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Detection Of New Strains Of Weeds Associated With Wheat For The First Time In Iraq

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Abstract

The expected losses in wheat crop due to the associated weeds can be the real threat to the production of this crop and the wellbeing of growers as more than 20 genera of weeds are spreading in all regions that growing by wheat in Iraq. However, the most widespread and harmful genera are those belonging to Poaceae, Brassicaceae and Fabaceae families. The geographical distributions, morphological identification confirmed by molecular identification were conducted to detect weed species associated with wheat crop in Babylon province. Results indicated that the average of density of weeds spread was 20.38% in 632 hectare cultivated with wheat. Eight strains belong to different families of weed were recorded for the first time and registered in GenBank (NCBI) under the accession numbers (OM069771 to OM069778). The phylogenetic tree and development of the genetic patterns of registered strains with forty compatible species were divided into several groups that showed different inherent in kinship and closely interdependence despite the difference in locations of groups.

Key words: Weeds, Strains, Phylogenetic tree, Poaceae, Wheat.

Introduction

Sustainable agricultural development occupies a special and exceptional position among the most important issues that many countries of the world including Iraq seek to achieve, which improves food security by working to increase production in quantity and quality as well as reduce negative effects on the environment and living beings thus ensuring that these countries have access to the food they need to enjoy a full healthy life. Wheat crop (Triticum aestivum L.) ranks first in the world in terms of production with an annual rate about 769.5 million ton (FAO 2021), and it has an important nutritional value represented by a good balance in its grains between proteins and carbohydrates, while, containing quantities of fats, vitamins B1 and B2, mineral salts as well as the essential amino acids that human needs in his diet (Costa et al 2013; Amangaliev et al 2021). Iraq is considered one of the first places for the emergence of wheat in addition to the availability of main production factors in the country such as soil, water and climate conditions but the productivity of this crop is still fluctuating compared to the global level in developed countries (Mohammed et al 2016). The reason for the low productivity in Iraq is due to the lack of good attention to the operations of servicing the crop and soil and its exposure to various agricultural pests including weeds which cause losses estimated at 15 to 50% depending on the density and type of weed as well as the accompanying period (El-Taif 2021; Manogna et al 2021). There are more than 16 species of broad-leaved and 12 species of thin-leaved weeds accompanying wheat crop in Iraq, but the most widespread and harmful are Melilotus indicus, Raphanus raphanistrum, Rapistrum rugosum, Avena fatua, Lolium rigidum, Lolium temulentum, Hordeum bulbosum and Phalaris minor which cause great losses in wheat yield (Safi 2016). Therefore, researchers and those interested in agriculture began to combat it in various methods including chemicals as it easy to use and have rapid effect which considered one of the best management practices to combat weeds and impressive results were achieved in eliminating it and limiting its damage (Flessner et al 2021). However, the dormancy of seeds for long periods and the excessive use of traditional weedicides with high rate of use and the repetition of their use for many years led to the emergence of a kind of resistance to these weedicides by many types of weeds due to the emergence of new environmental and genetic varieties that make them more tolerant of the deadly action of the chemical weedicide (Hussain et al 2017).

Weeds control is important in growth systems and requires the integration of various plant protection strategies and methods (Berbec´ et al 2020), which led to an urgent need to search for alternative methods that are economically feasible and environmentally stable such as early sowing of wheat, soil solarization, amendment of row spacing, weed-free seeds, adjustment of seeding rate, planting densities, crop rotation, competitive cultivars, effective nutrient management, appropriate irrigation scheduling, detection of the emergence of biological differences for weed species, mechanical control and selective weedicides (Verma et al 2021). Thus, current study aimed to identify the most prevalent types of weeds associated with wheat crop in Babylon province and determine the genetic differences between species as the study will provide the basis for the future research in biological, physiological and environmental studies and will contribute to finding the best IWM methods to increase wheat yield in Iraq.

Materials and Methods

Detection of spreading weeds associated with wheat in some regions of Babylon province

A field survey was conducted in some regions including Al Mahaweel, Niel, Hashemie and Jableh during 2020-2021 season. Sites of collecting weeds were determined by GPS as it collected from 2.5m^2 square using EM-CDTMR program and the percentage of weeds density was estimated according to Mohammed et al (2016) equation with some modifications:

Weeds density % = the number of weeds/the number of weeds + the number of wheat plants x 100

Weed plants at different growth stages were put in 10 L plastic bags and the seeds of weeds were put in 100ml sterile containers then labeled with information. Samples were transferred to the laboratory at 4-10 ^{0}C and seeds were kept at room temperature until used.

Morphological identification of weeds

Morphological identification was done in weeds lab/Faculty of Agriculture/University of Kufa for weed plants (mature plant and seeds) collected during field survey (total of 294 plants) as the most prevalent samples were washed and dried then installed on plant cards of phenotypic classification. A magnifying lens and dissecting microscope (Krüss) were used for the purpose of diagnosis according to the classification keys approved by the Iraqi National Herbarium (Chakravarty 1976; Barkly 2004; Shouliang 2006; Zheng-Yi et al 2010).

Molecular identification of weeds using polymerase chain reaction (PCR)

Eight samples of weeds and its seeds were selected to represent the most prevalent collected weeds, then the DNA of these samples were extracted following the method described in Al-Gburi et al (2019) using (ZR Plant/Seed DNA MiniPrep™) kit and the steps recommended by the company Zymo/USA as it identified based on ITS1 and ITS4 primers (Warwick and Sauder 2005). PCR products were injected in 1.5% agarose gel containing 5mg/ml of ethidium bromide stain, then electrophoreses was done at 50 v for one hour (Alsaadi et al 2016). Agarose gel was visualized using UV and VIVO camera, and then the PCR products were sent to Macrogen Korea for sequencing.

Studying the genetic affinity of weed strains

DNA sequences were analyzed to study the genetic affinity between identified weed strains of current study with wheat cultivars and weed strains from NCBI using Geneious Prime v. 19.4. alignment with cost matrix of 70% similarity (IUB) (5.0/_4.5), then the phylogenetic tree was conducted using Geneious Tree Builder with Tamura–Nei genetic distance model replications number was 1500, and support threshold >70% . rDNA sequences of Hordeum vulgare (KM040746) and Triticum aestivum (KP210655) from GenBank were used as out-groups for each tree.

Results and Discussion

Results of field survey showed that growers in studied regions were adapted traditional agricultural practices by using the chemical fertilizers with limited nutrients, in addition to lack of diversity of herbicides used in terms of the mechanism of influence on the density of weeds in the field. Field survey results also indicated a variation in the numerical density of the spread of weeds from 15% in Hashemie to 26% in Niel as the average of numerical density of the thirteen sites surveyed reached 20.38% (Fig 1).

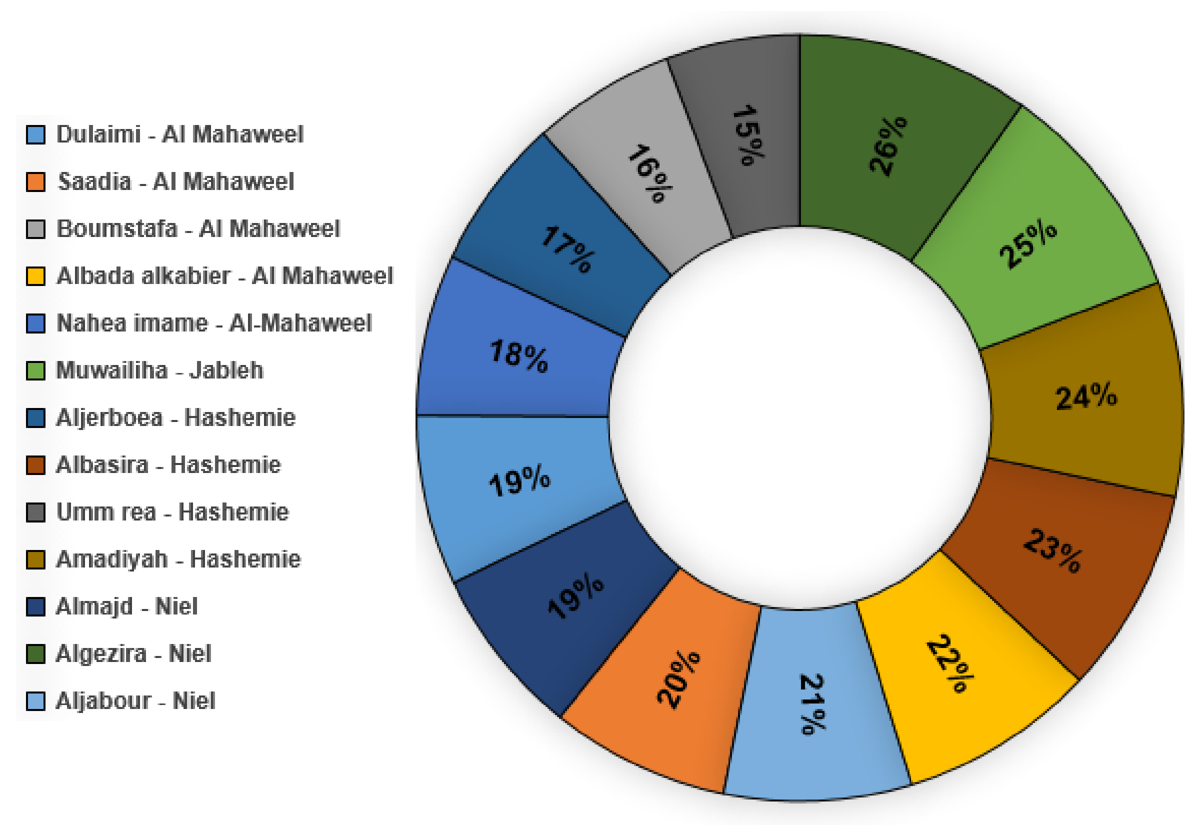

Fig 1. The percentage of numerical density of weeds in the sites surveyed.

Weeds tends to be a pioneering plant species spread widely using a variety of different mechanisms including seed dormancy and the production of a large amount of small weight seeds that are spread from one place to another by wind, water and organic fertilizers. These characteristics make weeds able to adapt to agricultural changes which leads to an increase in its spread in wheat fields (Koch et al 2016; Rühl et al 2016). The discrepancy in the percentage of weeds spread from one region to another is due to two reasons, the first is that the methods used to combat thin and broad- leaved weeds are varying in terms of efficiency in one field compare to another (Flessner et al 2021). The second reason is the difference in the levels of competition between weeds and wheat to obtain the largest possible amount of nutrients which affects the density of accompanying weeds (Singh and Saxena 3013). Failure to remove the weeds during the critical period in the first month of crop growth after sowing leads to reach the weeds the level of economic damage (Galon et al 2019; Hussein et al 2020).

Identification of weeds associated with wheat crop

The results of phenotypic diagnosis confirmed by molecular identification indicated that two strains of weeds belonging to Brassicaceae, one strain belonging to Fabaceae and five strains belonging to Poaceae, which were prevalent in terms of spread compared to other families (Fig 2). The obtained strains were varied in the degree of spread and accompanying wheat crop in areas covered by the survey as all eight strains were registered in NCBI (Table 1). These results are useful for facilitating and solving the difficulties of phenotypic difference between the genera and species of weeds spread in wheat fields.

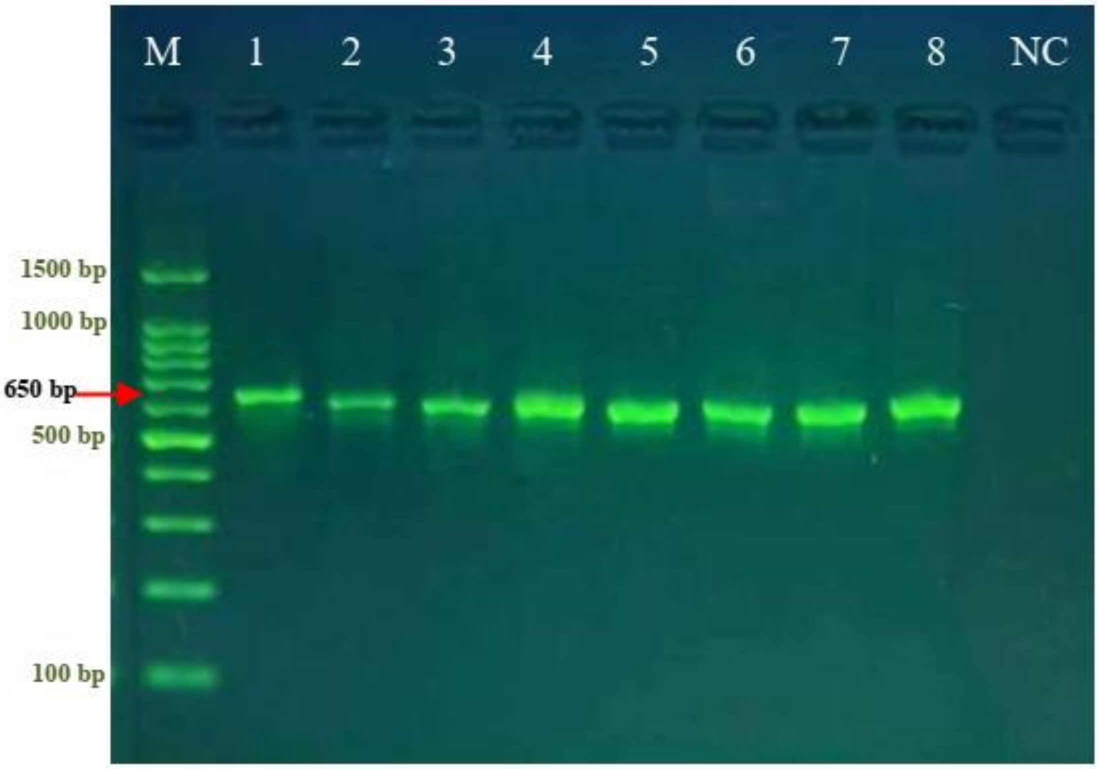

Fig 2. Agarose gel electrophoreses of PCR products (Band size 650bp) for species of weeds, 1500 bp

DNA Markers (M), 1,2 for Brassiceae, 3 for Fabaceae, 4, 5, 6, 7, 8 for Poaceae Negative control (NC) without adding DNA.

Table 1. Registered weed species with their families.

Accession number	Weeds spp.	Family name
OM069771	Rapistrum rugosum strain Babylon,SB01	Brassiceae
OM069772	Raphanus raphanistrum strain Babylon,SB02	Brassiceae
OM069773	Melilotus indicus strain Babylon,SB03	Fabaceae
OM069774	Avena fatua strain Babylon,SB04	Poaceae
OM069775	Lolium rigidum strain Babylon,SB05	Poaceae
OM069776	Lolium temulentum strain Babylon,SB06	Poaceae
OM069777	Hordeum bulbosum strain Babylon,SB07	Poaceae
OM069778	Phalaris minor strain Babylon,SB08	Poaceae

Weeds that belong to Poaceae, Fabaceae and Brassicaceae, grow and flourish before the germination of wheat as the increase in absorption of nutrients by weeds leads often to a great competitive advantage to increase its spread which reduces the density of wheat crop (Siddiqui et al 2010; Das et al 2017). The dominance of Poaceae weeds in spreading was due to the matching of the secretions of roots for both wheat and weeds that belonging to the same family, in addition to the fact that the herbicides of broad-leaved weeds have reduced the population density of many types of weeds belonging to different families (Vyvahare and Khose 2021; Kaur et al 2021). The emergence of new strains can be explained by the ability of weeds to develop rapidly which is the key to its permanence in the field as part of the natural evolution to counteract human management against it, and the differences in the successive breeding play a major role in the emergence of new genetic patterns (Clements and Jones 2021). These patterns affect the phenotypic, cellular and physiological features of weeds to produce strains that are more adapted to the changing environment, climate and the action of weedicides (Richards et al 2012).

The genetic affinity of identified weed strains

The phylogenetic tree of the genotypes of weeds strains showed that 100% of these genotypes are similar to those from GenBank as the tree was divided into two groups, the first for weed strains and the second for wheat cultivars with 48.3% matching percentage between the two groups. The percentage of congruence between the recorded strains varies in the percentage of genetic affinity due to genetic differences according to the genus of the strains in the first group (Fig 3). While, the phylogenetic tree of weed strains and the strains from GenBank showed that the species were genetically close and related despite the difference in their region (Fig 4). According to the arrangement of the groups in the tree, the percentage of genetic concordance between OM069771 and DQ249825 was 97.8%, OM069772 and GQ268068 was 98.6%, OM069773 and MK918726 was 96.2%, OM069774 and KU883489 was 97.5%, OM069775 and AF171163 was 95.4%, OM069776 and AF171167 was 97.9%, OM069777 and KM205214 was 96.8% and finally between OM069778 and KU883518 was 95.8%.

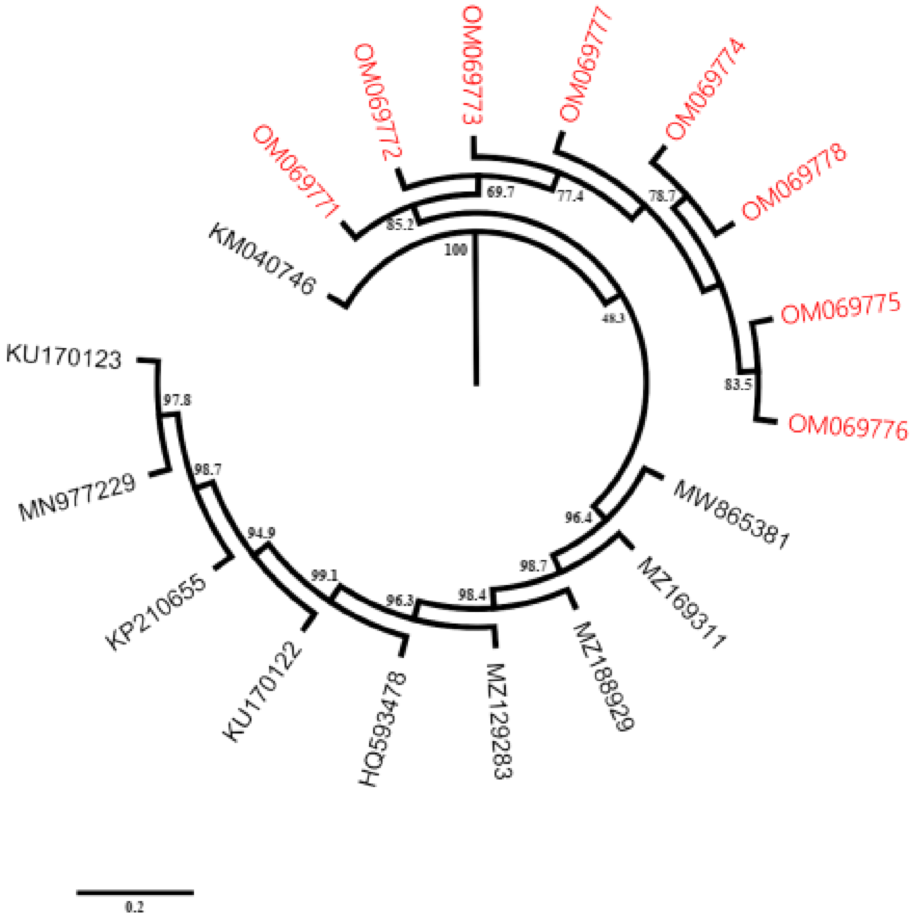

Fig 3. The phylogenetic tree of weed strains with wheat cultivars.

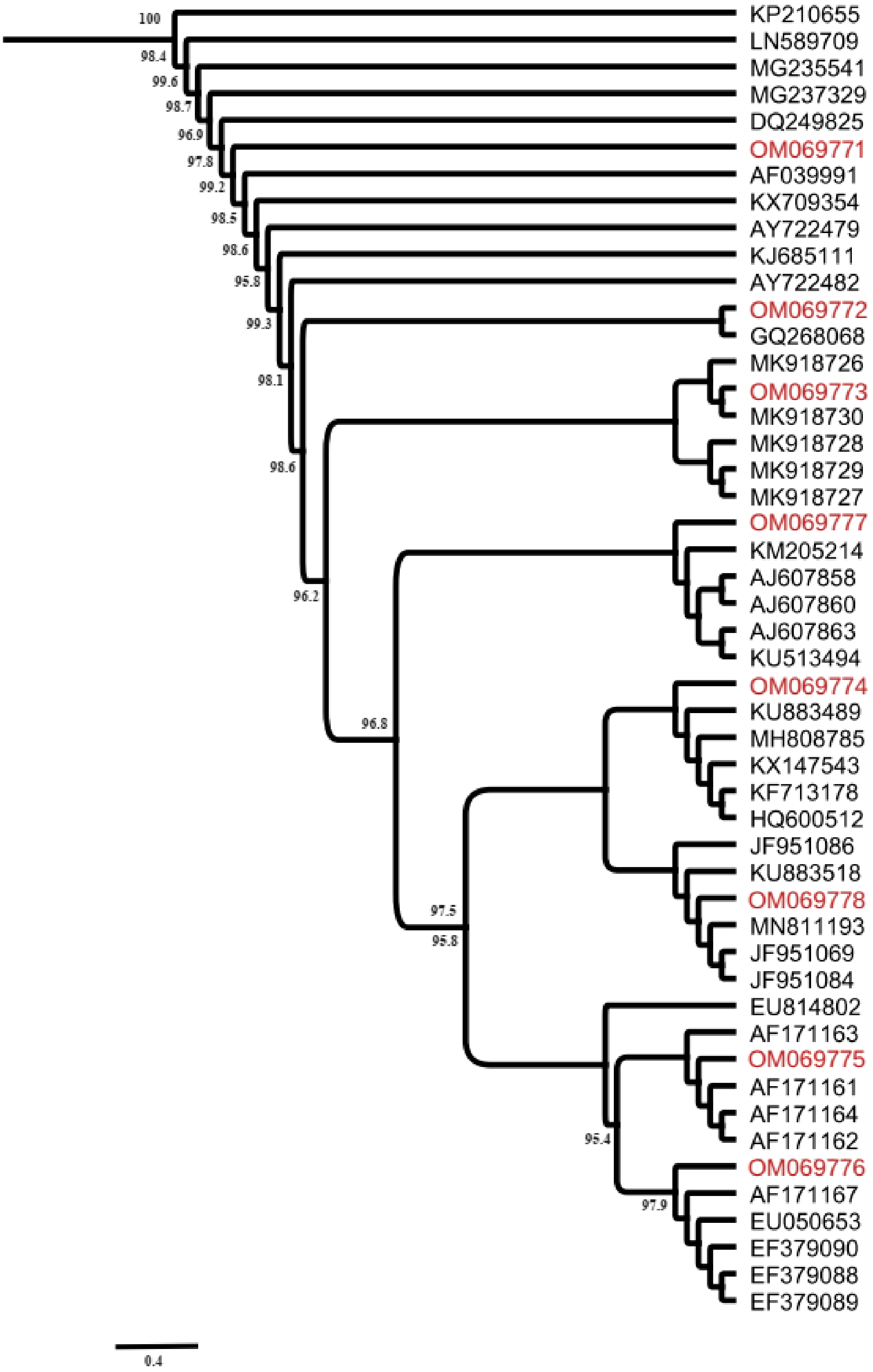

Fig 4. The phylogenetic tree of weed strains with the weed strains from GenBank.

The genetic structure is the decisive factor in the similarity and difference between plants which extinguishes the extreme phenotypic plasticity of the species and therefore, the percentage of genetic affinity between wheat cultivars and weed strains is rather small (Davidson et al 2011). The variation in the percentage of genetic affinity between weed strains is due to the different genera of weed belonging to different families that have a different genetic composition and work to preserve it, form its phenotypic traits (Darmency 2019). The genetic affinity between studied weed strains and the strains from GenBank appeared due to the increase in the percentage of similarity in the DNA sequence of the same species that undergoes a change in chromatin which leads to changes in the gene expression of the weed (Jones 2012). Chromatin and histone proteins that allow the DNA to fold into a more compact structure as this structure is more affected by abiotic factors including climate changes and herbicides (Peterson et al 2017; Asensi-Fabado et al 2018).

Conclusion

Current study indicated the possible economic damages due to the spread of various weeds belonging to Poaceae, Brassicaceae and Fabaceae in wheat fields within the geographical area of Babylon province. Eight strains of different species were diagnosed for the first time in Iraq and registered in GenBank (NCBI). Results of the phylogenetic tree weed strains with wheat cultivars on the one hand and weed strains from NCBI on the other hand were differed in genetic affinity. The outcome of this study will expand the identification of weed species associated with wheat crop which are most widespread in order to design effective integrated management programs for these species in Iraq.

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