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Detection of New Strains of Echinochloa Crus-Galli Resistant to ALS-AHAS and Accase Weedicides for the First Time in Iraq

Raad T. Toman[1] and Bashar K. H. Al-Gburi[2]*

[1]Medicinal plant Technologies and Natural Products, College of Agriculture Techniques, Al-Mustaqbal University, Babylon, Iraq. E-mail address: raad.talal.toman@uomus.edu.iq

[2]*Department of Plant Protection, Faculty of Agriculture, University of Kufa, Najaf, Iraq. Corresponding author: E-mail address: bashark.algbori@uokufa.edu.iq.

Abstract

The competition between weeds and rice crop is one of the most important restrictions that limit the success of production in the world. Despite the excessive and multiple use of herbicides, weeds still one of the biggest problems that facing rice cultivation in Iraq, as both farmers, and agricultural departments complained about the challenges of controlling it particularly the herbicide-resistant strains. Current study aimed to determine the reason/s for the spread of the barnyard grass and its resistance to ALS-AHAS and ACCase herbicides. A field survey was conducted in different areas of Najaf province where rice crop is cultivated. Weeds samples were collected and diagnosed phenotypically and molecularly. The outcomes of this research showed that the geographical distribution of weeds accompanying the rice crop indicated that the highest average numerical density of the spread of weeds is 43% in the Shallal- Al-Mishkhab area, and the barnyard grass weed was occupied about 36% of the percentage of weeds in the areas included in the field survey. Phenotypic diagnosis of Echinochloa crus-galli samples also revealed the presence of the variety (var. crus-galli) for the first time in Iraq, supported by molecular diagnosis that resulted in the registration of 19 new strains that were deposited at NCBI under the serial number (OQ591719 to OQ591737) for the first time in Iraq. The phenotypic characteristics of the seeds showed that their average size was 2.82 - 3.67 mm and their average weight was 0.0012 - 0.0038 g. Anatomical characteristics showed that the average thickness of the upper epidermis of the cochineal was 146.22 µM, the lower one was 181.76 µM, and the thickness of the shell was 136.48 µM, which indicates the great ability of seeds to spread and sustain growth. The phylogenetic tree the registered strains showed a genetic affinity with the barnyard grass strains registered in the NCBI database that distinguish by its resistance to herbicides.

Keywords: *Barnyard grass, rice, weedicide, resistance, phenotypic.*

1 | INTRODUCTION

Barnyard grass (*Echinochloa crus - galli*) is one of the most common types of weed closely related to rice, which belongs to the Poaceae family and is characterized by its thin leaves. It was first identified in Europe, and after that other types of barnyard grass were identified all over the world, including Asia, Australia, and America (Chauhan et al., 2019). Barnyard grass weed is most dangerous and harmful weed in rice fields and ranks as a third worst weed in the world. This weed characterized by being a short-day tropical weed and follows the C4 pathway in carbon fixation. It tends to grow in harsh climates, high adapted to various levels of temperature and humidity to complete its life cycle as the humid conditions promote early flowering of barnyard grass (Ali et al., 2017; Toman and Al-Gburi, 2023). The factor that have greatly contributed to the spread of barnyard grass in rice fields is the phenomenon of great similarity in appearance between this weed and rice at the seedling stage, which increases the difficulty of identifying them during mechanical control or manual removal (Chauhan et al., 2013). Barnyard grass weed is considered one of the most aggressive types of weed in the world and is widespread with rice, whether the method of cultivation used is wet or dry, characterized by all the competitive advantages and adaptive characteristics necessary for survival and successful competition under a range of geographical and climatic conditions (Bagavathiannan et al., 2012). Although the barnyard grass weed is considered an annual weed and has several ways to survive, including its ability to renew its growth again when the vegetative group is removed. Among its advantages is the ability to continue growing when partially submerged in water, it also has the ability to resist many of the chemical pesticides used in rice fields, such as (butachlor, propanil) herbicides, which can regain its activity again after the first irrigation of the crop after the control procedure (Chauhan et al., 2011). Nowadays, the barnyard grass weed has become the primary and main enemy in rice fields and the first source of nuisance for most farmers. Therefore, this study aimed to reveal the reason for the spread of the barnyard grass and its resistance to ALS-AHAS and ACCase herbicides in Iraq.

2 | MATERIALS AND METHODS

2.1 | Investigating the spread of barnyard grass weed accompanying the rice crop in Najaf province

A field survey was conducted on the types of jungles in some areas that are famous for rice cultivation, namely (Al-Manathera, Kufa, Al-Abassiya, Al-Hurriya, Al-Haydariyah, Al-Mishkhab, Al-Qadisiyah, Al-Heera) during the agricultural season (2017-2021), with samples of barnyard grass being taken for the 2022 season from Rice research Center in Najaf. The survey also included identifying the most widespread types of weeds and the pesticides most used by farmers, in addition to determining the area and cultivated varieties. The number of areas included in the survey was 19 agricultural areas with a total of 122 agricultural lands with different owners and farmers. The survey also included collecting samples of barnyard grass seeds from stores and mills in the survey areas. More than one sample was taken from each region and mixed together to obtain a sample representative of the region. After that, the seeds were stored in sterile boxes, and data was recorded on them indicating the area of collection of the barnyard grass weed seeds and the date of collection. Then the seeds were transported to the laboratory and kept at room temperature for the purpose of diagnosis and procedure subsequent studies.

2.2 | Phenotypic diagnosis of barnyard grass

Phenotypic diagnosis was carried out at the University of Kufa / Faculty of Agriculture / Weeds Laboratory, where seed samples of barnyard grass were taken from the field survey areas and their germination was 30 days old and their germination rate was determined, in addition to other samples of barnyard grass (whole plant) taken from Rice Research Center in Al-Mishkhab for the 2022 agricultural season, where representative models of the region were selected for the purpose of phenotypic diagnosis according to the classification keys approved by the Iraqi National Herbarium (Tahir et al., 2016).

2.3 | Anatomy of barnyard grass seeds

The anatomical study was conducted in the Weeds Laboratory / Faculty of Agriculture - University of Kufa on samples of barnyard grass seeds collected from stores, and the hand-sectioning method was used to prepare the slices according to what was stated in (Hamza et al., 2012) with some modifications.

2.4 | Molecular diagnosis of barnyard grass and DNA extracting using polymerase chain reaction (PCR) technology

Molecular diagnosis of the barnyard grass weed associated with the rice crop was carried out through the germination process of the barnyard grass seeds collected in the field survey. Samples (seedlings characterized by dense growth) representing the surveyed areas from the growing barnyard grass plants were taken. DNA was extracted using an extraction kit (ZR Plant/Seed DNA MiniPrep™) The instructions of the supplier company Zymo/USA were followed, and the diagnosis was made based on the forward primer *Matk*1 and the reverse primer *Matk*2 in the polymerase chain reaction (PCR) (Chen et al., 2017). The PCR product was injected into a 1.5% (wt/vol) agarose gel using RedSafe dye, and the electrophoresis process was carried out at 50 V for one hour. The *Matk*1 and *Matk*2 gene amplification products, as well as the forward primer and reverse primer, were sent to the Korean company Macrogene to analyze the sequences of nitrogenous bases of duplicated nucleic acids from seeds for the identification of weeds.

2.5 | Study of the genetic affinity of the identified barnyard grass weed strains

DNA sequences were analyzed to determine the genetic closeness between 19 strains of *Echinochloa crus-galli* var. *crus-galli* (OQ591719- OQ591737) in the current experiment with ten barnyard grass strains obtained from NCBI database that possess multiple herbicide resistance (MHR) and target and non-target site resistance (TSR & NTSR). MEGA11 program was used, and all DNA sequences were aligned regularly with the Maximum Likelihood matrix, then the sequences under study were matched using the Clustwal alignment type with the number of repetitions (1000) (Nah et al., 2015). Then the rDNA gene sequence of *Oryza sativa* (AK064112) was used as an out-group for the tree, ITOL program (https://itol.embl.de) was also used to improve the phylogenetic tree.

3 | RESULTS

3.1 | The percentage of weed population density and pesticides used

The results of the field survey of weeds showed variation in the spread in the areas included in the survey. The results of the survey also showed, according to the geographical distribution of weeds accompanying the rice crop in Najaf, that the highest percentage of weeds prevalence reached 43% in the Shallal- Al-Mishkhab area, compared to the lowest average in the Rice Research Center area in Al-Mishkhab reached 15% (Figure 1). In addition, the barnyard grass weed had the highest percentage of numerical density, reaching 36%, compared to the couch *Paspalum distichum*, which had a numerical density of 4% (Figure 2). Figure (3) shows that the weedicide Nominee is the highest in agricultural fields, as its use reached 39% compared to other herbicides.

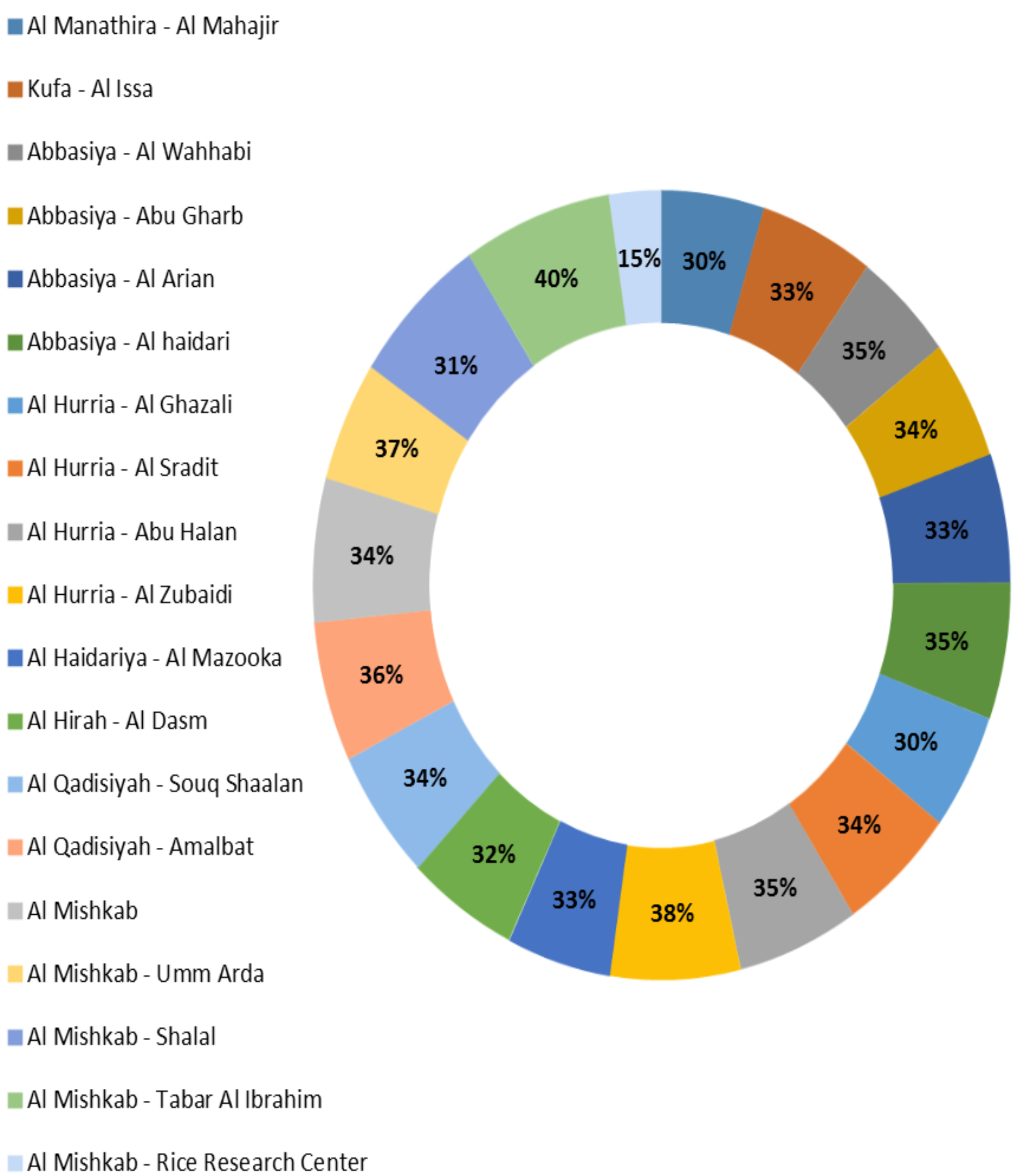

FIGURE 1 The percentage of numerical density of weeds in the areas covered by the field survey.

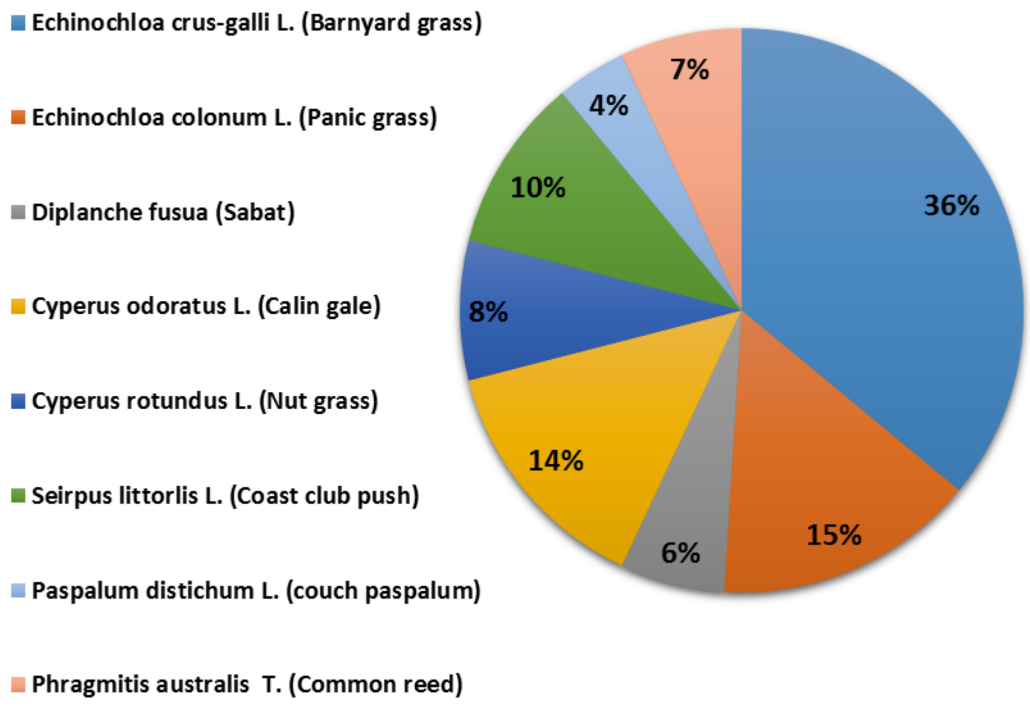

FIGURE 2 The most widespread weeds in the areas included in the field survey.

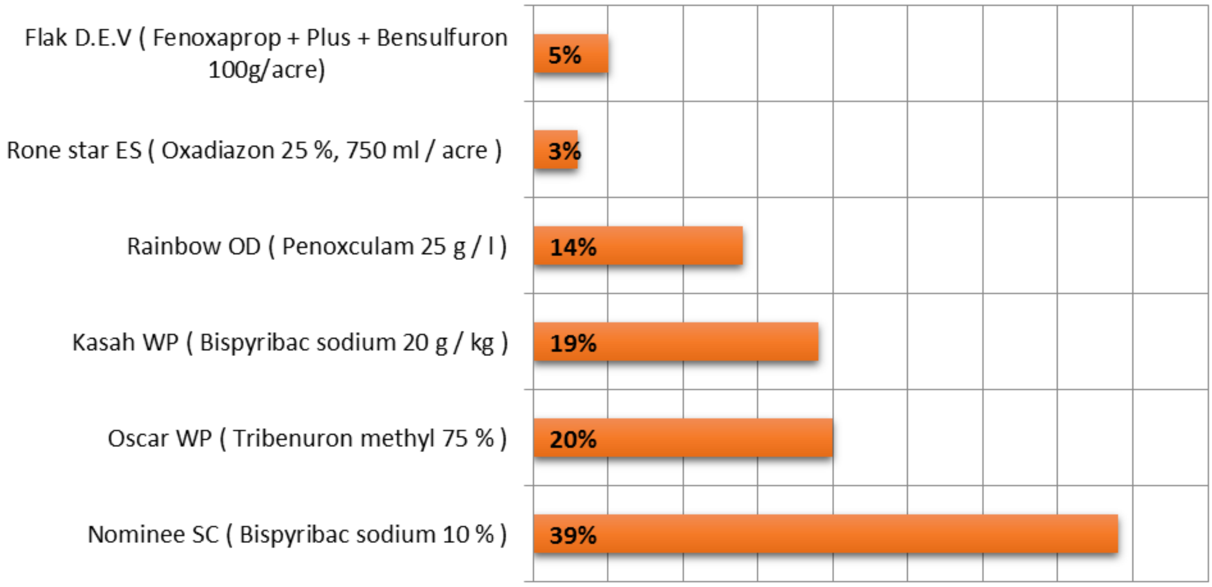

FIGURE 3 The most common weedicides used by farmers in rice fields in the areas covered by the survey.

3.2 | Diagnosis and anatomy of barnyard grass (*Echinochloa crus galli*)

The anatomical characteristics showed that the average thickness of the upper epidermis of the bran was 146.22 µM, while the lower was 181.76 µM, and the thickness of the cortex was 136.48 µM (Figure 4). Also, the average percentage of germination of barnyard grass seeds in the areas surveyed was (89-93%), which indicates the high susceptibility. The phenotypic characteristics of the seeds showed that their average size was 2.82 - 3.67 mm and their average weight was 0.0012 - 0.0038 grams per seed. The phenotypic diagnosis supported by molecular diagnosis resulted in the registration of 19 new strains for the first time in Iraq, which were deposited at NCBI under the accession numbers (OQ591719 to OQ591737) (Table 1).

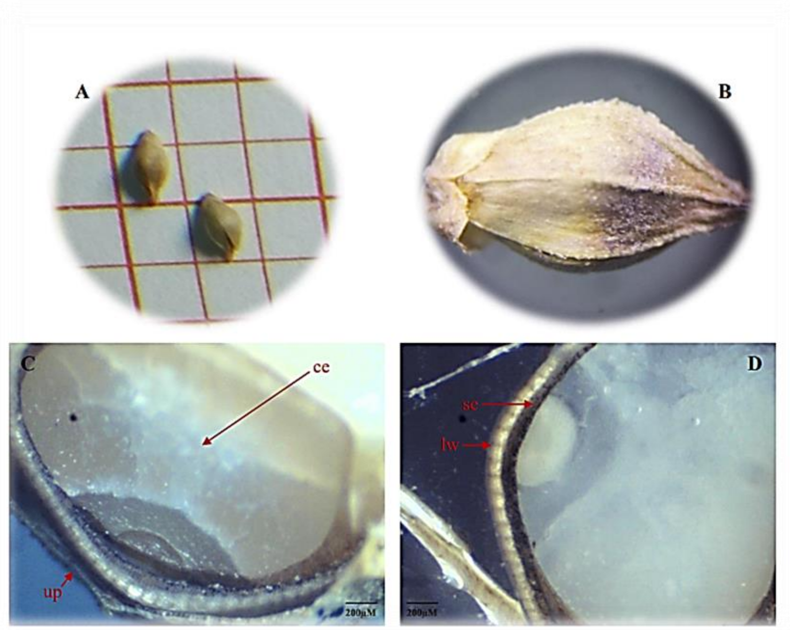

FIGURE 4 Anatomy of barnyard grass weed seeds. A, B *Echinochloa crus galli* seeds, C, D the anatomy of *Echinochloa crus galli* seeds. Up: upper epidermis of lemma ,ce: central endosperm ,lw: lower epidermis of lemma ,se: seed coat.

TABLE 1 Registered strains of barnyard grass weed *Echinochloa crus-galli* var. *crus-galli* .

Accession number	Strain	Sample source
OQ591719	RB-Ech1	Al-Mishkhab
OQ591720	RB-Ech2	Al-Hurriya
OQ591721	RB-Ech3	Al-Abassiya
OQ591722	RB-Ech4	Al-Hurriya
OQ591723	RB-Ech5	Al-Manathera
OQ591724	RB-Ech6	Al-Mishkhab
OQ591725	RB-Ech7	Al-Hurriya
OQ591726	RB-Ech8	Al-Mishkhab
OQ591727	RB-Ech9	Al-Hurriya
OQ591728	RB-Ech10	Al-Abassiya
OQ591729	RB-Ech11	Kufa
OQ591730	RB-Ech12	Al-Hayderiyh
OQ591731	RB-Ech13	Al-Qadisiyah
OQ591732	RB-Ech14	Al-Mishkhab
OQ591733	RB-Ech15	Al-Mishkhab
OQ591734	RB-Ech16	Al-Heera
OQ591735	RB-Ech17	Al-Abassiya
OQ591736	RB-Ech18	Al-Abassiya
OQ591737	RB-Ech19	Al-Qadisiyah

3.3 | Genetic affinity of the identified strains of barnyard grass (*Echinochloa crus-galli* var. *crus-galli*)

The phylogenetic tree for the genotypes of the barnyard grass strains (OQ591719 to OQ591737) shows that 100% of the genetic structure is similar to GenBank, as the tree was divided into several groups according to the degree of relatedness and dissimilarity (Figure 5). The strains varied in genetic affinity and closely relatedness, in addition to differences in group area. According to the arrangement of the groups in the genetic tree, it showed that there is genetic closeness between barnyard grass strains that have the characteristic of resistance to pesticides, as the two strains OQ591727 and OQ591734 had a genetic compatibility rate of 95% with the LC600900 strain from Japan, and the two strains OQ591723 and OQ591724 had a genetic compatibility rate of 96% with strain KF163623 from Korea.

Strain OQ591729 had a genetic compatibility rate of 96% with strain MH392229, which possesses the resistance gene EcAbcb4 (1). Also, strain OQ591732 had a genetic compatibility rate of 97% with strain MH392230, which possesses the resistance gene EcAbcb4 (9), as this group had the genetic compatibility rate reached 91% with the OK483200 strain, which possesses the Cyp81A68 gene, as this gene has the ability to metabolize ALS-AHAS and ACCase pesticides via O-demethylation. The genetic compatibility of OQ591736 strain had a rate of 98% with strain KY963554, which possesses the EcAs-like resistance gene, while this group had a genetic compatibility rate of 94% with strain JN241678, which possesses the EcGH3 gene, as this gene has the ability to reduce the toxicity of herbicides. Also, the OQ591728 and OQ591730 strains had a genetic compatibility rate of 98% with the HQ395760 strain, which possesses the EcAbcb4 (5) resistance gene. Therefore, it is clear from the phylogenetic tree of the genotypes that the identified barnyard grass strains from the field survey sites may have possessed some resistance traits (MHR) and (TSR & NTSR), which severely restricts control operations with herbicides of different chemical composition and its harmful effects on this weed.

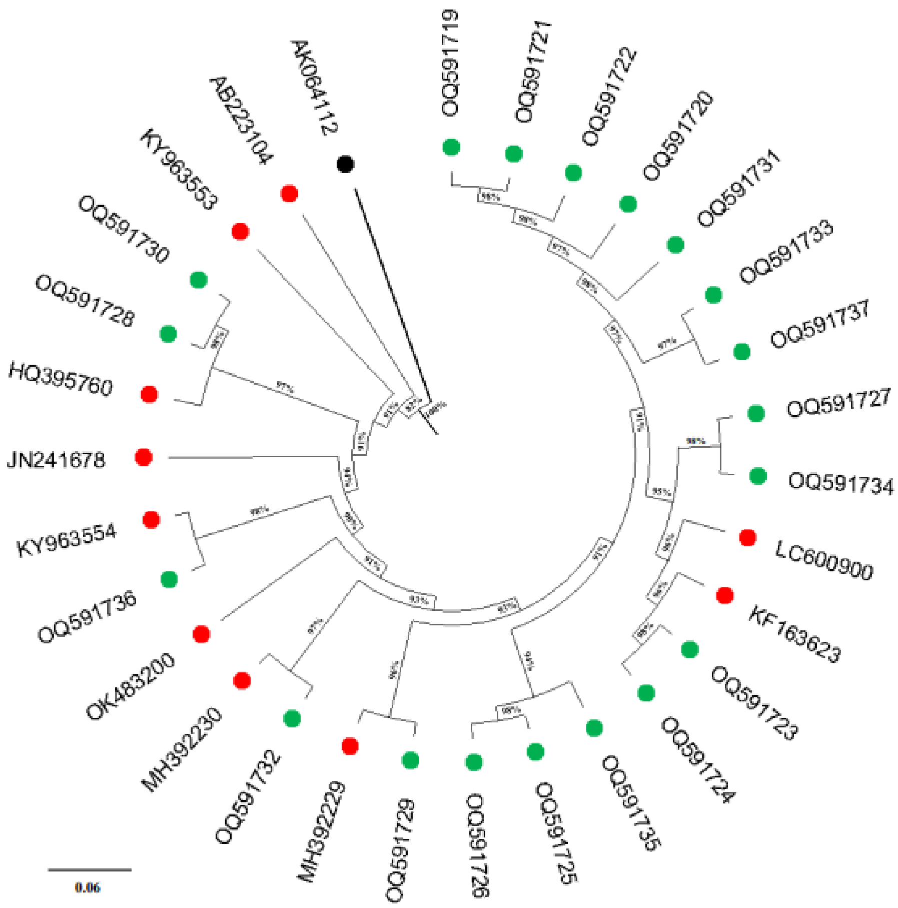

FIGURE 5 Phylogenetic tree of barnyard grass strains (OQ591719 to OQ591737) with strains from GenBank.

4 | DISCUSSION

The difference in the spread of weeds accompanying rice in the growing fields is due to many reasons, and the most important reason is the difference in methods of controlling weeds from one region to another, where control is carried out in some areas by relying mainly on mechanical and chemical control, and in other areas it is relied on chemical control only (Le et al., 2018). Other reasons are the use of non-specialized pesticides that have an impact on the density of weeds in the field, in addition to their significant impact on rice crop productivity (Iwakami et al., 2015). The spread of the barnyard grass weed in agricultural fields is attributed to many reasons, the most important of which is the resistance of the barnyard grass to many types of effective herbicides used including (ALS-AHAS and ACCase), as it can regain its activity again after the first irrigation of the crop after control due to the development of the genetic structure and the occurrence of mutations that enable the barnyard grass to metabolize the toxicity of herbicides or tolerate them, even if the amount of doses exceeds the recommended amount, which allows the weed to reproduce and spread in the field (Cai et al., 2022; Al-Gburi and Al-Gburi, 2024). The production of high quantities of seeds by barnyard grass, reaching 5,000 seeds per plant, in addition to the dormancy of the seeds for several years, as well as their small size and light weight, were among the reasons that led to the failure of barnyard grass control operations (Beltran et al., 2011). Also, the difference in sequential synthesis leads to the emergence of new genotypes that affect cellular, phenotypic, and physiological traits, which leads to the production of new strains that follow species characterized by high adaptation to changing in surrounding environmental conditions or due to herbicide control (Cai et al., 2022).

The emergence of new strains of the barnyard grass can also be explained by its ability to resist the action of herbicides at non-target sites (NTSR), which represents a great concern currently because it increases the opportunity for the barnyard grass to evolve to form new strains characterized by multiple herbicide resistance (MHR) (Damalas et al. 2023; Al-Gburi et al., 2024). The resistance of some barnyard grass varieties and strains to herbicides can be attributed to several reasons, their possession of (Cytochrome P450 monooxygenases) and (Glutathione-S-transferases) associated with enhanced metabolism to remove toxicity of herbicides (Dimaano et al., 2020; Yang et al., 2022). The weed also possesses (Allelochemical 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and (Phytoalexin momilactone) which are responsible for adaptation and correcting the biosynthesis of genes which in turn encode multiple genes for resistance to herbicides (Guo et al., 2018; Sen et al., 2022). What explains why barnyard grass possesses these characteristics is that the chromatin and histone proteins surrounding the DNA are more affected by abiotic factors, including climate change, in addition to competition for elements necessary for sustained growth and reproduction (Zhang et al., 2022). The main factor in possessing resistance characteristics are wrong processes and aspects used or the excessive use of herbicides by farmers and non-specialists through the use of non-specialized herbicides, increasing or decreasing the dose below the recommended rate (FRD), mixing several herbicides from a different chemical group. In addition to the effective effect without referring to the possibility of matching the mixing process and ensuring its effective effect on barnyard grass but not rice (Yang et al., 2021; Pan et al., 2022).

5 | CONCLUSION

The current study indicated that the potential agricultural economic damage occurred due to the spread of the weed species that belonging to the Poaceae and Cyperaceae families in rice cultivation fields within the geographical area of Najaf Province. The barnyard grass was the most widespread species in rice fields, as the presence of the variety (var. *crus-galli*) was recorded for the first time in Iraq, and 19 strains of the barnyard grass were identified under the accession numbers (OQ591719 to OQ591737) and registered at the National Center for Biotechnology Information (NCBI). Whereas, the results of the phylogenetic tree for the genotypes of *E. crus-galli*.var.*crus-gall* showed the presence of genetic convergence in resistance traits (MHR, TSR, NTSR) to herbicides.

AUTHOR CONTRIBUTIONS

Toman and Al-Gburi did the field survey, conducted all the experiments. Toman collected and analysed the data. Both authors wrote and approved the final version of the manuscript equally.

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CONFLECT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILIBILITY STATEMENT

The data of this study available upon reasonable request from the corresponding author.

REFERENCES

1. Al-Gburi, S.A.H., & Al-Gburi, B.K.H. (2024). Improving the nutritional content of wheat grains by integrated weeds management strategies and spraying with nano-micronutrient. *Journal of the Saudi Society of Agricultural Science*, 23(1), 88-92.
2. Al-Gburi, B.K.H., Lahmod, N.R., Al-Thabhawi, S.H., Al-Fallooji, S.A.K. (2024). Weed control in barley (*Hordeum vulgare*) via herbicides that inhibit ALS and ACCASE with increased seeding rate. Sabrao Journal of Breeding and Genetics. 56(5): 2136–2142.
3. Ali, M. B., & Tayeh, A. (2017). Climatic characteristics of Al-Najaf province and their relation with bush growth affecting rice protection. *Adab Al-Kufa*, 1(32).
4. Beltran, J. C., Pannell, D. J., Doole, G. J., & White, B. (2011). RIMPhil: a bioeconomic model for integrated weed management of annual barnyard grass (*Echinochloa crus-galli*) in Philippine rice farming systems. Working Paper 1112, School of Agricultural and Resource Economics, The University of Western Australia, Crawley, Australia. Pp.1-23.
5. Cai, X., Chen, J., Wang, X., Gao, H., Xiang, B., & Dong, L. (2022). Mefenacet resistance in multiple herbicide-resistant *Echinochloa crus-galli* L. populations. *Pesticide Biochemistry and Physiology*, 182, 105038.
6. Chauhan, B. S. (2013). Strategies to manage weedy rice in Asia. *Crop Protection*, 48, 51-56.
7. Chauhan, B. S., & Johnson, D. E. (2011). Ecological studies on *Echinochloa crus-galli* and the implications for weed management in direct-seeded rice. *Crop Protection*, 30(11), 1385-1391.
8. Chen, G., Zhang, W., Fang, J., & Dong, L. (2017). Restriction site-associated DNA sequencing allows for the rapid identification of simple sequence repeat markers in *Echinochloa crus-galli*. *Weed Biology and Management*, 17(2), 68-76.
9. Damalas, C. A., & Koutroubas, S. D. (2023). Herbicide-resistant barnyard grass (*Echinochloa crus-galli*) in global rice production. *Weed Biology and Management*, 23(1), 23-33.
10. Dimaano, N. G., Yamaguchi, T., Fukunishi, K., Tominaga, T., & Iwakami, S. (2020). Functional characterization of cytochrome P450 CYP81A subfamily to disclose the pattern of cross-resistance in *Echinochloa phyllopogon*. *Plant Molecular Biology*, 102(4), 403-416.
11. Hamza A , Derbalah A and El-Nady M. (2012). Identification and mechanism of *Echinochloa crus-galli* resistance to fenoxaprop-p-ethyl with respect to physiological and anatomical differences. *The Scientific World Journal*, 893204, 1-8.
12. Iwakami, S., Hashimoto, M., Matsushima, K. I., Watanabe, H., Hamamura, K., & Uchino, A. (2015). Multiple-herbicide resistance in *Echinochloa crus-galli* var. *formosensis*, an allohexaploid weed species, in dry-seeded rice. *Pesticide Biochemistry and Physiology*, 119, 1-8.
13. Le, D., Nguyen, C. M., Kumar, B. V., & Mann, R. K. (2018). Weed management practices to control herbicide-resistant *Echinochloa crus-galli* in rice in Mekong Delta, Vietnam. *Research on Crops*, 19(1), 20-27.
14. Lin, W., Guo, X., Pan, X., & Li, Z. (2018). Chlorophyll composition, chlorophyll fluorescence, and grain yield change in esl mutant rice. *International Journal of Molecular Sciences*, 19(10), 2945.
15. Marchesi, C., & Chauhan, B. S. (2019). The efficacy of chemical options to control *Echinochloa crus-galli* in dry-seeded rice under alternative irrigation management and field layout. *Crop Protection*, 118, 72-78.

16. Nah, G., Im, J. H., Kim, J. W., Park, H. R., Yook, M. J., Yang, T. J., Fischer, A.J., & Kim, D. S. (2015). Uncovering the differential molecular basis of adaptive diversity in three *Echinochloa* leaf transcriptomes. *PLoS One*, 10(8), e0134419.
17. Pan, L., Guo, Q., Wang, J., Shi, L., Yang, X., Zhou, Y., Yu, Q., & Bai, L. (2022). CYP81A68 confers metabolic resistance to ALS and ACCase-inhibiting herbicides and its epigenetic regulation in *Echinochloa crus-galli*. *Journal of Hazardous Materials*, 428, 128225.
18. Sen, M. K., Hamouzova, K., Košnarová, P., Roy, A., & Soukup, J. (2022). Herbicide resistance in grass weeds: Epigenetic regulation matters too. *Frontiers in Plant Science*, 13, 1040958.
19. Tahir, H. (2016). Characterization of *Echinochloa* spp. in Arkansas. University of Arkansas, USA.
20. Toman, R.T., Al-Gburi, B.K.H. (2023). First Record of Endophytic Fungi *Trichoderma asperellum* on Oryza sativa in Iraq. IOP Conference Series: Earth and Environmental Science.1262(3): 1-8, 032024.
21. Yang, Q., Yang, X., Zhang, Z., Wang, J., Fu, W., & Li, Y. (2021). Investigating the resistance levels and mechanisms to penoxsulam and cyhalofop-butyl in barnyard grass (*Echinochloa crus-galli*) from Ningxia Province, China. *Weed Science*, 69(4), 422-429.
22. Zhang, Y., Gao, H., Fang, J., Wang, H., Chen, J., Li, J., & Dong, L. (2022). Up-regulation of bZIP88 transcription factor is involved in resistance to three different herbicides in both *Echinochloa crus-galli* and *E. glabrescens*. *Journal of Experimental Botany*, 73(19), 6916-6930.