A Survey and Molecular Study for Ten Samples of Rice Weeds in Najaf Governorate

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Abstract. Ten samples of rice weeds from the Najaf Governorate were analyzed to determine their genetic polymorphisms. The samples of genetic polymorphisms were isolated from ten different sources of Cyperaceae and Poaceae families. Genes from the 18S, 5.8S ribosomal RNA, intron 1, intron 2, and 26S rRNA were amplified in this study. The amplified genetic locus' PCR amplicons were sequenced directly using the direct sequencing strategy. In order to determine the precise phylogenetic distances alongside relative sample sequences, a comprehensive phylogenetic tree was constructed using the observed variants. The results of this study show that the investigated samples and species of the Cyperaceae and Poaceae families have a variable homology. The sequenced results showed the presence of three variants distributed in the investigated samples. According to the phylogenetic data, In the currently generated comprehensive tree, the examined samples were distributed among nine major phylogenetic clades. These positions were represented by nine organisms with nine different distances, namely *Cyperus difformis, Eleocharis geniculata, Cressa cretica, Echinochloa crus-galli, Echinochloa colona, Diplachne fusca, Sesbania sesban, Paspalum dilatatum,* and *Paspalum distichum*. Most of the clades were distinctly positioned in separated places from each other in the tree. Based on the utilized rRNA amplicons, it was to be clear that all investigated samples were separated into distinctive phylogenetic positions. The different positions were generated due to the several ancestral differences which were found in the pattern and extent of the observed nucleic acid variations in the cyperaceae and poaceae families. In addition, the present tree is provided an inclusive tool for the guaranteed identity of the investigated samples due to the high similarity that notified with a variety of sequences. All DNA samples were identified using the PCR-sequencing strategy, and their phylogenetic distribution patterns were clearly visible.

Keywords. Molecular, Rice, Weeds, Echinochloa Crus-Galli, Echinochloa colona.

1. Introduction

The competition between weeds and rice plants on the nutrients begins simultaneously when growing the weeds and rice plants. The restriction of other required elements that are available in the environment when one of the minerals and plant nutrients less the required level to cease the growth. It was found that the need for nitrogen and the water for bushing the weeds is greater than that the need for the rice plants, The critical stage of competition begins at the first stage of spikes formation, where the competition for light intensifies *Echinochloa Crus-Galli*. The weeds are most harmful to compete with the rice crop [1].

Rice is very sensitive to weeds, especially at the early stages of the growth of rices. The results of previous study indicated that losses in this crop sometimes reach 70% of the outcome when it is not controlled. In addition to the poor quality of rices, the increase in the

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density of weeds reduces the rice grains by about 50% of the grains [2,3].
The current study aims to highlight the genetic map of rice weeds in the Najaf city. The aim was achieved by classifying the rice weeds morphology and genetics. All stages of the current study were performed in the field [4].

2. Methods

2.1. Survey of Rice Weeds

All of Najaf's rice fields were surveyed for the presence of rice weeds. A wooden square was used to divide each area into three equal parts. It was in this laboratory that a 1 m-long wooden square was constructed. Until the end of the agricultural season, each region was sampled five times. In order to calculate the percentage of each weed, we used a classification key to determine how the weeds looked[5,6].

2.2. DNA Sequencing of Multiplex PCR Machine

The resolved PCR amplicons were commercially sequenced from termini, forward, and reverse, according to the instructions reported by the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea), the clear chromatographs obtained from ABI sequence files were just furtherly analyzed. It is to ensure that the annotation and variations are not occured because of PCR or equencing artifacts. By comparing the observed nucleic acid sequences of the local samples with the retrieved reference sequences of genomic database, the virtual positions, and other details of the retrieved PCR fragments were identified.

2.3. Interpretation of Sequencing Data

Editing, aligning, and analyzing the sequencing results of PCR products from different samples using BioEdit Sequence Alignment Editor Version 7.1 was done using the software (DNASTAR, Madison, WI, USA). PCR amplicons and their corresponding positions within the reference genome were used to number the observed variations in each sequenced sample.

2.4. Comprehensive Phylogenetic Tree Construction

[7], neighbor-joining protocol was used to build a specific comprehensive tree in this study. The NCBI-BLASTn server was used to compare the observed variants to their neighboring homologous reference sequences [8]. In order to generate a circular cladogram, a neighbor-joining method was used to construct a full inclusive tree, which included the observed variant [9]. On the comprehensive tree, the sequences of each phylogenetic species-group were annotated in accordance[10].

3. Results

Table 1 shows the types of bushes and the percentage of occurrence in the studied areas. The percentage of Cyperus odoratus was recorded in the areas of Qadisiyah, Al-Meshkhab and Al-Abbasiya, with rates of 33.42%, 36.93% and 42.12%, respectively. The Eleocharis geniculata weeds in the area of Al-Hirah was 38.65%. The Echinochloa crus-galli in the areas of Almunadharat and Al-Hirah were 71.26% and 30.1%, respectively. The increase in the number of rice weeds with the crop increases due to its severe competition for the crop and its growth at the same time whithin the same environment (Table 1) [11].

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Table 1. Types of bushes and the percentage of occurrence in the studied areas.

No.	Region	*Echinochloa crus-galli*	*Echinochloa colona*	*Paspalum distichum*	*Diplachne fusca*	*Cyperus difformis*	*Cyperus odoratus*	*Eleocharis geniculat*	*Phragmites communis* L.
1	Qadisiyah	24.68	2.41	1.53	2.4	5.14	33.42	28.62	1.8
2	Al-Meshkhab	32.15	2.21	2.7	3.56	5.89	36.93	14.85	1.71
3	Eleocharis	14.42	0.72	1.43	3.66	5.82	34.18	38.65	1.12
4	Almunadharat	71.26	0.7	0.79	0.88	0.88	21.59	3.42	0.48
5	Al-Abbasiya	25.84	3.88	6.95	3.24	10.98	42.12	6.25	0.74
6	Al-Hirah	30.1	5.74	4.55	4.75	30.1	12.08	11.29	1.39

Ten samples were taken from this locus. They were shown a variety of amplicons with differing lengths of time. Ensure that all amplified amplicons show sharp, specific and clean bands before sending them to sequencing.

The results of the sequencing reactions showed that the amplified products' identities had been verified through the use of NCBI blastn. Sequence similarity between sequenced samples and their reference sequences was extremely high when using this engine. In the 18S, 5.8S ribosomal RNA, and 26S ribosomal RNA genes, the NCBI's BLASTn engine found about a high percentage of homology with the predicted target. These sections are .#."

critical in determining the species of interest's true identity. This can be done by looking at the observed and recovered DNA sequences from various samples. The precise locations of the PCR fragments that were retrieved were determined. The details of the amplified sequences were highlighted after the ribosomal bp amplicons' sequences were positioned within the reference sequences (Table 2).

For the purposes of this study, we focused on the 18S, internal transcript spacer 1, 5.8S, internal transcript spacer 2 (ITS 2 and 26S) and the internal transcript spacer 2 (ITS 1) regions of the genome. The sample numbers are denoted by the symbol "S No

Table 2. The position and length of the ribosomal PCR amp icons.

Sample number - Organis	Reference locus sequences (5' - 3')	length

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S1	MN718330.1 - *Cyperus difformis*	GGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG GATCATTGTTGTTGCCTTTGAACATGACTGTGAACATGTAACATAAA GCTACCGGGGAGGAGCTTCCTCCTCGGCCCCAACGGCCTTGGCCCT GTGGTCAGGTGTCGAAATACGGCGCGGATTGTCGCCAAGGAACACT GATTTGCTTAGGCAGACCGCATCATGCGGTCAGCTGAGGCCAAAAA AAAACAATATGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGA TGAAGAACGTAGCGAAATGCGATACGTGGTGTGAATTGCAGAATCC CGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAGGGATCCT CCCGAGGGCACGCCTGCCTGGGCGTTAGAAGCCCATCAACGCTCGG TCAAGTCCCCACGTATTGTGTGAGGAAATTTGGCCAGACGCGGACG TTGGCTCTCCGAGCTGTGAAGCGCGGTGGGCTTAAGAGCACGGCCG TTGACGGTTTCGGGAACGGCGAGTTGTGGGCTACAGCGAATGCCAA TCCCGACACATGTGTTGACATGTGGCCATTTTTGACCCCTGGACAAG TAGATTTGTCGCTGTAGCGTCTCGCTTTGCGACATCTTCGGACCGAT ACCCCAGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATCAATA AGCGGAGGAGAAGAAAC	710 bp
S2	AF190590.1 - *Eleocharis geniculata*	GGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG GATCATTGTTGTTGCCTTTTAAAAACACGACCAGCGAACTTGTAATG AAATGCTGTTGGGGAGGTCAGCCTCCTCGACTCCACCGACCCCGGA CGTTCTCCCGCCTTTCGGGGCATTGCCGGTCGAGGTGTCGGAACACG GCGCGGGTTGGCGCCAAGGAATACATGTTTGCTTAGGCAGGACTGC GATGCTTCGTCGTCGCATGGTCTGTCGAGGCCAAAGTAAGAAAAAA TGAGATGACTCCCGGCAACGGATATCTCGGCTCTCGCATCGATGAA GAACGTAGCGAAATGCGATACATGGTGTGAATTGCAGAATCCCGTG AACCATCGAGTCTTTGAACGCAAGTTGCGCCCAAGGGACCATCCCG AGGGCACGCCTGCCTCATGGGCGTTAGAAGCCCATCCACGCTCGGA GTCGGCGCCTTGCAGGGCCCGGCCTGATGCGGACAGTGGCCCTCCG AGCCGCAAGGCGCGACGGGCACAAGTGCACGGCCGTCGGTTGAGGT CGGGATCAGCGAGTGGTGGGCTACTGCGCACGCTGCATCAGCACCT CATGCCGACACAGGGCCAAGTTGGACCTCTAAACGAGGACTCCTGT CGTCGAGTGCGGCAGCCTCGGACCGATACCCCAGGTCAGGCGGGAC TACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGAGAAGAAAC	736 bp
S3	KJ004289.1 - *Cressa cretica*	GGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGACCTGCGGAAGG ATCATTGTCGAAAACCGCCCGGCGGAAAACCCGCGAACCAGTTCGA ATCCAGGTCCCCGCGCCGGGGAAGGGTCCCCTGGGCCCGCCCCCCG GCGCGAACATCGAACCCCACGGCGCGGAACGCGCCAAGGAATACC GAAACGGGACGGCCCGCTCCCCGCGCCCCCGTCCGCGGGGAACCCG GGGAGCGCCGGCGTCTTGGAGTACAAAGAAAAAACGACTCTCGGCA ACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCG ATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAA CGCAAGTTGCGCCCGAAGCCGTCAGGCCGAGGGCACGCCTGCCTGG GCGTCACGCATCGCGTCTCCCCCTCCCCCCCCGCAGCGCGGGACGGG CGGGGGAGGACGATGGCCTCCCGTGCCCCGATCCGGGACGCGGCCG GCCCAAACGCTGGTCCATGGCGACGGGCGTCGCGGCGAGTGGTGGT CGTACCCCGCGTGCAGTGTCTCCGCGCCGCGCCCCCGCCGTCCCGGG ACCGACGACCCTTCCGAGCCGACGGCTCTCCGACCGCGACCCCAGG TCAGGCGGGACCACCCGCTGAGTTTAAGCATATCAAAAGGGGGGGG GAAGAAAC	699 bp

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S4	AJ133707.1:1-687 *Echinochloa crus-galli*	GGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG GATCATTGTCGTGACCCTTAACCAAAACAGACCGTGAACGTGTCTCC AATGCCGCCGGGCTTCGGTCCGGCAAAGGCTCCCGACCTTCGTTAG GAGGAAAGGAGCCGCAAAAGAACCCACGGCGCCGAAGGCGTCAAG GAACACTAATATTGCCTTGCTCGGGACCGTGGCTGGCTTGCTAGCCA CTTCCCGTGCAGCGATGCTATACTAATCCACACGACTCTCGGCAACG GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATA CCTGGTGTGAATTGCAGAATCCCGCGAACCATCGAGTTTTTGAACGC AAGTTGCGCCCGAGGCCTTCTGGCCGAGGGCACGCCTGCCTGGGCG TCACGCCAAAAGACACTCCCACCCCATCATAGGGTTGGATGTGGCG TTTGGCTCCCCGTGCCTGAAGGCGCGGTGGGCCCGAAGTTGGGGCT GCCGGCATACCGTGTCGGGCACCGCACGTGGTGGGCGACTACAAGT TGTTCTCGGTGCAGCGCCCCGGCACGCAGCTAGCATGTTGGCCCTAA GGACCCATGTACAACCGAAGCGCATTGTCGCTCGGACCGCGACCCC AGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATCAATAAGCGG AGGAGAAGAAAC	706 bp
S5	KP878858.1 -*Echinochloa colona*	GGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG GATCATTGTCGTGACCCTTAACCAAAACAGACCGTGAACGTGTCTCC AATGCCGCCGGGCTTCGGTCCGGCAAAGGCTCCCGACCTTCGTTAG GAGGAAAGGAGCCGCAAAAGAACCCACGGCGCCGAAGGCGTCAAG GAACACTAATATTGCCTTGCTCGGGACCGTGGCTGGCTTGCTAGCCA CTTCCCGTGCAGCGATGCTATACTAATCCACACGACTCTCGGCAACG GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATA CCTGGTGTGAATTGCAGAATCCCGCGAACCATCGAGTTTTTGAACGC AAGTTGCGCCCGAGGCCTTCTGGCCGAGGGCACGCCTGCCTGGGCG TCACGCCAAAAGACACTCCCACCCCATCATAGGGTTGGATGTGGCG TTTGGCTCCCCGTGCCTGAAGGCGCGGTGGGCCGAAGTTGGGGCTG CCGGCATACCGTGTCGGGCACCGCACGTGGTGGGCGACTACAAGTT GTTCTCGGTGCAGCGCCCCGGCACGCAGCTAGCATGTTGGCCCTAA GGACCCATGTACAACCGAAGCGCATTGTCGCTCGGACCGCGACCCC AGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATAAATAAGCGG AGGAGAAGAAAC	705 bp
S6	MF353834.1 - *Diplachne fusca*	GGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG GATCATTGTCGTGACCCTGACCAAAACAGACCACGAACATGTCATC CATGCTGCCGGGTGATGGGGCTTGCACCCGTCTCCCGGTCTTGGCCA CCGACCTTCTTCGGGAGGGACGGGGGCCCAAAAGAACCCACGGCGC CGTTTGGCGTCAAGGAAAACTAATATTGCCTTGCCTGGGGCGACGT CCGGCCTGCTGGATGCACCCCCTGCAGCGATGCTATGTAGACACAC ATGACTCTCGGCAACGGATATCTTGGCTCTCGCATCGATGAAGAAC GTAGCAAAATGCGATACCTGGTGTGAATTGCAGAATCCCGTGAACC ATCGAGTTTTTGAACGCAAGTTGCGCCTGAGGCCTTCTGGCTGAGGG CACGTCTGCCTGGGCGTCACGCCAAAAGACACTCTGCACCTACCCT GGTGTGGACGTGGTGTTTGGCCCCTCATGCCGCAGGGTGTGGTGGG CCAAATTTGGGGCTGCCGGCGGTGCCGATCACAGCACAAGGTGGAT GACGCAAGTTGTTCTCGGTGTTATGATCCGGACCACTCCTGGTGATG TTATGGCCCTTTGGACCCATCGAGTGGAGCACGTGTTGCTCGGACCG CGACCCCAGGTCAGTCGGGACTACCCGCTGAATTTAAGCATATCAA TAAGCGGAGGAGAAGAAAC	713 bp

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S7	KY968939.1 - *Sesbania sesban*	GGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG GATCATTGTCGATGCCTCAAAAGCAATCTGACCCGTGAACTCGTTAG CAACACATCCACCAACGCTGGCCCCGTGTGCCCTGTGCCCAGGCTC AGKCACACGGGGCTGGCTAAAACACAAACCCCGGCGCTGAATGCGC CAAGGAACTACAACTCGTATGCTGCCCCCGTCGACCCGGAGACGGT GCTCGTGCGGGGGGAGCAACACGTCATTACTAAACACAATGACTCT CGGCAACGGATATCTCGGCTCTTGCATCGATGAAGAACGTAGCGAA ATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCT TTGAACGCAAGTTGCGCCCGAAGCCATTAGGCTGAGGGCACGCCTG CCTGGGTGTCACACATCGTTGCCCCAACGCCAACCACACACGTGTGT GTGTCGTGTCGTGAGGGGTGGAAGTTGGCCTCCCATGAGYGAGGCC TCATGGATGGCTGAAAATTGAGCCCTTGGTGCAGTCTGTGCCATGAC ATCCGGTGGATGAGTCATCACATGCTCGAGACCGATCATGCACAAA CCCACCTATTTTTGGCTCCAAGAGTAACCCACACGCGTCCCATTTCA TAGGAACGCTCTTAACGAGACCTCAGGTCAGGCGGGGCTACCCGCT GAGTTTAAGCATATCAATAAGCGGAGGAGAAGAAAC	731 bp
S8	KY968955.1 - *Paspalum dilatatum*	GGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG GATCATTGTCGTGACCCTTAAACAAAACAGACCGTGAATTTGTCACC AATGCCGCTGGACCTCGGTCCGGCTTTGGCCCCCGACCTTCGTCTAG GAGGGGAGGGGCCACAACAGAACCCACGGCGCCGAAGGCGTCAAG GAACACTGATATTGCCATGCGAGGGTGAGGGCTAGCTTGCTGGCCT AACCCCAAGCAGCGATCAACCATTAATCCACACGACTCTCGGCAAC GGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGAT ACCTGGTGTGAATTGCAGAATCCCGCGAACCATCGAGTTTTTGAAC GCAAGTTGCGCCCGAGGCCTTCTGGCTGAGGGCACGCCTGCCTGGG CGTCACGCCAAAAGACACTCCCAACCCATCATAGGGTGGGGACGTG GAGTTTGGCCTCTCGTGCCGTTGTGCACGGTGGGCCGAAGTCTTGGC TGCCGGCGTATCTTGCTGGGCACCGCACATGGTGGGCGACATCAAG TTGTTCTCAGTGCAGCGTCCCAGCATGTAGCTGGTACTATGGCCTTT AGGACCCATCATCGACCGCAGCGCTTGTACGCTCGGACCGCGACCC CAGGTCAGGCGGGATTACCCGCTGAGTTTAAGCATATAAATAAGCG GAGGAGAAGAAAC	706 bp
S9	KF163848.1 -*Paspalum distichum*	GGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGGTGAACCTGCGGAA GGATCATTGTCGTGACCCTTAAACAAAACAGACCGTGAATTTGTCA CCAATACCGCTGGACTTCGGTCCGGCTTTGGCCTCCGACTCTCTTCT TTGAGGGGAGGAGCCACAACAGAACCCACGGCGCCGAAGGCGTCA AGGAATACTGATATTGCCTTGCGAGGGTGAGGGCCGGCTTGCTGGC CTGACCCCAAGCAGCGATGATCCATTAATCCACACGACTCTCGGCA ACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCG ATACCTGGTGTGAATTGCAGAATCCCGCGAACCATCGAGTTTTTGAA CGCAAGTTGCGCCCGAGGCCTTCTGGCTGAGGGCACGCCTGCCTGG GCGTCACGCCAAAAGACACTCCCAACCCATCACAGGGTGCGGACGT GGAGTTTGGCCTCTCGTGCCATGCTGCACGGTGGGCCGAAGTCGTG GCTGCCGGCGTATCTTGCTGGGCACCGCACATGGTGGGCGACATGA AGTTGTTCTCGGTGCAGTGTCCTAGCATGTAGCTGGTACTATGGCCT TTAGGACCCATCATCGACCGTAGCGCTTGCCGCTCGGACCGCGACC CCAGGTCAGGCGGGATTACCCGCTGAGTTTAAGCATATAAATAAGC GGAGGAGAAGAAAC	706 bp

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S10	AM404346.1 - *Echinochloa crus-galli*	GGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG GATCATTGTCGTGACCCTTAACCAAAACAGACCGCGAACGTGTCTC CAATGTCGCCGGGCTTCGGTCCGGTAAAGGCTTCCGACCTTCATTTC GAGGGGGAGGAGCCGCAAAAGAACCCACGGCGCCGAAGGCGTCAA GGAACACTAATATTGCCTTGCTCGGGACCGTGGCTGGCTTGCCGGCC ACTGCCCGTGCAGCGATGCTATACTAATCCACACGACTCTCGGCAA CGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGA TACCTGGTGTGAATTGCAGAATCCCGCGAACCATCGAGTTTTTGAAC GCAAGTTGCGCCCGAGGCCTTCTGGCCGAGGGCACGCCTGCCTGGG CGTCACGCCAAAAGACACTCCCACCCCATCATCGGGTGTAGGATGT GGCGTTTGGCTCCCCGTGCCTGAAGGCGCGGTGGGCCGAAGTTGGG GCTGCCGGCATACCGTGTCGGGCACAGCACGTGGTGGGCGACTACA AGTTGTTCTCGGTGCAGCGTCCCGGCACGCAGCTAGCTTGATGGCCC TAAGGACCCATGTACAACCGAAGCGCACTGTCGCTCGGACCGCGAC CCCAGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATAAATAAG CGGAGGAGAAGAAAC	708 bp

In the alignment results of the 465-bp samples, it was found that each investigated sample had different nucleic acid variations in comparison to the corresponding referring sequence (Fig. 1). Referring to the NCBI reference sequences, the symbol "ref" should be noted, while "S No.#" refers to sample numbers in the table. To align each sample, the most similar sequences were used.

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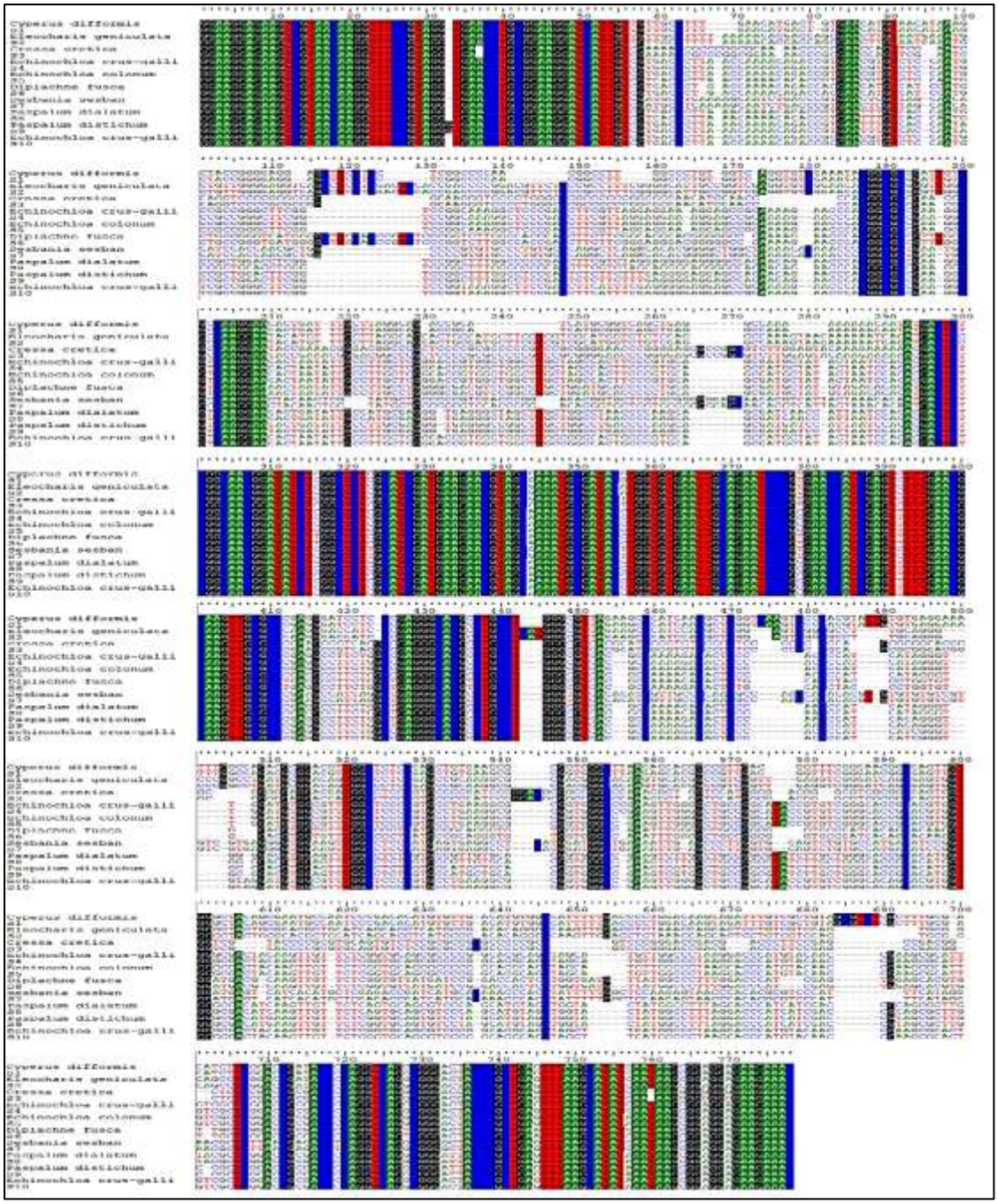

Figure 1. The rRNA sequences of ten samples were aligned with their corresponding reference sequences in the reference genomic DNA sequences.

Our samples were aligned one by one with the most closely related sequences in the NCBI database before being used to generate the sequences. In the samples currently being studied, three nucleic acid substitutions were observed, in which three nucleic acids were substituted in different positions in the samples. In this case, however, the sequence chromatograms of the discovered variations were verified and documented in great detail. Table 1 lists the sequence positions in relation to their respective chromatograms (Fig. 2), which are depicted in the same way. Substitution mutation is depicted by the ">" symbol in Fig. 2.

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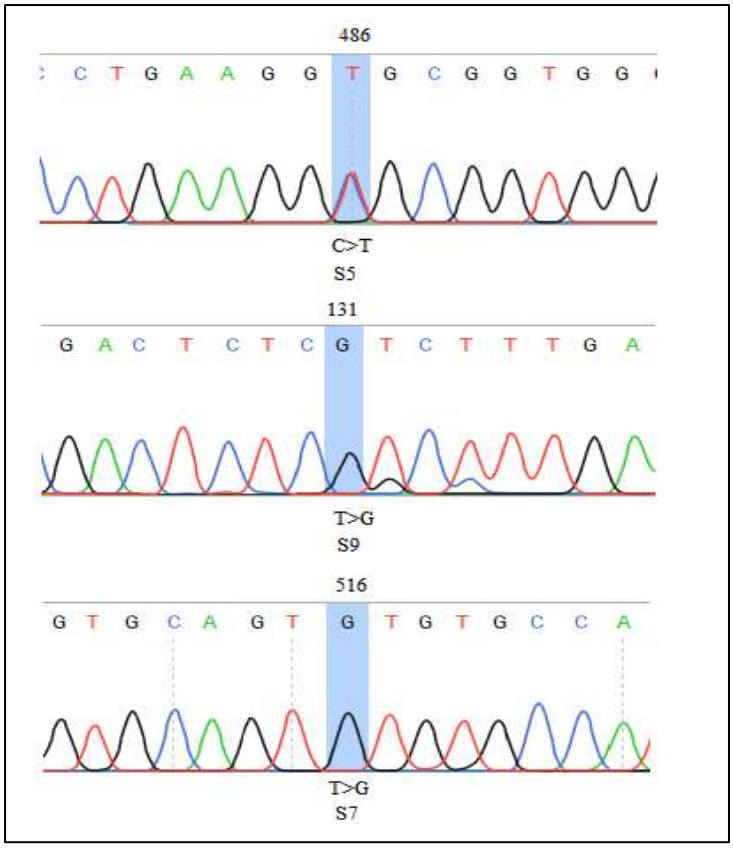

Figure 2. The DNA chromatogram of the targeted ribosomal amplicons of ten different Cyperaceae and Poaceae sequences showed a pattern of detected variants.

A comprehensive phylogenetic tree was generated based on the observed ribosomal nucleic acid sequences detected in the investigated samples of 18S, internal transcribed spacer 1, internal transcribed spacer 2, 5.8S ribosomal RNA gene and 26S rRNA within the Cyperaceae and Poaceae families. Along with the other deposited DNA sequences, this phylogenetic tree contained all currently investigated samples (S1 to S10) aligned with mostly their relative sequences in a neighbour-joining mode. In the current constructed cladogram, the total number of aligned nucleic acid sequences was 107 sequences. This comprehensive tree entailed the presence of nine different organisms, *Cyperus difformis*, *Eleocharis geniculata*, *Cressa cretica*, *Echinochloa crus-galli*, *Echinochloa colona*, *Diplachne fusca*, *Sesbania sesban*, *Paspalum dilatatum* and *Paspalum distichum*. The observed organisms represent only incorporated nucleic acid sequences within the tree. Based on the current analyzed ribosomal sequences, the sequences of the organisms were clustered into nine main clades, which presented a wide range of diversity of these organisms (Fig. 3). Furthermore, the considerable phylogenetic distances were observed among the incorporated organisms, which gives a further indication of the presence of high diversity among the sequences. Concerning *Cyperus difformis*, our investigated S1 resided between the GenBank accession numbers of MH711450.1 and the GenBank accession numbers of MH711466.1. They belonged to two Chinese samples of the same organism. Meanwhile, S2 was resided within the *Eleocharis geniculata* sequences, in the vicinity of two GenBank accession numbers (AF190590.1 and AF190589.1). Both AF190590.1 and AF190589.1 belonged to American samples of *Eleocharis geniculata* sequences. As in the case of S1 and S2 clades, the clade of S3 was clearly observed. Within this clade, the sample resided beside two GenBank accession numbers of *Cressa cretica*

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sequences. One of the accession numbers (MG256233.1) was deposited from Pakistan, while another one (KJ004289.1) was deposited from Saudia Arabia.

Both S4 and S10 resided beside each other in the same clade of the same organism, *Echinochloa crus-galli*. Both samples of S4 and S10 were positioned beside the GenBank accession numbers of AB353384.1 and AB353387.1. Both S4 and S10 originated from an Argentinian source of *Echinochloa crus-galli* sequences. Beside S4 / S10 clade, S5 was positioned within the *Echinochloa colona* sequences. This observation referred to the close phylogenetic distances between *Echinochloa crus-galli* and *Echinochloa colona*. The similarity between it was not unusual as both organisms belonged to the same genus (*Echinochloa*). However, it was found that S5 resided in the vicinity of GenBank accession number KP878858.1. It is a Brazilian variety of *Echinochloa colona* sequences. The Diplachne fusca sequences were incorporated into the current tree, and S6 was found in a new distinct clade there. The sample of belonged to this clade and was located near the GenBank accession number MF353834.1. It was found to be a member of the Sesbania sesban clade, which in turn belonged to the Diplachne fusca sequences from Mexico S7. Interestingly, it was found next to another specimen of the same organism, GenBank accession number KY968939.1, from China. An organism's scale range is indicated by a number "0.1" located at the top left portion of this diagram. The code for the samples under investigation is denoted by the symbols S1 through S10.

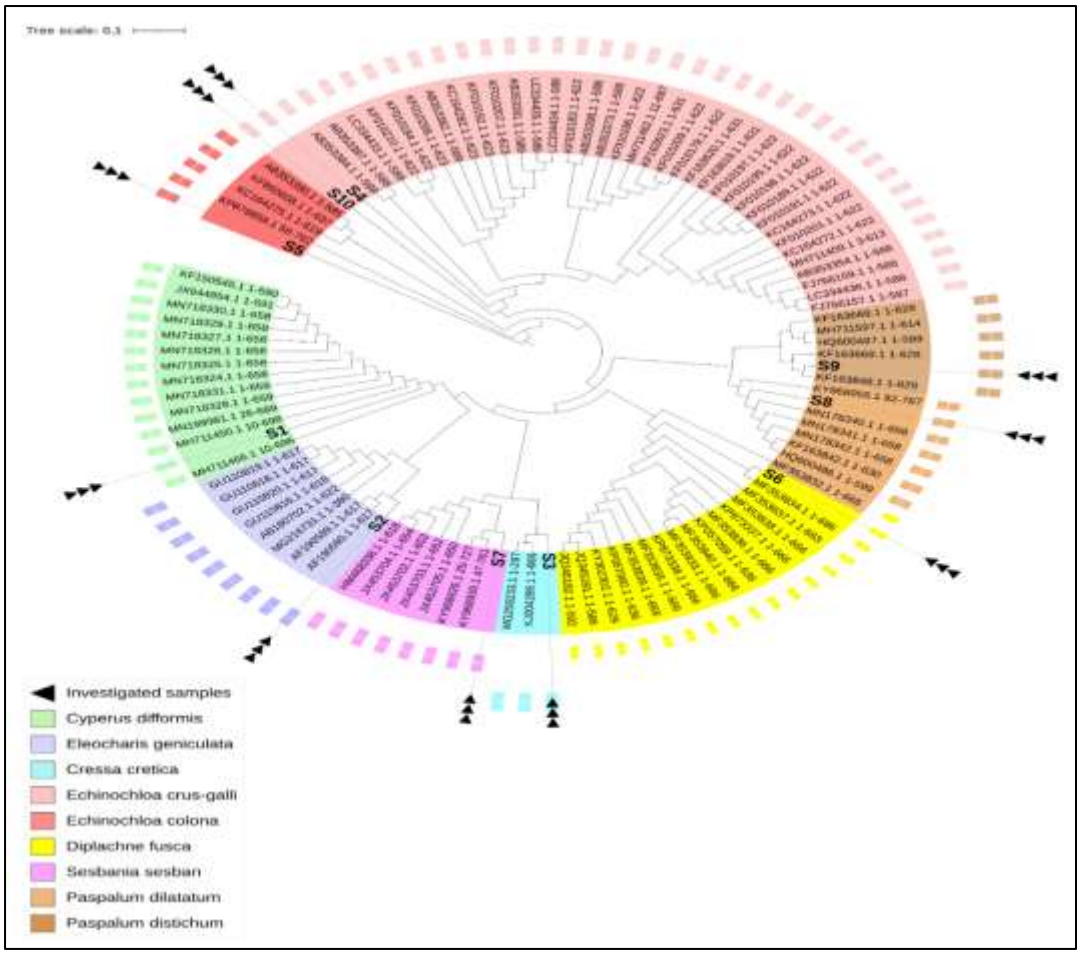

Figure 3. The comprehensive phylogenetic tree of ten samples of the ribosomal amplicons that partially covered 18S, internal transcribed spacer 1, internal transcribed spacer 2, 5.8S ribosomal RNA gene and 26S rRNA within the Cyperaceae and Poaceae families.

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Both S8 and S9 were positioned in two adjacent clades of *Paspalum dilatatum* and *Paspalum distichum*, respectively. The reason for the close distances of S8 and S9 to each other was due to the fact that both of Samples Both S8 and S9 belonged to the same genus, namely *Paspalum*. Accordingly, both observed clades of S8 and S9 were originated from the same biological ancestor. However, S8 was positioned in the vicinity of MN178340.1, an Uruguayan sample of *Paspalum dilatatum* sequences. Whereas S9 was positioned in the vicinity of KF163848.1, a Korean sample of *Paspalum distichum* sequences.It was inferred from the tree that our investigated samples were distributed into several clades originated from several geographical regions. The majority of investigated samples were included in distinct phylogenetic positions. This sort of S1–S10 genetic distribution referred to the sensitivity of the utilized rRNA-based amplicons in the accurate discrimination among the investigated samples. In addition, the observed multiple clades in the generated cladogram suggest the presence of a high level of diversity of the currently investigated sequences. Because of its unique role in identifying the analyzed samples, phylogenetic trees could not be ignored. Using this rRNA-based comprehensive tree, researchers have shown that these ribosomal sequences can effectively differentiate between different samples using the extremely high discriminative power provided by these rRNA sequences.

Conclusion

- study the weed in rice fields.
- Study of the density of the weed spread in Najaf.
- Study the molucular biology in the rice weed .
- Studying the presence of genetic mutations on some weed rice and the effect of herbicides

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