Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Molecular Characterization and Dose-Response to 2,4-D Herbicide in *Convolvulus arvensis* Populations in Türkiye

Yücel Karaman[a*], Nihat Tursun[a], Hikmet Murat Sipahioğlu[b]

[a]*Plant Protection Department, Agricultural Faculty, Malatya Turgut Ozal University, Malatya, 44210, TURKEY*

[b]*Plant Protection Department, Agricultural Faculty, Malatya Turgut Ozal University, Malatya, 44210, TURKEY (Retired)*

ARTICLE INFO

Research Article

Corresponding Author: Yücel Karaman, E-mail: yucel.karaman@ozal.edu.tr

Received: 19 December 2023 / Revised: 15 February 2024 / Accepted: 16 February 2024 / Online: 23 July 2024

Cite this article

Karaman Y, Tursun N, Sipahioğlu H M (2024). Molecular Characterization and Dose-Response to 2,4-D Herbicide in *Convolvulus arvensis* Populations in Türkiye . *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 30(3):584-593. DOI: 10.15832/ankutbd.1407008

ABSTRACT

Field bindweed (*Convolvulus arvensis* L.) is an important weed species in agricultural areas in Türkiye and worldwide. The study, conducted in 2018-2019, involved collecting seeds from 16 different provinces in Türkiye (Adana, Ankara, Çanakkale, Denizli, Diyarbakır, Erzurum, Hatay, İzmir, Karaman, Kayseri, Konya, Malatya, Samsun, Şanlıurfa, Tekirdağ and Uşak). These seeds were then germinated under greenhouse conditions (29/19°C day/night), and molecular characterization of the samples was performed. As a result of comparing the samples with a specific reference from NCBI GenBank, it was found that the similarity ratios were close to each other but formed different groups. The study revealed that samples from Adana, Konya and Samsun belonged to different groups in terms of similarity. Subsequently, the dose-response rates of these samples to 2,4-D, a herbicide licensed against field bindweed, were determined. In the dose-response analysis of the herbicides, ED_{50} values of 131.93, 115.42 and 141.89 g.a.i/ha were determined for Adana, Konya and Samsun, respectively. The study concluded that the dose-response of field bindweed in Adana, Konya and Samsun provinces, belonging to different molecular groups, to 2,4-D herbicide is close to each other but exhibits different values.

Keywords: Field bindweed, Weed, Molecular characterization, Herbicide

1. Introduction

Weeds in agricultural areas are one of the biggest problems in the world, as they lead to yield and product losses (Jabran et al. 2015). Around 1800 weed species in the world cause a 31.5% decline in agricultural productivity (Kubiak et al. 2022). Weeds in agricultural areas negatively impact crop development by competing for water, light, and nutrients with crops, ultimately reducing the quality and quantity of the product (Güncan & Karaca 2023). On the other hand, weeds also serve as hosts for plant diseases and pests (Mourelos et al. 2014; Üremiş et al. 2020).

Field bindweed (*Convolvulus arvensis* L.) was first classified by Linneaus in 1753 (Güncan 1979). Native to Europe and Asia and it thrives in temperate, tropical and Mediterranean climates (Lyons 1998; Gubanov et al. 2004). Originating from the European continent, field bindweed is widespread in temperate regions of Western Asia, North America and Europe. However, it causes more damage in Europe in terms of cultivated area (Holm et al. 1977). In Türkiye, it is widespread throughout the country and is observed in both temperate and arid regions (Coruh & Zengin 2007; Özkil & Üremiş 2020; Kuru & Üremiş 2021). Field bindweed competes with other species in the vicinity for water and nutrients. It climbs on surrounding plants, hindering their development and causing yield losses. The weed reduces the available water at a soil depth of 60 cm, leading to plants withering due to water deficiency. Additionally, it poses challenges in pruning by overgrowing shrubs and small trees (Vogelgsang 1998). Field bindweed serves a breeding ground for insect pests and an alternative host for viruses causing plant diseases (Tamaki et al. 1975). The seeds, leaves and especially the roots of field bindweed contain the glycoside convolvulin. This resinous, water-insoluble compound induces severe hyperemia (blood rush), peristalsis, and diarrhea in the stomach and intestinal tract of animals (Lubenov 1985).

Pesticides, commonly utilized for controlling diseases, pests and weeds in agricultural areas worldwide, play a significant role as pollutants. The continuous and improper use of these substances unnecessarily increases production costs, disrupts the balance of nature, diminishes sustainable agriculture, and negatively impact human health (Pimentel et al. 1992; Pimentel & Greiner 1997; Dogan et al. 2004). As human health is endangered by pollution, there has been an increasing pressure to reduce and ban the use of herbicides in agricultural areas (Matteson 1995). However, chemical control, specifically the use of herbicides

remains the primary method for easy and practical weed control in agricultural areas (Uygur & Şekeroğlu 1993). The application of herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D), dicamba and glyphosate in the chemical control of field bindweed can effectively manage this weed (Vogelgsang 1998). The primary objective of weed control in a given agricultural area is to maintain the weed population below the economic damage threshold rather than eradicating it completely. Even low doses of herbicide applications have been found to have a significant effect on weeds (Steckel et al. 1990). It has been proven that weeds can be controlled with lower those licensed (Salonen 1992; Zhang et al. 2000; Boström & Fogelfors 2002; Busi et al. 2016; Vranjes et al. 2019). Integrated weed control in agricultural areas should be carefully managed. If herbicides are needed in the integrated control method, determining the application dose is an important step (Kudsk & Streibig 2003).

The process of classifying living organisms into specific groups according to their degree of similarity and relatedness is called classification. The classification process helps us determine the relationships between weeds and crops and to obtain information about them. If the individuals of a particular species are similar in terms of their competitive ability and developmental characteristics, they also have similar habitats (Hoffmann & Frodsham 1993). Classifications based on the external appearance of living beings and their relationships with the environment are referred to as artificial or empirical classifications. However, this classification has lost its validity today. The scientific (natural or phylogenetic) classification is based on the physiological, anatomical and ancestral similarities of organisms and the degree to which they are related. The main feature of this classification is the use of molecular techniques (genetic markers), and it has recently taken an important place in the classification of living organisms (Hoffmann & Frodsham 1993). Chloroplast trnL-F and ribosomal ITS gene regions are frequently used to determine phylogenetic relationships in different plant groups (Brouat et al. 2001; Soejima & Nagamasu 2004). In recent years, in addition to morphological traits, molecular traits are increasingly used to identify plant species (Mummenhoff et al. 1997). In molecular characterization, many regions of genomic DNA (gDNA), chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) are used to classify plants. In phylogenetic analyses, molecular characters are employed when morphological characters are insufficient (Yokoyama et al. 2000). Internal transcribed regions (ITS) on nuclear DNA (nrDNA), intergenic regions (trnL-F) and protein coding (ndhF) regions on cpDNA are used to determine intraspecific diversity (Kellogg 1998). Since more variation can occur in the ITS region, it is possible to distinguish between closely related species and to characterize different populations of the same species by examining this region (White et al. 1990).

In this study, field bindweed seeds collected from different provinces of Türkiye (Malatya, Erzurum, Diyarbakır, Şanlıurfa, Kayseri, Konya, Karaman, Ankara, Samsun, Adana, Hatay, Denizli, İzmir, Uşak, Tekirdağ and Çanakkale) were grown in controlled greenhouses environments, and molecular characterization was performed with the obtained plant samples. After molecular processing, the aim of this study was to determine the phylogenetic similarity rates among the samples and to assess the responses of the samples found in different groups to the 2,4-D herbicide.

2. Material and Methods

2.1. Specimen collection

The seeds of field bindweed were collected between 2016 and 2017 from agricultural land in various provinces of Türkiye. (Malatya, Erzurum, Diyarbakır, Şanlıurfa, Kayseri, Konya, Karaman, Ankara, Samsun, Adana, Hatay, Denizli, İzmir, Uşak, Tekirdağ and Çanakkale). The collected seeds, which were 1-2 years old, were stored at +4 °C until the start of the studies. The studies were conducted in 2018-2019 in greenhouses and laboratories of Malatya Turgut Özal University Faculty of Agriculture, Malatya, Türkiye.

2.2. Genomic DNA isolation

For DNA isolation, field bindweed seeds collected from fields in different provinces were first subjected to a dormancy-breaking study (the seeds were immersed in water at 90 °C for 5 seconds and then removed (Karaman & Tursun 2021). Following this study, the seeds were sown in the greenhouse under conditions of 29 °C±1 14 hours during the day and 19 °C±1 10 hours at night. When the seedlings had reached the 4-6 leaf stage, 100 mg leaf samples were taken from each sample. The Bioline DNA extraction kit was used to isolate the plant DNAs. To obtain high-quality and high-density total DNA, the extraction methods specified by the respective company were used. The samples for the PCR application were stored at -20 °C and taken out of the freezer and used when needed.

2.3. PCR amplification of the ITS region and electrophoresis

The universal ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAG G - 3') primers were used to amplify the ITS regions on the isolated ribosomal DNA. The components for the PCR reaction, using the enzyme GoTaq G2 Flexi DNA Polymerase (Cat:M780B) from Promega with a total volume of 50 µl for the ITS region, are listed in Table 1.

Table 1- PCR components, amounts or ratios used in the amplification of ribosomal DNA by the PCR method

PCR Components	*For 1 sample (µl)*	*For 16 samples (µl)*
5X PCR Buffer	10	160
dNTP (20 mM)	1	16
$MgCl_2$ (25 mM)	3	48
Mould DNA	2	32
Primer F (ITS4) (100 mM)	1	16
Primer R (ITS5) (100 mM)	1	16
GoTaq G2 Flexi DNA poly. enzyme	0,4	6,4
Sterile water	31.6	505.6

After adding all PCR parameters to the sterile tubes, a brief cycle was performed to ensure that all liquids were collected at the bottom of the tubes. The prepared PCR mixture was tested in 36 thermal cycles (Table 2).

Table 2- PCR thermal cycles used in the amplification of ribosomal DNA

94 °C'de …2 min DNA double strand separation 94 °C'de …1 min DNA double strand separation 55 °C'de …1 min primer binding 72 °C'de …2 min DNA synthesis	Total 36 cycles
72 °C'de …10 min final elongation	

The PCR applications were conducted using the Prima-96plusTM Thermal Cycler device. The products obtained after the PCR process were prepared for electrophoresis as 1.5% agarose gel. For this, 100 mL of sterile distilled water and 3 µl of fluorescent dye were added to a suitable container and run at 85 V for 45 minutes. Imaging was performed using the Gel Imaging and Analysis System to visualize the stained DNA.

2.4. DNA sequencing of ITS regions

DNA sequencing of ITS regions, amplified from genomic DNA using universal primers, was carried outby BM Laboratory Systems (Ankara) in a single direction.

2.5. Phylogenetic analyzes and entry of DNA sequences into the GenBank

The phylogenetic analysis of the sequenced DNA samples was completed, and the DNA sequences were registered in the GenBank (NCBI). The access numbers can be found in the Results section. The programs Unipro UGENE, Unipro UGENE, MEGA11 and CLC Main Workbench 20 were used. A different function of each program was used for the sequence analysis and the creation of the phylogenetic tree. The creation of the phylogenetic tree was repeated 100 times using the neighbor-joining algorithm and the Bootstrap method (Felsenstein 1985; Saitou & Nei 1987; Nei & Kumar 2000; Tamura et al. 2021; Ateş 2022)

2.6. Dose-response studies of field bindweed (Convolvulus arvensis) samples to 2,4-D herbicide

In the study, the efficacy of the herbicide containing the active ingredient 2,4-D (Hektas Ester'h 480 g/l 2,4-D acid equivalent isooctylester), licensed for the control of field bindweed, was investigated for the samples in different groups resulting from phylogenetic analyses. For this purpose, the experiments were conducted in a fully automated greenhouse under controlled conditions at a temperature of 29 °C ±1 14 hours during the day and 19 °C±1 10 hours at night. The area of the glass greenhouse in the study is 25 m^2, and the humidity inside was maintained at around 60±10 % depending on the automation system.

2.7. Pot trials

Field bindweed seeds from three provinces (Adana, Konya and Samsun), which were in distinct groups based on molecular characterization, were used for the study. The seeds were immersed in 90 °C hot water for 5 seconds before sowing to break the dormancy of the seeds (Karaman & Tursun 2021). Following dormancy breaking, a prepared 1:1/3 peat:perlite mixture was placed in plastic pots measuring 25 cm height and 15 cm width, and sowing (5 seeds in each pot) was performed. Despite the humid greenhouse environment, irrigation was carried out at regular intervals to maintain moisture balance in the pots. After germination, the seedlings were thinned to one per pot at the stage of two true leaves. Since field bindweed tends to wrap 1 m long, stakes were driven into the pots to serve as controls. The study was conducted with four replications and two repetitions according to a randomized plots experimental design.

2.8. Effect of 2,4 D herbicide on convolvulus arvensis samples

Different doses of the herbicide containing the active ingredient 2,4 D-amine were applied post-emergence to field bindweed samples in Adana, Konya and Samsun provinces, which were categorized into different groups based on molecular studies, during the 2-6 leaf stage. The calibration of the electronic spray booth for herbicide application was set at 300 L of water per ha, 304 kPa pressure and 5 km/h. Various doses of herbicide 2,4-D were used in the trial: 0 g.a.i /ha (unsprayed control), N/4 (180 g.a.i /ha), N/2 (360 g.a.i /ha), N (recommended dose – 720 g.a.i /ha), 2N (1440 g.a.i /ha), 4N (2880 g.a.i /ha) and 8N (5760 g.a.i /ha). After the spraying process, the pots were again left under the same conditions. Percentage/symptom values (Table 3) were monitored weekly for 4 weeks after herbicide application (Bajwa et al. 2019). Finally, harvesting was conducted at the end of the 4th week (28th day). During harvesting, the aerial parts of the plants were removed. Subsequently, the plant materials were dried in paper bags in an oven set at 105 °C for 24 hours (Yusriah et al. 2012), their weight was determined using a precision scale.

Table 3- Herbicide damage assessment system based on morphological symptoms in the degree of damage in field bindweed (*Convolvulus arvensis* L.)

Damage rating	***Morphological responses***
0	Not evident
10	Negligible discoloration, distortion and/or stunting barely seen
20	Slight damage discoloration, distortion and or stunting clearly seen
30	Moderate damage: moderate discoloration, marked distortion and/or stunting. Recovery expected
40	Substantial damage: much discoloration, distortion, and stunting: some damage probably irreversible
50	Majority of plant tissue is damaged, mostly irreversibly: substantial necrosis; discoloration and distortion
60	Nearly all the plant tissue damaged, mostly irreversibly some plants killed (<40%): substantial necrosis and distortion
70	Sever damage, necrosis and wilting
80	Very severe damage, heavy necrosis and wilting
90	Extreme irreversible damage with <10% green tissue visible, most tissue discolored and distorted permanently or desiccated
100	Complete loss of plant

2.9. Statistical analysis

The significance of the differences in the recorded data was determined by analysis of variance (ANOVA) using IBM SPSS STATISTICS 25.0 software with a general linear model (GLM). Initially, the differences between treatments were analyzed with ANOVA and then with the Duncan test for a multiple-range test ($P \leq 0.05$).

Data on visual reduction (%) and dry matter (DM) reduction (%) of the effect of 2,4-D herbicide on field bindweed were subjected to nonlinear regression analysis on herbicide dose using the four-parameter log-logistic model (Knezevic et al. 2007; Ulloa et al. 2011):

$$Y = C + \frac{(D - C)}{\{1 + \exp[B(\log X - \log E)]\}}$$

Where; Y is the response (e.g., percent DM reduction), C is the lower limit, D is the upper limit, B is the slope of the line at the inflection point (also known as the rate of change), and E is the dose that results in a 50% response between the upper and lower limit (also known as the inflection point, ED_{50}).

The ED_{50} values were used to compare visual (%) and dry matter (DM) reduction of each field bindweed under 2,4-D (Matzenbacher et al. 2015; Chen et al. 2015). The analyzes of the dose-response curves were performed using the software R (R version 2.15.3, R Development Core Team 2013) using the statistical add-on package drc (dose-response curves) (Knezevic et al. 2007; Knezevic & Datta 2015). When determining the effect of 2,4 D on field bindweed, the ED_{50} (dose controlling 50% of field bindweed) and ED_{90} (dose controlling 90% of field bindweed) values were also determined.

3. Results and Discussion

3.1. Identification of samples of field bindweed (Convolvulus arvensis) from different provinces by molecular methods

For the phylogenetic studies, field bindweed the samples from different provinces (Malatya, Erzurum, Diyarbakır, Şanlıurfa, Kayseri, Konya, Karaman, Ankara, Samsun, Adana, Hatay, Denizli, İzmir, Uşak, Tekirdağ, and Çanakkale) were grown from seeds, and their relationships were determined by molecular methods. It has been found that morphological characteristics are not sufficient to determine the degree of similarity between plant species, and some sequence analyzes are useful for phylogenetic analyzes (Yokoyama et al. 2000). For example, one of the methods used to determine the relationships among plant species is ITS (Internal Transcribed Spacers) PCR of the nrDNA region (Baldwin et al. 1995). This method has become a commonly used in molecular systematic studies of plants, enabling the identification of plant species by determining species-specific gene regions (Baldwin et al. 1995; Tursun et al. 2021; Saric-Krsmanović et al. 2022). Ribosomal DNA internal transcribed spacers (rDNA ITS), known as one of the most reliable methods in phylogenetic studies, are used for plant systematics and identification due to genomic segments' functionally. The high percentage of conservative genes and their belonging to the ITS sections provide an advantage in their use (Baldwin et al. 1995).

The ITS has been widely utilized in systematic studies to determine genus and species levels in many plant varieties. The two internal regions ITS-1 and ITS-2 are located between the genes coding for the subunits of 5.8S, 18S and 26S nuclear ribosomal RNA (nrRNA). In this study, genomic DNA was isolated from each field bindweed in the provinces and universal primers ITS4 (5'- TCCTCCGCTTATTGATATGC-3') and ITS5 (5'GGAAGTAAAAGTCGTAACAAG G - 3') were used to amplify the ITS regions. Using these primers, the ITS1, 5,8S and ITS2 regions in the rDNA were amplified by PCR. The consensus sequences were determined by comparing the sequence data obtained with reference sequences. The genome information of the field bindweed species, for which DNA sequencing was performed was analyzed using the CLC Main Workbench 20.0.1 program. After DNA sequencing, a search for the cloned region in GenBank was performed with the BLAST program. The DNA sequences of field bindweed samples from different provinces were registered in the National Center for Biotechnology Information (NCBI) GenBank, making them available to researchers worldwide. The GenBank accession numbers and gene lengths (bp)are shown in Table 4.

Table 4- GenBank accession numbers of field bindweed samples

Provinces	*GenBank Accession Numbers*	*Length (bp)*
Malatya	MT071458	667
Erzurum	MT071459	678
Diyarbakır	MT071460	684
Şanlıurfa	MT071461	678
Ankara	MT071462	675
Karaman	MT071463	678
Kayseri	MT071464	673
Konya	MT071465	660
Samsun	MT071466	635
Adana	MT071467	677
Hatay	MT071468	677
Denizli	MT071469	678
İzmir	MT071470	669
Uşak	MT071471	656
Tekirdağ	MT071472	678
Çanakkale	MT071473	681

The relationships of field bindweed samples from different provinces of Türkiye both with each other and with other field bindweed individuals worldwide, were revealed through phylogenetic analysis. When comparing specimens from different provinces of Türkiye, it was found that the specimens from Adana and Diyarbakır provinces formed a distinct group due to the genetic differences. While specimens collected from other provinces were grouped together, it was determined that the plant sample collected from Samsun province, despite being in the same group, was genetically more distant from the plant samples collected from other provinces.

When comparing field bindweed samples from different provinces of Türkiye with other plant specimens worldwide, it was observed that the plant specimens in Türkiye were divided into two distinct groups. Specifically, Samsun, Konya, Kayseri, Uşak, Çanakkale, Karaman and Şanlıurfa were identified as forming one group, while Denizli, Ankara, Hatay, Adana, Diyarbakır,

Erzurum, Malatya, İzmir and Tekirdağ provinces constituted another group. Additionally, it was noted that the plant specimens belonging to other field bindweed species worldwide formed a separate group distinct from the plant specimens in Türkiye, indicating a significant degree of relatedness. The phylogenetic tree of the ITS gene region of the biotypes of *Convolvulus arvensis* was constructed by repeating 100 times with the distance-based method (neighbour-joining) and the bootstrap method in the programme CLC Main Workbench 20. As a result of the phylogenetic analyses, all studied *Convolvulus arvensis* biotypes were classified as I. and II. They are divided into two main groups: Group I is divided into subgroups Ia and Ib, and group II into subgroups Ib and IIb. Turkey's biotypes are distributed among subgroups Ia, Ib and IIb, in contrast to other countries (Figure 1). Sunar et al. (2015) utilized random amplified DNA markers (RAPD) in another molecular study on field bindweed. In this experiment, field bindweed samples were collected from five locations in Erzurum province (Aşkale, Erzurum (centre), İspir, Oltu and Tortum). Comparative analysis of populations revealed that the populations of Erzurum and Aşkale as well as the populations of Tortum and Oltu showed similar clustering. The study suggested that the similarity in these populations, forming a common group, could be attributed to geographical reasons. The findings indicated a significant correlation between the proximity of geographical regions and the genetic proximity of the populations within those regions. Several studies have also reported a significant correlation between geographical and genetic distance (Xia et al. 2005; Nianxi et al. 2006; Qian et al. 2008). The phylogenetically distant grouping of field bindweed samples collected from different locations in Türkiye and other field bindweed species worldwide aligns with the outcomes of these studies.

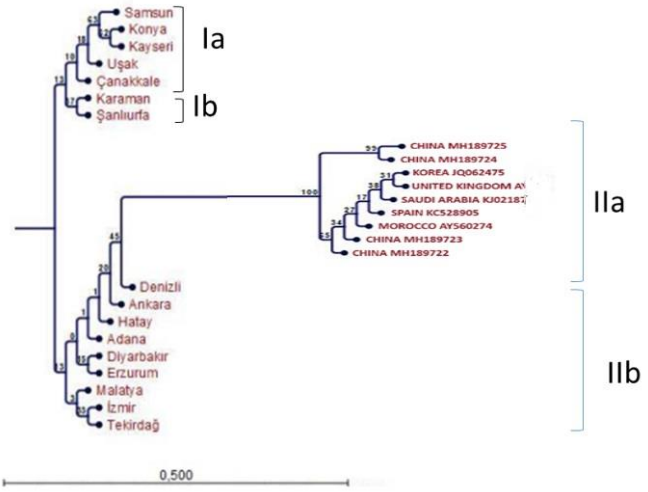

Figure 1- Phylogenetic analysis of field bindweed from different provinces in Türkiye and other field bindweed specimens from around the world

In the study, the CLC Main Workbench 20.0.1 program was used to determine the relationships among field bindweed species in different provinces of Türkiye and other field bindweed species in the world. It was observed that the degree of similarity among field bindweed samples in Türkiye varies among provinces and that their relationships to species globally are distant. Utilizing a specific reference from the NCBI GenBank, the similarities of field bindweed from different provinces were grouped in the study. As a result, it was determined that a total of four main groups were formed, except for the reference KJ021876.1 Saudi Arabia (Figure 2).

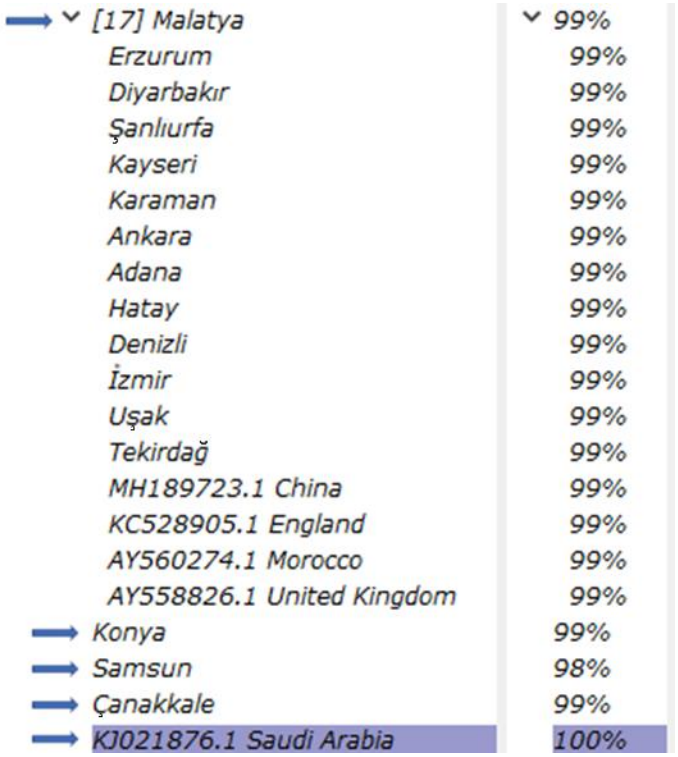

Figure 2- Similarities and grouping of field bindweed from different provinces with a specific reference via NCBI GenBank

Molecular diagnostic studies are used by researchers to identify species. Sarić-Krsmanović et al. (2022) entered the sequence results of the ITS1 and ITS2 5.8S ribosomal RNA gene regions of 24 of the 26 populations of *Cuscuta campestris* into NCBI GenBank. When compared with other samples in the database, all samples were found to belong to the species *Cuscuta campestris* Yunck. with a similarity level of 95%. When creating the phylogenetic tree, the genetic diversity between the species is divided into clusters. Keskin et al. (2017) conducted taxonomic identifications of species belonging to the genus *Cuscuta*. ITS primers were used to determine the relationships among the species of the genus *Cuscuta*, and the PCR products obtained were subjected to DNA sequencing. Phylogenetic analyzes were performed using CLC DNA Workbench software and Vector NTI programs. Plant samples with known DNA sequencing were registered in the GenBank of the National Center for Biotechnology Information (NCBI). The species registered in the GenBank included *C. approximata* Babington (GenBank accession no. KU686677), *C. lupuliformis* Krocker (GenBank accession no. KU707914), *C. campestris* Yuncker (GenBank accession no. KU725869), *C. babylonica* Aucherex Choisy (GenBank accession no. KU725870), *C. babylonica* (GenBank accession no. KU761258), as well as *C. approximata* (GenBank accession no. KU725873). The studies emphasized the importance of using the ITS region in species identification and in assessing the similarities of samples belonging to the species.

3.2. Effect of 2,4 D herbicide on field bindweed samples

ITS primers were used to identify the samples of field bindweed collected in different provinces for molecular characterization. All collected samples were identified as belonging to the field bindweed species, and the similarities between these samples were grouped using bioinformatics analysis. As a result, a total of four main groups were formed among the field bindweed samples of all provinces except the reference value. Since the samples of Adana, Konya and Samsun belonged to different groups, they were used in herbicide experiments. When the dose responses of Adana, Konya and Samsun samples to the herbicide 2,4-D were evaluated by their dry weight values 28 days after spraying, the ED_{50} values were 131.93, 115.42 and 141.89 g.a.i/ha, respectively, and the ED_{90} values were 728.0, 876.4 and 913.0 g.a.i/ha, respectively (Table 5 and Figure 3). In the trial, the herbicide 2,4-D produced similar dose-response results on three different samples of field bindweed (Adana, Konya and Samsun).

Table 5- Dose-response curves for the field bindweed (*Convolvulus arvensis* L.) grown under glass greenhouse conditions (dry matter) (2,4-D) (g.a.i /ha)

	Regression parameters (±SE)				
Locations	***B***	***C***	***D***	***ED_{50} (±SE)***	***ED_{90} (±SE)***
Adana	-1.29 (0.3)	-0.1 (2.3)	87.73 (2.12)	131.93 (15.3)	728.0 (98.3)
Konya	-1.08 (0.23)	-0.1 (1.9)	87.76 (2.43)	115.42 (14.7)	876.4 (30.5)
Samsun	-1.18 (0.17)	0.03 (2)	91.22 (1.94)	141.89 (13.1)	913.0 (81.2)

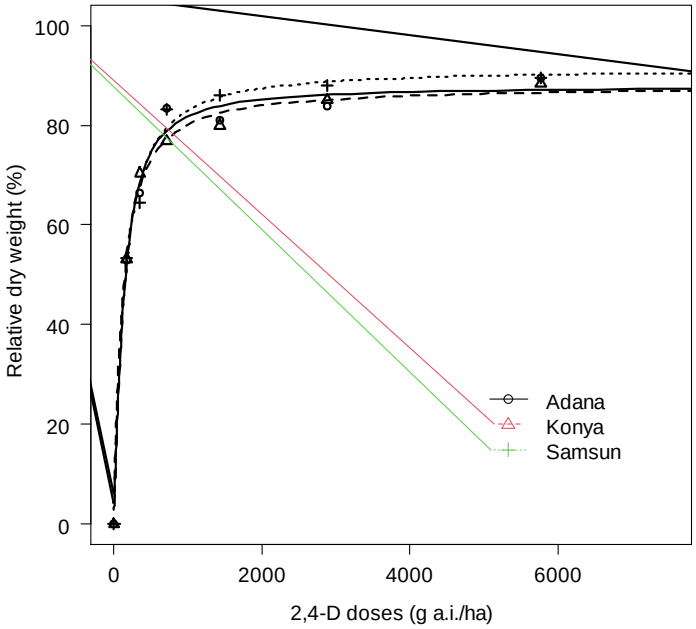

Figure 3- Dose-response curve of field bindweed in Adana, Konya and Samsun locations against herbicide with 2,4-D active ingredient

In addition to the dry matter of field bindweed, percentage symptom values were visually recorded each week, and differences between the samples of field bindweed in the provinces of Adana, Konya and Samsun were determined. When assessing the lethality of the normal dose of 2,4-D on the plants of Adana sample, results of 33%, 55%, 95% and 100% were obtained in the 1st, 2nd, 3rd and 4th weeks, respectively. Examining the lethality of the normal dose of 2,4-D on Konya sample plants yielded results of 22%, 70%, 90% and 95% in the 1st, 2nd, 3rd and 4th weeks, respectively. For the Samsun sample plants, results of 37%, 74%, 95% and 100% were observed in the 1st, 2nd, 3rd and 4th weeks, respectively. Mortality of 70% or more was observed in the Konya and Samsun samples in the 2nd week, while mortality in the Adana sample occurred slightly later and was determined to be 55%. In all three samples, a mortality rate of 90% or more was observed in the 3rd week. Other studies also indicate that the 2,4-D herbicide used in the experiment had a positive effect on the control of field bindweed (Flint & Barrett 1998; Bayat & Zargar 2020).

4. Conclusions

The result of the study indicates that the field bindweed populations originating from different provinces of Türkiye formed close groups in terms of their genetic similarity and also exhibited similar reaction to the herbicide 2,4-D, which was used for weed control. Based on these findings, it was determined that the herbicide 2,4-D had a positive effect on field bindweed as a weed control agent. Importantly, the genetic proximity or distance of field bindweed samples did not alter the efficacy of the herbicide.

References

Ateş A (2022). Determination of the critical period for weed control in wheat (*Triticum aestivum* L.) and investigation of control possibilities of wild mustard (*Sinapis arvensis* L.). PhD Thesis, Malatya Turgut Özal University, Malatya. 117pp (In Turkish)

Bajwa A A, Wang H, Chauhan BS & Adkins S (2019). Effect of elevated carbon dioxide concentration on growth, productivity and glyphosate response of parthenium weed (*Parthenium hysterophorus* L.). Pest Manag. Sci. 75: 2934–2941. https://doi.org/10.1002/ps.5403

Baldwin B G, Sanderson M J, Porter J M, Wojcıechowoski M F, Campell C S & Donoghue M J (1995). The ITS Region of Nuclear Ribosoma l DNA: Valuable Source of Evidence on Angiosperm Phylogeny. Annals of the Missouri Botanical Garden pp. 250-272. https://doi.org/10.2307/2399880

Bayat M & Zargar M (2020). Field bindweed (*Convolvulus arvensis*) control and winter wheat response to post herbicides application. *Journal of Crop Science and Biotechnology* 23: 149-155. DOI No. 10.1007/s12892-019-0213-0

Boström U & Fogelfors H (2002). Response of Weeds and Crop Yield to Herbicide Dose Decision-Support Guidelines. Weed Science 50:186-195. https://doi.org/10.1614/0043

Brouat C, Gielly L & Mckey D (2001). Phylogenetic relationships in the genus *Leonar doxa* (Leguminosae: Caesalpinioideae) inferred from chloroplast trnL intro nan dtrnL-trnF intergenic spacer sequences. *American Journal of Botany* 88: 143-149. https://doi.org/10.2307/2657134

Busi R, Girotto M & Powles S B (2016). Response to low-dose herbicide selection in self-pollinated *Avena fatua*. Pest Management Science 72(3): 603-608

Chen Z Y, Xiong Z J, Pan X Y, Shen S Q, Geng Y P, Xu C Y, Chen J K & Zhang W J (2015). Variation of genome size and the ribosomal DNA ITS region of *Alternanthera philoxeroides* (Amaranthaceae) in Argentina, the USA, and China. *Journal of Systematics and Evolution* 53(1): 82-87. DOI: 10.1111/jse.12118.

Coruh I & Zengin H (2007). Determination of economic threshold of field bindweed (*Convolvulus arvensis* L.) in spring wheat in Erzurum, Türkiye. *Journal of Ataturk University Agricultural Faculty* 38: 151-157

Dogan M N, Unay A, Boz O & Albay F (2004). Determination of optimum weed control timing in maize (*Zea mays* L.). Turk J Agric. 28: 349- 354

Felsenstein J (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791

Flint J L & Barrett M (1998). Effects of glyphosate combinations with 2, 4-D or dicamba on field bindweed. Weed Science, 37: 12-18. https://doi.org/10.1017/S0043174500055776

Gubanov I A, Kiseleva K V, Novikov V S & Tihomirov V N (2004). An illustrated identification book of the plants of Middle Russia, Vol. 3: Angiosperms (dicots: archichlamy deans). Moscow: Institute of Technological Researches; 520 pp In Russian

Güncan A (1979). Research on the Biology of Field Bindweed (*Convolvulus arvensis* L.) and its Control Possibilities in Wheat. Ataturk University Publications No. 515. Faculty of Agriculture Publications No. 234. Research Series No. 151 (In Turkish).

Güncan A & Karaca M (2023). Weed control. Selçuk University Publications 379pp (In Turkish)Hoffmann MP & Frodsham AC (1993).Natural enemies of vegetable insect pests. Cooperative Extension, Cornell University, Ithaca, NY 63 pp

Holm L G, Plunknett D L, Pancho J V & Herberger J P (1977). The World's Worst Weeds. University Press of Hawaii, Honolulu, Hawaii. USA. pp. 98-104

Jabran K, Mahajan G, Sardana V & Chauhan BS (2015). Allelopathy for weed control in agricultural systems. Crop. Prot. 72: 57–65

Karaman Y & Tursun N (2021). Germination biology of field bindweed seeds collected from different provinces. *Bulgarian Journal of Agricultural Science* 27(6): 1168-1177

Kellogg EA (1998). "Who's Related to Whom? Recent Results from Molecular Systematic Studies". Current Opinion in Plant Biology 1/2: 149. https://doi.org/10.1016/S1369-5266(98)80017-4

Keskin F, Kaya İ, Usta M, Demir İ, Sipahioğlu H M & Nemli Y (2017). Molecular Cloning and Sequence Analysis of The its Region of Nuclear Ribosomal DNA for Species Identification in Dodders (Cuscuta; Convolvulaceae). Internatıonal Journal of Agrıculture & Biology, Issn Print: 1560–8530; Issn Online: 1814–9596 17–0384/2017/19–6–1447–1451 Doi: 10.17957/Ijab/15.0442 http://www.fspublishers.org

Knezevic S Z, Streibig J C & Ritz C (2007). Utilizing R software package for dose-response studies: the concept and data analysis. Weed Technology 21: 840–848.https://doi.org/10.1614/WT-06-161.1

Knezevic S Z & Datta A (2015). The critical period for weed control: revisiting data analysis. Weed Science 63: 188–202.https://doi.org/10.1614/WS-D-14-00035.1

Kubiak A, Wolna-Maruwka A, Niewiadomska A & Pilarska A A (2022). The Problem of Weed Infestation of Agricultural Plantations vs. the Assumptions of the European Biodiversity Strategy. Agronomy, 12, 1808.

Kudsk P & Streibig J C (2003). Herbicides a two edged sword. Weed Research 43(2): 90–102. https://doi.org/10.1046/j.1365-3180.2003.00328.x

Kuru H H & Üremiş İ (2021). "

Determination of density and frequency of field muskmelon (*Cucumis melo* var. *agrestis* Naudin) and field bindweed (*Convolvulus arvensis* L.) in Çukurova Region" MKU Journal of Agricultural Sciences 26(2): 461-471 (In Turkish)

Lubenov Y (1985). Noxious Weeds are the Source of Death and Life. Çev, B. Makaklı, M. Dinçer. Cag Printing House. Ankara. (In Turkish)Lyons K E (1988). Element steward ship abstract for *Convolvulus arvensis* L. In: Field bindweed. Arlington: The Nature Conservancy pp.1-21

Matteson P C (1995). The 50% Pesticide cuts in Europe: A Glimpse of our future? American Entomology 41: 210-220. https://doi.org/10.1093/ae/41.4.210

Matzenbacher F O, Bortoly E D, Kalsing A & Merotto A Jr (2015). Distribution and analysis of the mechanisms of resistance of barnyard grass (*Echinochloa crus-galli*) to imidazolinone and quinclorac herbicides. *Journal of Agricultural Science* 153: 1044–1058.https://doi.org/10.1017/S0021859614000768

Mourelos C A, Malbran I, Balatti P A, Ghiringhelli P D & Lori GA (2014). Gramineous and non-gramineous weed species as alternative hosts of *Fusarium graminearum*, causal agent of *Fusarium* head blight of wheat, in Argentina. Crop Protection, 65: 100-104

Mummenhoff K, Franzke A & Koch M (1997). "Molecular phylogenetic of *Thlaspi*s. I. (Brassicaceae) Based on Chloroplast DNA Restriction Site Variation and Sequences of the Internal Transcribed Spacers of Nuclear Ribosomal DNA" Cand. J. Botany, 75, (1997): 469–487. https://doi.org/10.1139/b97-051

Nei M & Kumar S (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Nianxi Z, Yubao G, Wang J, Ren A & Xu H (2006). RAPD diversity of *Stipa grandis* populations and its relationship with some ecological factors. Acta Ecologica Sinica 26(5): 1312–1319. https://doi.org/10.1016/S1872-2032(06)60023-1

Özkil M & Üremiş İ (2020). "

The Situation Of Morningglory (*Ipomoea* spp.) and Field Bindweed (*Convolvulus* spp.) Species And Their Frequency And Density In The Agricultural Areas Of The Mediterranean Region" *Ege University Faculty of Agriculture Journal 57(2): 229-237* (In Turkish)

Pimentel D, Acquay H, Biltonen M, Rıce P, Silva M, Nelson J, Lipner V, Giordano S, Horowitz A & D'amore M (1992). Environmental and Economic Costs of Pesticide Use. Bioscience 42: 750-760. https://doi.org/10.2307/1311994

Pimentel D & Greıner A (1997). Environmental and Socio-Economic Costs of Pesticide Use. (Ed.:D. Pimentel), Techniques For Reducing Pesticide Use: Economic and Environmental Benefits. John Wiley and Sons, Chichester (UK), pp. 51-78

Qian Z Q, Xu L, Wang Y L, Yang J & Zhao G F (2008). Ecological genetics of *Reaumuria songorica* Pall. Maxim. population in the oasis-desert ecotone in Fukang, Xinjiang, and its implications for molecular evolution. Biochemical Systematics and Ecology 36: 593–601. https://doi.org/10.1016/j.bse.2008.01.008

Saitou N & Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425

Sarić-Krsmanović M, Zagorchev L, Gajić Umiljendić J, Rajković M, Radivojević L, Teofanova D & Vrbničanin S (2022). Variability in Early Seed Development of 26 Populations of *Cuscuta campestris* Yunck.: The Significance of Host, Seed Age, Morphological Trait, Light, Temperature, and Genetic Variance. Agronomy 12(3): 559

Salonen J (1992). Efficacy of Reduced Herbicide Doses in Spring Cereals of Different Competitive Ability. Weed Research 32(6):483-491. https://doi.org/10.1111/j.1365-3180.1992.tb01909.x

Soejima A & Nagamasu H (2004). Phylogenetic analysis of Asian Symplocos (Symplocaceae) based on nuclear and chloroplast DNA sequences. *Journal of Plant Research* 117(3):199-207

Steckel LE, Defelice MS & Sims BD (1990). Integrating Reduced Doses of Post Emergence Herbicides and Cultivation for Broad Leaf Weed Control in Soybeans (*Glycine max*). Weed Science 38: 541-545. https://doi.org/10.1017/S0043174500051456

Sunar S, Agar G & Nardemir G (2015). Analysis of genetic diversity in bindweed (*Convolvulus arvensis* L.) populations using random amplified polymorphic DNA (RAPD) markers. Journal of Biodiversity and Environmental Sciences (JBES). ISSN: 2220-6663 (Print) 2222-3045 (Online). Vol. 7, No. 1, pp. 197-204

Tamaki G, Moffitt H R & Turner J E (1975). The influence of perennial weeds on the abundance of the red backed cutworm on asparagi. Environmental Entomology 4: 274-276

Tamura K, Stecher G & Kumar S (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.

Tursun A O, Sipahioglu H M & Telci I (2021). Genetic relationships and diversity within cultivated accessions of *Salvia officinalis* L. in Turkey. Plant Biotechnology Reports 15(5): 663-672

Ulloa SM, Datta A, Bruening C, Neilson B, Miller J, Gogos G & Knezevic S Z (2011). Maize response to broadcast flaming at different growth stages: effects on growth, yield and yield components. *European Journal of Agronomy* 34: 10–19.https://doi.org/10.1016/j.eja.2010.09.002

Uygur N & Şekeroğlu E (1993). Agricultural Development and Nature Conservation in the Göksu Delta, Proceedings of the International Göksu Delta Environmental Development Seminar. Natural Life Protection Association, Istanbul, p. 162 (In Turkish)

Üremiş İ, Soylu S, Kurt Ş, Soylu E M & Sertkaya E (2020).

Evaluation of Weed Species, Their Frequencies, Densities and Current Status in Carrot Fields in Hatay Province. *Tekirdağ Faculty of Agriculture Journal 17(2): 211-228. https://doi.org/10.33462/jotaf.645336* (In Turkish)

Vogelgsang S (1998). Pre-emergence Elficacy of Phomopsis *C. arvensis* Ormeno to Control Field Bindweed (*C. arvensis* L.). Ph.D. thesis, Department of Plant Science, Macdondd Campus of McGU University Montr6al. QC. Canada.

Vranjes F, Vrbnicanin S, Nedeljkovic D, Savic A & Bozic D (2019). The response of *Chenopodium album* L. and *Abutilon theophrasti* Medik. to reduced doses of mesotrione. Journal of Environmental Science and Health, Part B, 54(7): 615-621

White TJ, Bruns T, Lee S & Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in M. A. Innis, D. H. Gelfland, J. J. Sninsky, and T. J. White, eds. PCR Protocols-A Guide to Methods and Application. San Diego: American Press.

Xia T, Chen SL, Chen SY & Ge XJ (2005). Genetic variation within and among populations of *Rhodiolaalsia* (Crassulaceae) native to the Tibetan Plateau as detected by ISSR markers. Biochemical Genetics 43(3–4): 87–101. DOI: 10.1007/s10528-005-1502-5

Yusriah L, Sapuan S M, Zainudin E S & Mariatti M (2012). Exploring the potential of betel nut husk fiber as reinforcement in polymer composites: effect of fiber maturity. Procedia Chemistry 4: 87-94

Yokoyama J, Suzuki M, Iwatsuki K & Hasebe M (2000). Molecular Phylogeny of *Coriaria* with Special Emphasis on the Disjunct Distribution, Molecular Phylogenetics and Evolution 14(1): 11. https://doi.org/10.1006/mpev.1999.0672

Zhang J, Weaver SE & Hamill AS (2000). Risks and Reliability of Using Herbicides at Below-labeled Rates. Weed Technology 14:106-115. https://doi.org/10.1614/0890-037X