Australian Journal of Crop Science AJCS
AJCS 6(6):1088-1093 (2012) ISSN:1835-2707

Genetic diversity and taxonomic relationships of some *Ipomoea* species based on analysis of RAPD-PCR and SDS-PAGE of seed proteins

Kadry Abdel Khalik[1, 2], Gamal Osman[1, 3] and Waeil Al-Amoudi [1]

[1]Biology Department, Faculty of Science, Umm-Al-Qura University, Mecca 673, Saudi Arabia
[2]Botany Department, Faculty of Science, Sohag University, Sohag 82524, Egypt
[3]Agricultural Genetic Engineering Research Institute (AGERI)-Giza, Egypt

***Corresponding author: kadry3000@yahoo.com**

Abstract

To elucidate the genetic diversity of ten *Ipomoea* species, seed proteins were analyzed by SDS-PAGE and RAPD analysis. According to SDS-PAGE analysis, in total 51 different bands were identified across the studied species. The number of bands varies from 16 bands in *Ipomoea cairica*, to 3 bands in *I. sinensis.* The similarity analysis based on the SDS-PAGE profile turned out to be a useful character for the inequity of *Ipomoea* species both on the subgeneric and sectional level. Analysis of RAPD-PCR of DNA provided more precise information concerning relationships between *Ipomoea* sections than SDS-PAGE analysis. A remarkable result from this study was identifying a close relationship between *Ipomoea purpurea* of section *Pharbitis* (subgenus Ipomoea) and species of the subgenus Quamoclit. Further support comes from the molecular data of RAPD which indicate that *Ipomoea cairica* should be considered a well separated section which may be related to section *Orthipomoea*. Our results suggest that section *Erpipomoea* is not a monophyletic group, whereas species of section *Orthipomoea* form a single monophyletic section.

Keywords: genetics, *Ipomoea*, RAPD-PCR, SDS-PAGE, taxonomy.
Abbreviations: RAPD-PCR, Randomly amplified polymorphic DNA, RFLP, Restriction fragment length polymorphic, SDS-PAGE, Sodium dodecyl sulphate polyacrylamide gel electrophoresis.

Introduction

Convolvulaceae are a large family, comprising approximately 50-60 genera with some 1600-1700 species (Mabberley, 1987), exhibiting a rich diversity of morphological characters and a wide range of ecological habitats. More than one-third of the species are included in two major genera, *Ipomoea* and *Convolvulus* (Cronquist, 1988). *Ipomoea* is a large and diverse genus in the Convolvulaceae, comprising over 600 species of vines and shrubs widely distributed throughout the tropics and subtropics (van Ooststroom, 1953; Austin, 1975a; Austin & Huáman, 1996; Miller et al., 1999). Choisy (1845), Hallier (1893 b), and House (1908a) provided early treatments recognizing subgenera and additional infrageneric subdivisions within *Ipomoea*. Moreover, the relationships among Old World *Ipomoea* species were further refined by van Ooststroom (1953), who recognized seven infrageneric taxa in his studies on Asian species. Verdcourt (1957, 1963) recognized eight infrageneric taxa in his treatment of African species. Austin's (1975a; 1979; 1997; Austin & Huáman, 1996) divided *Ipomoea* into three subgenera proposed from three major lineages within the genus: subgenus Eriospermum, subgenus Ipomoea and subgenus Quamoclit. McDonald & Mabry (1992) carried out phylogenetic analysis of chloroplast DNA and RFLPs for 31 New World *Ipomoea* species, and their study supported the monophyly of several traditionally recognized taxa of *Ipomoea*. Das & Mukherjee (1997) studied seedling morphology and the isozyme profile of 12 species of *Ipomoea*, and they revealed two broad clusters or groups. Miller et al. (1999) studied phylogenetic analysis of 40 species representing the three subgenera and nine sections within the *Ipomoea* using sequence data from the ITS region and waxy sequences. They determined a close relationship between species of section *Pharbitis* subgenus *Ipomoea* and species of subgenus Quamoclit. Willkin (1999) carried out a cladistic analysis of the tribe Ipomoeae based on 45 morphological and palynological characters, and suggested that the Ipomoeae is a monophyletic tribe. Manos et al. (2001) tested the phylogenetic relation of the genus *Ipomoea* with other genera from the tribe Ipomoeae based on morphology and phylogeny and found that *Ipomoea* is paraphyletic. Ogunwenmo (2003) investigated morphometric and qualitative characters of matured cotyledons of 18 taxa of *Ipomoea,* and he revealed that cotyledon characters provided are of taxonomic significance in the evolution of *Ipomoea*. Miller et al. (2004) investigated phylogenetic analysis of 36 *Ipomoea* species using sequence data from the ITS region. They settled that the molecular studies agree generally with the results from cpDNA and RFLP analyses in forming of two large clades of species. Molecular markers were used to determine genetic similarity by scientists in different fields more than a decade ago (Potokina et al., 1999; Cwiklinska et al., 2010). The objective of our specific study was to analyze the genetic diversity among some species of *Ipomoea,* to clarify the relationships among the species, and to verify whether these results match the results of Austin (1979, 1997), and Austin & Huàman (1996) for *Ipomoea* sections. We report the results of using two molecular techniques: randomly amplified polymorphic DNA (RAPD) of total genomic DNA and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed proteins.

Results

SDS-PAGE of seed proteins

Electrophoretic analysis of proteins exposed a total of 51 protein bands in the seeds of the 10 species under investigation. The analysis of the results reveals that some bands are characteristic and constant markers for each species and allow the unequivocal identification of their electrophoregrams. Other bands are shared by more than one species. The number of bands varies from one species to another, with the largest number (16) in *Ipomoea cairica*, and the lowest number (3) in *I. sinensis* (Fig. 1). For ease of comparison, the 51 protein bands were lumped together with molecular masses and the number of bands from each molecular mass was scored for every species. Three major clusters with about 0.50 similarity index were obtained (Fig. 2). The first clade includes *Ipomoea purpurea*. The second clade comprises *Ipomoea stolonifera*. The third group contains the rest of the taxa with about 0.52 similarity. Our SDS-PAGE of seed proteins analysis results provides useful information on relationships among closely related taxa. However, the relationships between the sections and subgenera of the genus *Ipomoea* remain unclear from SDS-PAGE analysis. To get a clearer picture of the situation we used the RAPD approach.

RAPD-PCR

Using five primers, a total of 48 bands were visualized; among these 16 were polymorphic in at least one pair wise comparison between species (Fig. 3). The mean number of polymorphic DNA fragments obtained per primer was 3.2. Primer OPO-09 generated the highest number of polymorphic amplification products (6 bands) with size ranged from 214 to 1360 bp (Table 2). Relations between the studied taxa are presented in a dendrogram built on the basis of similarity indexes (Table 3). For ease of comparison, the 48 bands were taken together and the number of bands from each size of DNA fragments (bp) was scored for every species. Two main clusters with about 0.91 similarity index were obtained (Fig. 4). The first group includes *Ipomoea purpurea, I. eriocarpa, I. sinensis* and *I. cairica* with 0.90 similarity. The second group comprises two subgroups with 0.93 genetic similarity: the first subgroup includes *Ipomoea carnea, I. triloba, I. heterotricha* and *I. stolonifera* with about 0.93 similarity; the second subgroup comprises *Ipomoea obscura* and *I. pes-caprae,* showing 0.94 genetic similarity.

Diversity and similarity between two methods

In our results the use of RAPD gave higher similarity coefficients than SDS-PAGE did for the same data, regardless of the higher number of polymorphic products generated by SDS-PAGE. To select a method for revising the genetic similarity, when the dendrogram obtained by both methods are different, as in Vaillancourt et al. (2008), and Nowosielski et al. (2002), Potokina et al. (2000), then the more exact, more replicable and more detailed method should be chosen. In our results a significant majority of the studied taxa were grouped in the RAPD method (Fig. 4).

Discussion

Scientists commonly use more than one method to consider genetic similarity. The purpose of such a procedure, besides comparison of methods, is to determine the number of the polymorphic amplification products. Matos et al. (2001) showed that the picture of genetic similarity can differ depending on the number of polymorphic bands generated by a given method. They said that the more polymorphic products are obtained, the smaller is the similarity between objects.
Generally, our results obtained from the RAPD analyses, suggested groups and partially confirm the subgenera and sectional classification of *Ipomoea* by Austin & Huaman (1996); Austin (1979, 1997) and Verdcourt (1957, 1963).

Subgenus Quamoclit-Group I

According to RAPD tree there is a close relationship between *Ipomoea purpurea, I. eriocarpa, I. sinensis* and *I. cairica* with 0.90 genetic similarity. The species - *Ipomoea eriocarpa,* and *I. sinensis* are morphologically similar to those that have been previously identified to belong to subgenus Quamoclit section *Orthipomoea* (Austin, 1979; Verdcourt, 1963), but *I. cairica* has been treated previously to belong to subgenus Quamoclit but indicating a section name. Although *Ipomoea purpurea* is the only representative of subgenus Ipomoea sect. *Pharbitis* in our study, this position clearly indicates that it is not related to subgenus *Ipomoea* (Table 1). However, *I. purpurea* is treated as a separate clade based on the analysis of seed proteins (Fig. 2) but with very low genetic similarity. Furthermore, Miller et al. (2004) presented a phylogenetic analysis of 36 species using sequence data from the ITS region. They determined that species of section *Pharbitis* subgenus Ipomoea (*I. purpurea*) were nested within species of subgenus Quamoclit. This result was shown previously by Miller et al. (1999) with a broader sample of *Ipomoea* species for both ITS and Waxy sequence data. Wilkin (1999) also observed this same result based on a morphological cladistics study. Moreover, Abdel Khalik & Osman (2007) studied the seed morphology of *Ipomoea* and they showed that *I. purpurea* shared the rest of the species of the subgenus Quamoclit with irregular, polygonal epidermal cells; undulate, raised, smooth to fine folds anticlinal boundaries, and flat to concave, folded periclinal cell walls. Conversely, *I. cairica* has unique characters such as leaf-blade palmate divided into 5-7 lobes; seed surface with micro reticulate periclinal cells. So, treatment of *Ipomoea eriocarpa* and *I. sinensis* in the section Orthipomoea is supported, and the inclusion of *I. purpurea* (sect.Pharbitis) within subgenus Quamoclit is upheld by our data**.** However*, I. cairica* should be treated as a separate section.
Generally these results are congruent with those of Miller et al. (1999; 2004), Wilkin (1999) and Abdel Khalik & Osman (2007).

Subgenus Eriospermum- Group II

Within Eriospermum group, two major clades were identified in Fig. 4. The first clade includes section *Erpipomoea.* The second clade includes section *Eriospermum* and one species from section *Erpipomoea*. Our results mainly support the taxonomic system of the subgenus *Eriospermum* proposed by Verdcourt, 1957, 1963; Austin, 1979, 1997; Austin & Huàman, 1996.

Section *Erpipomoea*

Our results do not support the monophyly of section *Erpipomoea* Choisy of subgenus Eriospermum. This is due to the placement of *I. obscura* and *I. pes caprae* within a separate subgroup with 0.95 genetic similarities, and *I. stolonifera* forming a species pair with *I. triloba* (sect. *Eriospermum*) within another cluster. Formerly treatments also pointed

Table 1. List of the studied species of *Ipomoea* cited according to Austin and colleagues (Austin, 1979, 1997; Austin & Huàman, 1996; Verdcourt, 1957, 1963).

N	Species	Voucher	Subgenera	Sections	Present study (RAPD)
1	*Ipomoea cairica* (L.) Sweet	Egypt: Ismailia canal at Abu Zaabal, El Hadidi & al. s. n. (CAI).	Quamoclit	-	Group I
2	*I. carnea* Jacq.	Egypt: Sohag, El Kola, canal bank, Abdel Khalik s. n. (SHG).	Eriospermum	*Eriospermum*	Group II
3	*I. eriocarpa* R.Br.	Egypt: El Khanka, A. Amer 1075 (CAI).	Quamoclit	*Orthipomoea*	Group I
4	*I. obscura* (L.) Ker-Gawl	Egypt: WadiAkaw, Gebel Elba, Abdel Khalik s. n. (SHG).	Eriospermum	*Erpipomoea*	Group II
5	*I. pes-caprae* (L.) R.Br.	Egypt: Ismailia, Täckholm, G. 4780 (CAI).	Eriospermum	*Erpipomoea*	Group II
6	*I. purpurea* (L.) Roth	Egypt:Idku, A. Amer 9720 (CAI).	Ipomoea	*Pharbitis*	Group I
7	*I. sinensis* (Desr.) Choisy	Egypt: Gebel Elba, Wadi Yahameib, Abdel Khalik& al. s.n. (SHG).	Quamoclit	*Orthipomoea*	Group I
8	*I. stolonifera* (Cyr.) Gmel.	Egypt: N. Sinai, Rafah, Tackholm et al. s.n. (CAIM).	Eriospermum	*Erpipomoea*	Group II
9	*I. triloba* L.	Millennium seed Bank, Kew herbarium number: 0223353	Eriospermum	*Eriospermum*	Group II
10	*I. heterotricha* F. Didr.	Millennium seed Bank, Kew herbarium, number: 0443926	Eriospermum	*Eriospermum*	Group II

Table 2. Characteristics of primers and amplification products generated by the ten decamer arbitrary RAPD-PCR primers (Operon model)

No	Primer name	Sequence	Number of polymorphic fragments of DNA	Size of DNA fragments (bp) min-max
1	OPO-09	5'-TCCCACGCAA-3'	6	214-1360
2	OPO-10	5'-TCAGAGCGCC-3'	1	196
3	OPB-11	5'-GTAGACCCGT-3'	3	197-508
4	OPA-13	5'-CAGCACCCAC-3'	3	439-518
5	OPZ-20	5'-ACTTTGGCGG-3'	3	190-1601

Table 3. Similarity matrix between all pairs of studied taxa based on the RAPD-PCR. Species names from 1-10 as in table 1.

Sp.	1	2	3	4	5	6	7	8	9	10
1	1									
2	0.93	1								
3	0.91	0.98	1							
4	0.89	0.91	0.93	1						
5	0.80	0.81	0.83	0.81	1					
6	0.76	0.82	0.84	0.86	0.95	1				
7	0.80	0.82	0.84	0.82	0.90	0.90	1			
8	0.82	0.80	0.81	0.80	0.92	0.88	0.93	1		
9	0.78	0.80	0.82	0.84	0.88	0.93	0.93	0.95	1	
10	0.82	0.84	0.86	0.84	0.92	0.93	0.98	0.95	0.95	1

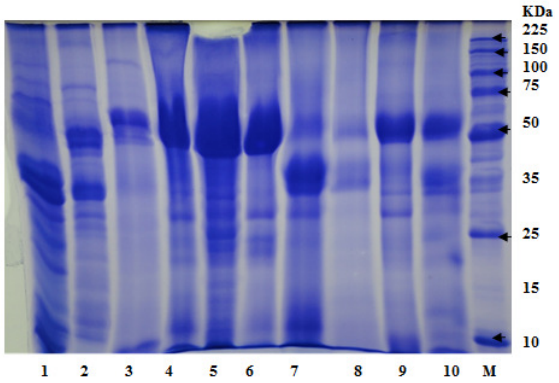

Fig 1. SDS-PAGE analysis of *Ipomoea* seed proteins numbered as in table 1. Species names are arranged and numbered as in Table 1.

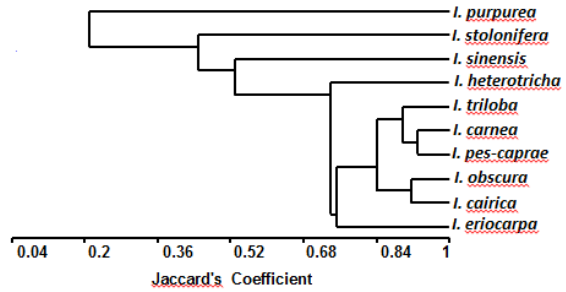

Fig 2. Clustering of the investigated taxa of *Ipomoea* based on the total seed protein profiles separated by SDS-PAGE.

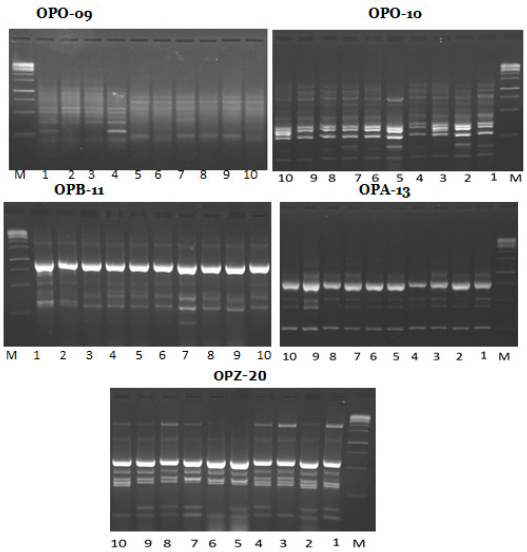

Fig 3. DNA polymorphism generated by five primers from the genomic DNA of the investigated species of *Ipomoea*. Species names are arranged and numbered as in Table 1.

towards the artificiality of section *Erpipomoea* (van Oostsroom 1953; Miller et al., 1999). Das & Mukherjee (1997) studied on seedling morphology and the isozyme profile of 12 species of *Ipomoea*, and they revealed two broad clusters: one includes *I. obscura* and other group includes *I. pes-caprae*. Our results agree with the results of phylogenetic and isozymes studies (Miller et al., 1999; Das & Mukherjee, 1997) which suggest that section *Erpipomoea* is not a monophyletic group.

Section *Eriospermum*

Within this subgroup, species of section *Eriospermum* have been recognized as a distinct group with 0.94 genetic similarities. These species can be clearly defined on the basis of various features: perennial woody habit, glabrous, hairy or glabrous seeds and two-locular gynoecia. Traditionally, some species of *Ipomoea* placed in subgenus Eriospermum on the basis of glabrous seeds (Austin, 1978; McDonald & Austin, 1990). McDonald & Mabry (1992) used chloroplast DNA restriction fragment length polymorphic (RFLP) to investigate the relationships among *Ipomoea* species and their studies led to the reclassification of series Batatas (*I. triloba*) from subgenus Quamoclit to subgenus Eriospermum. Our results show that *I. carnea* and *I. heterotricha* are sister species of *I. triloba* and *I. stolonifera*. Morphologically, both *I. carnea* and *I. stolonifera* share hairy seeds, while *I. triloba* and *I. heterotricha* have glabrous seeds. Our results suggest that species of section *Eriospermum* form a monophyletic group, and there are close relationships between section *Eriospermum* and *I. stolonifera* (sect. *Erpiopomoea*), and these data agree with those of McDonald & Mabry (1992); Austin (1979; 1997) and Austin & Huàman (1996), but disagree with Austin (1978) and McDonald & Austin (1990).

Materials and methods

Plant materials

The samples of *Ipomoea* seeds were taken from wild populations and some herbarium specimens. Voucher specimens of the populations studied are deposited in the herbarium of the Department of Botany of Sohag University (Table 1).

SDS-PAGE of seed protein

To extract seed proteins, 0.5 g of mature healthy seeds were ground on liquid nitrogen in 0.2 MTris pH 8, 2% w/v SDS, 10% sucrose and 1% BME (Wei et al., 2008). Proteins were separated by SDS-PAGE according to Laemmli (1970). Gel slab was scanned using gel proanalyzer ver. 3.3 (Media Cypermetics 93-97). The presence or absence of each band was treated as binary character in a data matrix i. e. coded 1 and 0 respectively Data were statistically analyzed by using gel Doc 2000 Bio-Rad system.

RAPD-PCR

Total genomic DNA was extracted from seeds. The seeds were first ground into a fine powder in liquid nitrogen using a pestle and mortar following the steps of CTAB protocol (Porebski et al., 1997; Hussien et al., 2003). RAPD was performed as described by Williams et al. (1993) with slight modification. PCR reactions were carried out in 25 µl volumes containing 25 ng of total genomic DNA, 10 pmol primer, 200µM dNTP, 2 mM MgCl2, 1X PCR buffer and 2 units

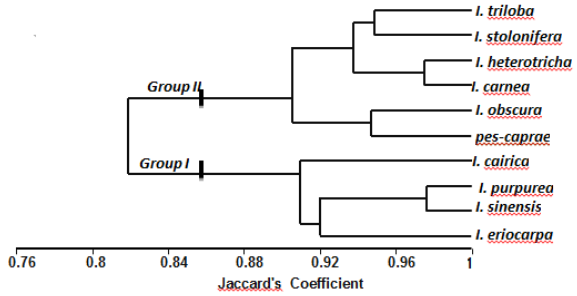

Fig 4. Dendogram of phylogenetic relationships between the investigated taxa of *Ipomoea* based on Jaccard's similarity coefficient obtained from 5 RAPD primers.

ampliTaq polymerase (RTS *Taq*DNA polymerase). Five random oligonuclotide primers OPA-13, OPB-11, OPO-09, OPO-10 and OPZ-20 were used in the experiment (Operon technologies, Alameda, USA) (Table 2). Amplification was performed in Perkin Elmer 9600 thermal cycler (Foster City, USA) with the following temperature profile: 94°C for 5 min followed by, 40 cycles of 94°C for 1 min, 36°C for 1 min, and extension at 72°C for 90s. The final extension step was carried out by 72°C for 5 min.

Statistical analysis

The gel profiles were visually scored by assigning a number to each distinctive band. The PCR reactions for polymorphic primers were repeated to verify reproducibility of results. The presence or absence of bands was scored as 1 or 0, respectively. Estimation of genetic similarity (GS) was calculated for all possible comparisons among the species was calculated by Jaccard's coefficient. An UPGMA phenogram was constructed by using the multivariate statistical Package supported by Kovach computing services. UPGMA was performed with matrix of GS estimates to measure the in formativeness of each marker (Sokal & Michener, 1958).

Conclusions

The analysis of variation between *Ipomoea* species studied showed that there are major differences in the frequencies of the electrophoretic profiles in the different species. RAPD-PCR of DNA is effective in determining relationships between sections and subgenera. We found that PCR-RAPD of DNA provided more precise information concerning relationships between *Ipomoea* sections than SDS-PAGE analysis. A remarkable result from this study was identifying a close relationship between *Ipomoea purpurea* of section *Pharbitis* (subgenus Ipomoea) and species of the subgenus *Quamoclit*. Further support comes from the molecular data of RAPD which indicate that *Ipomoea cairica* should be considered a well separated section which may be related to section *Orthipomoea*. Our results suggest that section *Erpipomoea* is not a monophyletic group.

Acknowledgements

We are grateful to the Director and Curator of Cairo University Herbarium (CAI) and Kew Herbarium (K). We are indebted to Prof. Dr. L.J.G. van der Maesen, professor of Plant Taxonomy in the Biosystematics group, Dept. of Plant Sciences, Wageningen University, the Netherlands for going through the manuscript and making valuable suggestions. Special thanks go to Prof. Dr. Rick Miller, professor of Plant Taxonomy, Department of Biological Sciences, South eastern Louisiana University, USA for his valuable suggestions and comments.

References

Abdel Khalik K, Osman KA (2007) Seed morphology of some species of Convolvulaceae from Egypt (Identification of species and systematic significance). Feddes Repert 1-2: 27-34.

Austin DF (1975a) Typification of the New World subdivisions of *Ipomoea* L. (Convolvulaceae). Taxon 24: 107–110.

Austin DF (1978) The *Ipomoea batatas* complex—I. Taxonomy. Bull Torrey Bot Club 105: 114–129.

Austin DF (1979) An infrageneric classification for *Ipomoea* (Convolvulaceae). Taxon 28: 359–361.

Austin D F (1982) Convolvulaceae. In: Harling G, Sparre B (eds.), Flora of Ecuador. Swedish Research Council, Stockholm, Sweden.

Austin D F (1997) Dissolution of *Ipomoea* series *Anisomerae* (Convolvulaceae). Taxon 28: 359-361.

Austin DF, Huáman Z (1996) A synopsis of *Ipomoea* (Convolvulaceae) in the Americas. Taxon 29: 501-502.

Choisy J D (1845) Convolvulaceae. In: De Candolle AP (ed.), Prodromus systematis naturalis regni vegetabilis, vol. 9, 323–462. Paris, France.

Cronquist A (1988) The Evolution and Classification of Flowering Plants. New York, The New York Botanical Garden press.

Ćwiklińska A, Zbigniew B, Bocianowski J, Dobrzycka A (2010) The usefulness of RAPD and AFLP Markers for determining genetic similarity in Rye (*Secale* L.) species and subspecies. Acta Biolog Cracov Ser Bot 52 (1): 19-25.

Das S, Mukherjee KK (1997) Morphological and biochemical investigations on *Ipomoea* seedlings and their species interrelationships. Ann Bot 79: 565-571.

Hallier H (1893b) Convolvulaceae Africanae. Bot Jahrb Syst 18: 81-160.

House HD (1908a) The North American species of the genus *Ipomoea*. Ann New York Acad Sci 18: 181-263.

Hussein Ebtissam HA, Abd-ala Nahla SM, Awad A, Hussien MS (2003) Genetic analysis in some *citrus* accessions using microstellities and AFLP based markers. Arab J Biotechnol 6(2): 180-201

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Mabberley DJ (1987) The Plant-book. Cambridge. Cambridge University Press.

Manos P S, Miller RE, Wilkin P (2001) Phylogenetic analysis of *Ipomoea*, *Argyreia*, *Stictocardia*, and *Turbina* suggests a generalized model of morphological evolution in morning glories. Syst Bot 26: 585-602.

Matos M, Pinto-Carnide O, Benito C (2001) Phylogenetic relationships among Portiguese rye based on isoenzyme, RAPD and ISSR markers. Hereditas 134: 299-336.

McDonald JA, Austin DF (1990) Changes and additions in *Ipomoea* section Batatas (Convolvulaceae). Brittonia 42: 116–120.

McDonald JA, TJ Mabry (1992) Phylogenetic systematics of New World *Ipomoea* (Convolvulaceae). Plant Syst Evol 180: 243-259

Miller RE, McDonald JA, Manos PS (2004) Systematics of *Ipomoea* subgenus *Quamoclit* (Convolvulaceae) based on ITS sequence data and a Bayesian phylogenetic analysis. Am J Bot 91: 1208-1218.

Miller RE, Rausher MD, Manos PS (1999) Phylogenetic systematics of *Ipomoea* (Convolvulaceae) based on ITS and *waxy* sequences. Syst Bot 24: 209-227.

Nowosielski J, Podyma W, Nowosielska D (2002) Molecular research on the genetic diversity of Pollish varieties and landraces of *Phaseolus coccineus* L. and *Phaseolus vulgaris* L. using the RAPD and AFLP method. Cell Mol Biol Let 7: 753-762.

Ogunwenmo KO (2003) Cotyledon morphology: an aid in identification of *Ipomoea* taxa (Convolvulaceae). Feddes Repert 114 (3-4): 198-203.

Porebski S, Baily LG, Baum R (1997) Modification of a CTAB DNA extraction protocol for plant containing high polysaccharide and polyphenol components. Plant Mol Biol Rep 15(1):8-15.

Potokina E, Tomooka N, Vaughan DA, Alexandrova T, Xu RQ (1999) Phylogeny of *Vicia* subgenus *Vicia* (Fabaceae) based on analysis of RAPDs and RFLP of PCR- amplified chloroplast genes. Gen Res Cr Evol 46: 149-161.

Potokina E, Vaughan DA, Eggi E, Tomooka N (2000) Population diversity of the *Vicia sativa* (Fabaceae) in the flora of the former USSR deduced from RAPD and seed protein analysis. Gen Res Cr Evol 47: 171-183.

Sokal R, Michener C (1958) A statistical evaluating systematic relationships. Unvi Kans Sci Bull 38: 1409-1438.

Vaillancourt A, Nkongolo KK, Michael P, Mehes M (2008) Identification, characterization, and chromosome locations of rye and wheat specific USSR and SCAR markers useful for breeding purposes. Euphytica 159: 297-306.

Van Ooststroom SJ (1953) Convolvulaceae. In: van Steenis CGGJ(ed.), Flora Malesiana, 458–489. Wolters-Noordhoff, Groningen, Netherlands.

Verdcourt B (1957) Typification of the subdivisions of *Ipomoea* L. (Convolvulaceae) with regard to the East African species. Taxon 6: 150-152.

Verdcourt B (1963) Convolvulaceae. In: Hubbard CE, Milne-Redhead E. (eds), Flora of tropical East Africa, 1–161. Whitefriars Press, London, UK.

Wilkin P (1999) A morphological cladistic analysis of the Ipomoeeae (Convolvulaceae). Kew Bull 54: 853-876.

Wei W, Fuju T, Shaoning C (2008) Optimizing protein extraction for plant tissues for enhanced proteomics analysis. J Sep Sci 31: 2032-2039.

Williams JGK, Hanafey MK, Rafalski JA, Tingey SV (1993) Genetic analysis using randomly amplified polymorphic DNA markers. Methods Enzymo 218: 704-740.