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Characterization of the rediscovered *Cyperus papyrus* L. dominating a stand on the bank of the Damietta branch of the Nile Delta, Egypt; based on isozyme studies

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Abstract – *Cyperus papyrus* L. (Cyperaceae) was rediscovered dominating a stand on the eastern bank of the Damietta Nile branch, Egypt by the second author in July, 2000. Isozyme variation among twelve *Cyperus papyrus* accessions were studied to evaluate the probable origin of the rediscovered stand. Among the studied accessions four were natural and eight cultivated. Three of the cultivated accessions represented the different genomic *C. papyrus* populations in Egypt – Egyptian, Ethiopian, and French. The other, five, accessions are represented by different soil type and water regime. Six enzymes were screened, three of with showing polymorphism between the studied accessions. The studied enzymes are: esterase (EST), peroxidase (PRX), and glutamate oxalo-acetate transaminase (GOT). The genetic variability components were studied by the Statistica computer program based on the variability on isozyme pattern. The results supported that the origin of the rediscovered natural *C. papyrus* stand is Ethiopian. However, divergent variations were observed between the rediscovered accessions and both of Egyptian and French genotypes. Although tremendous morphological diversity exists between the studied twelve *C. papyrus* accessions, no infra-specific grouping was recommended based on isozyme studies. Water stress and soil type are the major limiting factors for the *C. papyrus* growth.

Key words: isozyme, papyrus, Egyptian flora, estrase, peroxidase, glutamate oxalo-acetate transaminase.

1. Introduction

Cyperus papyrus L. is classified in the division of Magnoliophyta, class Liliopsida, order Cyperales, family Cyperaceae. According to Täckholm and Drar (1950), papyrus became almost extinct from Egypt more than 150 years ago. The last traveller to notice papyrus was Baroness V. Minutoli who recoreded it at Damietta and the shore of Lake Manzala during 1820–1821. On July 1968, El-Hadidi discovered a stand of 20 plants of *C. papyrus* growing among other reeds close to Lake Umm Rish, Wady Natroun depression, it was identified as *C. papyrus* subsp. *hadidii* (El-Hadidi 1971). At that time, it was believed to be the only known locality in Egypt and eventually in north Africa.

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The almost extinct *C. papyrus* had been universally used in ancient Egypt. It was the hieroglyphic symbol for lower Egypt and a common motif in art. The root was used for fuel, pith for food and had many uses in folk medicine. The stem was employed for sandals, boats, twine, boxes, mates, sails, cloth and the most notably writing material used in Egypt before the 8th century.

However, *C. papyrus* is almost extinct from Egypt. It formed vast stands in swamps, in shallow lakes, and along stream banks throughout Africa. Many African swamps known as the Sudd in Central Africa are dominated by papyrus thickets, which totally block navigation. It is considered a weed in the Sudan, Ethiopia, and Uganda. It also grown in Sicily, Syria, and Palestine. *C. papyrus* was first introduced to Sicily and a historical study has been carried out, based on available documentary material, to throw light on the origin of *C. papyrus* in Sicily and particularly of the papyrus of the river Caine (Basile and Di Natale 1996, 1997). The plant was also introduced from Egypt (*C. papyrus* subsp. *antiquorum*) to Syria and Palestine (*C. papyrus* subsp. *palaestina*) and Sicily (*C. papyrus* subsp. *siculus*), as mentioned by Täckhom and Drar (1950). *C. papyrus* subsp. *palaestina* and subsp. *siculus* are differentiated from the subsp. *antiquorum* by their exerted part of the connective 2–3 times the breadth of the anthers, while that of subsp. *antiquorum* is hardly exerted.

In 1872, twelve *C. papyrus* specimens were brought to Egypt from Luxembourg and Paris and planted in gardens in Cairo, as mentioned by Täckhom and Drar, (1950) and Serag (2000). In July 2000, Serag discovered a flourishing *C. papyrus* stand at Shrabas, the bank of the Damietta Nile branch, 24 km south of Damietta (Serag 2000).

Isozymes are distinguishable forms of an enzyme. The phenomena resulted from polyploidy found in most angiosperms (Crawford 1990). Isozymes are quite useful in plant breeding programs and taxonomic relationship studies. It is also considered a reliable method for cultivar identification (Barratt 1980), while Quiros (1980) claimed that zymograms of peroxidase, esterase, and acid phosphatase were very useful for the identification of alfalfa. Lange and Schifino-Wittmann (2000) used isozyme bands to distinguish between 36 accessions of 8 *Trifolium* species as well as species limitation. Isozymes were among the tools used by Potter and Doyle (1992) to study the genetic relationships between wild and cultivated accessions of *Sphenostylis stenocarpa* and its domestication centre. Isozymes are especially useful when several taxa, accessions, and individuals are to be compared as the assumption of homology is more accurate than with some DNA markers (Lange and Schifino-Wittmann 2000).

The aim of this study is to use isozymes to shed light on the probable origin of the rediscovered *C. papyrus* stand, and in addition to evaluate the probable reason for *C. papyrus* extinction through the different soil type and water regime.

2. Materials and methods

2.1. Plant materials

Twelve *C. papyrus* accessions (Table I), including the four wild accessions collected from the rediscovered site at Shrabas on the eastern bank of the Nile at Damietta (fig. 1) were studied. The four accessions represent the rediscovered *C. papyrus* stand with the different plant associations. Three accessions were chosen to represent the different *C. papyrus* genotypes in Egypt: Wady Natroun (Egypt),

Table I. The studied *Cyperus papyrus* L. accessions.

Accession	Symbol	Accession origin	Habitat
Wild	Wo	Shrabas, Egypt	open water, Nile branch side
Wild	Wp	Shrabas, Egypt	associated with *Phragmities australis*
Wild	Ws	Shrabas, Egypt	hydrosoil, soil bank side
Wild	We	Shrabas, Egypt	associated with *Eichhornia crassipes*
Cultivated	IF	Shrabas, Egypt	mud soil & fresh water current
Cultivated	1I	Shrabas, Egypt	mud soil & irrigated 3 days interval
Cultivated	2	Shrabas, Egypt	mud soil & drought stressed
Cultivated	3	Shrabas, Egypt	sandy soil & irrigated 3 days interval
Cultivated	4	Shrabas, Egypt	mud soil & flood irrigation / week
Cultivated	E	Ethiopia	cultivated in Jacob's Nile island, Egypt
Cultivated	Wn	Wady Natroun, Egypt	cultivated in Jacob's Nile island, Egypt
Cultivated	F	France	cultivated in Jacob's Nile island, Egypt

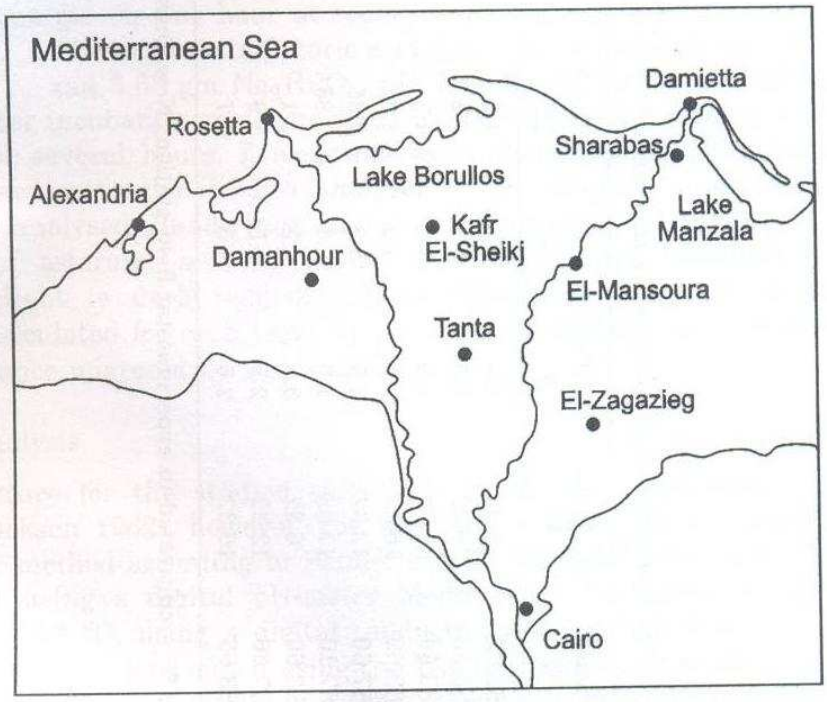

Fig. 1. Location of the rediscovered *Cyperus papyrus* L. stand in Egypt.

French and Ethiopian genotypes, all of them cultivated in Jacob's Nile island (Hassan Ragab, Pharahonic Village, Giza). The last five accessions were collected from the rediscovered wild stand at Shrabas, subject to different soil type and water regime at the Experimental Field Station of the Faculty of Science, Mansoura University, New Damietta, Egypt (Table II).

Table II. Physical and chemical properties of rhizosphere soil samples for the studied *Cyperus papyrus* L. accessions.

Accession symbol		pH	EC µS/cm	Sand %	Silt & clay %	$CaCO_3$ %	O.C. %	Total-P ***	Total-N ***	Na^+ ***	K^+ ***	Ca^{++} ***	Mg^{++} ***
Wo	*	6.92	404	83.62	16.32	2.4	4.5	1.3	5.1	3.1	1.7	1.7	2.6
We	*	6.89	403	83.23	16.30	2.5	4.2	1.3	5.2	3.2	1.8	1.6	2.7
Wp	*	7.10	405	83.64	16.33	2.5	4.2	1.3	5.0	3.5	1.9	1.9	2.9
Ws	*	6.93	405	83.62	16.35	2.5	4.2	1.3	5.1	3.4	1.5	1.8	2.5
1I	**	6.93	346	87.60	12.32	1.5	3.8	0.85	7.1	3.2	0.2	0.5	1.1
1F	**	6.92	349	87.10	12.32	1.5	3.5	0.85	7.1	3.1	0.3	0.5	1.3
2	**	7.27	562	93.60	6.32	3.5	1.8	1.1	5.3	3.2	0.4	0.6	1.2
3	**	7.39	140	87.66	12.32	2.5	1.5	5.9	2.1	1.7	2.0	1.2	2.7
4	**	6.65	582	83.65	16.32	2.5	3.8	0.7	9.8	0.87	5.2	1.6	3.8

* – rediscovered accessions at Shrabas, ** – cultivated in experimental field station, New Damietta, *** – mg/100 g dry soil

2.2. Methods

Esterase (EST), glutamate oxalo-acetate transaminase (GOT), and peroxidase (PRX) were performed on 100 mg freshly harvested umbel brabches for the studied *C. papyrus* accessions, according to Stegeman et al. (1983).

Gel preparation (6% PAGE); a polyacrylamide standard gel was prepared by dissolving 8.55 gram acrylamide and 0.45 gram bisacrylamide in 150 ml Tris-borate buffer 0.125 M Tris, pH 8.9). After filtration, 145 ml of this monomer solution was used to prepare the gel by adding 50 mg sodium sulphate, 0.1 ml TEMED, 2.8 ml ammonium persulphate. PAGE electrophoresis was performed in a Biometra apparatus using Slab gels (11 x 12 cm).

After sample application, electrophoresis was carried out at 20 mA and 120 Volt for 20 minutes and then at 40 mA and 200 Volt for 4 hours. Esterases were detected by incubating the gel for one hour at room temperature, in 200 ml phosphate buffer (0.15 M, pH 7.2) containing α–naphthyl acetate (40 mg) and fast blue RP salt (100 mg). Peroxidases was incubated for 5 minutes in a mixture of 15 ml benzedine (10 %); 85 ml ethanol (95 %); 100 mM K-acetate pH 4.67, and 0.2 ml H_2O_2 (30 %). Glutamate oxalo-acetate transaminases were detected by incubating the gel for one hour at room temperature in 25 ml substrate solution (200 ml H_2O, 146.1 mg, α-ketoglutaric acid, 532.4 mg L-aspartic acid, 2 mg PVP-40, 20 mg EDTA, and 5.68 gm Na_2HPO_4, pH 7.4), 25 ml water, and 50 mg fast blue BB salt. After incubation gels were fixed in 50 % glycerol for one hour and soaked in water for several hours. Zymograms were photographed. The resulting bands were analysed using the Gel-Pro Analyser V. 3.0 computer program. Only anodic bands were analysed. Bands were characterized by their rates of migration and in the case of esterase, also by colour (α-esterase dark brownish to blackish, β-esterase light to dark reddish, α/β intermediate). The rate of migration is generally calculated for each band by dividing the distance migrated by the band by the distance migrated by the front line.

2.3. Soil analysis

Soil texture for the studied soils (100 gram) was determined by the sieve method (Jackson 1962); however, the mud soil samples were analysed using the hydrometer method according to Palmer and Troeh (1995). The pH of the soil was determined using a digital pH-meter Model 6209. Conductivity (μS cm^{-1}) was measured at 20 °C, using a digital conductivity meter YSI Model 35. Oxidizable organic carbon was determined using the Walkely and Black rapid titration method as described by Black (1979) whereas, calcium carbonate was determined by titration against hydrochloric acid according to Jackson (1962). Total nitrogen was estimated using the persulphate method according to Adams (1990); the semi-automatic Kjeldhal Model Pro-Nitro I No. 4000627, was used for distillation and absorbance was measured using Spectrophotometer Model 340. Total phosphorus was estimated using the ignition method applied by Andresen (1976). Samples were diluted and analysed according to Murphey and Riley (1962). Cations (Na^+, K^+, Ca^{++} and Mg^{++}) were extracted using ammonium acetate at pH 7 and estimated using an Eppendorf flame photometer (Na^+ and K^+) and an Atomic Absorption Perkin-Elmer Model 560 (Ca^{++} and Mg^{++}), according to Allen et al. (1986).

2.4. Statistical analysis

Multivariate analysis for the isozymes zymograms data were carried out using Hierarchical clustering analysis. Isozymes bands were scored as presence or absence on the zymograms. If the band was present in another genotype it was designated 2, while not shared in another genotype it was designated 1, and 0 if absent. The genetic diversity was based both on shared and unique polymorphic bands, which had been used to construct the phylogenetic dendrogram. Statistica Programme for Windows Release 4.5, copyright by StatSoft, Inc. (1993) was used.

3. Results and discussion

3.1.Morphological investigations

C. papyrus grown in Egypt was mentioned by Täckholm and Drar (1950) as *C. papyrus* L. subsp. *antiquorum* (Willd.) Chiov. and that of Wady Natroun (El-Hadidi 1971) identified as subsp. *hadidii* Chrtek et Slavikovaá; however, Boulos (1995) claimed that all the papyrus grown in Egypt belongs to *C. papyrus* L. species and no infra-specific classification.

Morphological examinations of the studied accessions of papyrus revealed that there was a similarity between the accession of French origin (F), Wady Natroun (Wn), and the rediscovered wild accessions at Shrabas (Wo, We, Ws and Wp), in having 3–5 umbel bracteoles and 4 spikes. Ragab (1980) mentioned that Wady Natroun papyrus (Wn) is similar to that of Abyesinia in this respect.

However, a contradictory opinion was mentioned by Ragab (1980), that the accessions of French (F) origin have 3 bracteoles and 3 spikes. On the other hand, the present investigation revealed that Wady Natroun (Wn) accession is dissimilar to the other accessions in having stout erect umbel branches, which appeared pendulous and flexible in French (F), Ethiopian (E), and Shrabas accessions (Wo, We, Ws and Wp). The rigidity of umbel branches of Wn accession may be related to the environmental stress encountered in this stagnant water depression. The idea is supported by the data obtained from the field observations of the cultivated *C. papyrus* under drought stress conditions, such as 2 and 3 accessions compared with the wild accession, the stressed plants showing a culm length decrease from 3.5 m to 1 m; decrease in stem thickness (from 4 cm to 1.5 cm, in diameter); the number of umbel branches decreased from 120 to 20 branch; umbel height decreased from 45 to 17 cm (all these measures being the mean values of nine readings); in addition to the number of umbel branches, setting spikes decreased also in drought stressed accessions. These observations are quite notable in drought stressed accessions: 2, 3, 1I, 1F and 4, in decrease towards accession 4 which appeared giant as the natural stand.

3.2. Ecology

The most common species recorded in the stand at Shrabas include: *Saccharum spontaneum* L. var. *aegyptium* (Willd.) Hack., *Phragmites australis* (Cav.) Trin ex. Steud. (feeble growth), *Echinochloa stagnina* (Retz.) Beauv., *Persicaria tomentosa* Willd. and *Eichhornia crassipes* (Mart.) Solms-Laub. (on the stand borders).

The physical and chemical properties of the soil samples were collected from the rhizosphere of *C. papyrus* growing in the natural stand at Shrabas on the Nile bank of Damietta and at the Experimental Field Station at Damietta (Table II). The data outlined that *C. papyrus* grows in fresh water bottom soil with pH

varying from 6.6 to 7.4. The hydrosoil salinity is relatively low, ranging from 140 μS cm^{-1} in irrigated sandy soil to 582 μS cm^{-1} in flooded mud soil. Calcium carbonates varied from 1.5 to 3.5 %. Organic carbon (O.C.), ranged from 1.5 to 4.5 %. The highest value 5.9 mg/100 g dry soil of total-P was recorded for a soil sample which was collected from the irrigated sandy soil at the experimental field station. The Total–P ranged from 0.7 to 5.9 mg/100 gram dry soil. Total-N ranged from 2.1 mg/100 g dry soil in the irrigated sandy soil to 9.8 mg/100 g dry soil in the flooded mud soil (Table II).

The habitat conditions at the Shrabas site were favorable for flourishing papyrus. The muddy substratum is easily penetrable, water supply is adequate and stagnant, the light is intense, the air humid. Furthermore, the presence of rich nutrients are plentiful and flourishing for papyrus. Field studies indicated that the plant forms a dense mass of culms. The aerial branches are at different stages of development. Old branches dry out continually and are replaced by new sprouts. The rhizome of papyrus is layered, the uppermost layer being the latest formed, and bears the green culms. The lower rhizome layers have remnants of dry branches and are at different stages of decay. The rhizome mass rises to a height of one meter above the level of the substratum. Field observations indicated that *C. papyrus* showed that the optimum conditions for the papyrus growth is the presence of an adequate freshwater current, and continuous flooding of the root system. These field observations were supported by the early mentioned by Täckholm and Drar (1950) concerning the reasons of papyrus extinction from Egypt. They mentioned that the complete disappearance of certain branches of the Nile in the Delta by the silting process within the Christian and early Islamic periods, while the Nile changed its course and subsequent drying of the older channels, marshes and pools which were immediately fed by the Nile flood. The resulting stagnant water soon become of marked salinity and subsequently papyrus become extinct.

C. papyrus is a plant sensitive to water pollution, by biotic factors, as mentioned by Serag (2000), the dominance of *Phragmites australis* (Cav.) Trin. ex. Steud. and *Typha domingensis* Pers., *Saccharum spontaneum* L. var. *aegyptacum* (Willd.) Hack. and *Vossia cusipdata* Griff. may represent a stress retarding the papyrus growth. A similar conclusion was reported by Holm et al. (1977), where *Phragmites australis* (Cav.) Trin. ex. Steud., and *Vossia cusipdata* Griff. quickly suppress *C. papyrus* L. Biotic factor also affect the papyrus growth, as mentioned by Kaggwa et al. (2001), who claimed that the alum discharge in Lake Victoria at Gaba in Uganda notably reduced the productivity of *C. papyrus*.

3.3. Isozyme phenotypes and their variation

The esterase (EST), peroxidase (PRX), and glutamate oxalo-acetate transaminase (GOT) pattern show variability in the anodal region among the twelve *C. papyrus* accessions. The species shows a fixed heterozygous electrophoretic phenotype consisting of multi-isozyme variants specified by several genes. The number of bands observed on gel for each isozyme may be due to several factors: 1. The number of coding genes, 2. Their allelic states (homozygous or heterozygous), 3. Quaternary structure of the protein products, and 4. Their sub-cellular compartmentalization. The random association of sub-units into multimeric proteins results in an equal number of each type of molecule (Wendel and Weeden 1989).

Esterase (EST), esterases are a complex and heterogenous group of enzymes with multiple substrate specificity and one of the enzymatic systems with higher polymorphism in plants (Lange and Schifino-Wittmann 2000). A minor

intra-specific variability was noted between the twelve *C. papyrus* accessions. The variability was mainly noticed in α-, β- and α/β esterases bands (fig. 1). These variations were very informative, a total of 59 esterases bands were detected in the twelve accessions of *C. papyrus* species. Among them 26 α- and 20 β- and 13 α/β-esterases bands. Bands ranging from 9 in natural accessions as Wo, We, Wp, Ws and three bands in the drought stressed cultivated accessions as accessions 2 and 3.

Beta esterases were present in all the studied accessions in almost homogenous bands with equal concentrations. On the other hand, α-and α/β-esterases showed a notable variability. Esterase results showed that the detected bands in wild accessions (Wo, We, Wp, and Ws) are very similar to that of Ethiopian (E) accession. Variability between the rediscovered accession and that of Wady Natroun (Wn) and French (F) accession are notable, but the major bands are present in all the accessions (fig. 2).

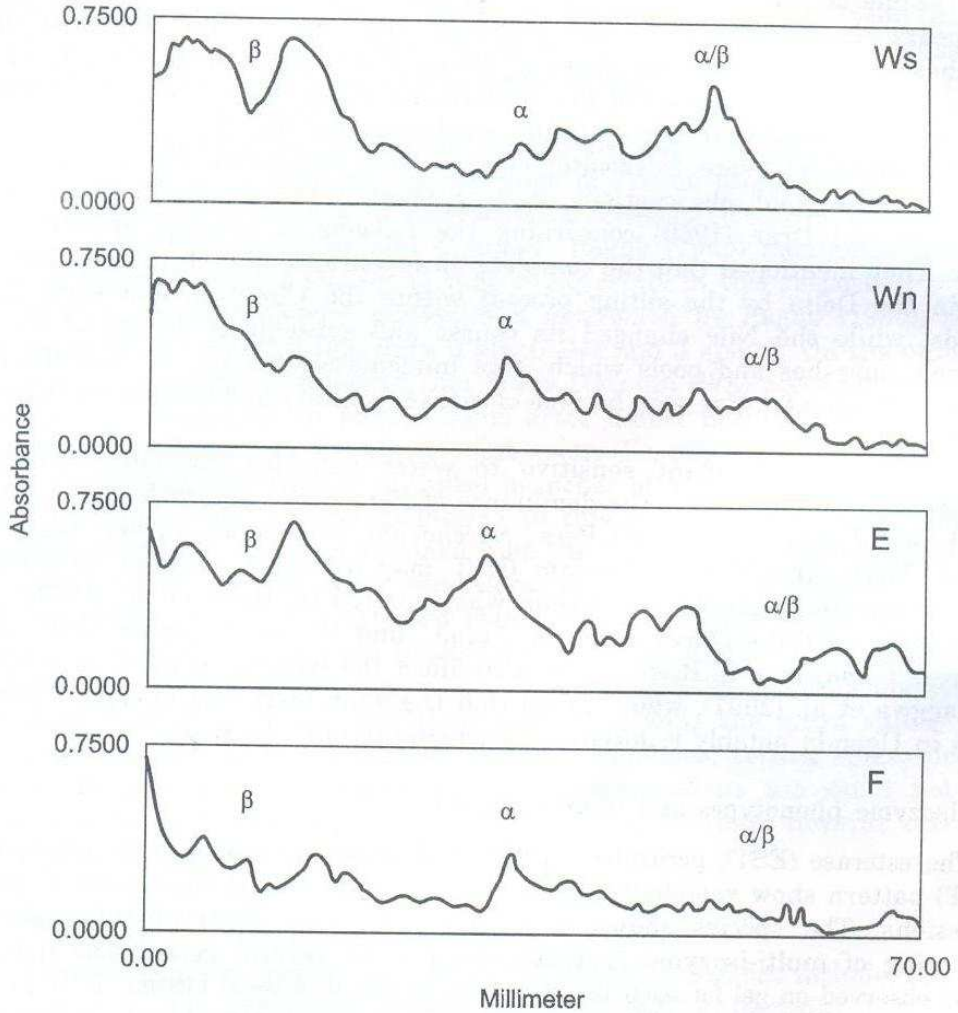

Fig. 2. Esterase zymogram spectra for *Cyperus papyrus* L. accessions: WS – rediscovered stand, Egypt; Wn – Wady Natroun, Egypt; E – Ethiopia; F – French.

On the other hand, α/β-esterases appeared with a notably high concentration in the treated accessions (1F, 1I, 2, and 3), and lower concentrations in accessions cultivated in normal habitats (Wn, E, F, and 4) and wild accessions (Wo, We, Wp

and Ws). Sawada and Yamauchi (1994) detected seven β-esterases in clones of *Trifolium repens* while Lange and Schifino-Wittmann (2000) detected 18 bands in a larger sample of *Trifolium repens* cultivars, while this study revealed 20 β-esterases bands, in the studied *C. papyrus* per accession.

Peroxidases (PRX), are a group of enzymes widely distributed in plant tissues, responsible for oxidation of phenolic compounds to a certain related compound using oxygen derived from peroxides. The zymogram of peroxidase indicated that there is minor polymorphism between the twelve studied *C. papyrus* accessions, normal cultivated (Wn, E, and F) and wild (Wo, We, Wp, and Ws) accessions. While the cultivated drought stressed accessions (1I, 2, and 3), showed disappearance of one of these bands, especially accession 2 grown in muddy soil and drought stress, and accession 3 grown in sandy soil and irrigated at three-day intervals.

Glutamate oxalo-acetate transaminase (GOT), the enzyme is an isomerase enzyme which catalyzes the transfer of an amino group from glutamic acid to oxaloacetic acid. Zymogram of the GOT indicated that there is a notable differences between the major bands detected in the drought-stressed accessions (1I, 2, and 3) which showed the disappearance of one of the common bands and the appearance of another band of a smaller molecular weight compared with the normal cultivated accession 4. No obvious variability was detected between the wild accessions (Wo, We, Wp, and Ws) and normal cultivated (Wn, E, and F), the major four isozyme bands being present in all accessions.

3.4. Statistical analysis of the three isozyme results

The phylogenetic tree (fig. 3) based on the bands polymorphism of the three studied isozymes (EST, PRX, and GOT) revealed distinct infra-specific similarity between the studied accessions. Generally, there is a notable genetic link between

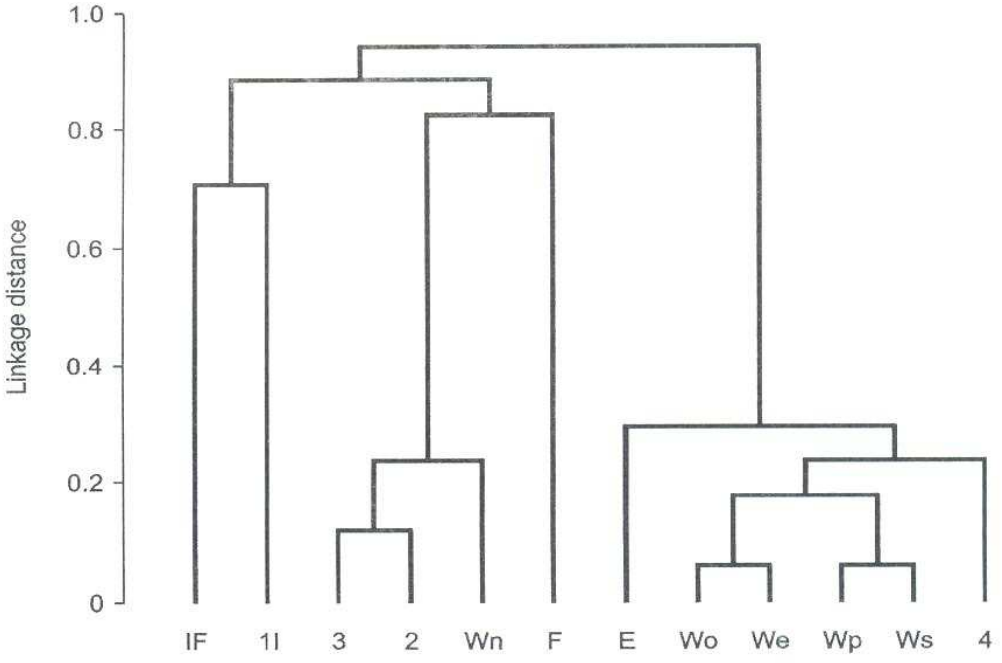

Fig. 3. Phylogenetic tree of the studied *Cyperus papyrus* L. accessions, constructed on the basis of the polymorphism of the isozyme bands.

French (F), Wady Natroun (Wn), and Ethiopian (E) accessions. The Ethiopian (E) accession showed a remarkable similarity to the rediscovered Egyptian stand, in Shrabas (Wo, We, Wp, and Ws). These results are supported by Klaas (1998), who mentioned that isozymes are especially useful when several accessions are compared as the assumption of homology is more accurate than with some DNA markers. The close similarity between the rediscovered *C. papyrus* stand and that of Ehtiopian origin provides scientific support for the historic information cited by Täckholm and Drar (1950) and Ragab (1980), concerning the African origin of *C. papyrus* in Egypt, which in turn was exported to Sicily, Palestine, Syria, and France; the phylogenetic tree (fig.2) also supported this idea.

The variability in EST, PRX, and GOT in drought stressed accessions revealed that isozymes can be used efficiently to differentiate between drought tolerant and drought sensitive accessions of *C. papyrus* by the disappearance of some bands and appearance of others. The drought stressed accessions 2 and 3 grouped in one fork with that of Wadi Natroun (Wn), similarly as the irrigated and flooded accessions (1F and 1I). The stress conditions of Wadi Natroun accession were earlier recorded by El-Hadidi (1971) and Fahmy et al. (1992).

In conclusion, no considerable inter-accession variability was noted by isozymes and morphological studies in the rediscovered *C. papyrus* accessions in Shrabas (Wo, We, Wp, and Ws), based on their common associate plant species (Table I). The negative impact of the associated plant species may be attributed to the presence of a continuous fresh water current, of the Damietta Nile branch, which remove the available water pollutants, and eliminate its harmful impact on *C. papyrus*.

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