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Phylogenetic Study of the Genus *Phalaris* L. (Poaceae) based on Nuclear Internal Transcript Region (ITS) in Iraq

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ABSTRACT

This study was conducted to investigate the genetic genotype pattern for the five species of the *Phalaris* (Poaceae) family in Iraq. By using polymerase chain reaction (PCR) and sequencing of the internal transcribed spacer (ITS) of nuclear ribosomal DNA technique. *Anthoxanthum monticola* is used as an out group. The result of the analysis of sequences and drawing the phylogenetic tree showed cluster analysis of the *Phalaris* species. The *P.brachystachys* form the basal lineage in the dendrogram. Sister clade to *P.brachystachys* is *P.arundinacea*, the three other *P.tuberosa*, *P.paradoxa*, and *P.minor* descendent in one main clade which divides into two secondary clades. *P.tuberosa* subclade and *P.paradoxa* & *P.minor* subclade. Finally, the monophylly of each clade is well supported.

KEYWORDS

Phylogenetic, *Phalaris*, ITS, Iraq.

Introduction

Poaceae encompass 10230 species throughout the world that scattered on 678 genera (Singh, 2010). Iraq has 251 species dispersed on 92 genera (Al-Rawi, 1964). In Iran, Rechinger (1970) pointed to that 5 species of the genus *Phalaris* existing, whereas Ghahreman and Attar (1999) specified 4 species. In Turkey, Baytop (1985) indicated 8 species of the genus *Phalaris*. Rechinger (1964) stated 3 species of the genus *Phalaris* in the low lands of Iraq, while (Al-Rawi, 1964) indicated 4 species In Iraq, furthermore, Bor (1968) mentioned 22 species and Ridda and Daood (1982) declared 5 species. Khalaf, (1980) and Faris (1983) didn't mention any species in Sinjar and Piramagrun mountains respectively, Ahmad (2013) mentioned 3 species in Hawraman mountain. Fatah (2003), Ahmed (2010), Hameed (2016) and Darwesh (2017) stated 1 species in Haibat Sultan, Gomaspan, Hujran, and Choman respectively.

Analysis of the morphological data places Poaceae in an unresolved relationship relative to several other taxa, including *Joinvillea* and *Ecdeiocolea*, while analysis of the molecular and combined data resolves *Ecdeiocolea* as a sister of Poaceae, with *Joinvillea* the sister of this group. (Fabian, *et. al*., 2003).

A strongly supported clade of ((Eriachneae, *Isachne*) *Micraira*) was recovered as a sister subfamily to Arundinoideae and excluded from Panicoideae. *Arundinaria* was strongly united with Bambusoideae. The position of *Streptogyna* was weakly supported among Ehrhartoideae and is still unresolved. (Melvin, *et. al*., 2007). All grass species appear to have an incompatibility mechanism controlled by two unlinked loci, *S* and *Z*. Sequencing of the PCR-amplified *S* thioredoxin region from wheat, barley, rye and *Dactylis* revealed that this is a highly conserved gene with 94–97% sequence similarity with the corresponding *Phalaris S* gene. The conservation of sequence and ubiquitous expression of the gene across the grass family strongly suggest that the *S*-related gene is carrying out a significant biological function in the Poaceae. (Melvin, *et. al.*, 2007). The study of (Stephanie, *et. al.*, 2011) demonstrated a single origin of the x = 6 chromosome number and revealed the sister relationship of this lineage to the monophyletic $x = 7$ lineages. The clades recovered in the analyses display geographic affiliations and demonstrate diploid-polyploid associations. The current study intended to conduct a molecular study for the species of the genus *Phalaris* in Iraq.

Materials and Methods

1). Taxon Sampling

The plant taxa used in the present study were collected from the different districts of Iraq preserved in the Herbarium of College of Sciences, University of Baghdad. Five distinct taxa consist of Five ingroup taxa and one outgroup

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Anthoxanthum monticola (Bigelow) Veldkamp was used in the analysis because of documented close phylogenetic affinity to Phalaris (Voshell *et al.*, 2011).

2). DNA Extraction

Total DNA was extracted from the collected specimens. The extraction method was based on the CTAB protocol of - Doyle & Doyle (1990) with some modification (1X CTAB: 10 mL of 1.0 M Tris-HCl, PH 8; 4 mL of 0.5 M EDTA, PH 8; 28 mL of 5 M NaCl; 2% CTAB; 2 g PVP; and 158 ddH2O), the washing process of the DNA pellet has been conducted twice with 0.5 mL of 80% ethanol, then DNA was dissolved in 25 µl TE-buffer.

3). PCR and DNA Sequencing

The nuclear region ITS was amplified by using the primers ITS A and ITSB of (White *et al*., 1990) (Table 1). The primers were ordered from (IDT) company-Skokie, Illinois-USA. The total volume of amplification reactions was 25 µl and Master Mix made up of 10.8 µl of ddH2O, 2.5 µl ThermoPol reaction buffer, 2.5 µl MgCl2, 5 µl dNTPs, 2 µl template, 1 µl from each primer, 0.2 µl DNA polymerase (Taq polymerase). The PCR-Thermal cycler started with 2 min for initial denaturation at 94 C° followed by 39 cycles: 30 sec. ITS1 for denaturation at 94 C°; 60 sec. for annealing at 52 C°, Extension 90 sec at 72 C° The resultant PCR products were checked on 1.5% agarose gel run in TAE buffer. The gel was stained with EtBr and photographed under a UV transilluminator.

Table 1. List of primers and their sequences that have been used in the study

Primer	Direction	Sequence 5'---- 3'	Resources
ITS A	Forward	5'GGAAGGAGAAGTCGTAACAAGG 3	(white *et al*., 1990)
ITS B	Reverse	5'-CTTTTCCTCCGCTTATTGATATG-3	(white *et al.*, 1990)

PCR products were purified by using Kits (Promega company-Madison-USA). The purified PCR products were sent to the National Science and Technology Development Agency (NSTDA) in Thailand for sequencing.

4). Sequence Alignment

Sequences were manually aligned with Quick Align version 1.6.0. (Muller,2004), there were accessions for each ITS, including the out-group species.

5). Phylogenetic Analyses

The reconstruction of the phylogenetic relationships was based on UPGMA methods. UPGMA analysis was performed by using Bio Editor, Version 7.o.4.1. (Hall,2001) Using heuristic search with 100 replicates of random taxon additions, Tree-Bisection-Reconnection (TBR) branch swapping, MulTrees on, and steepest descent off was performed. The maximum numbers of saved trees were 100 for each replicate. The bootstrap values were calculated from 100 replicates.

Results and Discussion

Phylogenetic relationships within *Phalaris* species, the alignment of the ITS data sets was 956 characters in length. We also used different methods for analysis *Phalaris* genus in Iraq and our findings got the best phylogenetic dendrogram through UPGMA analysis. Our phylogenetic study demonstrates unequivocally the origin of species belong the *phalaris* genus in Iraq and If we follow Figure 1, which shows the phylogenetic tree based on UPGMA analysis methods, we observe that *P. brachystachys* had its clade at the base of the phylogenetic tree, which consider as a sister with the clade of the *P. aurndinacea* with good bootstrapping value (bs =88%), while the three other species in the genetic dendrogram consider a sister with *P. aurndinacea* clade (bs = 74%), the remaining three species included in one main clade and distributed in a secondary subclade. The *P. tuberosa* clade descended in a single clade in the base of these main secondary clades with a full bootstrapping value (bs = 100%) with the clade that gathered the *P. paradox* and *P. minor*, Our current results on the distribution of *Phalaris* species with this topology by UPGMA analysis did not agree with the results of (Al -Edhari,2017) when studied of *Phalaris* by *matK*

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gene sequencing where the species differed in their distribution in the phylogenetic tree that he drew depending on a Bayesian analysis.

It is observed that *Phalaris* species are closely related to each other with a high degree of reliability of their data. This confirms the common monophyletic of the *Phalaris* species, as well as the great similarity in the other taxonomic characters such as morphological and anatomical especially in *Phalaris* and generally in Poaceae. This is convenient with the results of the study of (Voshell & Hilu,2014) Which confirmed the monophyletic of 21 species belonging to the *Phalaris* spread in different regions of the world despite giving them different genetic patterns.

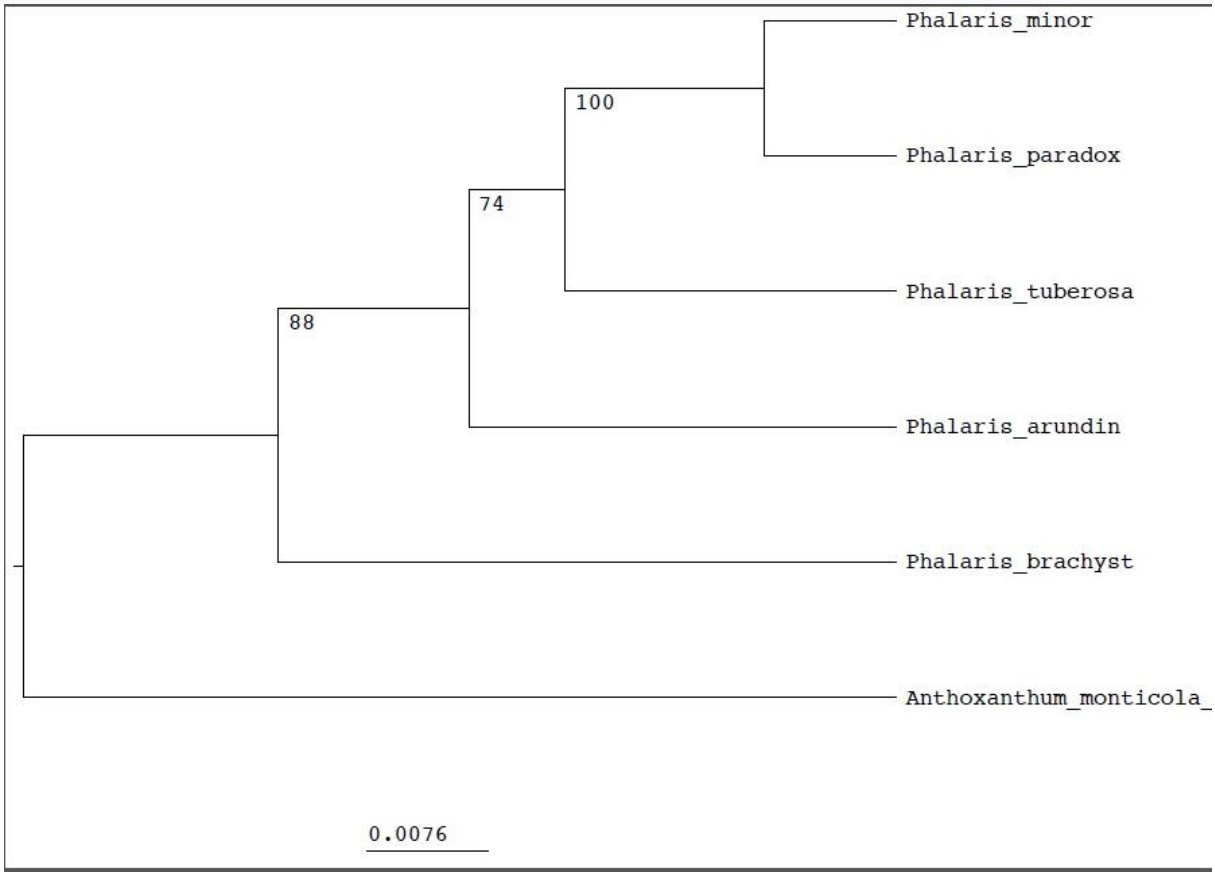

Fig. 1. Strict consensus tree of most UPGMA trees resulting from phylogenetic analysis of the nr DNA ITS. The number of branches indicates bootstrap support

Conclusion

In the present study three clade within the species of *Phalaris* were identified in nuclear ITS region. The *P. paradox* and *P. minor* in the same sub clade, while the *P. brachystachys, P.aurndinacea* & *P. tuberosa* arranged alone in a single clade individually with high reliability of data.

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