RESEARCH PAPER

Molecular Systematic Study for the Two Genera Lophochloa Rchb. & Schismus P. Beauv. (Poaceae) in Iraq

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ABSTRACT:
This study was conducted to identify genetic genotype patterns for the species *Lophochloa berythea* (Boiss. et Blanche) Bor, *L. obtusiflora* (Boiss.) Gontsch., *L. phleoides* (Vill.) Rchb., *L. pumila* (Desf.) Bor, *Schismus arabicus* Nees. and *S. barbatus* (L.) Thell. from family Poaceae in Iraq by using polymerase chain reaction (PCR) and sequencing of chloroplast gene *trnL*-F technique. The result of the analysis of sequences and drawing the phylogenetic tree showed cluster analysis of the species and descendant species of the genus *Schismus* in a single clade alone in the base of phylogenetic tree and associated as a sister clade to the *Lophochloa* clade. The *Lophochloa* clade also subdivided into two secondary clades. The first one gathered the *L. phleoides* and *L. obtusiflora* while the second gathered *L. pumila* and *L. berythea* with high support (bootstrapping) for all clades in cladogram. It seems that the sequences technique of chloroplast *trnL*-F gene has highly efficient in determination, separation and comparison between the two genera under study and support their descendant from one common ancestral origin.

KEY WORDS: Molecular, Lophochloa, Schismus, Poaceae, Iraq.
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1. INTRODUCTION:

Poaceae family contain 10230 species throughout the world that distributed on 678 genera (Singh, 2010). In Iraq contain 251 species distributed on 92 genera (Al-Rawi, 1964). In Turkey, Dogan and Tan (1985) indicated 4 species of the genus *Lophochloa* (= *Rostraria* Trin.) and 1 species of the genus *Schismus* which is *S. arabicus* In Iran, Rechinger (1970) pointed out that 4 species of the genus *Lophochloa* and 2 species of the genus *Schismus* present. Rechinger (1964) mentioned 3 species of the genus *Lophochloa* and 2 species of the genus *Schismus* in the low lands of Iraq. In Iraq, Al-Rawi (1964) indicated 2 species for each of the two genera, while Ridda and Daood (1982) and Bor (1968) mentioned 4 species of the genus *Lophochloa* (which are: *L. berythea*, *L. obtusiflora*, *L. phleoides* and *L. pumila*) and 2 species of the genus *Schismus* (which are: *S. arabicus* and *S. barbatus*). Each of Khalaf (1980) and Faris (1983) mentioned 1 species of the genus *Lophochloa* which is *L. phleoides* in Sinjar and Piramagrun mountains respectively, while Miller et al., 2015) did not state any species of the two genera in Peramagroon mountain. Chakravarty (1976) stated that the species *L. phleoides* which present in Iraq use as a grazing grass for animals, it undoubtedly contributes an important fodder, especially in summer months. Its value as a spring forage has also been stressed upon by many field collectors.
During the past decade, molecular genetics variation had played an essential role in separation living organism and enabling to understand speciation & evolutionary processes (Baumung et al., 2004).

Recently molecular systematic sciences have been developed rapidly in term of revolution informatics. The uses of DNA sequences which mean determination of Nucleotides Adenine = A, Cytosine = C, Guanine = G & Thymine = T in a particular gene or region of the DNA of a taxon under study. Other Marker such as DNA restriction sites, Alloenzyme, Microsatellites, RAPDS (Random Amplified Polymerase DNA) & AFLPS (Amplified Fragment Length Polymerase) is a typical modern and reliable methods in inferring phylogenetics relationships in distinguish between living organism related to each other (Simpson, 2006).

Mathematical principals and computer program can be used to analyze the differences in the nucleotide sequences and other characters and showing evolutionary changes at the molecular levels and compared among living organism in different species to establish phylogenetics relationships (Singh, 2010).

The present study aimed to conduct a molecular study for the species of the two genera under study in Iraq.

2. MATERIALS AND METHODS

2.1. Taxon sampling and DNA Isolation

The molecular study was carried out in the department of biology, University of Kufa and the National Science and Technology Development Agency. The entire chloroplast gene (trnL-F) was sequenced for 6 species from the two genera Lophocholoa and Schismus of poaceae family in Iraq. Streptochaeta sodiroana Hack. ex Sodiro was used as out group because of documented close phylogenetic affinity to Lophocholoa and Schismus (GPWG II, 2012)

2.2. DNA Extraction

Total cellular DNA was extracted from dried leaves of species from herbarium specimens which belong to National Herbarium of Iraq (BAG). The total DNA was extracted in CTAB (cetyltrimethyl ammonium bromide) isolation buffer according to the protocol of (Doyle and Doyle, 1990). Turn on water bath (60° C), Tubes were labeled, and prepared CTAB+BME first.

1. β-mercaptoethanol (BME) was added to the CTAB at a ratio of 1mL CTAB/1 μl. This step was done in the hood because BME has a noxious odor.
2. Leaf materials ground into a fine powder using a microcentrifuge tube pestle or a mortar and pestle and 300 μl of CTAB+β-mercaptoethanol was added. Thoroughly grind the material and a sterile pipette tip or spatula used to transfer the material to a labeled tube. The procedure was repeated by using a new mortar and pestle for each sample.
3. All samples incubated at 60°C for 30 minutes. The tubes were gently agitated every 5 minutes to make sure the CTAB was contacted with all of the plant material.
4. The samples were centrifuged for 2 minutes and transferred the top layer to a new tube. For a double extraction 300 μl of CTAB+ β-mercaptoethanol was added to the plant material and repeat step 4. The two top layers was combined when we were done.
5. Add 600 μl of chloroform to the tube containing the top layer(s) and shake to mix.
6. Spin this down and transfer the top layer to a new tube. If the top layer does not appear clean, repeat the chloroform rinse step.
7. 400 μl of cold 100% isopropanol (stored in freezer) was added to the tube.
8. Place the tube in the freezer overnight for maximum precipitation.
9. Spin the samples down for 5 minutes to pellet the precipitated DNA. It is helpful to align the hinge on the lid a certain way so that you know where to expect the pellet to form in the tube.
10. Pour off the liquid and add 0.5 mL of cold 80% ethanol (stored in freezer). Gently roll the tube so the ethanol washes the sides. 11. Spin the samples for 2 minutes and pour off the liquid. Dab the top with a chem wipe and try to pipette as much liquid away from the pellet as possible without disturbing it.
12. Lay the tubes out to dry horizontally (this can take 10-20 minutes).
13. Once you are certain the ethanol has evaporated you can add 25 μl of TE buffer. Store the samples in the freezer when you are finished. The gene trnL-F was enlarged by using the primers trn-C and trn-F, and the primers information have been mentioned in (table 1).
2.3. DNA alignment and tree reconstruction

Sequences were manually aligned with QUICKALIGN, Version 1.6.0 (Müller, 2004). There were 7 accessions in the trnL-F alignment, including the outgroup species. The JMODELTEST, version 2.1.1 (Darriba et al., 2012) was used to determine the optimal substitution model for the Bayesian analysis based on the AIC criterion. The HKY+H model was recommended for trnL-F data sets. BEAUTI version 1.6.2 (Drummond and Rambaut, 2007) was used to prepare files for BEAST, version 1.6.2 (Darriba et al., 2012), and to generate phylogenetic trees using Bayesian inference. TRACER, version 1.5 (Rambaut and Drummond, 2009), was used to calculate the effective sample size (ESS) of each run in BEAST and check for convergence through visual examination of plotted posterior probability estimates.

Table 1: Primers and their sequences that have been used in the study

| Primer | Direction | Sequence 5'--- 3' | Length (base) |
|--------|-----------|------------------|---------------|
| trn-C  | Forward   | CGAAATCGGTAGACGCTACG | 20-MER       |
| trn-F  | Reverse   | ATTTGAACCTGGTGACACGAG | 20-MER       |

3. RESULTS AND DISCUSSION

The phylogenetic tree drawing can be determinate on the basis of the alignment the trnL-F data sets in the species examined ranges in length from 972 bp (L. berythea) – 1068 bp (L. phleoides) characters in length.

The result of DNA sequences analysis showed a good tool for inferring phylogenetic interpretation among taxa, topology of trnL-F trees designed based on the Bayesian inference or strict phylogenetic trees.

The species S. barbatus and S. arabicus was the most basal lineages in the base of trees which consider the first main clade in the tree and gathered the species of the genus Schismus with great support (bootstrap value 0.999) (figure1). Lophochloa as the sister second main clade included the species of the genus and well support bootstrap 0.8865 which subdivided in two secondary clades (high support 0.999), the first one gathered the L. phleoides and L. obtusiflora while the Second sister clade gathered L. pumila and L. berythea species with the same strong supported (0.999). The importance of use DNA sequencing data in general and especially trnL-F data sets is to visualize the best grass (Poaceae) systematics and taxonomy of these two genera Schismus and Lophochloa and analysis of this data demonstrate comparable reliable study in the Poaceae systematics and plant systematic especially with the use modern software in representation phylogenetic trees. Similar findings were concluded by Qader (2014) in his study on the genus Cousinia, Al-Mousawi (2015) in separation some Papaveraceae genera in Iraq and (Al-Edhari, 2015) on the tribe Aristideae and Stipeae from Poaceae family in Iraq.
4. CONCLUSIONS
In the current study two major clades within species of the two genera *Lophochloa* and *Schismus* were recognized. The species *S. arabicus* and *S. barbatus* placed in a single clade, and the species of *Lophochloa* located in two secondary clades, the first clade involved *L. berythea* and *L. pumila*, while the second clade contained *L. phleoides* and *L. obtusiflora* with high support (bootstrapping) for all clades, as well as the genus *Schismus* connected as a sister clade with the *Lophochloa* clade. The trnL-F gene has vastly effective in separation the two genera under study.

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