Bangladesh J. Plant Taxon. **22**(1): 1–8, 2015 (June)

MOLECULAR PHYLOGENETIC ANALYSES OF INTERNAL TRANSCRIBED SPACER (ITS) SEQUENCES OF NUCLEAR RIBOSOMAL DNA INDICATE MONOPHYLY OF THE GENUS *PHYTOLACCA* L. (PHYTOLACCACEAE)

M. AJMAL ALI[1], JOONGKU LEE[2], SOO-YONG KIM[2], SANG-HONG PARK[2,3] AND FAHAD M.A. AL-HEMAID

Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia

Keywords: ITS; nrDNA; Phytolaccaceae; Phylogeny.

Abstract

Relationships within the family Phytolaccaceae *sensu lato* were examined based on internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA (nrDNA). The study revealed *Phytolacca* L. as taxonomically the most difficult genus in the family with completely unknown phylogeny. Molecular evidence was used from nrDNA ITS sequences of about 90% of the species for maximum parsimony analyses, and the molecular phylogenetic analyses defined a monophyletic *Phytolacca*. This first molecular phylogenetic study of *Phytolacca* concludes that the relationships among the species within the genus do not show harmony with the generic classification based on morphology. These results set the stage for a more detailed phylogenetic analysis of *Phytolacca*.

Introduction

The angiosperm family Phytolaccaceae *sensu lato* comprises a weedy, and polyphyletic genera (APGIII, 2009) of largely tropical and subtropical plants that have been placed, almost without exception, in Centrospermae under either the order Chenopodiales or Caryophyllales (Nowicke, 1969). The genus *Phytolacca* L. (family Phytolaccaceae) is commonly known as '*pokeweeds*' comprises about 20 species (Nowicke, 1969) of perennial herbs, shrubs and trees, nearly cosmopolitan, mostly native to South America, with a few species in Africa and Asia (Shu, 2003). The genus *Phytolacca* possess alternate, simple leaves, pointed at the end, with entire or crinkled margins; the leaves can be either deciduous or evergreen; the stems are green, pink or red; the flowers are greenish-white to pink, produced in long racemes at the ends of the stems; they develop into globose berries 4−12 mm in diameter, green at first but dark purple to black after ripening (Nowicke, 1969).

The generic name is derived from the Greek word *phyton*, meaning plant, and the Latin word *lacca*, meaning a red dye (Umberto, 2000). Phytolaccatoxin and phytolaccigenin, which are poisonous, are present in many species of the genus *Phytolacca*. The active principles for analgesic, anti-inflammatory, bactericidal, fungicidal, mitogenic and molluscicide action have been reported from several species of *Phytolacca* (Hernández *et al*., 2013). The active principles have also been found in methanolic extracts of fruit of *P. tetramera* Hauman, which is a source of saponins with fungicidal action (Escalante *et al*., 2002; Santecchia *et al*., 2002). The African soapberry plant, *P. dodecandra* L'Her., locally called *endod*, produces a range of triterpenoid

[1]Corresponding author. Email: alimohammad@ksu.edu.sa
[2]International Biological Material Research Center, Korea Research Institute of Bioscience and Biotechnology, 125 Gwahak-ro, Yuseong-gu, Daejeon 305-806, South Korea. Email: joongku@kribb.re.kr
[3]Present address: Division of Plant Management, National Institute of Ecology, Choongnam, Secheon-gun, Maseo-myeon, Geumgang-ro, 1210, 325-813, South Korea

saponins possessing very potent and useful biological properties, including antifungal, anti-protozoan, spermicidal and insecticidal activities (Lemma *et al.*, 1979). Because of its fast-growing nature, *P. dioica* L. is frequently planted as a shade tree in the tropics. Nowicke (1969) reported the use of berries and the young sprouts, and leaves of some species of *Phytolacca* as an adulterant of red wine and poke salad, respectively.

The generic composition and phylogeny of Phytolaccaceae have long been controversial. The phylogenetic studies have substantially added new results to our knowledge of phylogeny of the family Phytolaccaceae (Brown and Varadarajan, 1985; Downie *et al*., 1997; Cuenoud *et al*., 2002; Lee *et al*., 2013). Nowicke (1969) referred *Phytolacca* as the most difficult genus in the family Phytolaccaceae *sensu lato*, and classified under three subgenera and six sections (Table 1). However, comprehensive information on phylogeny of the genus *Phytolacca* is lacking.

Table 1. Infrageneric classification of the genus *Phytolacca* L. by Nowicke (1969). Taxa included in the present study are marked with asterisk.

Subgenus	Section	Species
Pircunia	*Pircunia*	**Phytolacca acinosa* Roxb.
		**P. heptandra* Retz.
	Pircunioides	**P. dodecandra* L'Her.
Pircuniopsis	*Pircuniophorum*	**P. sanguinea* H. Walter
		**P. rugosa* Br. & Bouche
		P. chilensis (Miers *ex* Moq.) H. Walter
	Pircuniopsis	**P. tetramera* Hauman
		**P. dioica* L.
		**P. weberbaueri* H. Walter
Phytolacca	*Phytolacca*	**P. icosandra* L.
		**P. octandra* L.
		**P. thyrsiflora* Fenzl *ex* J.A. Schmidt
		**P. heterotepala* H. Walter
		**P. meziana* H. Walter
		**P. rivinoides* Kunth & Bouchk
		**P. purpurascens* A. Br. & Bouche
		**P. brachystachys* Moq.
		**P. bogotensis* H.B.K.
		**P. americana* L.
	Phytolaccoides	*P. pruinosa* Fenzl.

During the last two decades, the internal transcribed spacers (ITS) sequences of nuclear ribosomal DNA (nrDNA) have gained wide attention, not only because of its efficacy in understanding phylogeny of the plants at lower taxonomic level, but also to be considered as the most conserved markers, because, even after facing criticism of its utility, this marker stands parallel to the smartest genes available for the molecular phylogeny and plant DNA barcoding (Ali *et al*., 2013, 2014). The nrDNA ITS sequences have, therefore, provided a useful source of phylogenetic information in many genera and families of flowering as well as non-flowering plants (Ali *et al*., 2015), including Phytolaccaceae (Lee *et al*., 2013). Hence, as such the nrDNA ITS are appropriate to analyze for the genus *Phytolacca* too.

Materials and Methods

Taxa examined

Twenty taxa representing five sections (i.e. *Phytolacca*, *Pircunia*, *Pircunioides*, *Pircuniophorum* and *Pircuniopsis*) under three subgenera (i.e. *Phytolacca*, *Pircunia* and *Pircuniopsis*) of *Phytolacca* and two outgroup taxa (namely *Petiveria alliacea* F. Muell. and *Monococcus echinophorus* L.) were sampled from specimens deposited in the Herbarium of University of California (UC), Berkeley, USA (Table 2). *Petiveria alliacea* and *M. echinophorus* were chosen as outgroup taxa because of their close affinity to *Phytolacca* (Lee *et al*., 2013).

Table 2. Accessions of the genus *Phytolacca* L. examined in this study.

Taxon	Voucher	Locality	GenBank Acc. No.
Ingroup			
Phytolacca acinosa	*M.T. Yu et al. s.n.*	Tibet	EU239681
P. americana	*D.W. Taylor 7922* (UC/JEPS)	California, USA	JX232573
P. bogotensis	*H.L. Mason 23712* (UC)	Colombia, South America	KM491868
P. brachystachys	*F.R. Fosberg 9004* (UC)	Hawaiian Island, USA	KM491869
P. dioica	*Marquez et al. 38645* (UC)	Mexico, North America	JX232571
P. dodecandra	*R.E.S. Tanner 572* (UC)	Tanganyika, Africa	KM491870
P. heptandra	*L.C.C. Libeoberg 5830* (UC)	South Africa	KM491871
P. heterotepala	*Sally Pugh s.n.* (UC)	California, USA	KM491872
P. icosandra	*J.H. Beaman* 2749 (UC)	Mexico, North America	JX232570
P. meziana	*Edward 89055* (UC)	Mexico, North America	KM491873
P. octandra	*G.J. Martin 468* (UC)	Oaxaca, Mexico, North America	KM491874
P. purpurascens	*W.H. Wagher 5027* (UC)	Hawaiian Island, USA	KM491875
P. rivinoides	*J. Nowicke 874* (UC)	Panama, Central America	KM491876
P. rugosa	*A. Weston 5981* (UC)	Costa Rica, Central America	KM491877
P. sanguinea	*J. H. Langenneim* 3576 (UC)	Colombia, South America	KM491878
P. tetramera	*N. Tur 1329* (UC)	Argentina, South America	KM491879
P. thyrsiflora	*C. Chung 4248* (UC)	California, USA	KM491880
P. weberbaueri	*C.H. Dodson 6481* (UC)	Ecuador, South America	KM491881
Outgroup			
Monococcus echinophorus	*Franch 1130* (UC)	New Caledonia	JX232579
Petiveria alliacea	*C.A. Purpus 2272* (UC)	Mexico, North America	JX232580

Molecular methods

Total genomic DNA was extracted by use of the DNeasy Plant Mini Kit from Qiagen (Valencia, CA, USA). The nrDNA ITS regions were amplified using the primers ITS1 and ITS4 (White *et al*., 1990). The DNA amplification for 35 cycles was carried out through PCR. Initial denaturation was carried out at 94°C for 5 min, followed by denaturation at 94°C for 1 min, annealing at 48°C for 1 min, extension at 72°C for 1 min, and the final extension at 72°C for 5 min. The PCR products were purified using SolGent PCR Purification kit-Ultra (SolGent, Daejeon, South Korea). For sequencing, the Big Dye Terminator chemistry (ABI) and an ABI 3100 Avant capillary sequencer were used. All sequences were BLAST-searched in GenBank.

Sequence alignments and phylogenetic analyses

Sequences were edited using the ABI Sequence Navigator (Perkin-Elmer/Applied Biosystems, USA). Sequence alignment was performed using Clustal X version 1.81 (Thompson *et al*., 1997), and subsequently adjusted manually using BioEdit (Hall, 1999). Information on sequence alignment can be made available from the corresponding author. Data were exported as a nexus file and subsequently analyzed using Maximum Parsimony (MP) in PAUP* 4.0b10 (Swofford, 2002). The MP analysis was performed with the following settings: heuristic search algorithms with tree bisection reconnecting (TBR) branch swapping, MULPARS in effect, all characters equally weighted, gap treated as missing characters, zero-length branches collapsed, random addition sequence set to 1000 replicates, and branch swapping limited to 10,000,000 rearrangements per replicate. When maximum parsimony trees were saved, a strict consensus tree was constructed. Bootstrap analysis was performed using 1000 replicates, with the random addition sequence set to 10, and branch swapping limited to 10,000,000 rearrangements per replicate.

Results and Discussion

Sequence characteristics

The combined length of the entire ITS region (ITS1, 5.8S and ITS2) from taxa analyzed in the present study ranged from 609−631 nucleotides (nt). The length of the ITS1 region and GC contents ranged from 220−232 nt and 56%−63%, the 5.8S gene was 166 nt long, the length of the ITS2 region and the GC content ranged from 221−235 nt and 55%−63%, respectively. Data matrix has a total number of 654 nt characters of which 423 nt characters were constant, 88 nt characters were variable but parsimony-uninformative, and 143 nt characters were parsimony-informative.

Phylogenetic analyses

The parsimony analysis of the entire ITS region resulted a total number of four maximally parsimonious trees (MPTs) with a total length of 252 steps, a consistency index (CI) of 0.7110, a homoplasy index (HI) of 0.2890, rescaled consistency index (RC) of 0.5361 and a retention index (RI) of 0.7540 (Fig. 1).

The rooted bootstrap strict consensus parsimony tree (Fig. 1) revealed that the monophyly of *Phytolacca* species is supported with 100% parsimony bootstrap support (BS). All trees resulted from the analysis of ITS sequences resolve three major clades (Clades I−III, Fig. 1). The Clade I consists of *P. heptandra*, the Clade II (96% BS) consists of members of subgenus *Pircuniopsis* (i.e. *P. dioica, P. tetramera* and *P. weberbaueri*), and the Clade III (56% BS) consists of [*P. americana* + (*P. dodecandra* - *P. acinosa* - *P. purpurascens*) + *(P. rivinoides* - {*P. rugosa* - *P. thyrsiflora* + *P. icosandra* - *P. brachystachys* - *P. heterotepala* + *P. octandra* - *P. meziana* - *P. sanguinea* - *P. bogotensis*})].

The generic composition of *Phytolacca* has long been controversial principally due to common occurrence of intraspecific variability and hybridization (Fassett and Sauer, 1950; Sauer, 1951). Walter (1909) placed 26 species of *Phytolacca* into three subgenera based on the degree of connation of the carpels: free, connate at the base with the apices free, or completely united carpels. The subgenus *Pircunia* (Moq.) H. Walter contains *P. heptandra* Retz., *P. esculenta* van Houtte, *P. acinosa* Roxb., *P. latbenia* (Buch.-Ham.) H. Waiter and *P. cyclopetala* H. Walter under the Sect. *Pircuniastrum* Moq. characterized by hermaphroditic flowers, and *P. dodecandra*, *P. goudotii* Briq. and *P. nutans* H. Walter under the Sect. *Pircunioides* H. Walter characterized by dioecious plants. The subgenus *Pircuniopsis* H. Walter characterized by carpels connate at the base with the apices free, contains a hermaphroditic group, the Sect. *Pircuniophorum* H. Walter,

with three species, *P. chilensis* (Miers *ex* Moq.) H. Walter, *P. rugosa* Br. & Bouche and *P. sanguinea* H. Walter, and the Sect. *Pseudolacca* Moq., with two dioecious species, *P. dioica* and *P. weberbaueri* H. Walter. The subgenus *Euphytolacca* Moq., the largest group characterized by carpels completely united contains a very large hermaphroditic flower, has the Sect. *Phytolaccastrum* H. Walter with *P. americana* L., *P. australis* Phil., *P. brachystachys* Moq., *P. heterotepala* H. Walter, *P. icosandra* L., *P. meziana* H. Walter, *P. micrantha* H. Walter, *P. octandra* L., *P. polyandra* Batalin, *P. purpurascens* A. Br. & Bouche, *P. rivinoides* Kunth & Bouchk and *P. thyrsiflora* Fenzl *ex* J.A. Schmidt, and a monotypic dioecious Sect. *Phytolaccoides* H. Walter containing *P. pruinosa* Fenzl. Later on Heimerl (1934) noted approximately 35 species of *Phytolacca*; however, Nowicke (1969) did not consider the names assigned to hybrid origin. Nowicke (1969) recognized a total of 20 species in the genus *Phytolacca* and classified them into

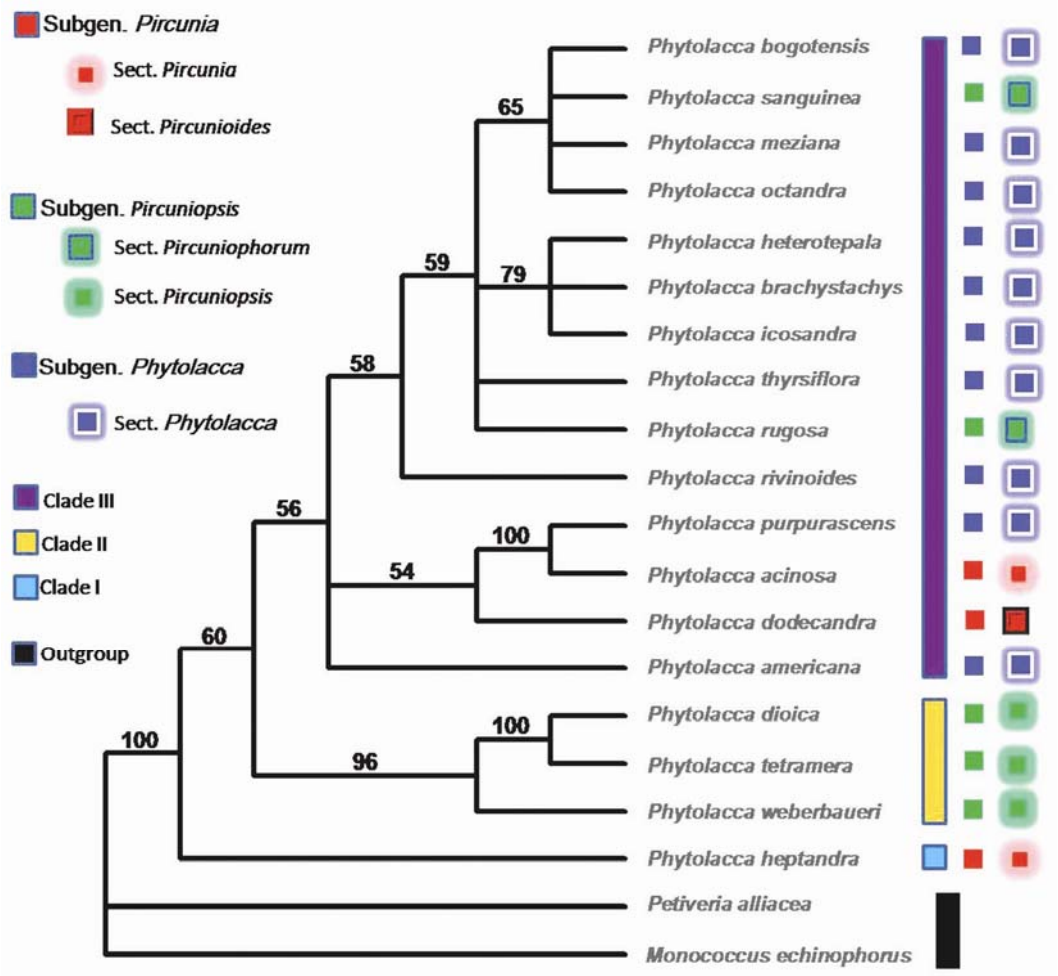

Fig. 1. The bootstrap strict consensus of four maximally parsimonious trees of *Phytolacca* L. species based on the ITS sequence with gaps being treated as missing data (252 steps, CI= 0.71, HI= 0.28, RC= 0.53 and RI= 0.75). Bootstrap values greater than 50% in 1000 replicates are shown above lines.

three subgenera, i.e. *Pircunia* (carpels completely free), *Pircuniopsis* (carpels more or less united) and *Phytolacca* (carpels completely united, the styles more or less connivent). Based on characteristic of flowers, Nowicke (1969) divided the subgenus *Pircunia* into two sections: *Pircunia* (*P. acinosa* and *P. heptandra*) and *Pircunioides* (*P. dodecandra*); *Pircuniopsis* into two sections: *Pircuniophorum* (*P. chilensis*, *P. rugosa* and *P. sanguinea*) and *Pircuniopsis* (*P. dioica*, *P. tetramera* and *P. weberbaueri*); and *Phytolacca* into two sections: *Phytolacca* (*P. americana*, *P. bogotensis* H.B.K., *P. brachystachys, P. heterotepala, P. icosandra, P. meziana, P. octandra, P. purpurascens, P. rivinoides* and *P. thyrsiflora*) and *Phytolaccoides* (*P. pruinosa*).

In our study, the Clade I, which occupies independently at the basal position in MPT, consists of only *P. heptandra. Phytolacca heptandra* was treated along with *P. esculenta*, *P. acinosa*, *P. latbenia* and *P. cyclopetala* under the subgenus *Pircunia*, Sect. *Pircuniastrum* (Walter, 1909). Nowicke (1969) also treated *P. heptandra* along with *P. acinosa* under the subgenus *Pircunia* Sect. *Pircunia.*

The Clade II (96% BS) consists of *P. dioica, P. tetramera* and *P. weberbaueri*. In Walter's (1909) classification *P. dioica, P. tetramera* and *P. weberbaueri* are under the subgenus *Pircuniopsis*, Sect. *Pseudolacca*. Nowicke (1969) also treated these under the subgenus *Pircuniopsis* Sect. *Pircuniopsis.*

The Clade III (56% BS) consists of members mainly belonging to subgenus *Phytolacca* Sect. *Phytolacca* (i.e. *P. americana, P. brachystachys, P. bogotensis*, *P. heterotepala, P. icosandra, P. meziana*, *P. octandra*, *P. purpurascens*, *P. rivinoides* and *P. thyrsiflora,*), and those treated under subgenus *Pircunia* Sect. *Pircunioides* (*P. dodecandra*), subgenus *Pircunia* Sect. *Pircunia* (*P. acinosa*) and subgenus *Pircuniopsis* Sect. *Pircuniopsis* (*P. sanguinea* and *P. rugosa*) of Nowicke (1969). In the Walter (1909) treatment, *P. americana, P. brachystachys, P. heterotepala, P. icosandra, P. meziana, P. octandra*, *P. purpurascens*, *P. rivinoides* and *P. thyrsiflora,* were treated under subgenus *Euphytolacca* Sect. *Phytolaccastrum*, while *P. dodecandra* under subgenus *Pircunia* Sect. *Pircunioides, P. acinosa* under subgenus *Pircunia* Sect. *Pircuniastrum,* and *P. sanguinea* and *P. rugosa* under subgenus *Pircuniopsis* Sect. *Pircuniophorum.*

The Clade III further bifurcates into four subclade, namely (IIIa) *P. americana*; (IIIb) *P. acinosa, P. dodecandra* and *P. purpurascens*; (IIIc) *P. rivinoides*; and (IVd) *P. bogotensis*, *P. brachystachys, P. heterotepala, P. icosandra, P. meziana, P. octandra, P. rugosa, P. sanguine* and *P. thyrsiflora*. Under the subclade IVd, *P. bogotensis*, *P. meziana* & *P. sanguinea*, and *P. brachystachys*, *P. heterotepala* & *P. icosandra*, are grouped together, and these two groups show polytomic relationships with *P. rugosa* and *P. thyrsiflora*.

It is interesting to note that *P. rugosa* and *P. sanguinea*, [subgenus *Pircuniopsis* Sect. *Pircuniopsis* of Nowicke (1969) and subgenus *Pircuniopsis* Sect. *Pircuniophorum* of Walter (1909)] are nested within the Clade III, while the other members, namely *P. dioica, P. tetramera* and *P. weberbaueri* [subgenus *Pircuniopsis* Sect. *Pseudolacca* of Walter (1909) and subgenus *Pircuniopsis* Sect. *Pircuniopsis* of Nowicke (1969)] form a separate Clade II with strong bootstrap support (96% BS).

Phytolacca acinosa [subgenus *Pircunia* Sect. *Pircuniastrum* of Walter (1909) and subgenus *Pircunia* Sect. *Pircunia* of Nowicke (1969)] and *P. dodecandra* [subgenus *Pircunia* Sect. *Pircunioides* of Walter (1909) and subgenus *Pircunia* Sect. *Pircunioides* of Nowicke (1969)] grouped together with *P. purpurascens* (54% BS), while *P. heptandra* [Subgen. *Pircunia* Sect. *Pircuniastrum* of Walter (1909) and subgenus *Pircunia* Sect. *Pircunia* of Nowicke (1969)] occupies basal most position in the MPTs as a separate clade.

In conclusion, this is the first inclusive study using molecular nrDNA ITS sequences to estimate phylogenetic relationships of *Phytolacca*. It is clearly evident that the phylogenetic trees

resulting from the analysis of nrDNA ITS sequences are strongly supported as a monophyletic group (100% BS). However, the relationships among the species within the genus do not show harmony with the previous generic classification based on morphology. In the present analysis, a total number of 143 out of 654 (21%) sites of sequence data set were phylogenetically informative, so further sampling of additional taxon and addition of more regions are needed for the robust phylogeny of the genus *Phytolacca*. We herein based on the present analysis hypothesize that the intraspecific classification of *Phytolacca* should be recircumscribed into subgenus *Phytolacca* (*P. acinosa*, *P. americana*, *P. bogotensis*, *P. brachystachys*, *P. dodecandra*, *P. heterotepala*, *P. icosandra*, *P. meziana*, *P. octandra*, *P. purpurascens*, *P. rivinoides*, *P. rugosa*, *P. sanguinea* and *P. thyrsiflora*), subgenus *Pircuniopsis* (*P. dioica*, *P. tetramera* and *P. weberbaueri*), and *P. heptandra* should be treated under an independent subgenus. This treatment, as a hypothesis, however, needs testing and further data would help to clarify their true intraspecific affinities.

Acknowledgements

The first and second authors provided an equal contribution to this paper. Grant support (#2011-00402) from the Ministry of Education, Science and Technology, Government of South Korea to the second author is thankfully acknowledged. Authors thank to the curators of The University and Jepson Herbarium (UC), University of California, Berkeley, USA for providing plant materials for the study. The first and last authors acknowledge research supported by the King Saud University, Deanship of Scientific Research, College of Science, Research Center.

References

Ali, M.A, Al-Hemaid, F.M., Choudhary, R.K., Lee, J., Kim, S.Y. and Rub, M.A. 2013. Status of *Reseda pentagyna* Abdallah & A.G. Miller (Resedaceae) inferred from combined nuclear ribosomal and chloroplast sequence data. Bangladesh J. Plant Taxon. **20**(2): 233–238.

Ali, M.A., Gábor, G., Norbert, H., Balázs, K., Al-Hemaid, F.M.A., Pandey, A.K. and Lee, J. 2014. The changing epitome of species identification - DNA barcoding. Saudi J. Biol. Sci. **21**(3): 204–231.

Ali, M.A., Pandey, A.K., Al-Hemaid, F.M.A., Lee, J., Pandit, B., Kim, S.Y., Gyulai, G. and Rahman, M.O. 2015. Nuclear Sequences in Plant Phylogenetics. *In*: Ali, M.A., Gábor, G. and Al-Hemaid, F.M.A. (Eds), Plant DNA Barcoding and Phylogenetics. Lambert Academic Publishing, Germany, pp. 37–52.

APG III 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. **161**:105–121.

Brown, G.K. and Varadarajan, G.S. 1985. Studies in Caryophyllales I: Re-evaluation of classification of Phytolaccaceae *s.l.* Syst. Bot. **10**: 49–63.

Cuenoud, P., Savolainen, V., Chatrou, L.W., Powell, M., Grayer, R.J. and Chase, M.W. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbc*L, *atp*B, and *mat*K DNA sequences. Am. J. Bot. **89**: 132–144.

Downie, S., Katz-Downie, D. and Cho, K. 1997. Relationships in the Caryophyllales as suggested by phylogenetic analyses of partial chloroplast DNA ORF2280 homolog sequences. Am. J. Bot. **84**: 253–273.

Escalante, A.M., Santecchia, C.B., López, S.N., Gattuso, M.A., Gutiérrez, A., Delle Monache, F., González Sierra, M. and Zacchino, S.A. 2002. Isolation of antifungal saponins from *Phytolacca tetramera*, an Argentinean species in critic risk. J. Ethnopharmacol. **1**: 29–34.

Fassett, N. and Sauer, J. 1950. Studies of variation in the weed genus *Phytolacca*. 1. Hybridizing species in northeastern Colombia. Evolution **4**: 332–339.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. **41**: 95–98.

Heimerl, A. 1934. Phytolaccaceae. *In*: Engler, A. and Prantl, K. (Eds), Die naturlichen Pflanzenfamilien. 2nd edition. Leipzig, Wilhelm Engelmann, **4**: 135–164.

Hernández, M., Murace, M., Ringuelet, J., Petri, I., Gallo, D. and Arambarri, A. 2013. Effect of aqueous and alcohol extracts of *Phytolacca tetramera* (Phytolaccaceae) leaves on *Colletotrichum gloeosporioides* (Ascomycota). Bol. Soc. Argent. Bot. **48**(2): 201–209.

Lee, J., Kim, S.Y., Park, S.H. and Ali, M.A. 2013. Molecular phylogenetic relationships among members of the family Phytolaccaceae *sensu lato* inferred from internal transcribed spacer sequences of nuclear ribosomal DNA. Genet. Mol. Res. **12**(4): 4515–4525.

Lemma, A., Heyneman, D. and Kloos, H. (Eds). 1979. Studies on the molluscicidal and other properties of the endod plant, *Phytolacca dodecandra*, with special emphasis on the epidemiology of Schistosomiasis in Ethiopia and the possibility of localized control using endod as a molluscicide on a community self-help basis. Addis Ababa University, Ethiopia; University of California, San Francisco.

Nowicke, J.W. 1969. Palynotaxonomic study of the Phytolaccaceae. Ann. Miss. Bot. Gard. **55**: 94–363.

Santecchia, C., Escalante, A., Gattuso, M., Zacchino, M., Gutierrez Ravelo, A., Delle Monache, F. and Gonzalez Sierra, F. 2002. *Phytolacca tetrámera*, una fuente de saponinas triterpenoides. Revista Lat. Am. Quim. **28**: 246–247.

Sauer, J. 1951. Studies of variation in the weed genus *Phytolacca*. II. Latitudinally adapted variants within a North American species. Evolution **5**: 273–279.

Shu, S.L. 2003. *Phytolacca*. *In*: Wu, Z.Y., Raven, P.H. and Hong, D.Y. (Eds), Flora of China. Vol. **5**. Science Press, Beijing and Missouri Botanical Garden Press, St Louis, pp. 435–436.

Swofford, D.L. 2002. PAUP: Phylogenetic Analysis using Maximum Parsimony (and Other Methods). Version 4.0b 10. Sinauer, Sunderland, Massachusetts.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997. The Clustal X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **24**: 4876–4882.

Umberto, Q. 2000. CRC World Dictionary of Plant Names: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology. CRC press Taylor & Francis, New York, pp. 1–2065.

Walter, H. 1909. Phytolaccaceae. *In*: Enler, A. Pflanzenr. IV **83** (Heft 39): 1–154.

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninksky, J.J. and White, T.J. (Eds), PCR Protocols: A Guide to Method and Amplifications. Academic Press, San Diego, California, pp. 315–322.

(Manuscript received on 22 March 2015; revised on 29 April 2015)