bundle sheaths; the outer one is parenchymatous and the inner one is sclerenchymatous cells, forming a sclerenchymatous cap that is present at the phloem pole. The studied C3 plants do not have this Kranze anatomy. There were regions of sclerenchyma extend from each vascular bundle sheath towards both epidermises. The number of bundle sheath cells and number of xylem vessels per each vascular bundle are different between the studied species in the two habitat (Fig. 3) (Fig. 4) (Table 2).

Table 2: Morphological and anatomical characters of the studied species. Character number is preceded by # and states of the same character are assigned serial numbers.

Plants characters	Plant species													
	Saccharum sp. aquatic	Saccharum sp. terrestrial	S. spontaneum aquatic	S. spontaneum terrestrial	E. stagnina aquatic	E. stagnina terrestrial	E. colona aquatic	E. colona terrestrial	E. crusgalli aquatic	E. crusgalli terrestrial	P. australis aquatic	P. australis terrestrial	L. hexandra aquatic	L. hexandra terrestrial
#1.< Plant duration>/ 1.annual/2.perennial	2	2	2	2	2	2	1	1	1	1	2	2	2	2
#2. <plant length>/ 1.<100 cm/2.>1oo cm	2	2	2	2	2	2	1	1	1	1	2	2	1	1
#3 .<root>/1.fibrous/ 2.spongy	1	1	1	1	2	2	1	1	1	1	1	1	1	1
#4.<rhizome>/ 1.rhizomatous/2.non rhizomatous	1	1	1	1	1	1	2	2	2	2	1	1	1	1
#5.<culm habit>/ 1.erect/2.decumbent/ 3.prostrate/4.erect or decumbent/ 5.decumbent or prostrate	5	5	1	1	5	5	1	1	4	4	1	1	4	4
#6.<culm internal appearance>/1.hollow/ 2.solid	2	2	2	2	1	1	1	1	1	1	1	1	1	1
#7.<culm color>/ 1.reddish purple/ 2.green/3.grey/4.green and grey	4	4	4	4	1	1	1	1	1	1	2	2	4	4
#8.<culm surface>/ glabrous	1	1	1	1	1	1	1	1	1	1	1	1	1	1
#9.<culm nodes>/ noded	1	1	1	1	1	1	1	1	1	1	1	1	1	1
#10.<node surface>/ 1.glabrous/2.hairy	1	1	2	2	2	2	1	1	1	1	2	2	2	2
#11.<culm diameter (mm)>/	2.9-3.5	1.5-1.9	4.8-5.3	1-1.3	0.5-0.9	1.5-2.2	0.31-0.36	0.31-3.7	0.1-0.2	0.7-1.1	2.1-2.5	2-3.2	1.1-1.6	0.19-0.24
#12.<internode length (cm)>/	9-13	7-11	16-21	8-12	12-16	20-24	14-18	9-14	11-16	9-13	15-19	19-23	10-14	6-10
#13. <leaf surface>/ 1.glabrous/ 2.scaberulous	2	2	1	1	1	1	1	1	1	1	1	1	2	2
#14.<leaf blade apex>/1.Acute/ 2.Acuminate/3.attenuate	2	2	2	2	2	2	1	1	1	1	3	3	1	1
#15.<leaf blade shape>/1.linear/ 2.lanceolate/3.linear and lanceolate	1	1	1	1	1	1	1	1	1	1	3	3	1	1
#16.<leaf sheath with involute margine>/ Flat	1	1	1	1	1	1	1	1	1	1	1	1	1	1
#17.<leaf length (cm)>/	80-84	81-84	129-134	62-66	39-43	53-55	25-28	152-154	27-31	18-22	59-63	53-57	16-20	10-14
#18.<leaf width (cm)>/	1.9-2.9	0.86-	2-2.8	0.3-0.6	0.75-	2-3	0.5-0.8	0.5-0.8	1-2	1-2	2-2.5	3.47-	0.94-	1-2

		0.97			0.89							3.95	0.98	
#19.<leaf blade color>/1.lightgreen/2.dark green/3.grayish blue/4.light green or grayish blue	2	2	2	2	2	2	1	1	2	2	4	4	1	1
#20.<leaf margin> 1. scabrous/2.smooth/ 3.rough	3	3	2	2	3	3	1	1	1	1	3	3	1	1
#21.<ligule>/1.present/2.absent	1	1	1	1	1	1	2	2	2	2	1	1	1	1
#22.<ligule surface>/ 1. Ligule an eciliate membrane/2.a ciliate membrane/ 3.a ciliolate membrane/ 4.a fringe of hairs	4	4	3	3	4	4					2	2	1	1
#23.<Inflorscence type>/ Racemose			1	1	1	1	1	1	1	1	1	1	1	1
#24.<inflorescence shape>/1.head to cluster per culm/ 2.linear to ovate			1	1	1	1	1	1	2	2	1	1	1	1
#25.<inflorescence branches>/1.2-10 branches/2.more than 10			2	2	2	2	1	1	1	1	2	2	2	2
#26.<spikelet >/1.sessile/2.sub sessile/3.pedicellate/ 4.sessile or sub sessile			4	4	4	4	2	2	4	4	2	2	2	2
#27.<spikelet surface>/1.hairy/ 2.hispid/3.pubescent to hispid			1	1	3	3	2	2	2	2	1	1	2	2
#28.<spikelet shape>/1.ovate/ 2.ovate to elliptic / 3.elliptic to oblong / 4.cuneate/ 5.lanceola te			5	5	1	1	2	2	2	2	4	4	3	3
#29.<spikelet type>/ Bisexual			1	1	1	1	1	1	1	1	1	1	1	1
#30.<glume>/Present			1	1	1	1	1	1	1	1	1	1	1	1
#31.<glume surface>/ 1.glabrous/2.hairy			1	1	2	2	1	1	2	2	1	1	2	2
#32.<glum nerves>/4-7 nerves			1	1	1	1	1	1	1	1	1	1	1	1
#33.<lemmaapex>/acute or acuminate			1	1	1	1	1	1	1	1	1	1	1	1
#34.<lemma surface>/1.membranous/ 2.glabrous/3.glabrous to scabrous hispid/4.hispid			1	1	2	2	3	3	2	2	2	2	4	4
#35.<lower lemma shape>/1.ovate/ 2.ovate to acuminate/ 3.acuminate/4.o vate tolanceolate/5.linear lanceolate to linear oblong/6.pectinate			4	4	3	3	1	1	2	2	5	5	6	6
#36.<upper lemma shape>/1.elliptic/ 2.ovate to lanceolate/ 3.linear to oblong/ 4.linear to lanceolate/ 5.pectinate/ 6.acuminate			3	3	6	6	1	1	2	2	4	4	5	5
#37.<palea>/Present			1	1	1	1	1	1	1	1	1	1	1	1
#38.<palea shape>/ 1.ovate to oblong/ 2.elliptic to oblong/ 3.elliptic to acuminate/ 4.linear to oblong			1	1	3	3	1	1	2	2	3	3	4	4
#39.<flower>/Bisexual			1	1	1	1	1	1	1	1	1	1	1	1
#40.<style number>/ Two fid			1	1	1	1	1	1	1	1	1	1	1	1
#41.<style branches>/ Deepl y two branched			1	1	1	1	1	1	1	1	1	1	1	1
#42.<stigma number>/ Two			1	1	1	1	1	1	1	1	1	1	1	1
#43.<ovary shape>/ 1.oblong/2.elliptic			1	1	1	1	1	1	1	1	1	1	2	2
#44.<stigma color>/ 1.pink/2.white/3.violet/4.cream coloured			2	2	3	3	1	1	2	2			4	4
#45.<fruit>/Caryopsis			1	1	1	1	1	1	1	1	1	1	1	1
#46.<presence of culm aerenchyma>/1.present/2.absent	1	1	1	1	1	1	2	2	2	2	1	1	1	1
#47.<shape of culm parenchyma>/1.armed/2.isodiametric/3.Ly sigenous aerenchyma	3	2	3	3	1	1					3	3	2	3
#48.<presence of culm fibers>/Present	1	1	1	1	1	1	1	1	1	1	1	1	1	1
#49.<arrangement of culm fibers>/ 1.cylinder/2.patches	1	2	1	1	1	1	1	1	1	1	1	1	1	1
#50.< number of culm vascular bundle layers>/1.two/2.three/ 3.four/4.five	4	4	4	4	5	5	3	3	3	5	4	4	2	2
#51.< number of leaf bundle sheath cell>/	18	26	17	30	25	30	12	19	12	19	7	22	21	27
#52.< no of leaf inter vascular bundle cell>/	10	12	9	11	12	13	5	7	8	7	3	8	3	8
#53.< no of leaf vascular bundle per 1.378 mm>/	21	16	15	19	17	17	18	14	16	15	7	8	12	10
#54.< leaf thickness>/	2	2	2	2	2	5	4	2	3	3	2	2	2	3
#55.< no of leaf xylum vessels per V.B>/	4	4	4	4	4	4	4	3	5	4	4	3	3	4
#56.< no of leaf bulliform cells per 1.378 mm>/	12	10	12	2	24	6	10	4	17	2	12	3	2	3
#57.< no of leaf main vascular bundle per	2	2	2	2	2	2	2	2	2	2	2	1	2	1

1.378 mm>/														
#58.< no of leaf bundle sheath layers soil>/	2	2	1	1	2	1	1	1	1	1	1	1	1	1
#59. <nature of leaf inner bundle sheath layer soil>/1.lignified/ 2.non lignified	1	1	2	2	1	2	1	2	2	2	2	2	2	2
#60.< nature of leaf outer bundle sheath layer soil>/ Lignified	1	1			1									

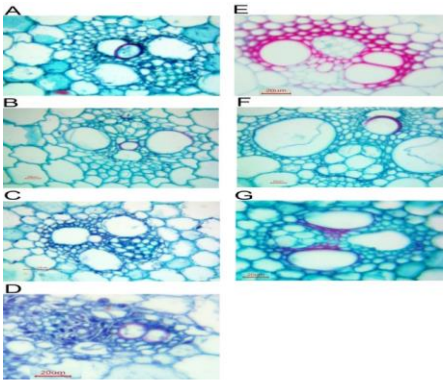

Fig. 1: T.S. in culms vascular bundles in the aquatic habitat, A: *Saccharum sp*., B: *S. spontaneum*, C: *E. stagnina*, D: *E. colona*, E: *E. crusgalli*, F: *P. australis* and G: *L. hexandra*.

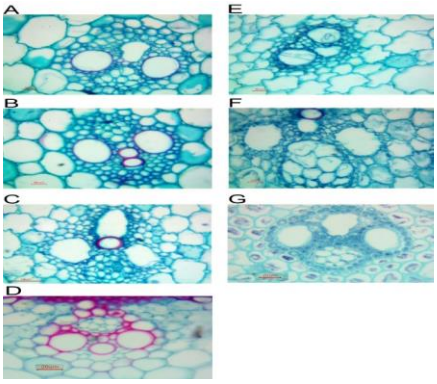

Fig. 2: T.S. in culms vascular bundles in the terrestrial habitat, A: *Saccharum sp*., B: *S. spontaneum*, C: *E. stagnina*, D: *E. colona*, E: *E. crusgalli*, F: *P. australis* and G: *L. hexandra*.

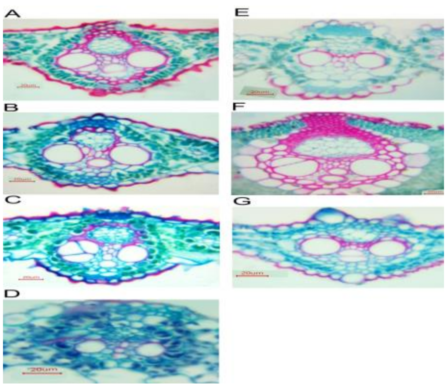

Fig. 3: T.S. in leaves vascular bundles in the aquatic habitat, A: *Saccharum sp*., B: *S. spontaneum*, C: *E. stagnina*, D: *E. colona*, E: *E. crusgalli*, F: *P. australis* and G: *L. hexandra*.

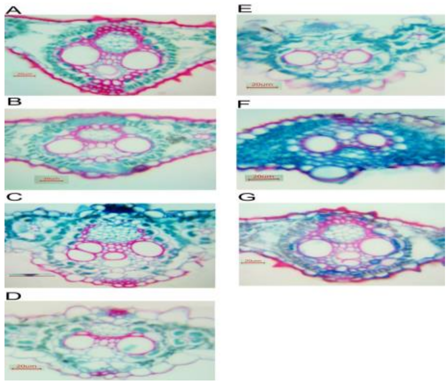

Fig. 4: T.S. in leaves vascular bundles in the terrestrial habitat, A: *Saccharum sp*., B: *S. spontaneum*, C: *E. stagnina*, D: *E. colona*, E: *E. crusgalli*, F: *P. australis* and G: *L. hexandra*.

Discussion

The results obtained from this study concluded that the difference in habitats between the aquatic and terrestrial one has no significant effect on morphological characters but the growth rate of the studied plants changed. In addition, most species have higher growth rate in the aquatic habitat than in the terrestrial one, this may be due to the higher photosynthetic rate in the aquatic habitat than in the terrestrial one.
Based on the morphological characters of the studied species in aquatic habitat (Fig. 5A), the studied taxa are divided into two groups, the first group includes *Saccharum sp.* and *S. spontaneum.* The second group includes *E. stagnina*, *E. colona*, *E. crusgalli*, *P. australis* and *L. hexandra*. *P. australis* separates first from the rest of group two followed by *E. stagnina*.
This finding is not agree with the phylogenetic classification of Poaceae (Soreng *et al.*, 2015). As *P. australis* is more related to *L. hexandra* as they are C3 plants and *E. stagnina*, *E. colona*, *E. crusgalli* related to each other as they belong to sub tribe Boivinellinae and they are C4 plants.

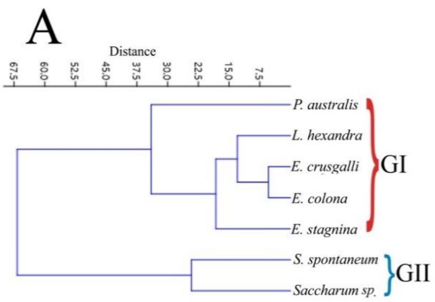

Fig. 5A: Dendogram show the relationship between the seven studied species in the aquatic habitats based on the morphological characters

Regarding to the morphological characters in the terrestrial habitat (Fig. 5.B), the studied species are divided into two groups. The first group contains *Saccharum sp.*, *S. spontaneum*, *E. stagnina* and *P. australis*. The second one contains *E. colona*, *E. crusgalli* and *L. hexandra*.

This not agreed with the phylogenetic classification of Poaceae (Soreng et al., 2015). In contrast, this may be agree with the view that environmental changes may affect speciation and may cause the presence of new species (Taia, 2005).

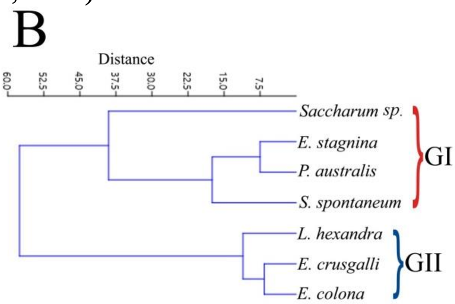

Fig. 5B: Dendogram show the relationship between the seven studied species in the aquatic habitats based on the morphological characters

Vascular system of stem and leaf is considered as important feature in phylogeny and taxonomy. The anatomy of the leaves were less than that of stems (Taia, 2005).

From the present study, the anatomical characters showed some changes between both habitats and they differ between some studied species in the same habitat and this agree with Tayal (2004) who was considered that the anatomical characters of plants changed by changing the environment.

The important charcters are; culm aerenchyma present in all studied species except for *E. colona* and *E. crusgalli*, cylindrical arrangement of culm fibers present in all studied species except *Saccharum sp*. The number of culm vascular bundle layers differ in *E. crusgalli* in the two habitats. Regarding to the leaf anatomy the important characters are the number of intervascular bundle cells, number of vascular bundle, number of main vascular bundle, number of bulliform cells, number of bundle sheath cells and number of xylem vessels per each vascular bundle. These characters are different in the studied plants in the two habitat (Table 2) (Fig. 1,2,3,4).

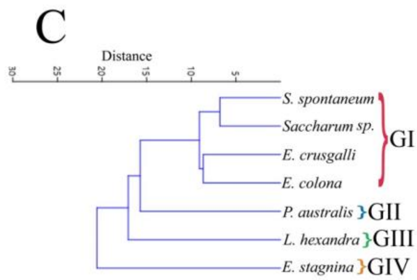

Fig. 5C: Dendogram show the relationship between the seven studied species in the aquatic habitats based on the anatomical characters

Cluster analysis according to the anatomical characters in the aquatic habitat showed that: *Saccharum sp.* and *S. spontaneum* shared common ancestor that could be different from that of *E. stagnina*, *E. colona*, *E. crusgalli*, *P. australis* and *L. hexandra*, *P. australis* is more related to *L. hexandra*, *E. colona* is closely related to *E. crusgalli* (Fig. 5C).

Cluster analysis according to the anatomical characters in the terrestrial habitat showed that: *P. australis* and *L. hexandra* (C3) is separated in one subgroup. (Fig. 5D). This is agree with the fact that C4 trait occurred within the PACMAD clade, whereas the BEP clade contains only C3 taxa (Christin, 2013).

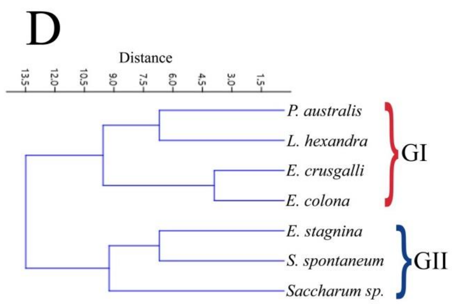

Fig. 5D: Dendogram show the relationship between the seven studied species in the terrestrial habitats based on the anatomical characters

It was known that the C4 photosynthetic pathway has evolved as an adaptation to high photorespiratory pressures which resulted from various combinations of stresses such as low atmospheric CO_2, high temperature, aridity and salinity (Ehleringer et al., 1991; Sage, 2001, 2004; Tipple and Pagani, 2007). Therefore, aquatic habitat may cause disturbance in the growth of these C4 plants, but not (data not shown) where the conditions in this habitat may generate photorespiration (Sage, 2004). Although there were many questions about the unusual presence of C4 plants in the aquatic habitat, whether it is adaptive, or is the result of phylogenetic inertia (Pearcy and Calkin 1983; Pearcy et al. 1987). In the same path, Orcuttieae grasses (tribe of Poaceae) spend a significant part of their life cycle as submerged aquatics, which considered as an unusual habitat for C4 plants (Ehleringer and Monson 1993).

The relationship between the studied species depending on morphological characters different from that based on the anatomical characters. Also, changing the habitat from aquatic to terrestrial one affects the relationship between C3 and C4 species.

Our result is agreed with the suggestion of Keeley (1998) that there are a transition state from C4 to C3 photosynthesis or there are characteristics of the aquatic environment that provide selective advantage to intra-cellular separation of C4-PCA and C3-PCR pathways, as opposed to inter-cellular separation. Also, it was considered that some terrestrial plants have expanded their habitats into aquatic environments. As, submerged aquatic macrophytes, which are thought to derive from terrestrial ancestors, have acquired morphological and physiological characteristics adapted for living in aquatic habitat. The presence of culm aerenchyma in the terrestrial habitat of most studied species along with the high growth rate in the aquatic habitat may suggest that these species were evolved in the aquatic habitat (Roalson et al. 2010).

It was concluded that the morphological characters of the studied species was not distinguish these species according to the habitat type or C3-C4 categorize. This may resulted from the high similarity of the morphological characters of grasses.

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الملخص العربي

عنوان البحث : تأثير اختلاف البيئة على بعض الأنواع ثلاثية ورباعية الكربون للفصيلة النجيلية في دلتا النيل.

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تم عمل رحلات ميدانية إلى سبع مواقع لجمع عينات لنباتات ثلاثية ورباعية الكربون من العائلة النجيلية من بيئات مائية وأرضية. تشمل النباتات رباعية الكربون على قصب الماء والامشوط والباشفت والدنيبة. تشمل النباتات ثلاثية الكربون على البوص والشليح. تم قياس الصفات المظهرية والتشريحية للساق و الورقة في البيئتين. معدل النمو والصفات التشريحية للنباتات تتأثر بتغير البيئة من مائية إلى أرضية. تم تحليل هذه الخواص باستخدام برنامج Past Software V. تم عمل التصنيف التفرعي اعتمادا علي الصفات المورفولوجية والتشريحية كلا علي حدة. العلاقة بين النباتات التي تم دراستها اعتمادا علي الصفات المورفولوجية مختلفة عن العلاقة بين النباتات اعتمادا علي الصفات التشريحية. أيضا العلاقة بين النباتات ثلاثية ورباعية الكربون تتأثر بتغير البيئة من مائية اللي أرضية.