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POTENTIAL MUTATION IN PDS GENE THAT CAUSES HERBICIDE RESISTANCE IN WILD RADISH GROWS WITH WHEAT CROP

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SUMMARY

A field experiment set out during the winter of 2022–2023 availed one of the Holy Kerbala Governorate's agricultural experimental fields. The study aimed to evaluate the efficacy of some herbicides in controlling the wild radish weeds accompanying the wheat crop, as well as, diagnosing genetic mutations in the PDS gene responsible for resistance to herbicides in wild radish. The experiment employed a randomized complete block design (RCBD) with a split-plot arrangement and three replications. The main plots included five groups of wild radish seeds taken from five Iraqi Governorates, i.e., Najaf, Kerbala, Babel, Diwaniyah, and Wasit. The subplots contained four herbicides: Navigator, Tatsteler, Mark zone, and Decimate, in addition to the treatment of spraying with water only. Wild radish seeds serve for artificially infecting wheat seeds after field preparation. The study also measured wheat yield components, with the weeds appraised twice after 60 and 90 days of management. Genomic alterations in the PDS gene caused herbicide resistance in wild radish. The global herbicide-resistant cultivar has two missense mutations in codons 69 and 330 (TGT → TAT and CGT → GGT), encoding for Cys → Tyr and Arg → Gly. These alterations were identical in all weeds studied, but some Governorates had new mutations, such as, in the seeds of the Babylon Governorate's weed. The evaluation of the efficiency of chemical pesticides comes after 60 and 90 days. Wild radish seeds in the targeted Governorates differed in all features. The herbicides Navigator and Decimate are vital in giving the best percentages of control and inhibition in wild radish weeds. Chemical herbicides have enhanced wheat production by eradicating weeds, specifically difficult ones. Genetic changes in wild radish weeds in wheat crops make some chemical herbicides less effective. Herbicide-resistant radish weed seeds are genetically mutated. Thus, herbicides can target gene variants and be diversified to prevent weed resistance.

Keywords: *Triticum aestivum* L., weeds resistance, gene inhibition PDS, chemical herbicide, *Raphanus raphanistrum* L.

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Key findings: The Navigator herbicide eliminated wheat crop weeds better than other chemical herbicides, with 423.57 spike m^2, 15.09 tons ha^{-1} biological yield, 5.83 tons ha^{-1} grain yield, and 36.92 harvest index. Missense mutations in the target area make weeds herbicide-resistant.

INTRODUCTION

Wheat (*Triticum aestivum* L.) It is the staple food for most of the world's population, as about 55% of the world's population depends on wheat for 20% of daily calorie consumption. Wheat contains approximately 8.51 g of carbohydrates and 23.15 g of protein per 100 g (López-Barón *et al*., 2017). The wheat crop is widespread in temperate regions and is a relevant source of income for the farming community. Wheat crop ranked first in terms of total arable area and global production (AL-Fatlawi *et al*., 2022), as the cultivated area for this crop reached approximately 218 million ha, with an estimated productivity of 777.5 million tons globally (FAO, 2022). However, its productivity began to decline, especially in the past few years, making it necessary to understand how technology interacts with agricultural production systems in different growing environments to ensure optimum grain yield. Developing an integrated approach based on weather conditions and agronomic inputs could sustain production. Agriculture practices need application at an economically optimal level, integrating genetic supplication when management options become necessary where breeders cannot provide genetic solutions soon appropriate to increase the potential yield (Farhood *et al*., 2022; Ismail *et al*., 2023).

In fulfilling the food needs of the growing population, there must be an increase in production by 30%–40% to meet the demand for wheat by 2050 **(**Westwood *et al*., 2018**).** However, this challenge has become more complex in light of imbalanced production conditions, heat waves, drought, and water scarcity as the foremost factors expected to have a significant impact on crop productivity with concomitant effects on the safety of food security on the part of abiotic factors (Helman and Bonfil, 2022). In vital constraints, weeds come at the forefront, considered one of the most critical factors faced by wheat production, which also cause losses by 15%–50%, depending on the density and type of the weeds (Al-Gburi and Al-Gburi, 2021). Among the weeds, the most important is the wild radish, spreading along various crops, especially wheat.

The wild radish belongs to the family Cruciferae, which includes approximately 388 genera and more than 3700 species (Shankar *et al*., 2019). The word *Raphanus* is of Greek origin, as it derives from the word "Ra," meaning fast, and "phanomai," which implies germination, a plant with rapid germination. The weeds originated in the Mediterranean regions with a current wide distribution in most countries, such as, Australia, England, Kenya, the USA, Canada, and South Africa. Herbicide use is one of the most efficient methods for controlling and reducing the prevalence of this weed type. However, herbicides' repeated application led to the emergence of some resistant individuals (Zhang *et al*., 2021).

Wild radish resistance emerged when Picolinafen herbicides' application began to a group of weeds in Western Australia, and their resistance rate reached 35%. When applying Diflufenican, the field's survival rate after control ranged from 70% to 80%, which was evidence of resistance development to this herbicide. Herbicide resistance to phytoene desaturase (PDS) inhibition first appeared in wild radishes in Australia in 1998 (Heap, 2019). The importance of suppressing the enzyme phytoene desaturase (PDS) comes through the inhibition of carotenoid production, which assists in photosynthesis as chlorophyll helps in receiving light and protecting the crop from ultraviolet radiation (Lu *et al*., 2020).

Phytoene synthase catalyzes the first stage in synthesizing carotenoids in the plastids, which results in the hydrocarbon phytoene. Then, the PDS enzyme starts functioning in the second phase, turning phytoene into B-carotene. The herbicide that prevents the PDS enzyme from working also prevents the synthesis of carotenoids, resulting in a malfunction in the photosynthesis process, the yellowing of the leaves, and the plant's death (Dayan *et al*., 2014). This herbicide's release in the market started in the 1970s, with a small portion of the herbicide used in global agricultural systems (Kahlau *et al*., 2020). Therefore, the study aims to evaluate the efficacy of some herbicides in the control of the wild radish weeds accompanying the wheat crop, as well as, diagnose genetic mutations in the PDS gene responsible for resistance to herbicides of the wild radish weed.

MATERIALS AND METHODS

Genetic material and experimental procedure

A field experiment set out during the winter of 2022–2023 at the College of Agriculture, University of Kerbala, Kerbala, Iraq. Wheat seeds (Al-Wafia variety), sown on November 15 in 10 lines comprising a longitude of 2 m each line, had the distance between each plot at 20 cm for each experimental unit. At the same time, the wheat seed contamination proceeded by sowing 0.5 g per experimental unit of *Raphanus* weed seeds to five diverse groups obtained from different Iraqi Governorates. When the weeds reached the appropriate stage for control, herbicide spraying ensued, as recommended. Wheat crop harvesting occurred on May 10, 2023. The experiment included two factors: the first factor was five groups of wild radish seeds taken from five Iraqi provinces, i.e., Najaf Governorate, Holy Kerbala Governorate, Babel Governorate, Diwaniyah Governorate, and Wasit Governorate. Meanwhile, the second factor was four types of herbicides, i.e., Navigator, Tatsteler, Mark zone, and Decimate, plus the control treatment (spraying with water only) (Table 1). The experiment, set up in a randomized complete block design (RCBD), had a split-plot arrangement and three replications.

RNA extraction

Following the implementation of the control procedure, every treatment had a total of 20 plants selected from each group of wild radishes. These groups consisted of 10 plants that exhibited resistance to herbicides and the other 10 that were sensitive to herbicides. The selection purpose was to investigate the molecular identification of the PDS gene, which is accountable for the plant's carotenoid synthesis, and all the seeds obtained from the Governorates under study. The said process ran at the Department of Field Crop Sciences, College of Agriculture, University of Kerbala, Kerbala, Iraq.

A sample taken from each plant acquired labeling for extraction of RNA at the beginning of the control results and then monitoring the control results. For the purpose of distinguishing in the wild radish weeds the resistant and sensitive ones to herbicides, the study of the molecular diagnosis of the PDS gene continued using the primers of the mRNA sequence of the PDS gene (kit prepared by the American company Zymo) (Table 2). Carrying out the Reverse transcription polymerase chain reaction (RT-PCR) extracted RNA samples from leaves and for all studied groups of wild radish, using the 2X AddScript RT-PCR SYBR Master kit, with the final volume of the sample supplemented with distilled water to 25 μl. The reaction mixture's concocting in a sterile tube (a tube for each gene with a tube free of nucleic acid negative control), had its components mixed using a micropipette, then, placed in a centrifuge to maintain the final volume of the reaction mixture, afterward, placing it in the instantaneous thermal polymerization device, and then implementing the special program with the PDS gene (Table 3).

Table 1. Herbicides used in the study as recommended.

Code	Treatments	Recommendation	Origin
T0	Weedy (Control)	-------	-------
T1	Navigator	1250 ml ha^{-1}	Syngenta
T2	Tatsteler	400 g ha^{-1}	China
T3	Decimate	2400 ml ha^{-1}	BCT
T4	Mark zone	500 ml ha^{-1}	China

Table 2. Primers used in reverse transcription polymerase chain reaction (RT-PCR) specific for PDS gene diagnosis.

Gene	Name	Forward (5'---3')	Reverse (5'---3')
PDS	PDS1	TCTCTGTGGGAGTGTTTCTGC	CAATAAACACCTCCTCGGTCAC
	PDS2	GGCATTCTTGGATGGCAAC	CGACATCAGTTGATGCTTGC

Table 3. Program of reverse transcription RT-PCR conditions for PDS gene amplification.

Stage	Temperature (°C)	Time	Number of courses
cDNA synthesis	50	20 min	Hold
Denaturation Initial	95	10 min	Hold
Denaturation	95	30 s	
Annealing	***60***	30 s	
Extension	72	2 min	40
Extension	72	5 min	Hold

Reverse transcriptase polymerase chain reaction (RT-PCR)

Researchers identified and detected a PDS gene responsible for resistance to herbicides that inhibit carotenoid synthesis using the primers they designed using NCBI listed in Table 2. The reaction mixture, prepared in a sterile tube (one for each genotype with a test tube free of DNA, a negative control) and its components mixed using a micropipette, contained the following: 10 µl of Taq PCR Premix, 1 µl of forward and 1 µl of reverse primer of the target gene, 5 µl of DNA and 8 µl of distilled water, then placed in a centrifuge to maintain the final volume (25 µl) of the reaction mixture. Then, putting in a PCR device, the reaction continued to amplify. In determining the diameters of the fragment for PCR products and the DNA Ladder marker, electrophoresis progressed after combining 1 g of agarose and dissolving in 100 ml of TBE (1X), with the mixture heated until it reached boiling point.

Reducing the temperature between 40 °C and 50, the mixture incurred two µl of a red safe dye. Concurrently, combining 3 µl of PCR products with 5 µl of loading buffer took place. After preparing the gel casting tray and placing the comb to create wells within the agarose gel layer, the dissolved agarose poured into the prepared tray underwent hardening at room temperature. When the agarose had solidified completely, the comb’s careful removal followed without causing any deformation or breaking any of the wells. Placing back the tray in the electrophoresis device, pouring TBE into the electrophoresis chamber went on until the agarose layer’s total submergence at a height of approximately 1 mm. Finally, PCR products’ inoculation into each well of the agarose gel continued, with a 5 microliter (1 Kbp) ladder marker placed on the wells on the left side of the additional samples for a more accurate determination of the size of PCR products. Then, electrophoresis ran with the power supply of 120 mA for 1.5 h. After electrophoresis, placing the agarose layer on a UV transilluminator ensued.

Data recorded

The data recording noted the control and inhibition ratios, spikes per meter square, biological yield, 1000-grain weight, grain yield, and harvest index. The control ratio constituted the percentage of the number of weeds after 60 and 90 days of control by the following equation (Safi, 2016):

$$\text{Control percentage} = (\text{The number of Weed in the comparison treatment} - \text{The number of weeds in the control treatment})/(\text{The number of Weed in the comparison treatment}) \times 100$$

The inhibition ratio calculation used the following equation:

$$\text{Inhibition percentage} = (\text{Dry weight of weed in comparison treatment} - \text{Dry weight of weeds in control treatment})/(\text{Dry weight of weed in comparison treatment}) \times 100$$

For the number of spikes (spikes m^2), a square meter allotted for each experimental unit attained calculation based on the number of spikes from the group of plants gathered from the middle lines. Biological yield (tons ha^{-1}) calculation was the yield of dry matter (grain and straw) per the square meter area harvested from each experimental unit, with the weight converted to tons per hectare. For 1000-grain weight (g), a random sample of grains taken for each experimental unit counted 1000 grains and then weighed on an electric scale. For grain yield (tons ha^{-1}), the grains and hay isolated per square meter harvested in each experimental unit had the grains weighed and converted into tons per hectare. The harvest index (%) computation employed the following equation (Salim *et al*., 2019):

Harvest index = grain yield / biological yield × 100

RESULTS AND DISCUSSION

Diagnosis and analysis of the PDS gene

For the study and characterization of the PDS gene in the wild radish, and after creating the reaction conditions of the RT-PCR technology, the reaction products' transfer to the agarose gel ensued. The results in fig 1 and fig 2 show the presence of bands with a molecular weight of 776 and 808 bp for weed samples studied from all the groups of wild radish collected from different Governorates of Iraq (Najaf, Kerbala, Babel, Diwaniyah, and Wasit).

Diagnosis of genetic mutation at the PDS gene level

The results showed the presence of mutations in weeds that are resistant and sensitive to herbicide action that act on the PDS gene responsible for the biosynthesis of carotenoids (Table 4). Two missense mutations were evident in the resistant global cultivar in codon 69 and 330 (TGT → TAT and CGT → GGT) encoding acids with other amino (Cys → Tyr and Arg → Gly), and these results also agree with the findings of Chamovitz *et al*. (1991). These mutations were also identical in the wild radish seeds of the study provinces. However, some of them recorded with new mutations as in the seeds of the Babylonian province, diagnosing two missense mutations in codons 47 and 543 (AGG → TGG and CGT → GGT) which encode the acids Arg → Trp and Ser → Cys, sequentially.

In the seeds of the Kerbala Governorate, silent mutations emanated in codons 116 and 140 (GGT → GGC, GGA → GGG) that code for amino acids Gly and Gly, respectively. In the wild radish population of Wasit province, a missense mutation was apparent in codon 93 (CCA → ACA), which codes for another amino acid (Pro → Thr). Likewise, silent mutations diagnosed in the wild radish seeds of the Najaf province are in codons 44 and 66 (TTT → TTC and CCA → CCG) that code for the amino acids Phe and Pro, respectively, which also showed sensitivity to the action of herbicides.

The percentage of control in the wild radish weeds

Significant differences appeared among the different weed groups of the studied Governorates (Table 5). Reaching the highest percentage of control was after 60 days in the

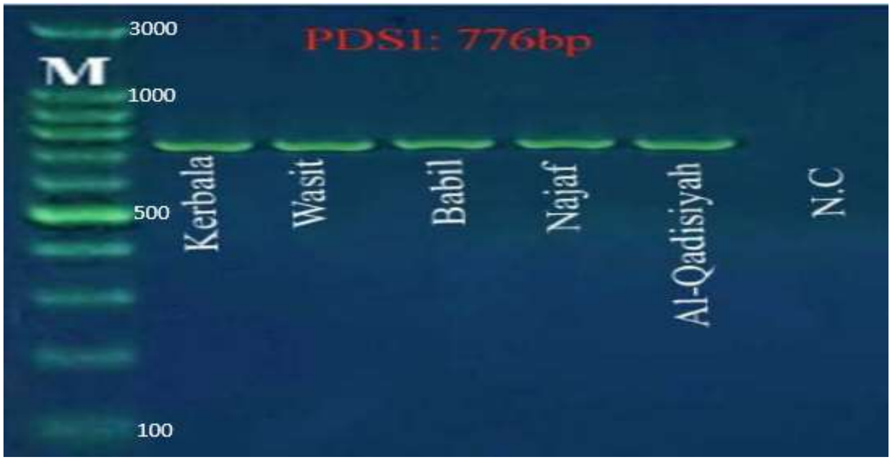

Figure 1. Electrophoresis of PCR products of PDS gene amplification results in wild radish plants from five Iraqi provinces with comparison treatment (N.C.) in addition to the DNA ladder size attached on the right side of the figure.

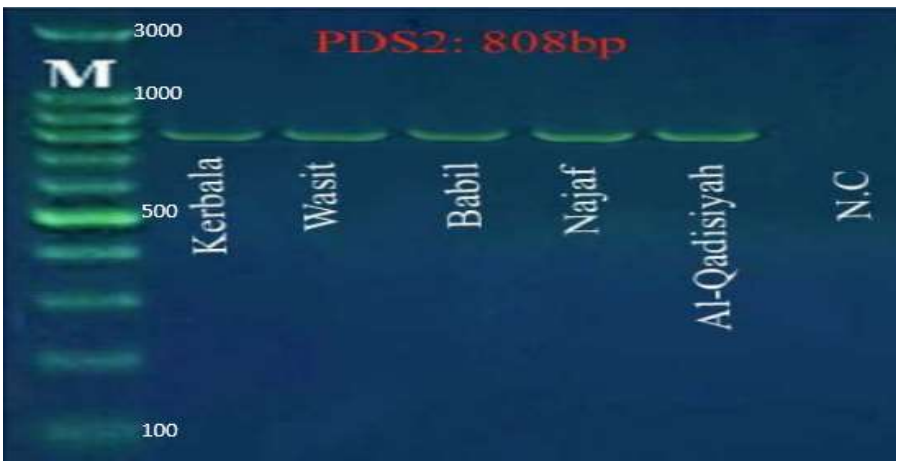

Figure 2. Electrophoresis of PCR products of PDS gene amplification results in wild radish plants from five Iraqi provinces with comparison treatment (N.C.) in addition to the DNA ladder size attached on the right side of the figure.

weed seeds of Kerbala Governorate, with an average of 41.63%, which did not differ significantly from the seed averages of the two other Governorates, i.e., Diwaniyah and Wasit, amounting to 41.21% and 41.26%, respectively. However, the Governorates of Najaf and Babylon averaged 31.97% and 32.91%, respectively. By measuring the percentage of control after 90 days, it was evident that the highest percent emerged in the weed seeds of the Governorate Diwaniyah, with an average of 39.91%, but did not differ significantly from the weed averages of three other Governorates, i.e., Kerbala, Babil, and Wasit, with percentages of 35.65%, 39.71%, and 38.20%, respectively. Al-Najaf Governorate weeds seed showed the lowest average rate of 29.23%.

The differences among the weed seeds of various Governorates may explain the variation in their control response, and it may be due to the percentage of genetic variations in the genes responsible for resistance among the weed populations of the provinces. The

Table 4. Diagnosis of genetic mutations in PDS genes of the resistant and sensitive wild radish weeds to the action of chemical herbicides.

Position / Weed provinces	Missense mutation									
	47		69		93		330		543	
	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid
MH165308.1: Global susceptible plants	AGG	Arg	TGT	Cys	CCA	Pro	CGT	Arg	TCT	Ser
Babil susceptible plants	AGG	Arg	TGT	Cys	CCA	Pro	CGT	Arg	TCT	Ser
XM_047198410.1 Resistant	AGG	Arg	TAT	Tyr	CCA	Pro	GGT	Gly	TCT	Ser
Babil Resistant plants	TGG	Trp	TAT	Tyr	CCA	Pro	GGT	Gly	TGT	Cys
Kerbala Resistant plants	AGG	Arg	TAT	Tyr	CCA	Pro	GGT	Gly	TCT	Ser
Najaf Resistant plants	AGG	Arg	TAT	Tyr	CCA	Pro	GGT	Gly	TCT	Ser
Wasit Resistant plants	AGG	Arg	TAT	Tyr	ACA	Thr	GGT	Gly	TCT	Ser
Al-Qadisiyah Resistant plants	AGG	Arg	TAT	Tyr	CCA	Pro	GGT	Gly	TCT	Ser
Silent mutations										
Position / Weed provinces	44		66		93		116		140	
	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid
MH165308.1: Global susceptible plants	TTT	Phe	CTC	Leu	CCA	Pro	GGT	Gly	GGA	Gly
Najaf susceptible plants	TTC	Phe	CTA	Leu	CCA	Pro	GGT	Gly	GGA	Gly
XM_047198410.1 Resistant	TTT	Phe	CTC	Leu	CCA	Pro	GGT	Gly	GGA	Gly
Babil Resistant plants	TTT	Phe	CTC	Leu	CCA	Pro	GGT	Gly	GGA	Gly
Kerbala Resistant plants	TTC	Phe	CTC	Leu	CCA	Pro	GGC	Gly	GGG	Gly
Najaf Resistant plants	TTT	Phe	CTC	Leu	CCA	Pro	GGT	Gly	GGA	Gly
Wasit Resistant plants	TTT	Phe	CTC	Leu	ACA	Thr	GGT	Gly	GGA	Gly
Diwaniyah Resistant plants	TTT	Phe	CTC	Leu	CCA	Pro	GGT	Gly	GGA	Gly

Table 5. Effect of chemical herbicides on control percentage after 60 and 90 days of spraying in weeds of wild radish for five Iraqi Governorates.

After 60 days of control						
Treatments	Study provinces					Means
	Najaf	Kerbala	Babel	Diwaniyah	Wasit	
Control	0.00	0.00	0.00	0.00	0.00	0.00
Navigator	52.73	53.19	52.41	54.70	55.08	53.62
Tatsteler	31.26	42.96	39.65	38.76	56.94	41.91
Decimate	45.95	51.15	44.93	54.42	52.44	49.77
Mark zone	29.91	60.86	27.59	58.17	41.83	43.67
$LSD_{0.05}$	10.32					3.97
Means	31.97	41.63	32.91	41.21	41.26	
$LSD_{0.05}$	7.41					
After 90 days of control						
Treatments	Study provinces					Mean
	Najaf	Kerbala	Babel	Diwaniyah	Wasit	
Control	0.00	0.00	0.00	0.00	0.00	0.00
Navigator	46.92	50.22	49.61	57.48	55.45	51.94
Tatsteler	32.38	36.40	46.48	36.77	48.58	40.12
Decimate	35.19	51.00	47.08	52.74	48.49	46.90
Mark zone	31.65	56.94	35.07	51.56	38.46	42.74
$LSD_{0.05}$	9.38					3.61
Means	29.23	38.71	35.65	39.91	38.20	
$LSD_{0.05}$	6.73					

PDS gene was responsible for the synthesis of carotenoids, with these variations represented in the weed seeds of the Babylon province with two missense mutations in codons 47 and 543 (AGG → TGG and CGT → GGT) that encode the acids Arg → Trp and Ser → Cys sequentially. The weed population of Wasit province had the presence of a missense mutation in codon 93 (CCA → ACA) that encodes for the amino acid Pro → Thr (Table 4).

The results further revealed that all the used herbicides caused significant differences in the percentage of control after 60 days of spraying (Table 5). The highest control percent was noteworthy in the two herbicides, Navigator and Decimate, with averages of 53.62% and 49.77%, respectively. The two other herbicides, i.e., Tatsteler and Mark zone, showed means of 41.91% and 43.67%, respectively. After 90 days of sprayings, the herbicide Navigator excelled with the highest average of 51.94%, while the three herbicides, viz., Tatsteler, Decimate, and Mark zone, provided the medians of 40.12%, 46.90%, and 42.74%, respectively. The herbicide Decimate, which inhibits the process of the second photosynthetic system, in addition to inhibiting the action of the enzyme phytoene desaturase responsible for the synthesis of pigment carotenoids, and thus eventually, affected the chloroplasts leading to yellowing of the weeds foliage and death of sensitive ones. The amino and fatty acids, which caused an imbalance in the biosynthesis of chloroplasts, affected the efficiency of the photosynthesis process and decreased the decomposition of chlorophyll, leading to plant death (Dayan *et al*., 2014).

The percentage of inhibition of wild radish weeds

The results of Table 6 showed that Governorates from which collected wild radish seeds showed significant differences in the percentage of inhibition after 60 and 90 days of control, with the highest average recorded in the weed seeds of Governorate Diwaniyah, reaching 28.72% and 26.92% for two different periods, respectively. However, these percentages differed nonsignificantly from the averages of the wild radish weed seeds of Kerbala, Babylon, and Wasit at 24.21%, 26.11%, and 25.46% for the two periods, respectively. The lowest mean for weed seeds had recordings in the Governorate Najaf with percent values of 21.61% and 22.30% for two periods, respectively.

Table 6. Effect of chemical herbicides on inhibition ratio after 60 and 90 days of spraying in weeds of wild radish for five Iraqi Governorates.

After 60 days of control						
Treatments	Study provinces					Means
	Najaf	Kerbala	Babel	Diwaniyah	Wasit	
Control	0.00	0.00	0.00	0.00	0.00	0.00
Navigator	37.19	39.06	39.80	33.81	39.47	37.87
Tatsteler	22.39	21.68	22.26	36.48	23.59	25.28
Decimate	34.73	31.21	38.97	35.21	35.15	36.01
Mark zone	17.18	29.13	29.53	42.11	29.09	29.41
$LSD_{0.05}$	5.81					2.24
Means	22.30	24.21	26.11	28.72	25.46	
$LSD_{0.05}$	4.17					
After 90 days of control						
Treatments	Study provinces					Means
	Najaf	Kerbala	Babel	Diwaniyah	Wasit	
Control	0.00	0.00	0.00	0.00	0.00	0.00
Navigator	37.04	38.84	37.12	33.59	35.61	36.62
Tatsteler	20.97	18.58	19.91	31.01	19.89	25.07
Decimate	33.70	35.76	38.17	34.97	32.53	35.03
Mark zone	15.46	26.96	28.96	35.06	22.74	26.24
$LSD_{0.05}$	4.04					2.16
Means	21.61	24.03	24.83	26.92	22.15	
$LSD_{0.05}$	5.62					

The resistance was due to emerging genetic mutations in the target genes, indicating that herbicides have no association with the target enzyme, resulting from the variation in the sequence of the nitrogenous bases of the genes responsible for manufacturing these enzymes. However, it may be attributable to the decomposition of herbicide inside the plant and its metabolism into nontoxic substances before reaching the target enzyme in the plant; thus, the plant does not die (Jugulam and Shyam, 2019). It also may refer to the nature of genetic mutations in these weed seeds, as a section of seed groups showed silent mutations (Table 4), which do not affect the essence of herbicide action. It can be an indicator of the occurrence of resistance in them in the coming seasons.

All the sprayed herbicides caused a significant difference in the inhibition percentage of wild radish weeds after 60 and 90 days of control (Table 6). However, the excelling treatments were Navigator, which gave averages of 37.87% and 36.01%, and Decimate, which had 36.62% and 35.03% for the two periods. The herbicides Tatsteler and Mark zone showed the averages of 25.28%, 29.41%, 25.07%, and 26.24% for the two periods, respectively. The reflection of the effect of herbicides in the dry weight inhibition percentage amounted to 36.62% and 35.03% for the herbicides Navigator and Decimate, respectively. It revealed the ability of these herbicides to reduce the height of weeds and their superiority in percentage control, which explains their dominance in the inhibition percentage.

Wheat characteristics

Spikes number (Spike m^{-2})

Research outcomes indicated that herbicides and control treatments showed significant differences in spike number, and due to better weed control, the herbicide treatments also increased substantially in spike number compared with the control (Table 7). The herbicide Navigator produced the highest average of spikes m^{-2} (423.57) versus the control treatment, which had the lowest average (314.25 spikes m^{-2}). However, the three herbicides Tatsteler, Decimate, and Mark zone gave means of 417.02, 409.10, and 400.39 spikes m^{-2}, respectively. It may refer to the lack of weeds allowing the crop to grow well without environmental stress, as reflected in the increased efficiency of the photosynthesis process, thus a productive crop performance. Therefore, the absence of the competition factor at this stage positively impacted the number of branches and enhanced the number of spikes per unit area. These results are analogous to past findings indicating that the highest number of spikes may be attainable when the competition between the crop and the accompanying weeds decreases (Muhammad *et al*., 2018).

Table 7. Effect of herbicides on some traits of the wheat crop.

Herbicides	Spikes number (spike m^{-2})	Biological yield (tons ha^{-1})	1000-grain weight (g)	Grain yield (tons ha^{-1})	Harvest index (%)
Weedy (Control)	314.25	13.12	34.22	3.09	26.55
Navigator	423.57	15.09	35.61	5.83	36.92
Tatsteler	417.02	15.06	37.20	5.61	35.25
Decimate	409.10	15.13	36.57	5.50	35.16
Mark zone	400.39	14.91	40.10	5.47	35.52
$LSD_{0.05}$	6.92	0.23	3.34	0.15	1.61

Biological yield (tons ha^{-1})

The herbicides caused a significant increase in the biological yield, with the herbicide Navigator treatment giving the highest average of 15.09 tons ha^{-1}, compared with the control treatment (13.12 tons ha^{-1}) (Table 7). The herbicides Tatsteler, Decimate, and Mark zone treatments produced a biological yield of 15.06, 15.13, and 14.91 tons ha^{-1}, respectively. The rise in the natural harvest of the herbicide treatments versus the control treatment may be due to the activity of the root system as a result of the weeds inhibition, which led to the efficiency of root growth and the ability of the plant to absorb more water and nutrients (Al-Hayali *et al*., 2018). The increase in the biological yield could also refer to the upsurge in the crop and its components, which are also essential components of the biological yield (El-Taif, 2021).

1000-grain weight (g)

In terms of 1000-grain weight, the herbicide Mark zone was superior by showing the highest average (40.10 g); however, it did not differ significantly from the herbicide Tatsteler (37.20 g) compared with the control (34.22 g) (Table 7). The herbicides Navigator and Decimate emerged with average 1000-grain weights of 38.87 and 36.57 g, respectively. The superiority of the herbicide Mark zone in 1000-grain weight can be due to the decrease in grain number with this herbicide, causing a negative relationship between the grains per spike and the grain weight per spike, also called the phenomenon of compensation (Al-Mutrafi *et al*., 2014).

The grain weight is one of the vital characteristics of the grain yield, as it is an indicator of considerable efficiency in transferring stored materials to plant parts from the source to downstream, represented by the grain and the ability of the origin to supply the grains with the products of representation. Photosynthesis is the final downstream by pushing the plant resulting from the control to use water and nutrients effectively (Al-Ayadi, 2014). The positive role of the herbicide in affecting the growth of the weeds and reducing its competition with the crop provided the opportunity to grow and increase the leafy area exposed to light. These results align with past findings enunciating that a significant increase in the 1000-grain weight may point to the performance ability of the crop to grow due to the efficiency of the photosynthesis and absence of weed competition (Al-Dulaimi, 2013; Al-Wahili and Al-Haidari, 2018).

Grain yield (tons ha^{-1})

Using herbicides for wild radish control caused a significant increase in grain yield compared with the control treatment (Table 7). The herbicide Navigator showed the maximum average grain yield of 5.83 tons ha^{-1} versus the weedy treatment (3.09 tons ha^{-1}). The herbicides Tatsteler, Decimate, and Mark zone provided average grain yields of 5.31, 5.50, and 5.37 tons ha^{-1}, respectively. The grain yield increase is associated with the improvement in yield components, such as, the increase in spike number, as the grain yield depends on yield components (Al-Latif, 2022). The rise in yield also correlates to the absence of broad and narrow leaf weeds in the experimental treatments from the beginning of crop growth until the physiological maturity, causing the wheat crop to make optimal use of the essential growth requirements. The increase in photosynthesis and growth rates also reflected more dry matter accumulation in the grains (Jadoua, 2012).

Harvest index (%)

The herbicide treatments triggered significant increases in the harvest index versus the control treatment (Table 7). The herbicide Navigator displayed the highest harvest index (36.92%) but did not differ significantly from the herbicide Mark zone (35.52%), compared with the weedy treatment with the lowest percentage (26.55%). The herbicides Tatsteler and Decimate showed a harvest index of 35.25% and 35.16%, respectively. The high proportion of the harvest index in some treatments might refer to the increase in the wheat grain yield relative to the biological harvest (Al-Ziyadi, 2015). The herbicides caused a significant reduction in the inhibition rate, reducing the chance of weeds, hence, the lack of competition between the crop and the weeds on the resources of photosynthesis, thus improving the performance of the vital processes involved in the transfer of material stored in the stem and leaves and, eventually, increasing grain yield in the overall biological produce (Mohammed *et al*., 2021).

CONCLUSIONS

The results revealed that the silent mutations do not affect the nature of the herbicide action. The observed missense mutations inhibited the effectiveness of herbicides through their effect on the herbicide-binding regions in the target gene. The superiority of two herbicides, Navigator and Decimate, was also evident in the control and inhibition percentages of the wild radish weeds, in addition to the dominance of some herbicides in yield components and grain yield, as well as, the overall biological yield and harvest index.

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