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Article

Vegetative Compatibility and Virulence Diversity of *Verticillium dahliae* from Okra (*Abelmoschus esculentus*) Plantations in Turkey and Evaluation of Okra Landraces for Resistance to *V. dahliae*

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Abstract: Forty-four *V. dahliae* isolates were collected from symptomatic vascular tissues of okra plants each from a different field in eight provinces located in the eastern Mediterranean and western Anatolia regions of Turkey during 2006-2009. Nitrate-nonutilizing (*nit*) mutants of *V. dahliae* from okra were used to determine heterokaryosis and genetic relatedness among isolates. All isolates from okra plants were grouped into two vegetative compatibility groups (VCGs) (1 and 2) and three subgroups as 1A (13.6%, 6/44), 2A (20.5%, 9/44) and 2B (65.9%, 29/44) according to international criteria. Pathogenicity tests were performed on a susceptible local okra (*A. esculentus*) landrace in greenhouse conditions. All isolates from VCG1A and VCG2B induced defoliation (D) and partial defoliation (PD) symptoms, respectively. Other isolates from VCG2A gave rise to typical leaf chlorosis symptoms without defoliation. The obtained data showed that the virulence level of *V. dahliae* isolates from okra was related to their VCG belongings. Eighteen okra landraces from diverse geographical origins were screened for resistance to VCG2B and VCG1A of *V. dahliae*. The results indicated that all landraces were more susceptible to highly virulent VCG1A-D pathotype displaying D or PD symptoms depending on their susceptibility levels with a mean disease severity index of 3.52 than to less virulent VCG2B-PD pathotype of *V. dahliae* displaying PD and ND symptoms with a mean disease severity index of 2.52. Significant differences were observed among the landraces; however, none of them exhibited a level of resistance. Okra landraces; Çorum, Hatay Has and Şanlıurfa displayed the lowest level of susceptibility or little tolerance to both D and PD pathotypes. VCG2B of PD was prevailing in the surveyed areas and VCG1A of D was the most virulent of the VCGs identified. Introduction of resistant genotypes to Turkish okra germplasm from different sources and breeding new resistant okra cultivars are critical for the sustainability of okra production.

Keywords: Landraces; Malvaceae; *nit* mutants; pathotypes; VCGs; defoliating; wilt

1 Introduction

Okra [*Abelmoschus esculentus* (L.) Moench], or Lady's finger, a flowering perennial plant belonging to the family Malvaceae, is one of the important and popular vegetables of subtropical and tropical areas, and other warm temperate regions around the world with 9.6 million tons production on 2.4 million hectares [1]. In Turkey, it is cultivated for its edible green seedpods in 5.559 hectares area with the production of 28.536 tones [1]. This plant can be grown anywhere by smallholders, although it bears most abundantly in regions with long, hot summers. Its high-fiber, nutritious fruit is consumed as dried, frozen, fresh, canned and in pickled forms in Turkey [2]. Soil-borne plant pathogen; *Verticillium dahliae* (Kleb) causes vascular wilt in a large number of dicotyledonous plants [3] as in okra. The pathogen causes serious yield losses especially on olive, cotton and vegetables in Turkey [3]. Okra *Verticillium* wilt is also well known in many okra growing regions throughout the country and is one of the largest plant diseases [3].

Vegetative compatibility groups (VCGs) is a popular method for assessing fungal population structures and genetic diversity of several plant pathogenic fungi worldwide. Vegetative compatibility (VC) is a genetically defined capability of a particular fungi to generate viable heterokaryons with hyphal anastomosis and highly related to asexually reproducing fungi which are only able to interchange genes by parasexual recombination when both genotypes are vegetatively compatible [4]. VC has been recognized as a sub specific classification in many plant pathogenic fungi, including *V. dahliae*. Five VCGs have been characterized in this species [5,6] by the use of nitrate-nonutilizing (*nit*) mutants, which do not use nitrate as a nitrogen source. In *V. dahliae* populations, nine clonal lineages, with several lineages distributed worldwide in agricultural crops, were described by molecular genetic techniques [7,8], e.g., haplotypes of the *V. dahiae* defined by amplified fragment length polymorphisms (AFLPs) and single nucleotide polymorphisms (SNPs), and they were correlated with VCGs [8]. In *V. dahliae*, two pathotypes were also correlated with VCGs as well as with clonal lineages. *V. dahliae* isolates belonging to the defoliating (D) pathotype of cotton, olive and okra were merely in VCG1A and 1A clonal lineage, not in other VCGs which mostly cause wilting without defoliation [9–12].

So far, very few studies included *V. dahliae* isolates from okra or okra as a test plant to explore this pathosystem. In 2007, Hadizadeh and Banlhashemi examined VCG diversity within *V. dahliae* populations isolated from pistachio in Iran. Pathogenicity tests in the greenhouse with 22 isolates on cotton and okra indicated that all cotton and pistachio VCG1 isolates were highly virulent causing D symptoms on both hosts. However, VCG2B and VCG4B comprised weak to high virulent isolates, independent from their host origins. Lineage 1A/D causing leaf defoliation on olive, okra and cotton is widely distributed and it can infect at least eight more crop plants (artichoke, celery, eggplant, flax, safflower, tomato, sunflower, and watermelon) with different virulence ranges but not defoliation [13,14].

Although the importance of *Verticillium* wilt in Turkey has increased, there is no information about the genetic variation in *V. dahlia*e populations from okra. Therefore, the aims of this study were: (i) to evaluate genetic relatedness among 44 *V. dahliae* isolates obtained from okra plants in Turkey; (ii) to determine if VCGs could be useful to correlate VCGs affiliations with the isolates' geographic origin and/or virulence; (iii) to screen or evaluate okra landraces from diverse origin for resistance to *V. dahliae* under greenhouse conditions, aiming to identify sources of resistance to *V. dahliae*.

2 Materials and Methods

2.1 Collection of Isolates

Branch segments of the okra plants revealing wilting symptoms and brown discoloration in vessels were collected to determine the causative agent from the major okra-growing provinces of the east Mediterranean and western Anatolia regions in Turkey (Fig. 1 and Tab. 1). These collections were made in the summers of 2006 to 2009. Samples from branch segments were cut into small pieces (approx. 2 cm), dipped in ethyl alcohol (70%), surface-disinfected with NaOCl (1%) for 1 minute. Infected plant pieces were then rinsed

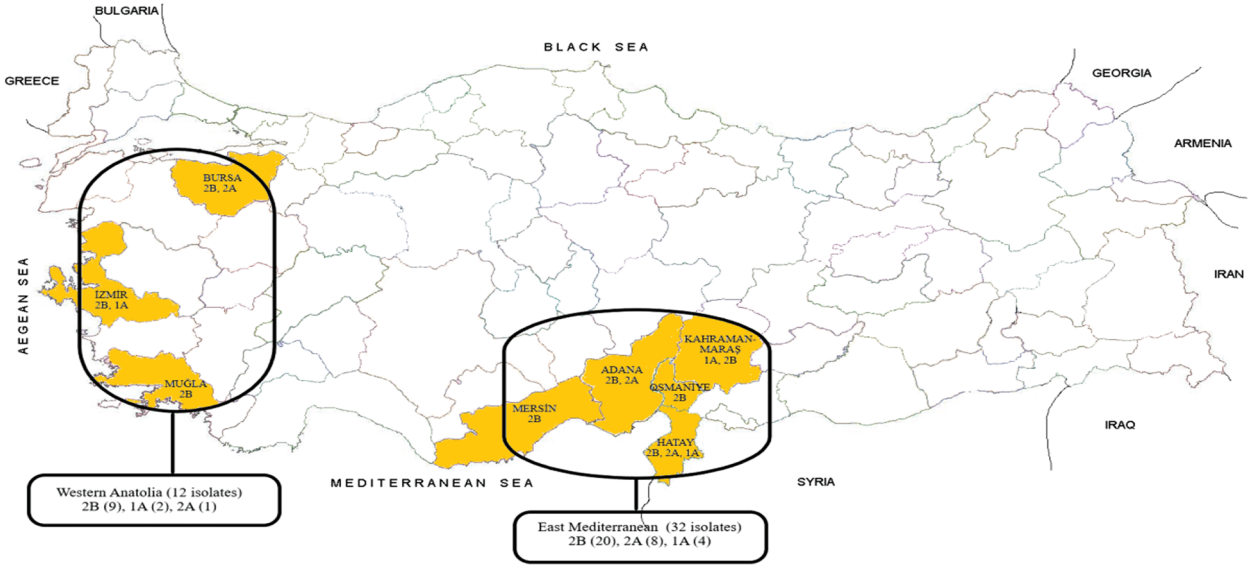

Figure 1: Geographic distribution of vegetative compatibility groups (VCGs) of *Verticillium dahliae* from okra in Turkey. 1A, 2A and 2B refer to *V. dahliae* VCG1A, VCG2A and VCG2B, respectively, infecting okras within each surveyed province (colored) and region (circled). VCG abbreviations are written in the order of isolate number of VCGs found in each province and region

Table 1: Isolates of *Verticillium dahliae* from okra in Turkey, listed by geographic origin (regions, provinces and locations in Turkey), vegetative compatibility group (VCG), virulence, and pathotype

Region	Province	Locality	VCG[a]	DI[b]	Pathotype[c]	Isolates
East Mediterranean	Adana	Ceyhan	2B	2.70 fg[d]	PD	OkVd03
			2B	2.80 ef	PD	OkVd16
		Karataş	2A	2.00 h	ND	OkVd21
		Kozan	2A	2.00 i	ND	OkVd22
			2A	2.00 i	ND	OkVd25
			2A	2.50 h	ND	OkVd26
			2B	2.60 gh	PD	OkVd28
		Yüreğir	2B	3.00 d	PD	OkVd27
			2B	2.80 ef	PD	OkVd40
	Hatay	Altınözü	2B	2.90 de	PD	OkVd33
			2B	2.65 g	PD	OkVd35
		Antakya	2B	3.00 d	PD	OkVd30
			2B	3.00 d	PD	OkVd31
			2B	2.90 de	PD	OkVd32
		Hassa	2A	1.90 i	ND	OkVd23
			2A	1.90 i	ND	OkVd24

(Continued)

Table 1 (continued).

Region	Province	Locality	VCG[a]	DI[b]	Pathotype[c]	Isolates
		Karaali	2A	2.50 h	ND	OkVd08
		Kırıkhan	1A	3.30 c	D	OkVd20
			2B	3.00 d	PD	OkVd39
		Serinyol	2A	1.95 i	ND	OkVd17
			2B	2.60 gh	PD	OkVd29
			2B	2.80 ef	PD	OkVd36
			2B	2.90 de	PD	OkVd37
			2B	2.85 e	PD	OkVd38
	Kahramanmaraş	Kerhan Bademli	1A	3.90 a	D	OkVd18
		Merkez	1A	3.50 b	D	OkVd19
		Muradiye	2B	2.80 ef	PD	OkVd14
			2B	2.80 ef	PD	OkVd41
		Sekamer	1A	3.40 b	D	OkVd13
	Mersin	Yenice	2B	2.80 ef	PD	OkVd15
		Silifke	2B	2.65 g	PD	OkVd34
	Osmaniye	Kadirli	2B	2.60 gh	PD	OkVd44
Western Anatolia	Bursa	İznik	2B	2.60 gh	PD	OkVd42
		Mudanya	2A	2.50 h	ND	OkVd43
	İzmir	Bergama	2B	2.60 gh	PD	OkVd01
			2B	2.65 g	PD	OkVd02
			2B	3.00 d	PD	OkVd12
		Yenikent	1A	3.90 a	D	OkVd04
			1A	3.50 b	D	OkVd05
			2B	3.00 d	PD	OkVd06
			2B	2.70 fg	PD	OkVd07
		Tire Belevi	2B	2.65 g	PD	OkVd10
			2B	3.00 d	PD	OkVd11
	Muğla	Milas	2B	2.90 de	PD	OkVd09

Notes: [a]Vegetative compatibility was determined using nitrate-nonutilizing mutants generated and pairing them with complementary mutants of international and Turkish tester strains of previously described VCGs. [b]A mean disease index (DI) was calculated by summing both vascular discoloration and foliar disease severity indices of 30 plants (five replicates of three plants per isolate, two experiments) by using the 0-4 scales. [c]D = okra defoliating PD = okra partial-defoliating ND = okra nondefoliating. [d]Numbers with different letters are significantly different statistically ($P \leq 0.01$).

in sterilized distilled water and dried on sterilized filter papers. Sample pieces were then transferred onto potato dextrose agar (PDA) (Difco Laboratories, Detroit) with an antibiotic (100 mg l^{-1} of streptomycin sulphate). Plates were then incubated at 25 ± 1°C for 5 to 7 days. Colonies growing in the plates were identified as *V. dahliae* by cultural characteristics and microscopic morphology of conidiophores and microsclerotia following the practice of Smith [15]. One isolate from each site was single-spore cultured and kept for the experiments in this study (Tab. 1).

2.2 Development and Classification of Nitrate-Nonutilizing (Nit) Mutants

Nit mutants of *V. dahiae* isolates were generated using the previously described techniques [16]. To generate *nit* mutants, water agar chlorate (WAC) medium including agar (2%), glucose (0.02%) and potassium chlorate (2.5-5%) were used in this study. Ten mycelia plugs (approx. 1 mm) from single-spored colonies of each isolate were placed on WAC medium and then Petri plates were incubated at 27°C in the dark conditions. After 10-28 days, growing edges of thin, fast growing mycelial chlorate-resistant sectors were transferred onto Czapek-Dox Agar medium (CDA; Merck, Damstradt, Germany) and incubated for five days. Thin mycelial colonies on the CDA were presumed as *nit* mutants. Mycelial plugs from each isolate were transferred to CDA, CDA supplemented with hypoxanthine (0.02%) and CDA supplemented with 0.05% nitrite to phenotype *nit* mutants (*nit*1, *nit*3 and NitM). The plates were then kept in an incubator at 27°C for 5 days in the dark conditions. Colonies growing on CDA amended with hypoxanthine/sodium nitrite in a wild-type phenotype, and on CDA in a thin mycelium were categorized as *nit*1 mutants. Colonies growing on CDA amended with hypoxanthine in a thin mycelium were categorized as NitM mutants. Mutants growing like wild-type on CDA medium supplemented with hypoxanthine but thinly on CDA medium supplemented with sodium nitrite were determined as *nit*3. After phenotyping, all the *nit* mutants were stored at −80°C in the facilities of Department of Plant Pathology, Mustafa Kemal University, Turkey.

2.3 Complementation of Nit Mutants and Testing for Vegetative Compatibility

Phenotypically categorized *nit* mutants of all isolates were paired with complementing *nit* mutants obtained from international and Turkish tester isolates on CDA. Phenotypically distinct *nit* mutants of an isolate were also paired among themselves to reveal self-compatibility. *Nit* mutants derived from 70-21 and 131M international reference *V. dahliae* isolates were used as tester isolates for identifying VCG3 and VCG4A, respectively [17]. Turkish *nit* testers OVd19 (VCG1A), OVd211 (VCG2A), OVd60 (VCG4B) [18] and Ch03 (VCG2B) [19] were used for identifying VCG1A, VCG2A, VCG4B and VCG2B, respectively. These latter testers were generated in previous studies from local *V. dahliae* isolates and correlated with the American origin (VCG1A, T9 from cotton) [17] and the Israeli origin *nit* testers (VCG2A, ep8 and ep52 from eggplant; VCG2B, cot11 and cot256 from cotton; and VCG4B, Pt15M and Pt9G from potato) of *V. dahliae* VCGs. Briefly, each three mutants were located 1 cm apart in in a triangular patternin 5-cm-diameter Petri plate, and plates were kept in an incubator for 28 days at 25°C in the dark conditions. After 7-28 days, plates were scored for prototrophic growth. The formation of a prototrophic heterokaryon, a dense, aerial growth, and the development of microsclerotia between an unidentified and a tester isolate indicated positive complementation reactions and allowed the assignment of unidentified isolates to the VCG of the tester isolate.

2.4 Pathogenicity Tests and Evaluation of Resistance of Okra Landraces to Verticillium Dahliae

Pathogenicity tests were conducted in two parts. In the first part of experiments, virulence of all isolates (44 isolates) was tested on a local landrace of okra (*A. esculentus*) by root dip inoculation. In the second stage, 18 okra landraces originated from diverse geographical origins (Tab. 3) were screened aiming to identify sources of resistance to *V. dahliae* (Tab. 3). All landraces used in this study were named according to their geographic origin in Turkey (16) and Iran (2). In the second assay, two highly virulent *V. dahliae* isolates [(OkVd18, defoliating pathotype (D) of okra belonging to VCG1A; and OkVd12, partial-defoliating pathotype (PD) of okra belonging to VCG2B)] were selected according to the results of first set of experiments.

Before sowing, okra seeds were dipped in ethanol, washed in running tap water and rubbed down with paper towels to remove excess water while conducting both experiments. The seedlings were grown in 128-celled Styrofoam trays containing sterilized sandy soil, sowing one seed in each cell with 30 seeds

Table 2: Comparison of VCGs of *Verticillium dahliae* isolates from okra in Turkey

VCG	Isolate ID Codes[a]	Mean DI[b]
VCG1A	OkVd04, OkVd05, OkVd13, OkVd18, OkVd19, OkVd20	3.58 a[c]
VCG2B	OkVd01, OkVd02, OkVd03, OkVd06, OkVd07, OkVd09, OkVd10, OkVd11, OkVd12, OkVd14, OkVd15, OkVd27, OkVd28, OkVd29, OkVd30, OkVd31, OkVd32, OkVd33, OkVd34, OkVd35, OkVd36, OkVd37, OkVd38, OkVd39, OkVd40, OkVd41, OkVd42, OkVd43, OkVd44	2.80 b
VCG2A	OkVd08, OkVd16, OkVd17, OkVd21, OkVd22, OkVd23, OkVd24, OkVd25, OkVd26	2.14 c
Pairwise comparison (P)[d]		<0.001
VCG1A vs VCG2A		<0.001
VCG1A vs VCG2B		<0.001
VCG2A vs VCG1A		<0.001
VCG2A vs VCG2B		<0.001
VCG2B vs VCG1A		<0.001
VCG2B vs VCG2A		<0.001

Notes: [a]All isolate ID codes begin with the prefix 'OkVd'. [b]Mean disease index (DI) was calculated from each treatment by summing the scores of plants on a scale of 0 to 4, where 0 = no symptoms and 4 = dead plant. [c]Different letters shows that mean disease index numbers are significantly different according to SNK multiple comparison test ($P < 0.01$). [d]P = probability for the statistic of linear single-degree-of-freedom contrasts.

per isolate or landrace. After sowing, trays were kept in a greenhouse for 21 days. They were uprooted from the soil; roots were then washed to remove soil and trimmed with sterile scissors and then dipped in an inoculum suspension of *V. dahliae* (4×10^6 conidia ml^{-1}) for 3 min. Some okra seedlings which only dipped into sterilized distilled water were kept as a negative control. After inoculation, okra seedlings were transferred into plastic pots (three seedlings per pot) having 12-cm-diameter (five pots were used for each treatment) containing sterile potting mixture (clay loam/peat; 2:1, vol:vol), and kept in greenhouse conditions in a completely randomized plot design. Plants were watered on demand and kept in a greenhouse at 25 ± 5°C with 50 to 90% relative humidity. Plants were observed for symptoms of wilt and browning of the vessels after 5 weeks. Severity of symptoms was calculated in each test plant with the vascular (Fig. 2) and foliar (Fig. 3) 0–4 scales: 0 = vascular discoloration is not present in the cross-section of the stem, absence of foliar symptoms; 1 = 1 to 25% discolored vascular area and 1 to 33% aerial part affected with slight wilting or chlorosis, 2 = 26 to 50% discolored vascular area and 34 to 66% aerial part affected with foliar necrosis, 3 = 51 to 75% discolored vascular area and 66 to 100% aerial part affected with partial defoliation, and 4 = 76 to 100% discolored vascular area with severe defoliation and stunting (Figs. 2 and 3). Re-isolations were made from tested plants to prove Koch's postulates. Means were computed when vascular discoloration and foliar symptom severity scales were different and

Table 3: Mean disease index (DI) values recorded on 18 different okra landraces by VCG1A and VCG2B isolates of *Verticillium dahliae*

Landraces	VCG1A DI[a]	Symptom type[b]	VCG2B DI[a]	Symptom type[b]
Batı Trakya	4.00 a[c]	D	3.00 a	PD
Burdur	4.00 a	D	3.00 a	PD
Bursa	4.00 a	D	3.00 a	PD
Iran 2	4.00 a	D	3.00 a	PD
Karaburun	4.00 a	D	3.00 a	PD
Mersin	4.00 a	D	3.00 a	PD
Amasya	3.83 b	D	3.00 a	PD
Balıkesir	3.67 c	D	2.90 a	PD
Denizli	3.67 c	D	2.00 de	ND
Marmara	3.67 c	D	3.00 a	PD
Dalaman	3.50 d	D	2.20 bc	ND
Iran 1	3.42 d	PD	2.10 cd	ND
İzmir	3.25 e	PD	2.20 bc	ND
Hatay	3.11 f	PD	2.30 b	ND
Adana	2.89 g	PD	2.20 bc	ND
Çorum	2.83 g	PD	1.80 f	ND
Hatay Has	2.78 g	PD	1.90 ef	ND
Şanlıurfa	2.78 g	PD	1.80 f	ND
Mean DI	3.52		2.52	

Notes: [a]A mean disease index (DI) was calculated by summing both vascular discoloration and foliar disease severity indices of 30 plants (five replicates of three plants per landrace, two experiments) by using the 0-4 scales. [c]D = defoliating type symptom PD = partial-defoliating type symptom. [c]Different letters shows that mean disease index numbers are significantly different according to SNK multiple comparison test ($P \leq 0.01$).

Figure 2: Vascular disease severity scale for *Verticillium* wilt on okra (from right to left 0 = no vascular discoloration, 1 = 1 to 25% vascular area discolored, 2 = 26 to 50% vascular area discolored, 4 = 76 to 100% vascular area discolored, and 3 = 51 to 75% vascular area discolored)

Figure 3: Foliar disease severity scale for *Verticillium* wilt on okra (0 = no sign of wilting, 1 = slight wilting or chlorosis, 2 = foliar necrosis, 3 = partial defoliation 4 = severe defoliation and stunting)

rounded to the nearest scale values. The experiment was repeated once and the data were presented as the mean of the two experiments.

2.5 Statistical Analyses

A mean disease index (DI) was calculated by summing both vascular discoloration and foliar disease severity indices of 30 plants (three plants per isolate in five replicates, two different experiments). Mean diseases severities of VCG groups were compared by SNK multiple comparison test using JMP statistical software (version 12.0; 100 SAS Campus Drive Cary, NC 27513-2414, USA). As to statistical analyses results, isolates in VCGs 2A, 2B, 1A that caused a disease severity of < 1.5, $1.5 \leq - < 2.6$, $2.6 \leq - > 3.3$, and $3.3 \leq -4$ were grouped as ND, PD, and D pathotypes, respectively. Virulence of 44 *V. dahliae* isolates and landrace reactions against VCGs1A and 2B were compared by subjecting disease severity indices to SNK test using JMP statistical software (version 12.0; 100 SAS Campus Drive Cary, NC 27513-2414, USA).

3 Results

3.1 Collection of Isolates

A total of 44 *V. dahliae* isolates were obtained from okra plants originating from 32 sites in five provinces (Adana, Hatay, Kahramanmaraş, Mersin and Osmaniye) in the east Mediterranean region, 12 sites in three provinces (İzmir, Muğla and Bursa) in the eastern Anatolia of Turkey from 2006 to 2009. The 44 isolates each from a different site were used in the vegetative compatibility study (Tab. 1).

3.2 Development and Classification of Nitrate-Nonutilizing (Nit) Mutants

Chlorate-resistant sectors developed from *V. dahliae* isolates displayed a fast-growing and thin or almost hyaline mycelium on WAC medium. Recovery from WAC onto CDA was optimum after 15 days of incubation. In 10 to 25 replications, each isolate formed 3 to 14 chlorate-resistant sectors. On CDA medium, nit mutants came into view as thin mycelium. The mean occurrence of nit mutants from sectors was 48.3%. Among the nit mutants, the nit1 made up 82.6% of the total number of mutants, while NitM and nit3 mutants made up 14.9 and 2.5%, respectively.

3.3 Complementation of Nit Mutants and Testing for Vegetative Compatibility

After complementation tests with the tester isolates, two VCGs (1 and 2) and three subgroups (1A, 2A and 2B) were characterized among the 44 isolates (Tab. 1). Tester isolates were able to generate a stable

heterokaryon only with the isolates from their own VCG. Thirty isolates for which we recovered both nit1 and NitM mutant were self-compatible. Compared to others, VCG2B was the largest component and included 65.9% of the total number of isolates (Tab. 1); VCG2A and VCG1A included 20.5 and 13.6% of all isolates, respectively. VCG1A was only recovered among the isolates from İzmir (2), Kahramanmaraş (3) and Hatay (1) (Tab. 1). Nine isolates belonging to VCG2A were recovered from Adana (4), Hatay (4) and Bursa (1). VCG2B isolates of *V. dahliae* (29) were detected from all eight provinces sampled with a similar frequency of recovery between provinces (Tab. 1).

3.4 Pathogenicity Tests and Evaluation of Resistance of Okra Accessions to Verticillium Dahliae

All 44 *Verticillium dahliae* isolates were virulent to local landrace of okra in different levels. Seven to ten days after inoculation, inoculated plants by isolates belonging to different VCGs began wilting, much like the symptoms observed in field plants. Wilting was observed on all replicates of the treatments but no evidence of disease was observed on non-inoculated plants. Disease expression levels showed three major profiles: D, PD and ND; and isolates belonging to distinct VCGs were never in the same pathotype. In test plants inoculated with VCG1A isolates, plants showed symptoms earlier and the disease on them was more severe with defoliation. Most of the test plants died much earlier than others that were inoculated with VCGs 2B and 2A isolates. After five weeks of inoculation, the plants were totally defoliated and had the highest stem colonization levels. Plants inoculated with isolates in VCG2B showed partial defoliation symptoms. In contrast, plants inoculated with VCG2A isolates showed a typical leaf chlorosis symptom without defoliation (Tab. 1). Control plants were healthy with no disease symptoms. There was no significant difference between the virulence levels of geographic origin of the isolates, irrespective of their VCG belongings.

Compared to others, VCG1A was the most virulent group with 3.58 mean disease severity index and all of the isolates in this group were causing defoliating on okra. VCG2B isolates were moderately virulent, caused 2.80 mean disease severity index and the isolates in the group were partially defoliating on the okra seedlings. Isolates from VCG2A were slightly virulent, caused 2.14 mean disease severity index and they all were non defoliating on the okra seedlings (Tabs. 1 and 2).

In the second set of bioassays, both VCGs 1A and 2B did affect all landraces at different levels. VCG1A isolate was more aggressive and induced D (11) or PD (7) symptoms, whereas VCG2B isolate induced PD (9) and ND (9) symptoms on 18 landraces tested depending on the susceptibility levels of landraces. Although virulence varied among VCG1A and VCG2B infecting the same okra landrace, Batı Trakya, Burdur, Bursa, Iran 2, Karaburun and Mersin were identified as the most susceptible landraces for both VCGs 1A and 2B of *V. dahliae*, with disease severity indices being 4 and 3 for these landraces, respectively. They were followed by Amasya (VCG1A: 3.83; and VCG2B: 3), Marmara (VCG1A: 3.67 and VCG2B: 3) and Balıkesir (VCG1A: 3.67 and VCG2B: 2.9). Only Çorum, Hatay Has and Şanlıurfa landraces appeared to have little tolerance to both VCGs 1A and 2B of *V. dahliae* (Tab. 2). In these landraces, disease severity indices for VCG1A and VCG2B were recorded as between 2.78-2.83 and 1.8-1.9, respectively (Tab. 3).

4 Discussion

VCG diversity within *V. dahliae* population from okra was demonstrated for the first time during this study. We identified three multimember VCGs among 44 isolates of *V. dahliae* from okra. Isolates from VCG2B were recovered from all eight provinces surveyed, whereas VCG3, VCG4A and VCG4B were not recovered from the limited number of samples from Turkey. Forty-four isolates obtained from all provinces surveyed were compatible with the selected testers used in the present study and could be allocated to one of the three VCGs. The assignment of isolates from okra to VCG2B (65.9%), VCG2A (20.5%) and VCG1A (13.6%) (Tab. 1) may suggest a possibility of adaptation of these VCGs to okra in

particular growing areas where a diverse range of previous crop history of locations may be an important source of *V. dahliae* inoculum in soil.

The disease severity evaluation made 35 days after inoculation through observations of leaves (yellowing, wilting and defoliation) and vascular discoloration symptoms to determine the level of colonization revealed differences on the aggressiveness of isolates. The occurrence of more than one pathotype of *V. dahliae* was observed. As to disease indices results, VCGs 1A, 2B and 2A isolates were classified as D, PD and ND pathotypes, respectively, so that the former was the most virulent on okra. This study has concurring results with those of previous studies in terms of correlations between VCGs and virulence on certain hosts [20,21]. For instance, VCG4A was the most aggressive pathotype causing severe disease symptoms on potato, but VCG4B and VCG2B resulted in mild to moderate symptoms. Johnson and Santo [22] showed that VCG2B was the most aggressive pathotype infecting mint, nevertheless VCG4A caused only mild to moderate symptoms. VCG2B isolates were more virulent to artichoke those in VCG1A. Similarly, isolates of the D pathotype of cotton and olive were exclusively in VCG1A and lineage 1A (1A/D) whereas those in other VCGs caused either partial defoliation or just wilting but not defoliation [23,24].

All landraces derived from the most important okra cultivated provinces of Turkey were susceptible in different degrees depending on the VCG1A and VCG2B belongings of the isolates (Tab. 2). Virulence was also related to the differential susceptibility of the 18 landraces tested. Overall, VCG1A isolate was more virulent than VCG2B isolate on all landraces. Çorum, Hatay Has and Şanlıurfa landraces showed the lowest level of susceptibility when inoculated with virulent isolates of VCG1A and VCG2B.

Previously, out of nine clonal lineages that were identified by genotyping by sequencing in the asexual fungus *V. dahliae*, lineage 1A concordant with VCG1A was highly virulent and caused leaf defoliation in olive, cotton and okra (denoted 1A/D to indicate both VCG and pathotype), whereas others in different lineages caused wilting without defoliation (ND). The present study confirmed that D isolates obtained from okra were solely in VCG1A as those from olive and cotton were in the same VCG [25].

In conclusion, this is the first detailed study of *V. dahliae* VCGs focusing on populations from okra plants. Our results suggest that it is a highly destructive and genetically diverse pathogen of okra and an important inoculum source of the pathogen may be from established populations in fields that get distributed along with diverse previous host plants. This study demonstrated that the expression of symptoms in okra plants revealed substantial differences to VCGs 1A, 2B and 2A of *V. dahliae*. Therefore, VCG characterization of *V. dahliae* isolates may help predict the disease severity on okra plants. These results also indicate the necessity of using genetically known *V. dahliae* isolates, while screening okra cultivars for genetic resistance against *Verticillium* wilt. As seen in the results, no resistance was found among the landraces tested. Resistant donor genotypes from different sources should be introduced into Turkish okra germplasm and new resistant cultivars should be bred for a permanent eco-friendly solution. On the other hand, we can also advise to the growers not to grow their okra in *V. dahliae* infested fields with previous history of *Verticillium* wilt.

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References

1. FAOSTAT (2017). FAOSTAT data collection. *http://www.fao.org/faostat/en/#data/QC*.

2. Calisir, S., Ozcan, M., Haciseferogullari, H., Yildiz, M. U. (2005). A study on some physico-chemical properties of Turkey okra (*Hibiscus esculenta* L.) seeds. *Journal of Food Engineering, 68(1),* 73–78. DOI 10.1016/j.jfoodeng.2004.05.023.
3. Pegg, G. F., Brady, B. L. (2002). *Verticillium wilts*. Oxford: CAB International.
4. Leslie, J. F. (1993). Fungal vegetative compatibility. *Annual Review of Phytopathology, 31(1),* 127–150. DOI 10.1146/annurev.py.31.090193.001015.
5. Strausbaugh, C. A. (1993). Assessment of vegetative compatibility and virulence of *Verticillium dahliae* isolates from Idaho potatoes and tester strains. *Genetics, 83,* 1253–1258.
6. Chen, W. (1994). Vegetative compatibility groups of *Verticillium dahliae* from ornamental woody plants. *Phytopathology, 84(2),* 214–219. DOI 10.1094/Phyto-84-214.
7. Collado-Romero, M., Mercado-Blanco, J., Olivares-García, C., Valverde-Corredor, A., Jiménez-Díaz, R. M. (2006). Molecular variability within and among *Verticillium dahliae* vegetative compatibility groups determined by fluorescent amplified fragment length polymorphism and polymerase chain reaction markers. *Phytopathology, 96(5),* 485–495. DOI 10.1094/PHYTO-96-0485.
8. Milgroom, M. G., Jiménez-Gasco, M. M., Olivares-García, C., Drott, M. T., Jiménez-Díaz, R. (2014). Recombination between clonal lineages of the asexual fungus *Verticillium dahliae* detected by genotyping by sequencing. *PLoS One, 9(9),* 730–740. DOI 10.1371/journal.pone.0106740.
9. Daayf, F., Nicole, M., Geiger, J. P. (1995). Differentiation of *Verticillium dahliae* populations on the basis of vegetative compatibility and pathogenicity on cotton. *European Journal of Plant Pathology, 101(1),* 69–79. DOI 10.1007/BF01876095.
10. Korolev, N., Pérez-Artés, E., Mercado-Blanco, J., Bejarano-Alcázar, J., Rodríguez-Jurado, D. et al. (2008). Vegetative compatibility of cotton-defoliating *Verticillium dahlia* in Israel and its pathogenicity to various crop plants. *European Journal of Plant Pathology, 122(4),* 603–617. DOI 10.1007/s10658-008-9330-1.
11. Dervis, S., Mercado-Blanco, J., Erten, L., Valverde-Corredor, A., Pérez-Artés, E. (2010). Verticillium wilt of olive in Turkey: a survey on diseases importance, pathogen diversity and susceptibility of relevant olive cultivars. *European Journal of Plant Pathology, 127(2),* 287–301. DOI 10.1007/s10658-010-9595-z.
12. Jiménez-Díaz, R. M., Olivares-García, C., Landa, B. B., Jiménez-Gasco, M. M., Navas-Cortés, J. A. (2011). Region-wide analysis of genetic diversity in *Verticillium dahliae* populations infecting olive in southern Spain and agricultural factors influencing the distribution and prevalence of vegetative compatibility groups and pathotypes. *Phytopathology, 101(3),* 304–315. DOI 10.1094/PHYTO-07-10-0176.
13. Schnathorst, W. C., Mathre, D. E. (1966). Host range and differentiation of a severe form of *Verticillium albo-atrum* in cotton. *Phytopathology, 56,* 1155–1161.
14. Jiménez-Díaz, R. M., Mercado-Blanco, J., Olivares-García, C., Collado-Romero, M., Bejarano-Alcázar, J. et al. (2006). Genetic and virulence diversity in *Verticillium dahliae* populations infecting artichoke in eastern-central Spain. *Phytopathology, 96(3),* 288–298. DOI 10.1094/PHYTO-96-0288.
15. Smith, H. C. (1965). The morphology of *Verticillium albo-atrum*, *V. dahliae*, and *V. tricopus*. *New Zealand Journal of Agricultural Research, 8,* 450–478.
16. Korolev, N., Katan, T. (1997). Improved medium for selecting nitrate non-utilizing (nit) mutants of *Verticillium dahliae*. *Phytopathology, 87(10),* 1067–1070. DOI 10.1094/PHYTO.1997.87.10.1067.
17. Joaquim, T. R., Rowe, R. C. (1990). Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. *Phytopathology, 80(11),* 1160–1166. DOI 10.1094/Phyto-80-1160.
18. Dervis, S., Erten, L., Soylu, S., Tok, F. M., Kurt, S. et al. (2007). Vegetative compatibility groups in *Verticillium dahlia* isolates from olive in Turkey. *European Journal of Plant Pathology, 119(4),* 437–447. DOI 10.1007/s10658-007-9183-z.
19. Dervis, S., Bicici, M. (2005). Vegetative compatibility groups in *Verticillium dahliae* isolates from cotton in Turkey. *Phytoparasitica, 33(2),* 157–168. DOI 10.1007/BF03029975.
20. Bhat, R. G., Subbarao, K. V. (1999). Host range specificity in *Verticillium dahliae*. *Phytopathology, 89(12),* 1218–1225. DOI 10.1094/PHYTO.1999.89.12.1218.

21. Joaquim, T. R., Rowe, R. C. (1991). Vegetative compatibility and virulence of strains of *Verticillium dahliae* from soil and potato plants. *Phytopathology, 81(5),* 552–558. DOI 10.1094/Phyto-81-552.
22. Johnson, D. A., Santo, G. S. (2001). Development of wilt in mint in response to infection by two pathotypes of *Verticillium dahliae* and co-infection by *Pratylenchus penetrans*. *Plant Disease, 85(11),* 1189–1192. DOI 10.1094/PDIS.2001.85.11.1189.
23. Dervis, S., Kurt, S., Soylu, S., Erten, L., Soylu, E. M. et al. (2008). Vegetative compatibility groups of *Verticillium dahliae* from cotton in the Southeastern Anatolia region of Turkey. *Phytoparasitica, 36(1),* 74–83. DOI 10.1007/BF02980750.
24. Milgroom, M. G., Jiménez-Gasco, M. M., Olivares-García, C., Jiménez-Díaz, R. M. (2016). Clonal expansion and migration of a highly virulent, defoliating lineage of *Verticillium dahliae*. *Phytopathology, 106(9),* 1038–1046. DOI 10.1094/PHYTO-11-15-0300-R.
25. Korolev, N., Katan, T., Katan, J. (2009). Physiological races and vegetative compatibility groups among *Verticillium dahliae* isolates from tomato in Israel. *II International Symposium on Tomato Diseases, 808,* 57–64.