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Exploring Resistant Sources of Chickpea against *Fusarium oxysporum* f. sp. *ciceris* in Dryland Areas

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Abstract: *Fusarium* wilt is a fungal disease that has a significant impact on chickpeas worldwide. This study examined the response of 58 chickpea genotypes to *Fusarium* wilt. The experiment was conducted over two growing seasons at the Sararoud and Maragheh research stations at the Drylands Agricultural Research Institute of Iran. Genotype resistance was screened through wilt incidence records and nonparametric stability statistic evaluation. The identified resistant genotypes were then evaluated in the greenhouse for their response to four isolates of *Fusarium oxysporum* f. sp. *ciceris* (races 1/BC, 2, 4, and 6). Out of 58 genotypes, 32 exhibited moderate resistance, while 24 showed strong resistance abilities. Under warmer conditions, disease severity was higher, with scores at the Sararoud location being higher than those at the Maragheh location. Of the total genotypes across all locations and years, 41.4% were resistant, 55.17% were moderately resistant, 1.72% were susceptible, and 1.72% were highly susceptible. The nonparametric stability measures $S^{(1)}$, $S^{(2)}$, and $S^{(3)}$ identified FLIP 05-42C and FLIP 05-43C as stable and resistant genotypes. The study found that Azad/Hashem K3 was stable based on the non-parametric stability measure $S^{(6)}$. Other resistant genotypes were identified using stability parameters $NP^{(1)}$, $NP^{(3)}$, and $NP^{(4)}$, while FLIP 05-104C was identified by $NP^{(2)}$. The genotypes selected by nonparametric stability parameters showed resistance against at least two *Fusarium oxysporum* f. sp. *ciceris* races. The screening method and nonparametric stability statistics used in this study were effective in identifying sources of resistance to *Fusarium oxysporum* f. sp. *ciceris*.

Keywords: *Fusarium* wilt; chickpea germplasm; nonparametric stability measures; agricultural microbiology; sustainable biotic stress management

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1. Introduction

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world. This crop is cultivated mainly in the Middle East, Central and South Asia, the Mediterranean basin, East Africa, North America, and Australia [1]. Chickpea is a cheap and valuable source of protein (16.7–30.6%), especially for people in developing countries or for people following a vegetarian diet [2]. It also contains high quantities of lipids, carbohydrates,

minerals, vitamins, and non-nutritive health-beneficial compounds such as phenolics, tannins, sterols, and saponins [3]. Furthermore, chickpea, as a legume plant, plays an important role in aerial nitrogen fixation in the soil via a microbiological process and improving soil physicochemical properties, hence sustaining the agricultural systems [4].

Chickpea cultivation faces numerous challenges, including fungal diseases that creates significant threats to yield and quality. Among these, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo & K. Sato (FOC) stands out as a major constraint, particularly in dryland regions where environmental stressors exacerbate disease pressure [5]. This fungus colonizes the surface of the host's roots, grows intracellularly in the root cortex, penetrates the xylem vascular system, and colonizes the stem and root system, causing yellowing and wilting symptoms [6]. *Fusarium* wilt results in a reduction of both seed yield and weight, with an estimated annual loss ranging from 10% to 100% [7]. The pathogen displays pervasive pathogenic variability, with eight distinct races identified (0, 1A, 1B/C, 2, 3, 4, 5, and 6) [8]. Races 0 and 1A only result in foliar yellowing and late death, while other races can cause rapid and severe chlorosis, flaccidity, and early plant wilt, ultimately leading to the host plant's death [9]. Races 1B/C and 6 are common in the Mediterranean basin, while earlier findings in the western part of Iran evidenced the presence of pathogenic groups; among them, three were identified as FOC 1, 2, and 4 based on the disease symptoms in chickpea plants [10].

FOC can survive for up to several years in infested soil, even in the absence of a host plant [11]. To control the disease, several methods have been recommended, including soil solarization, fungicidal chemicals, biocontrol agents, agronomic practices, and resistant/tolerant cultivars [12]. The most practical and cost-efficient method for controlling FOC in chickpea crops is using resistant cultivars [13]. These cultivars can be identified and selected through screening campaigns of chickpea genotypes in diseased field conditions [13]. The current distribution of FOC races is unclear due to the large exchange of germplasm and climate variability, as well as the existence of multiple races in one region [14].

The dynamic nature of *Fusarium* wilt, characterized by pathogenic variability, and uncertain distribution patterns, necessitates a comprehensive understanding of resistance mechanisms and their stability over time and across different environments. Multi-environmental trials play a pivotal role in elucidating the resilience of chickpea genotypes to *Fusarium* wilt and informing breeding strategies for developing resilient cultivars [15]. Within this study, we hypothesized that a multi-environment field trial screening and nonparametric stability statistics approach could be useful to identify chickpea genotypes resistant to *Fusarium*. By employing this screening approach, this study aims to contribute valuable insights into the identification of resistant chickpea genotypes and their suitability for sustainable disease management. The experiment was conducted over two growing seasons at the Sararoud and Maragheh research stations at the Drylands Agricultural Research Institute of Iran. Genotype resistance was evaluated by screening natural (Sararoud) and induced (Maragheh) FOC wilt incidence records and assessing nonparametric stability statistics. To confirm the suitability of the approach, the resistant genotypes were tested in the greenhouse to confirm their response to four *C. arietinum* common isolates of *Fusarium oxysporum* f. sp. *ciceris* (race 1/BC, 2, 4, and 6). The findings from this research hold practical implications for chickpea breeding programs, agronomic practices, and ultimately, the resilience and productivity of chickpea-based agricultural systems worldwide.

2. Materials and Methods

2.1. Plant Material

This study tested a suite of chickpea genotypes, including 12 commercial cultivars and 46 advanced lines, for resistance to *Fusarium* wilt. This plant materials were prepared kindly via the Drylands Agricultural Research Institute of Iran (DARII) and The International Center for Agricultural Research in the Dry Areas (ICARDA). Full names and origins of studied genotypes are presented in Table S1. The susceptible check, cultivar Kaka[b], is a

local desi-type chickpea that has been identified as highly susceptible in screening projects over the last decade at the Drylands Agricultural Research Institute (DARI).

2.2. Multi-Environmental Field Tests

The field experiments were conducted over two consecutive years (2019–2020 and 2020–2021) at two different locations: the Sararoud (34°19′55″ N; 47°17′53″ E, elevation 1351 m) and Maragheh (37°18′10″ N; 46°28′24″ E, elevation 1710 m) research stations in the west and northwest of Iran, respectively. Each field trial consisted of four randomized complete block design plots with three replications. A natural infection occurred in the experimental plots at Sararoud, while at Maragheh, the plots were artificially infected through soil inoculation with 4 FOC isolates. Infections were repeated once yearly. Artificial infections were carried out following the steps summarized in Figure 1. Both the Sararoud and Maragheh research stations are part of DARI and cover a wide range of agro-climatic zones (Table 1).

Figure 1. Preparing the *Fusarium oxysporum* f. sp. *ciceris* FOC sick plot in DARI, Maragheh research station (**A**,**B**). Growing the fungal pathogen on chickpea-sand substrate in different vessels in the germinator (**C**). Scaling up the inoculum using 20L black plastic bags (**D**). The inoculum was applied in a single application, mixing it with soil at sowing (**E**). The farrows established to sowing chickpea with the inoculum in their bottom (**F**). Established sick plot after 10 years with appropriate amounts of FOC inoculum in the soil enhances 100% mortality on susceptible chickpea cultivar Kaka (assigned by the arrow). The rows around it are other chickpea genotypes.

Table 1. The climatic data from the two-year open field experiment, recorded in Maragheh and Sararoud locations during the growing seasons 2019–2020 and 2020–2021.

		Maragheh		Sararoud	
		2019–2020	**2020–2021**	**2019–2020**	**2020–2021**
Total evaporation (mm)		867.5	686.9	528.5	797.5
Average relative humidity (%)		61.9	61.3	51.8	51.5
No. of days below 0 °C		132	103	78	59
Temperature (°C)	Average	4.6	6.9	11.3	12.5
	Average Max	9.4	11.6	19	18.8
	Average Min	−0.5	2.3	3.6	6.2
	Absolute Max	18.8	20.5	25	25.9
	Absolute Min	−9	−5.8	−3.2	−1.2
Total Rain Fall (mm)		262.9	423.2	492.1	521.3

2.3. Wilt Incidence Screening

Each genotype was sown in two adjacent one-meter rows with 25 cm space, and the distance from the neighbor genotype was fixed at 50 cm. The susceptible check was sown after each genotype, with all genotypes surrounded on both sides by the susceptible check. Chickpea genotypes were sown in early March and April during both growing seasons of the studied years at the Sararoud and Maragheh research stations, respectively. Scoring began when the mortality percentage of the susceptible check reached 50% during the reproductive stage and was performed three times with one-week intervals. To correct any possible heterogeneity of the pathogen distribution across the plots, the final mortality value obtained for each accession was divided by the average value of two susceptible checks surrounding the treatment. Wilt incidence was assessed by recording plant mortality due to *Fusarium* wilt using a five-digit scoring system. The wilt incidence scoring system categorizes plant mortality as High Resistant (HR), Resistant (R), Moderately Resistant (MR), Susceptible (S), or High Susceptible (HS) based on the following percentages and digits: 0–5% = 1, 5.1–20% = 3, 20.1–40% = 5, 40.1–80% = 7, and >80.1% = 9.

2.4. Greenhouse Tests

2.4.1. Fungal Pathogens

The FOC races 1/BC, 2, 4, and 6 were obtained from the collection of the Agricultural Microbiology Laboratory of the University of L'Aquila. The pure cultures of FOC races were grown in potato dextrose broth (PDB, 200 g potato: 20 g dextrose: 1 L water) at 28–30 °C and 150 rpm for 3–4 days. The medium was filtered through a four-layer cheesecloth and centrifuged at 10,000 rpm for 10 min. The harvested spores were used to prepare the suspensions, which were adjusted to 1×10^6 conidia mL^{-1} using a hemocytometer.

2.4.2. Seed Sterilization and Seedlings Infection

Seeds of the genotypes identified as resistant and stable were surface sterilized in 0.5% NaOCl for 10 min, rinsed twice in sterile distilled water, and sown in plastic pots (5 × 7 × 8 cm) filled with sterile perlite. The pots were kept at 25 °C with a 12 h dark/12 h light photoperiod of 200 μE m^{-2} S^{-1} for germination. Two seeds were sown in each pot, and five pots were prepared. The seedlings were inoculated via the standard root-dip method at the 4–6 leaf stage, which occurred approximately 2 weeks after sowing. The roots were cut and immersed in a spore suspension for 1 min before being replanted in the pots. The control seedlings were treated using the same method but were dipped in sterile water instead.

2.4.3. Pot Experiment

The inoculated and control seedlings were treated with Hogland's solution (5 μM—Sigma-Aldrich, Saint Louis, MO, USA) and kept in a growth chamber with a 12 h dark/12 h light photoperiod of 300 μE m^{-2} S^{-1} at 26 °C and 22 °C, respectively. The pots were arranged inside

plastic trays containing a 10 cm depth of sterilized riverbed sand on the floor. Tap water was used for irrigation, which was conducted through the bottom of the pots by soaking up the sand layer twice a week. Disease incidence was estimated by calculating the percentage of dead plants. According to Sharma et al. [16], plants exhibiting wilt of 10% or less were considered resistant, those with wilt between 11–89% were considered to have an intermediate response, and those with wilt of 90% or more were considered susceptible.

2.5. Data Analysis

Genotypes with susceptible/resistant responses were identified based on their disease severity score and the mean over years and locations. Nonparametric stability statistics, proposed by Sabaghnia [17], were computed for the studied genotypes, including nonparametric measures of Huehn [18], based on original ranks, and nonparametric measures of Thennarasu [19], based on the corrected ranks. The data processing and nonparametric stability statistics were computed using Microsoft Excel ver. 2019. The genotypes were ranked based on nonparametric statistics. Factor analysis was then performed using Statistica software ver. 10 (Informer Technologies Inc, Shingle Springs, CA, USA) to assess the interrelationship between them, as shown in the graph of the first two factors.

3. Results

3.1. Screening for Resistance

The main objective of this study was to identify the sources of resistance against the *Fusarium* wilt in chickpea collections belonging to NCBP, DARI. Herein, a wide range of genetic variability across two growing seasons was explored against the *Fusarium* wilt. As presented in Table 2, most of the genotypes presented moderate resistance, followed by the resistant type. Genotypes with susceptible responses were rare. During the 2019–2020 growing season at the Maragheh location, G26 (FLIP 09-24C) was identified as susceptible. The remaining 56 genotypes exhibited varying levels of resistance, with the majority being highly resistant (45 HR vs. 10 R vs. 1 MR). At Sararoud during the same growing season, only genotype G19 (FLIP 07-244C) was found to be susceptible, while the other genotypes were resistant or moderately resistant (27 R vs. 28 MR). G29 (FLIP 86-06C) and G33 (FLIP 98-121C) were the only genotypes that exhibited high resistance. At Maragheh, genotype G47 (FLIP97-530C X94TH103//FLIP91-186C/FLIP 91-96C/3/FLIP 90-109C) was identified as susceptible and G58 (Kaka^{b}) as highly susceptible during 2020–21. Out of the other 56 genotypes, the majority were identified as resistant or moderately resistant (21 MR vs. 26 R vs. 9 HR). At Sararoud, 11 genotypes were identified as either susceptible (7, including G3, G7, G10, G11, G17, G26, and G45) or highly susceptible (4, including G23, G41, G51, and G58). Based on the mean wilt incidence across locations and years, 41.4% of the total genotypes were resistant, followed by 55.17% moderately resistant, 1.72% susceptible, and 1.72% highly susceptible.

Figure S1 shows the graphical representation of the studied germplasm based on their severity scores, indicating a skewed distribution. In the same figure, it is evident that the incidence of wilt was more pronounced at the Sararoud site compared to the Maragheh site.

Table 2. Wilt incidence results (five-digit scoring system) recorded at the Maragheh and Sararoud locations during the 2019–2020 and 2020–2021 growing seasons.

Genotype No.	Maragheh		Sararoud		Wilt Incidence (Mean)	Disease Resistance *
	2019–2020	2020–2021	2019–2020	2020–2021		
G1	1	5	5	3	3–5	MR
G2	1	5	5	5	4	MR
G3	3	5	5	7	5	MR
G4	1	1	5	5	3	R
G5	3	3	5	3	3–5	MR
G6	3	1	5	3	3	R
G7	1	3	5	7	4	MR
G8	1	5	5	3	3–5	MR
G9	1	1	5	5	1–5	R
G10	1	5	3	7	4	MR
G11	3	3	5	7	4–5	MR
G12	1	1	3	3	2	R
G13	1	1	3	3	2	R
G14	1	1	3	5	2–5	R
G15	1	3	5	5	3–5	MR
G16	5	5	3	5	4–5	MR
G17	1	3	3	7	3–5	MR
G18	1	3	5	5	3–5	MR
G19	1	3	7	5	4	MR
G20	1	3	3	3	2–5	R
G21	1	3	3	5	3	R
G22	3	1	3	5	3	R
G23	1	5	3	9	3–6	MR
G24	1	5	5	5	4	MR
G25	1	3	3	3	2–5	R
G26	7	5	3	7	5–5	S
G27	1	1	3	3	2	R
G28	1	3	5	3	3	R
G29	1	3	1	3	2	R
G30	1	3	3	5	3	R
G31	1	1	5	5	3	R
G32	1	3	3	5	3	R
G33	1	5	1	5	3	R
G34	1	5	5	5	4	MR
G35	1	5	3	5	3–5	MR
G36	1	5	3	5	3–5	MR
G37	1	3	3	5	3	R
G38	1	3	5	3	3	R
G39	1	5	3	3	3	R
G40	1	5	5	3	3–5	MR
G41	1	3	3	9	4	MR
G42	1	3	3	5	3	R
G43	1	5	5	5	4	MR
G44	1	3	3	5	3	R
G45	1	3	5	7	4	MR
G46	1	3	5	5	3–5	MR
G47	1	7	3	3	3–5	MR
G48	1	5	3	5	3–5	MR
G49	1	5	3	3	3	R
G50	3	3	5	3	3–5	MR
G51	3	3	5	9	5	MR
G52	3	3	3	5	3–5	MR
G53	1	5	5	3	3–5	MR
G54	1	5	5	5	4	MR
G55	3	3	3	3	3	R
G56	1	3	5	5	3–5	MR
G57	3	5	5	5	4–5	MR
G58	9	9	5	9	8	HS

* Disease resistance reaction have determined regarding mean value of wilt disease across locations and years.

3.2. Nonparametric Stability Analysis

The Huehn and Thennarasu nonparametric stability measures are presented for the 58 genotypes in Table 3. Using the Huehn nonparametric stability measures, the $S^{(1)}$, $S^{(2)}$, and $S^{(3)}$ indices were detected for genotypes G12 (FLIP 05-42C) and G13 (FLIP 05-43C). These genotypes were then considered as stable resistant. Considering only the $S^{(6)}$ value, genotype G3 (Azad/Hashem K3) was also calculated to be stable. Using the Thennarasu's (1995) nonparametric stability parameters, $NP^{(1)}$, $NP^{(3)}$, and $NP^{(4)}$, genotype G3 (Azad/Hashem K3) was introduced as a stable resistant genotype. Using $NP^{(2)}$, G11 (FLIP 05-104C) was also identified as a suitable resistant genotype.

Table 3. Summary of nonparametric stability statistics for the 58 genotypes.

Genotype No.	$S^{(1)}$	$S^{(2)}$	$S^{(3)}$	$S^{(6)}$	$NP^{(1)}$	$NP^{(2)}$	$NP^{(3)}$	$NP^{(4)}$
G1	22.33	347.33	61.29	3.76	19.25	1.03	1.16	1.31
G2	19.17	234.92	32.4	2.07	13	0.45	0.66	0.88
G3	10.67	72	5.4	0.7	5.75	0.37	0.2	0.27
G4	17.67	208.67	48.15	3.69	16	1.76	1.44	1.36
G5	25.83	408.25	56.31	2.99	18.75	0.81	0.91	1.19
G6	27.33	499	76.77	3.79	24	1.19	1.24	1.4
G7	26.83	441.58	59.54	3.01	18.25	0.84	0.89	1.21
G8	22.33	347.33	61.29	3.76	19.25	1.03	1.16	1.31
G9	17.67	208.67	48.15	3.69	16	1.76	1.44	1.36
G10	29	558	76.09	3.64	21	1.03	0.98	1.32
G11	21.67	310.33	27.79	1.61	14.25	0.36	0.48	0.65
G12	1	1	2	2	12.75	23.5	9.36	0.67
G13	1	1	2	2	12.75	23.5	9.36	0.67
G14	9.83	84.92	40.76	4.4	14.25	13.88	2.6	1.57
G15	16.17	156.92	30.87	2.56	10.75	0.73	0.77	1.06
G16	29.17	511.58	53.38	2.4	18.25	0.62	0.7	1.01
G17	24.67	484.33	93.74	4.19	12.5	2.38	1.2	1.59
G18	16.17	156.92	30.87	2.56	10.75	0.73	0.77	1.06
G19	30.17	628.25	84.71	3.21	16.25	1.12	0.97	1.36
G20	4.83	18.25	14.6	3.33	8.75	13.63	2.7	1.29
G21	10.67	73.67	26	3.06	5.75	2.54	0.96	1.25
G22	25.33	433.67	74.34	3.54	18.75	1.63	1.15	1.45
G23	32.5	691.58	87.36	3.66	21.75	1.12	0.97	1.37
G24	19.17	234.92	32.4	2.07	13	0.45	0.66	0.88
G25	4.83	18.25	14.6	3.33	8.75	13.63	2.7	1.29
G26	29	558	46.5	1.83	17.25	0.55	0.57	0.81
G27	1	1	2	2	12.75	23.5	9.36	0.67
G28	16	187	53.43	3.71	11	3.09	1.5	1.52
G29	4.5	20.25	18.69	4.15	15.5	25.75	4.99	1.38
G30	10.67	73.67	26	3.06	5.75	2.54	0.96	1.25
G31	17.67	208.67	48.15	3.69	16	1.76	1.44	1.36
G32	10.67	73.67	26	3.06	5.75	2.54	0.96	1.25
G33	20.67	285.67	59.1	3.72	16.25	1.83	1.31	1.43
G34	19.17	234.92	32.4	2.07	13	0.45	0.66	0.88
G35	20.33	268.67	53.73	3.47	14	1.15	1.04	1.36
G36	20.33	268.67	53.73	3.47	14	1.15	1.04	1.36
G37	10.67	73.67	26	3.06	5.75	2.54	0.96	1.25
G38	16	187	53.43	3.71	11	3.09	1.5	1.52
G39	17.83	295.58	86.51	5.02	14.25	8.88	1.75	1.74
G40	22.33	347.33	61.29	3.76	19.25	1.03	1.16	1.31
G41	28.17	648.25	112.74	4.38	14.75	2.35	1.32	1.63
G42	10.67	73.67	26	3.06	5.75	2.54	0.96	1.25
G43	19.17	234.92	32.4	2.07	13	0.45	0.66	0.88
G44	10.67	73.67	26	3.06	5.75	2.54	0.96	1.25
G45	26.83	441.58	59.54	3.01	18.25	0.84	0.89	1.21
G46	16.17	156.92	30.87	2.56	10.75	0.73	0.77	1.06
G47	28.33	766.33	148.32	5.35	15	9.13	1.44	1.83
G48	20.33	268.67	53.73	3.47	14	1.15	1.04	1.36
G49	17.83	295.58	86.51	5.02	14.25	8.88	1.75	1.74
G50	25.83	408.25	56.31	2.99	18.75	0.81	0.91	1.19
G51	25.17	390.25	33.21	1.73	16.25	0.53	0.57	0.71
G52	23.17	354.92	53.91	2.68	13.5	0.97	0.79	1.17
G53	22.33	347.33	61.29	3.76	19.25	1.03	1.16	1.31
G54	19.17	234.92	32.4	2.07	13	0.45	0.66	0.88
G55	23.67	442	88.4	4.13	13.75	2.77	1.14	1.58
G56	16.17	156.92	30.87	2.56	10.75	0.73	0.77	1.06
G57	14	118.67	10.79	0.97	8.75	0.24	0.31	0.42
G58	14.5	184.25	11	0.81	19.5	0.38	0.42	0.29

To better understand the relationships among the nonparametric stability parameters, factor analysis was performed based on the rank correlation matrix. The relationships are represented graphically by plotting the scores of the first two factors as shown in Figure 2.

Using factor analysis, the first two factors explained 89% of the total variance (51% by Factor 1 and 38% by Factor 2). The Factor 1 axis did not distinguish the nonparametric stability parameters, while the Factor 2 axis separated $S^{(6)}$, $NP(^2)$, $NP(^3)$, and $NP(^4)$ from the other parameters ($S(^1)$, $S(^2)$, $S(^3)$, and $NP(^1)$). It is clear that the parameters $S(^1)$, $S(^2)$, and $NP(^1)$ were grouped close to each other and had no significant correlation with the parameters $NP(^2)$ and $NP(^3)$ due to the nearly perpendicular vectors ($r = \cos 90° = 0$).

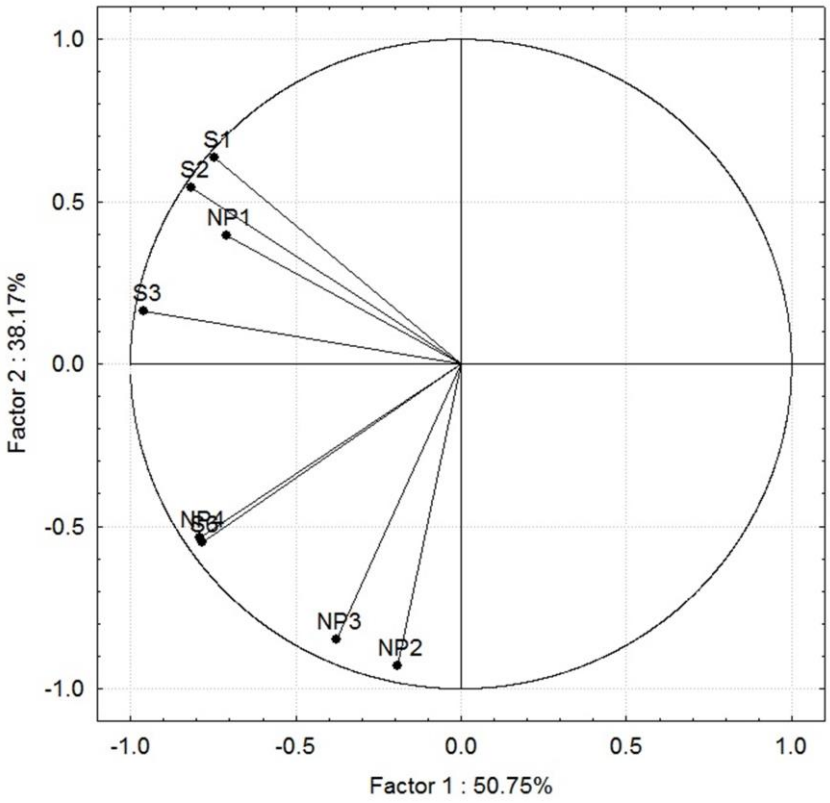

Figure 2. Factor analysis plot of ranks of stability, estimated by several nonparametric methods.

A visualization of the variation in resistance response is provided by the Factor 1 vs. Factor 2 plot in Figure 3. The plot shows that the *Fusarium* wilt scores for the germplasm were significantly different. The best favorable genotypes with stable disease resistance and high resistance should be on the far left of the plot with large negative values for Factor 1 scores. This is the case for genotypes G2, G3, G11, G12, G13, G15, G18, G20, G21, G24, G25, G29, G51, G57, and G58, which were also identified as having low disease scores and no environment-specific interactions. Similarly, the most unfavorable genotypes (low disease resistance and high instability) should be located as far to the right as possible in the plot, with a large positive value for the scores of Factor 1. On the other hand, Factor 2 could separate genotypes into two distinct groups with respect to their disease resistance and stability characteristics.

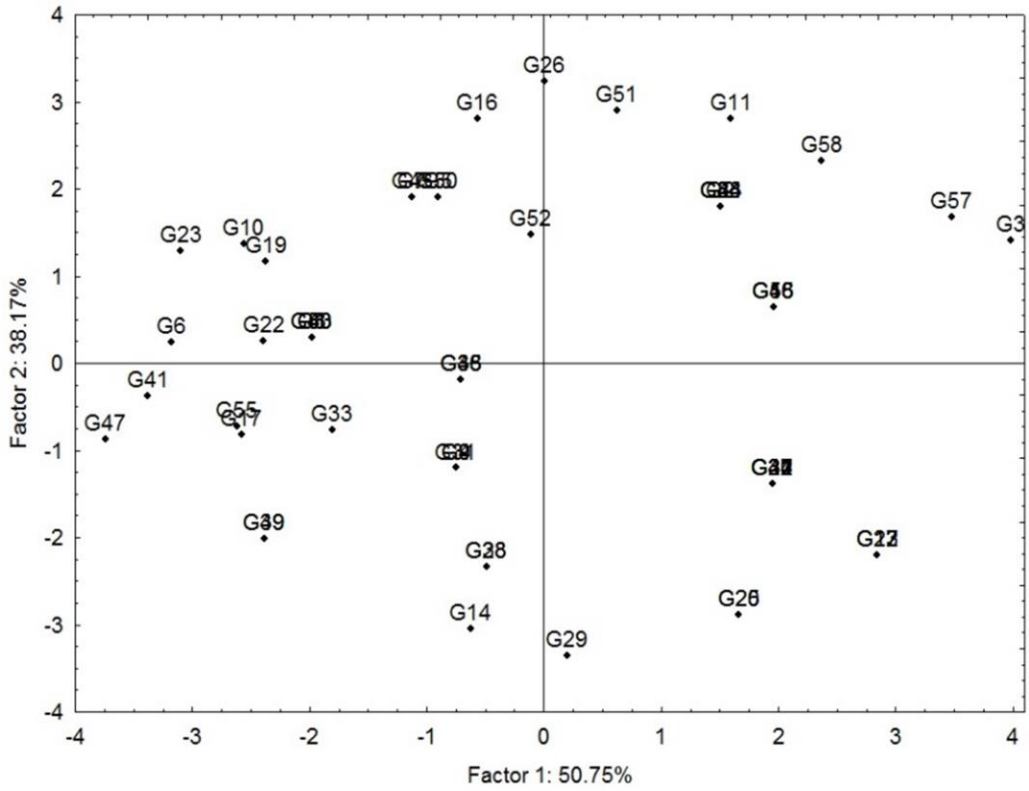

Figure 3. Factor analysis plot of variation in resistance response, estimated by several nonparametric methods.

Genotypes G3, G11, G12, and G13, which showed good levels of stable and durable resistance under field conditions, were further evaluated for resistance to four common races of FOC, including 1B/C, 2, 4, and 6, under controlled conditions. The results, presented in Table 4, show that G12 (FLIP 05-42C) was resistant to all races tested, while G11 (FLIP 05-104C) showed an intermediate response to race 4 and resistance to the others. G13 (FLIP 05-43C) and G3 (Azad/Hashem K3) were ranked in the lower resistance levels. G13 showed resistance to only three races (2, 6, 1B/C), while G3 showed resistance to only two races (6, 1B/C).

Table 4. Response of field-stable/resistant chickpea genotypes to four prevalent races of FOC under greenhouse conditions.

	Total	Killed	Percent	Response	Total	Killed	Percent	Response *
		Race 2 (C-133)				Race 6 (C-158)		
FLIP 05-104C (G11)	8	0	0	R	7	0	0	R
Azad/Hashem K3 (G3)	4	4	100	S	4	0	0	R
FLIP 05-42C (G12)	3	0	0	R	9	1	11.1	R
FLIP 05-43C (G13)	6	0	0	R	5	0	0	R
Kaka[b] (G58)	5	5	100	S	6	6	100	S
		Race 4 (C-101)				Race 1/BC (C-126)		
FLIP 05-104C (G11)	5	2	40	I *	6	1	16.6	R
Azad/Hashem K3 (G3)	5	5	100	S	4	0	0	R
FLIP 05-42C (G12)	3	0	0	R	8	0	0	R
FLIP 05-43C (G13)	7	6	85.7	S	6	0	0	R
Kaka[b] (G58)	6	6	100	S	6	6	100	S

* Immune reaction.

4. Discussion

Fusarium wilt is a biotic stress that reduces the quality and quantity of chickpea yields. The effects of FOC on the host plant has been shown to be enhanced under dry and warm conditions [20]. Accordingly, several studies have been conducted to elucidate the genetic

control of resistance to *Fusarium* wilt in chickpea [16,21–23]. Soil-born attributes of *Fusarium* wilt make field practices a nonapplicable method in controlling this disease; therefore, the identification of field-stable/resistant chickpea sources of disease resistance with novel defense mechanisms is unavoidable. These sources will play a crucial role in developing genotypes with long-lasting resistance [12]. Several studies have reported that breeding resistant cultivars is the most effective, environmentally friendly, and economical approach to control *Fusarium* wilt in crops [21,24,25]. Therefore, this study was carried out to identify sources of resistance to FOC, which also had stable resistance in semi-arid regions of Iran.

In line with the previous reports [26], no immune genotype was observed in the chickpea germplasm examined in this work. In the present study, the disease severity score in Sararoud diseased plots was higher than that in Maragheh, which paralleled the findings of Pande et al. [27], who reported widespread *Fusarium* wilt under dry and warm conditions. As Maragheh and Sararoud are considered the main stations of cold and temperate regions of DARI, respectively, this was expected. Wilt incidence data showed differences among the studied genotypes in their response to *Fusarium* wilt for each region over two years. Moreover, in the naturally infested plots, the increasing of the amount of *Fusarium* wilt among the years could probably be influenced by the temperature and the rainfall parameters. The infection dynamics observed can be attributed to other several factors, such as the survival and persistence of the pathogen and variation of other environmental conditions [25]. FOC has the capability to endure in the soil for extended periods, often in the form of chlamydospores or mycelium. These structures can persist in the soil for multiple years, acting as potential sources of inoculum for subsequent growing seasons [14]. Environmental factors such as temperature, humidity, and soil moisture can significantly impact the severity of *Fusarium* wilt [25]. Landa and collaborators, for example, showed that the early stages of the wilt appeared faster as temperature increased and, during the development of the disease, rainfall became the relevant driver for the wilt progression. Further studies revealed that changes in soil moisture and temperature could be the cause of the yearly variation in *Fusarium* wilt incidence [17]. Chickpea genotypes with S and HS responses were very rare in the studied chickpea gene pool. Higher frequencies of R and MR resistance types and high wilt incidence in susceptible genotypes, especially the susceptible check, in all environments indicated adequate disease pressure in diseased plots. The increased natural selection pressure of the pathogen over the years may have resulted in an increased frequency of resistant alleles/QTLs in the chickpea germplasm [15]. Furthermore, the disease response of a genotype was found to vary from one screening location to the other; therefore, it appears that such genotypes that were found to be resistant in both regions have a broader resistance base that could be controlled by multiple factors or genes.

Many researchers have identified elite genotypes through field screenings of chickpea germplasm for resistance to *Fusarium* wilt [28]. These studies are mostly based on evaluation of limited germplasm at one or a few locations. As a result, resistance is often limited to wilt races prevalent in a particular region, and the donor can only be used for breeding programs in that region. Location-specific variation in wilt incidence could be due to differences in pathogen virulence or random distribution of resistance gene(s) within chickpea genotypes, or the influence of both factors [14]. In this research, the variation in the response of the studied chickpea genotypes (G) to the environment (E) represented the influence of the environment on the instability of wilt incidence. According to Lillemo et al. [29], a visualization of the G × E interaction is given by the data plotting, and it shows that the *Fusarium* wilt scores of the genotypes varied between locations. The high contribution of the G × E interaction indicates a high level of environmental variability, i.e., variable pathogen races at different locations and the effect of variation in local weather conditions over years on *Fusarium* wilt incidence [30]. In this context, the nonparametric stability parameters of Huehn ($S(^1)$, $S(^2)$, $S(^3)$, $S(^6)$) and Thennarasu ($NP(^1)$, $NP(^3)$, $NP(^4)$) were adopted for our multi-environmental data of *Fusarium* wilt. A literature review proved that nonparametric stability statistics are easy to compute and do not require assumptions

about the distributions of the data [31]. Here, using the aforementioned nonparametric indices, the genotypes G3 (Azad/HashemK3—S(^{6}), NP(^{1}), NP(^{3}), NP(^{4})), G12 and G13 (FLIP 05-42C and FLIP 05-43C—S(^{1}), S(^{2})), and G11 (FLIP 05-104C—NP(^{2})) were selected from the 58 studied genotypes.

Efficient screening methods for *Fusarium* wilt of chickpea have been adapted and developed under field, greenhouse, and laboratory conditions [32]. Greenhouse and laboratory tests can be conducted to confirm data obtained from field experiments [12,33]. Soil is a very complicated environment, and the presence of other microorganisms that can potentially affect the final response of chickpea plants to FOC is highly probable [34]. On the other hand, the use of known pathogens or races of pathogens, along with the establishment of optimal environmental conditions such as temperature, light, and humidity, allows the plant material to be challenged with high levels of accuracy and efficiency under controlled conditions [35]. Race interactions found in greenhouse experiments may be valuable to understand naturally infested plots occurrence [35]. The race-specific interaction investigated in the greenhouse experiments have made it possible to confirm the selected genotypes resistance stability. Furthermore, these outcomes validated the chickpea genotype-specific responses previously documented in studies [16,21–23]. A considerable number of genes are typically involved in the observed genetic resistance to FOC, and there may be intricate interactions between these genes and specific pathogen races [14]. It is plausible that distinct FOC races have distinctive virulence characteristics that permit them to overcome specific resistance mechanisms in diverse genotypes of chickpeas. Consequently, resistance in one genotype of chickpeas against a specific race of FOC does not necessarily imply resistance against another race. [24]. In the present study, four chickpea genotypes out of 58 accessions were selected as stable/resistant genotypes through a multi-locational trial for two consecutive years, and their evaluation under controlled conditions showed that they were resistant to at least two races. The results obtained through greenhouse studies indicate that our screening methods and the statistical approaches used in this study were effective and useful for identifying sources of resistance to FOC. Identification of highly stable genotypes with low disease incidence is the main source for resistant breeding programs [32]. Therefore, the genotypes identified in the current study could be used as resistant donors for chickpea breeding programs in such locations.

5. Conclusions

Today, with climate change and its effects on seasonal rainfall and global warming, there is a greater likelihood of creating an environment conducive to disease spreading. Therefore, it is strategically important to screen chickpea germplasm for biotic stresses such as *Fusarium* wilt and identify sources of resistance. The present study offers a practical method for screening and identifying chickpea accessions that are resistant to multiple races of *Fusarium* wilt through multi-locational testing. The suitability of the approach was confirmed through greenhouse tests with known FOC races. The genotypes FLIP 05-42C and FLIP 05-104C were selected based on multiple nonparametric stability parameters and demonstrated resistance to FOC races under controlled conditions. Resistance sources can be utilized in chickpea breeding programs by mapping genomic regions that control resistance and pyramiding resistance genes in a desired genotype. The genotypes identified in this project are novel and valuable genetic resources that can be directly used for the above-mentioned purposes against *Fusarium* wilt. As far as we know, this is the first study that shows resistance reaction of new and identified field stable genotypes against known FOC races.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14060824/s1, Table S1: Full names and origins of the 58 chickpea genotypes investigated. Figure S1. Classification of chickpea germplasm based on their response to FOC disease. The graphical representation includes 58 genotypes for two years (2019–2020 to 2020–2021) at the Maragheh and Sararoud locations.

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References

1. Marteau-Bazouni, M.; Jeuffroy, M.-H.; Guilpart, N. Grain Legume Response to Future Climate and Adaptation Strategies in Europe: A Review of Simulation Studies. *Eur. J. Agron.* **2024**, *153*, 127056. [CrossRef]
2. Arriagada, O.; Cacciuttolo, F.; Cabeza, R.A.; Carrasco, B.; Schwember, A.R. A Comprehensive Review on Chickpea (*Cicer arietinum* L.) Breeding for Abiotic Stress Tolerance and Climate Change Resilience. *Int. J. Mol. Sci.* **2022**, *23*, 6794. [CrossRef] [PubMed]
3. Wood, J.A.; Grusak, M.A. Nutritional Value of Chickpea. In *Chickpea Breeding and Management*; CABI: Wallingford, UK, 2007; pp. 101–142.
4. Ghadirnezhad Shiade, S.R.; Fathi, A.; Kardoni, F.; Pandey, R.; Pessarakli, M. Nitrogen Contribution in Plants: Recent Agronomic Approaches to Improve Nitrogen Use Efficiency. *J. Plant Nutr.* **2024**, *47*, 314–331. [CrossRef]
5. Hamida, D.; Gowda, V.T.; Kundu, A.; Kaur, R.; Bag, T.K. Effect of Culture Filtrate Containing Fusaric Acid of *Fusarium oxysporum* f. Sp. *Ciceris* on Defence Enzymes in Chickpea. *Indian. Phytopathol.* **2024**, *77*, 61–69. [CrossRef]
6. Jiménez-Fernández, D.; Landa, B.B.; Kang, S.; Jiménez-Díaz, R.M.; Navas-Cortés, J.A. Quantitative and Microscopic Assessment of Compatible and Incompatible Interactions between Chickpea Cultivars and *Fusarium oxysporum* f. sp. *ciceris* Races. *PLoS ONE* **2013**, *8*, e61360. [CrossRef] [PubMed]
7. Sankar, P.M.; Shreedevasena, S.; Karthiba, L.; Raju, P.A.; Vanitha, S.; Kamalakannan, A.; Jeyakumar, P. Ecology, Biology and Management of Fusarium Wilt in Chickpea (*Cicer arietinum* L.): A Review. *Agric. Rev.* **2022**, *2481*, 343–358. [CrossRef]
8. Jimenez-Diaz, R.M. Use of Fungicide Treatments and Host Resistance to Control the Wilt and Root Rot Complex of Chickpeas. *Plant Dis.* **1985**, *69*, 591. [CrossRef]
9. Singh, C.; Vyas, D. The Trends in the Evaluation of Fusarium Wilt of Chickpea. In *Diagnostics of Plant Diseases*; IntechOpen: London, UK, 2021.
10. Younesi, H.; Bazgir, E.; Darvishnia, M.; Chehri, K. Selection and Control Efficiency of Trichoderma Isolates against *Fusarium oxysporum* f. Sp. *Ciceris* in Iran. *Physiol. Mol. Plant Pathol.* **2021**, *116*, 101731. [CrossRef]
11. Haware, M.P.; Nene, Y.L.; Natarajan, M. The Survival of *Fusarium oxysporum* f. sp. *Ciceri* in the Soil in the Absence of Chickpea. *Phytopathol. Mediterr.* **1995**, *35*, 9–12.
12. Yadav, R.K.; Tripathi, M.K.; Tiwari, S.; Tripathi, N.; Asati, R.; Patel, V.; Sikarwar, R.S.; Payasi, D.K. Breeding and Genomic Approaches towards Development of Fusarium Wilt Resistance in Chickpea. *Life* **2023**, *13*, 988. [CrossRef]
13. Hotkar, S.; Jayalakshmi, S.K.; Suhas, P.D. Screening for Resistant Sources in Chickpea Entries against Fusarium Wilt. *J. Pharmacogn. Phytochem.* **2018**, *7*, 663–665.
14. Sharma, M.; Kiran Babu, T.; Gaur, P.M.; Ghosh, R.; Rameshwar, T.; Chaudhary, R.G.; Upadhyay, J.P.; Gupta, O.; Saxena, D.R.; Kaur, L.; et al. Identification and Multi-Environment Validation of Resistance to *Fusarium oxysporum* f. sp. *Ciceris* in Chickpea. *Field Crops Res.* **2012**, *135*, 82–88. [CrossRef]
15. Gayacharan; Rani, U.; Singh, S.; Basandrai, A.K.; Rathee, V.K.; Tripathi, K.; Singh, N.; Dixit, G.P.; Rana, J.C.; Pandey, S.; et al. Identification of Novel Resistant Sources for Ascochyta Blight (*Ascochyta rabiei*) in Chickpea. *PLoS ONE* **2020**, *15*, e0240589. [CrossRef]
16. Sharma, K.D.; Chen, W.; Muehlbauer, F.J. Genetics of Chickpea Resistance to Five Races of Fusarium Wilt and a Concise Set of Race Differentials for *Fusarium oxysporum* f. sp. *Ciceris*. *Plant Dis.* **2005**, *89*, 385–390. [CrossRef] [PubMed]
17. Sabaghnia, N. Nonparametric Statistical Methods for Analysis of Genotype × Environment Interactions in Plant Pathology. *Australas. Plant Pathol.* **2016**, *45*, 571–580. [CrossRef]
18. Huehn, M. Nonparametric Measures of Phenotypic Stability. Part 1: Theory. *Euphytica* **1990**, *47*, 189–194. [CrossRef]
19. Thennarasu, K. *On Certain Non-Parametric Procedures for Studying Genotype-Environment Inertactions and Yield Stability*; Indian Agricultural Research Institute: New Delhi, India, 1995.
20. Landa, B.B.; Navas-Cortés, J.A.; Hervás, A.; Jiménez-Díaz, R.M. Influence of Temperature and Inoculum Density of *Fusarium oxysporum* f. Sp. *Ciceris* on Suppression of Fusarium Wilt of Chickpea by Rhizosphere Bacteria. *Phytopathology* **2001**, *91*, 807–816. [CrossRef]

21. Cachinero, J.M.; Hervás, A.; Jiménez-Díaz, R.M.; Tena, M. Plant Defence Reactions against Fusarium Wilt in Chickpea Induced by Incompatible Race 0 of *Fusarium oxysporum* f. Sp. *Ciceris* and Nonhost Isolates of *F. oxysporum*. *Plant Pathol.* **2002**, *51*, 765–776. [CrossRef]
22. Kumar, S. Inheritance of Resistance to *Fusarium* Wilt (Race 2) in Chickpea. *Plant Breed.* **1998**, *117*, 139–142. [CrossRef]
23. Das, A.; Mondol, B.; Singh, P.; Thakur, S. Genetic Improvement of Nutraceutical Traits in Chickpea (*Cicer arietinum* L.). In *Compendium of Crop Genome Designing for Nutraceuticals*; Kole, C., Ed.; Springer Nature: Singapore, 2023; pp. 639–659. ISBN 978-981-19-4169-6.
24. Sabbavarapu, M.M.; Sharma, M.; Chamarthi, S.K.; Swapna, N.; Rathore, A.; Thudi, M.; Gaur, P.M.; Pande, S.; Singh, S.; Kaur, L.; et al. Molecular Mapping of QTLs for Resistance to Fusarium Wilt (Race 1) and Ascochyta Blight in Chickpea (*Cicer arietinum* L.). *Euphytica* **2013**, *193*, 121–133. [CrossRef]
25. Sharma, M.; Ghosh, R.; Tarafdar, A.; Rathore, A.; Chobe, D.R.; Kumar, A.V.; Gaur, P.M.; Samineni, S.; Gupta, O.; Singh, N.P.; et al. Exploring the Genetic Cipher of Chickpea (*Cicer arietinum* L.) Through Identification and Multi-Environment Validation of Resistant Sources Against Fusarium Wilt (*Fusarium oxysporum* f. sp. *Ciceris*). *Front. Sustain. Food Syst.* **2019**, *3*, 78. [CrossRef]
26. Mohamed, O.E.; Hamwieh, A.; Ahmed, S.; Ahmed, N.E. Genetic Variability of *Fusarium oxysporum* f.Sp. *Ciceris* Population Affecting Chickpea in the Sudan. *J. Phytopathol.* **2015**, *163*, 941–946. [CrossRef]
27. Pande, S.; Siddique, K.H.M.; Kishore, G.K.; Bayaa, B.; Gaur, P.M.; Gowda, C.L.L.; Bretag, T.W.; Crouch, J.H. Ascochyta Blight of Chickpea (*Cicer arietinum* L.): A Review of Biology, Pathogenicity, and Disease Management. *Aust. J. Agric. Res.* **2005**, *56*, 317. [CrossRef]
28. Dubey, S.C.; Singh, B.; Srinivasa, N. Evaluation of Chickpea Genotypes against Fusarium Wilt for Resistant Sources. *Indian. Phytopathol.* **2017**, *70*, 254–255. [CrossRef]
29. Lillemo, M.; Singh, R.P.; van Ginkel, M. Identification of Stable Resistance to Powdery Mildew in Wheat Based on Parametric and Nonparametric Methods. *Crop Sci.* **2010**, *50*, 478–485. [CrossRef]
30. Ejaz, M.R.; Jaoua, S.; Ahmadi, M.; Shabani, F. An Examination of How Climate Change Could Affect the Future Spread of Fusarium Spp. around the World, Using Correlative Models to Model the Changes. *Environ. Technol. Innov.* **2023**, *31*, 103177. [CrossRef]
31. Pour-Aboughadareh, A.; Khalili, M.; Poczai, P.; Olivoto, T. Stability Indices to Deciphering the Genotype-by-Environment Interaction (GEI) Effect: An Applicable Review for Use in Plant Breeding Programs. *Plants* **2022**, *11*, 414. [CrossRef] [PubMed]
32. Yadav, S.; Kumar, S. Screening and Evaluation of *Cicer arietinum* Genotypes against Fusarium Wilt under Sick Field and Artificial Condition. *Asian J. Microbiol. Biotech. Env. Sc.* **2019**, *21*, 1068–1075.
33. Halila, M.H.; Strange, R.N. Screening of Kabuli Chickpea Germplasm for Resistance to Fusarium Wilt. *Euphytica* **1997**, *96*, 273–279. [CrossRef]
34. Farda, B.; Djebaili, R.; Bernardi, M.; Pace, L.; Del Gallo, M.; Pellegrini, M. Bacterial Microbiota and Soil Fertility of Crocus Sativus L. Rhizosphere in the Presence and Absence of *Fusarium* spp. *Land* **2022**, *11*, 2048. [CrossRef]
35. Velásquez, A.C.; Castroverde, C.D.M.; He, S.Y. Plant–Pathogen Warfare under Changing Climate Conditions. *Curr. Biol.* **2018**, *28*, R619–R634. [CrossRef] [PubMed]

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