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Article

Determination of Susceptibility of Some Hybrid Grapevine Genotypes to Powdery Mildew

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Abstract: The purpose of this study was to identify grape genotypes resistant to powdery mildew to reduce pesticide use in vineyards. A total of 70 hybrid genotypes from Narince x Regent and Narince Kishmish Vatkana crosses, along with four grape varieties (Narince, Regent, Kishmish Vatkana, and Isabella), were evaluated for their susceptibility to powdery mildew. Inoculations were carried out under controlled greenhouse and laboratory conditions between 2021 and 2022. The study assessed the severity of infection by measuring mycelium and sporulation density on the leaves. Results showed significant variation in susceptibility, with genotypes exhibiting differences in infection severity, ranging from resistant (Regent) to highly susceptible (Narince). Genotypes NRG-7, NRG-146, NRG-174, NRG-195, NRG-196, NRG-197, and NRG-200, as well as cultivars Regent, Kishmish Vatkana, and Isabella, showed resistance to the disease, while Narince was highly sensitive. These resistant genotypes have potential for use in organic farming, offering an opportunity to reduce fungicide applications and enhance sustainable viticulture practices.

Keywords: hybrid genotypes; powdery mildew; artificial inoculation; susceptibility

1. Introduction

The grapevine (*Vitis vinifera*) is a highly diverse plant species, enabling it to thrive under a variety of soil and climatic conditions. *V. vinifera* is the primary source for most table, dried, and wine grape varieties, which are crucial to the wine, juice, and table grape industries [1,2]. However, *V. vinifera* is highly susceptible to powdery mildew (*Erysiphe necator*), a disease that poses significant challenges to viticulture [3,4]. Powdery mildew, originating in North America, spread to Europe in the 1850s and led to devastating crop losses, reaching up to 100% in European vineyards [4]. In response to this threat, breeding programs were initiated to develop grape varieties resistant to powdery mildew [5]. The identification of resistance in certain American and Asian grapevine species (Alleweldt and Possingham [4], Eibach and Töpfer [7]) led to the importation of North American grapevine species in the late 19th century, facilitating the transfer of resistance traits to the *V. vinifera* genome. This process resulted in the development of several interspecific vinifera-American hybrids [8]. Additionally, resistance was identified in Chinese wild species such as *V. bryoniifolia*, *V. davidii*, and *V. piasezkii*, which were incorporated into breeding programs for improved disease resistance [9].

As the global population rapidly increases, so too does the demand for agricultural production, accompanied by a rise in pesticide use. The widespread use of pesticides in agricultural settings, although necessary for crop protection, poses significant threats to ecological balance and human health [10]. Pesticides can harm not only pathogenic species but also beneficial organisms such as parasitoids and predators, leading to long-term environmental damage. Moreover, these chemicals can enter the human body through the skin, mouth, or respiratory system, resulting in potential poisoning. As a result, there is an increasing global emphasis on developing environmentally

sustainable agricultural practices [11,12]. In addition, consumer awareness has shifted toward the importance of consuming healthy, high-quality products [13]. To minimize pesticide usage and its negative impacts on both human health and the environment, the development of disease-resistant crop varieties has become a priority in agricultural research. The development of grape varieties resistant to fungal diseases, particularly powdery mildew, has emerged as a key focus of modern breeding programs [14,15]. Identifying grape genotypes with high resistance to pathogens through the use of resistant parental sources is crucial for sustainable viticulture practices [16]. The primary objective of this study was to identify grape genotypes with tolerance to powdery mildew to reduce reliance on chemical fungicides. To achieve this, hybridization studies were conducted using Narince as the maternal parent and Regent and Kishmish Vatkana as the paternal parents, producing a set of hybrid genotypes. These genotypes were tested for powdery mildew resistance under both greenhouse and laboratory conditions, with the severity of infection and mycelium growth evaluated to assess susceptibility. The goal of the study was to determine the degree of resistance in these genotypes, contributing to the development of grape varieties that can reduce pesticide applications in vineyards.

2. Materials and Methods

2.1. Plantal Material

The study material consisted of the Narince grape variety as the female parent, crossed with male parents from a breeding program initiated in 2019, using Isabella, Regent, and Kishmish Vatkana grape varieties [17]. The study included 66 Narince x Regent hybrids (NRG), 4 Narince x Kishmish Vatkana hybrids (NKV), and the parent varieties: Narince, Regent, Kishmish Vatkana, and Isabella. Narince is a white grape variety with seeds and a mid-season ripening period, used for both table and wine production [18,19]. Regent, a German wine variety (Diana x Chambourcin), is resistant to powdery and downy mildew, with Ren3, Ren9, and Rpv3.1 loci [20]. Kishmish Vatkana, from Uzbekistan, is resistant to powdery mildew [21,22]. Isabella (*V. labrusca*) is also tolerant to both powdery and downy mildew [23] and represents a valuable genetic resource for breeding in Turkey [24].

2.2. Inoculation, Counting and Evaluation of Powdery Mildew

To obtain the inoculum of powdery mildew, grapevine leaves and fruits heavily infected with spores were collected and transported to the laboratory in June. A suspension was prepared by adding glucose (0.78%) and Tween-20 (0.05%) to one liter of sterile water to maintain spore viability and ensure suspension homogeneity. The spore density was adjusted to 10^5 spores/ml [9,24,25], (Figure 1 and Figure 2). The spore suspension, prepared in early July, was applied as a fine spray to the potted genotypes. Afterward, excess water on the leaves was removed using a fan. Initial infection counts were taken one-week post-inoculation, with seven subsequent evaluations conducted at weekly intervals (Figure 3 and Figure 4). Infection severity was assessed using the Wang et al. [9] scale, which ranges from 0 to 7, indicating the percentage of leaf area colonized by the pathogen (Figure 5, Table 1).



Figure 1. Source of inoculum.



Figure 2. Preparation of the suspension.



(a)



(b)

Figure 3. The inoculation of powdery mildew (a) and (b).



(a)



(b)

Figure 4. The enumeration of powdery mildew infection densities (a) and (b).

Table 1. The infection rates according to the severity of powdery mildew.

The scale value	The infection rate (%)
0	absent
1	- 5.0
2	5.1–15.3
3	15.1–30.4
4	30.1–45.5
5	45.1- 65.6
6	65.1- 85.7
7	85.0–100.0



Figure 5. The scale values according to the colonization rate on the surface of the leaves.

2.3. The Calculation of Disease Severity

The disease susceptibility level of the varieties/genotypes was determined by calculating the disease severity (Severity Index, SI) [26].

$$\frac{\sum (\text{The scale value} \times \text{The number of leaves falling within the specified scale value})}{\text{The total number of leaves} \times \text{The highest scale value}} \times 100$$

The classification of genotypes according to the severity of the disease;

SI = 0 ; Immune

SI= 0.1-5.0 ; Highly Resistant (HR)

SI = 5.1-25.0 ; Resistance (R)

SI = 25.1-50 ; Susceptible (S)

2.4. The Inoculation, Counting and Evaluation of Powdery Mildew Under Laboratory Conditions

The nutrient medium used for the experiment was water agar, supplemented with benzimidazole (Sigma Aldrich: 8.21956.0100) at a concentration of 30 mg/l. After treatment with a 2.6% sodium hypochlorite solution, grapevine leaves were placed in Petri dishes containing agar, ensuring the veins were in contact with the agar surface (Figures 6). Infected leaves were used to inoculate the new leaves by transferring spores onto them. The Petri dishes were sealed with parafilm Blanc [27] and incubated in a climate chamber set at 24°C, 70% relative humidity, and a 12-hour light/dark cycle [28]. Inoculation evaluations were conducted one week after the first signs of infection, using five leaves per genotype. The methodology followed the guidelines of Miclot et al. [29] with mycelium development and sporulation density assessed under a binocular microscope (x25 magnification). The genotypes' resistance or susceptibility to powdery mildew was determined using the sensitivity/tolerance scale of Miclot et al. [29] with control groups including Narince (sensitive) and Regent (tolerant) varieties. Mycelium and sporulation development were scored on a 1–9 scale, where 1 indicated high sensitivity and 9 indicated high resistance.

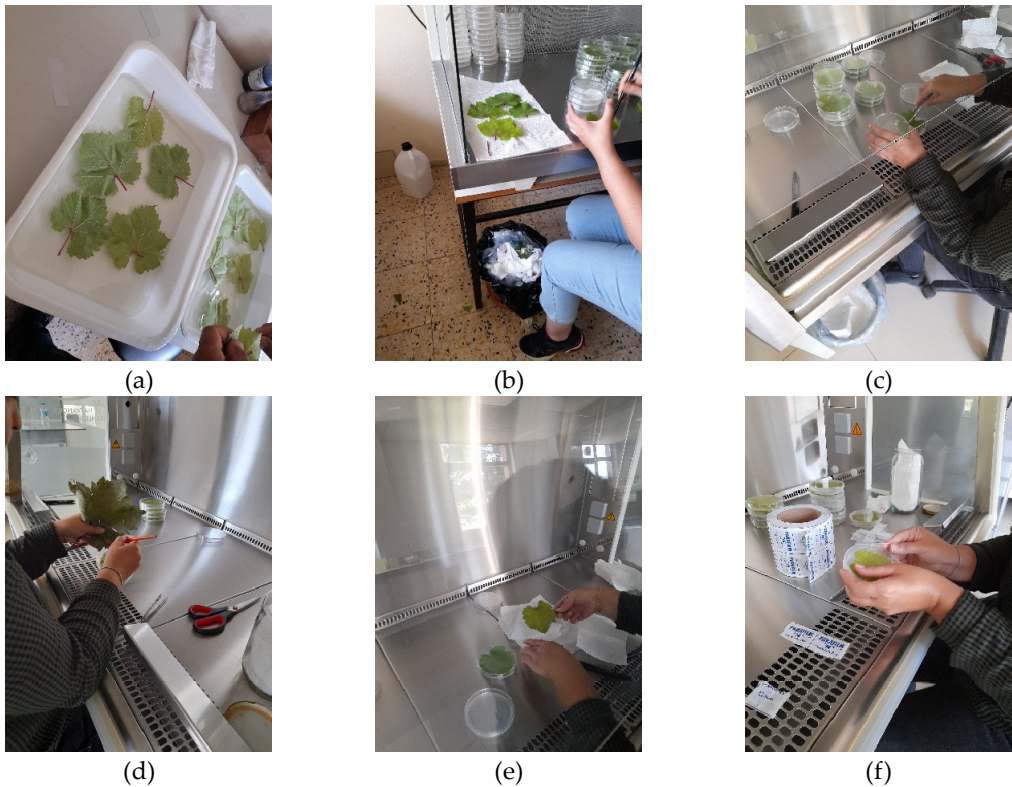


Figure 6. Inoculation stages on leaves (a) The process of sterilising leaves; (b) The process of drying the leaves in a sterile cabinet; (c) The procedure for placing leaves in petri dishes; (d) The inoculation process on leaves; (e) The procedure for placing leaves in petri dishes; (f) The process of wrapping the leaves with stretch film.

2.5. Statistical Analysis

The JMP Pro 13.0.0 program was employed for the purpose of statistical analysis. The data were subjected to variance analysis and the LSD (0.05) test was applied to determine the differences.

3. Results

3.1. The Scale Values and Disease Severity in Genotypes in Terms of Powdery Mildew Infection

The powdery mildew infection scale values and disease severity percentages for the genotypes in 2021 and 2022 are summarized in Table 2. In the first year, scale values ranged from 1 to 7, with disease severity ranging from 0.86% (NRG-200) to 100% (Narince and NRG-33). In the second year, scale values ranged from 2.6 (Regent) to 7, with disease severity ranging from 8.7% (Regent) to 76.30% (Narince), indicating higher infection intensity in the second year.

Table 2. The scale values of the genotypes and the percentage of disease severity.

Genotype	The year 2021				The year 2022			
	The scale value	The severity of the disease	SI (%)	SI	The scale value	The severity of the disease	SI (%)	SI
NRG-002	7	80.00	50-100	HS	7	62.00	50-100	HS
NRG-004	2	6,29	5.1-25	R	5	31.43	25.1-50	S
NRG-005	7	68.00	50-100	HS	7	64.00	50-100	HS
NRG-007	3	8.57	5.1-25	R	4	18.29	5.1-25	R
NRG-009	4	14.86	5.1-25	R	5	35.71	25.1-50	S
NRG-012	6	44.57	25.1-50	S	7	62.00	50-100	HS
NRG-013	7	84.00	50-100	HS	7	64.00	50-100	HS

NRG-025	6	53.14	50-100	HS	5	22.86	25.1-50	S
NRG-028	5	28.57	25.1-50	S	5	35.71	25.1-50	S
NRG-033	7	100.00	50-100	HS	7	72.00	50-100	HS
NRG-060	4	20.57	5.1-25	R	7	68.00	50-100	HS
NRG-061	7	64.00	50-100	HS	7	66.00	50-100	HS
NRG-062	3	10.29	5.1-25	R	7	68.00	50-100	HS
NRG-063	3	12.00	5.1-25	R	7	84.00	50-100	HS
NRG-064	3	10.29	5.1-25	R	7	48.00	25.1-50	S
NRG-065	4	12.57	5.1-25	R	7	56.00	50-100	HS
NRG-066	2	4.57	0.1-5	HR	5	25.71	25.1-50	S
NRG-067	4	17.14	5.1-25	R	7	68.00	50-100	HS
NRG-068	4	20.57	5.1-25	R	7	76.00	50-100	HS
NRG-075	7	46.00	25.1-50	S	7	64.00	50-100	HS
NRG-080	2	2.29	0.1-5	HR	7	70.00	50-100	HS
NRG-085	7	68.00	50-100	HS	7	74.00	50-100	HS
NRG-088	3	11.14	5.1-25	R	7	74.00	50-100	HS
NRG-089	2	4.57	0.1-5	HR	7	39.43	25.1-50	S
NRG-092	3	6.86	5.1-25	R	5	32.86	25.1-50	S
NRG-093	4	14.86	5.1-25	R	7	60.00	50-100	HS
NRG-094	4	28.57	25.1-50	S	5	27.14	25.1-50	S
NRG-098	3	12.00	5.1-25	R	5	30.00	25.1-50	S
NRG-102	7	92.00	50-100	HS	7	72.00	50-100	HS
NRG-104	5	32.86	25.1-50	S	7	60.00	50-100	HS
NRG-109	6	41.14	25.1-50	S	7	56.00	50-100	HS
NRG-110	4	13.71	5.1-25	R	5	25.71	25.1-50	S
NRG-115	3	9.43	5.1-25	R	5	25.71	25.1-50	S
NRG-120	4	28.57	25.1-50	S	7	64.00	50-100	HS
NRG-128	3	15.43	5.1-25	R	6	48.00	25.1-50	S
NRG-137	5	37.14	25.1-50	S	6	36.00	25.1-50	S
NRG-146	2	5.71	5.1-25	R	5	24.29	5.1-25	R
NRG-147	4	41.14	25.1-50	S	5	27.14	25.1-50	S
NRG-161	4	18.29	5.1-25	R	5	32.86	25.1-50	S
NRG-162	2	6.86	5.1-25	R	5	40.00	25.1-50	S
NRG-164	4	16.00	5.1-25	R	7	64.00	50-100	HS
NRG-165	3	5.14	5.1-25	R	6	54.86	50-100	HS
NRG-167	3	12.86	5.1-25	R	6	49.71	25.1-50	S
NRG-170	3	19.71	5.1-25	R	7	70.00	50-100	HS
NRG-174	3	12.86	5.1-25	R	4	18.29	5.1-25	R
NRG-175	1	2.29	0.1-5	HR	5	38.57	25.1-50	S
NRG-176	3	7.71	5.1-25	R	7	64.00	50-100	HS
NRG-177	3	20.57	5.1-25	R	7	68.00	50-100	HS
NRG-178	5	34.29	25.1-50	S	7	68.00	50-100	HS
NRG-179	4	18.29	5.1-25	R	5	25.71	25.1-50	S
NRG-181	2	3.43	0.1-5	HR	7	46.00	25.1-50	S
NRG-182	6	60.00	50-100	HS	7	72.00	50-100	HS
NRG-183	1	3.71	0.1-5	HR	5	24.29	25.1-50	S
NRG-193	4	27.43	25.1-50	S	5	24.29	25.1-50	S
NRG-195	2	5.14	5.1-25	R	4	17.14	5.1-25	R
NRG-196	3	13.71	5.1-25	R	4	16.00	5.1-25	R
NRG-197	2	9.14	5.1-25	R	4	18.29	5.1-25	R
NRG-198	2	6.29	5.1-25	R	6	37.71	25.1-50	S
NRG-199	1	2.29	0.1-5	HR	5	25.71	25.1-50	S
NRG-200	1	0.86	0.1-5	HR	5	22.86	5.1-25	R
NRG-201	1	2.29	0.1-5	HR	5	27.14	25.1-50	S
NRG-211	5	28.57	25.1-50	S	7	66.00	50-100	HS
NRG-213	3	12.00	5.1-25	R	7	64.00	50-100	HS

NRG-217	6	48	25.1-50	S	6	48.00	25.1-50	S
NRG-218	5	30	25.1-50	S	7	70.00	50-100	HS
NRG-219	2	4	0.1-5	HR	6	44.57	25.1-50	S
NKV-004	7	50	50-100	HS	7	64	50-100	HS
NKV-010	7	60	50-100	HS	7	72	50-100	HS
NKV-016	7	76	50-100	HS	7	100	50-100	HS
NKV-017	7	66	50-100	HS	7	80	50-100	HS
Narince	7	100	50-100	HS	6.6	76.3	50-100	HS
Regent	3	9.7	5.1-25	R	2.6	8.7	5.1-25	R
K.Vatkana	3	9.7	5.1-25	R	2.6	11	5.1-25	R
Isabella	2,6	6	5.1-25	R	2	5.8	5.1-25	R

3.2. The Mycelium and Sporulation Densities of the Genotypes Were Determined Under Laboratory Conditions

To evaluate the powdery mildew resistance of the genotypes, the sensitive variety Narince and the resistant varieties Regent, Isabella, and Kishmish Vatkana were considered. The relative mycelium and spore density on the leaves are presented in Table 3. Statistical analysis revealed significant differences ($p < 0.01$) in mycelium and sporulation density among the genotypes. The highest scale values were recorded for Isabella (7.4), Regent (6.8), NRG-92 (6.6), Kishmish Vatkana (6.6), and NRG-181 (6.2). Other genotypes, such as NRG-201, NRG-161, and NRG-183, had scale values of 5.8, with remaining genotypes ranging from 1.8 to 5.4.

Table 3. The mycelium and sporulation density calculated in the leaves of the genotypes.

Genotype	The density of mycelium and sporulation	Genotype	The density of mycelium and sporulation
NRG-002	4.6 d-g	NRG-170	4.2 e-g
NRG-004	3.8 f-h	NRG-174	5.4 b-e
NRG-005	1.8 j	NRG-176	3.8 f-h
NRG-007	3.8 f-h	NRG-177	4.2 e-g
NRG-012	2.2 ij	NRG-179	3.8 f-h
NRG-013	2.6 h-j	NRG-181	6.2 a-c
NRG-028	5.4 b-e	NRG-183	5.8 b-d
NRG-033	1.8 j	NRG-193	5.8 b-d
NRG-061	3.8 f-h	NRG-194	5.0 c-f
NRG-064	4.6 d-g	NRG-195	5.8 b-d
NRG-066	5.0 c-f	NRG-196	5.0 c-f
NRG-075	3.4 g-i	NRG-197	4.6 d-g
NRG-089	4.2 e-g	NRG-198	3.8 f-h
NRG-092	6.6 ab	NRG-199	4.2 e-g
NRG-095	1.8 j	NRG-200	4.2 e-g
NRG-098	4.2 e-g	NRG-201	5.8 b-d
NRG-102	4.2 e-g	NRG-217	4.2 e-g
NRG-104	4.2 e-g	NRG-219	5.0 c-f
NRG-110	3.8 f-h	NKV-004	2.6 h-j
NRG-115	4.6 d-g	NKV-010	2.2 ij
NRG-137	4.6 d-g	NKV-016	1.8 j
NRG-146	4.6 d-g	NKV-017	1.8 j
NRG-147	3.8 f-h	Narince	1.8 j
NRG-161	5.8 b-d	Regent	6.8 ab
Isabella	7.4 a	K. Vatkana	6.6 ab

4. Discussion

The susceptibility of 74 grape genotypes to powdery mildew was evaluated through artificial inoculation conducted from June 2021 to June 2022 under greenhouse conditions. Over a seven-week period, no symptoms were observed in May of either year. Following inoculation, infection onset was noted within 7 to 10 days, with a marked increase in infection by July, peaking in late August. In the seventh week, genotypes were categorized into four groups based on their response to the disease: highly sensitive (HS), sensitive (S), resistant (R), and highly resistant (HR). In the first year, 13 genotypes were classified as highly sensitive, 14 as sensitive, 33 as resistant, and 10 as highly resistant. In the second year, six genotypes remained resistant, while others exhibited varying levels of susceptibility. Seven genotypes—NRG-7, NRG-146, NRG-174, NRG-195, NRG-196, NRG-197, and NRG-200—demonstrated consistent resistance across both years.

Research on grapevine susceptibility to powdery mildew has highlighted significant variation across species, varieties, and genotypes [24,30–35]. These findings align with existing literature, reinforcing genotype-specific differences in susceptibility and severity. The genetic structures of the genotypes likely account for these differences, as noted by Karbalaee Khiavi and Davoodi [36], who emphasized the role of genetic factors in mildew resistance. For instance, the Narince variety, a *Vitis vinifera* species, exhibited high susceptibility to powdery mildew, corroborating the findings of Kozma et al. [21], who identified *V. vinifera* varieties as generally susceptible, despite some variation. Similarly, Vojtovic et al. [37] and Pospisilova [38] observed genetic diversity within *V. vinifera* that influenced mildew resistance. In contrast, paternal varieties like Regent, Kishmish Vatkana, and Isabella demonstrated resistance consistent with earlier studies. Salotti et al. [39] found high mildew resistance in Regent and other varieties, while Schwab et al. [40] reported minimal infection in Regent after five years of evaluation. Eibach and Töpfer [41] confirmed Regent's resistance, though it was not immune. Kishmish Vatkana and Isabella were also noted for their resistance to powdery mildew in various studies [22,42–44].

The results from this study mirror these findings, as both the genotypes and parental varieties exhibited expected patterns of resistance and susceptibility. The density of mycelium and sporulation in the genotypes supported the mildew scoring results. Susceptible genotypes showed low-scale values, while the most resistant genotypes, such as Regent, had higher scale values. However, no genotype, including the parental varieties, attained the maximum scale value of 9, which would indicate complete resistance. In genotypes such as Isabella, Regent, Kishmish Vatkana, and others like NRG-92 and NRG-181, necrosis was observed at specific points along the mycelial hyphae at magnifications above x50, though sporulation was absent. In contrast, more pronounced necrosis and sporulation were seen in other genotypes, indicating a higher level of infection. These findings are consistent with Blanc [27], who suggested that the degree of mycelial development on the leaf surface correlates with sporulation, and that genotypes restricting fungal mycelial growth may prevent sporulation, thereby disrupting the fungal lifecycle.

In this study, sporulation was absent in genotypes with mycelium density scale values above 6, and was limited in those with values above 7. Additionally, necrosis was observed in both resistant and susceptible genotypes, with a higher intensity in the latter. Localized necroses were noted in all genotypes, regardless of susceptibility, confirming that necrosis is a common response to powdery mildew infection. The degree of sporulation was mainly associated with genotypes having scale ratings below 6. Regent, although resistant, did not achieve the highest resistance level of 9, which aligns with previous research by Eibach and Töpfer [41], who emphasized that resistance does not equate to immunity. Salotti et al. [39] also advocated for using leaf disc screening under laboratory conditions as an efficient, cost-effective method for selecting resistant genotypes, while stressing the importance of integrating visual data from field conditions to complement laboratory assessments.

These findings enhance our understanding of grapevine powdery mildew resistance and provide valuable insights for breeding programs aimed at improving resistance and optimizing disease management strategies. By identifying genotypes with consistent resistance, such as NRG-7

and NRG-200, this study contributes to the development of more robust grape varieties capable of withstanding powdery mildew, a significant concern in viticulture.

5. Conclusions

Seven genotypes from the NRG group were identified as resistant to powdery mildew based on general scoring results. While these genotypes are not fully resistant, they exhibit a delayed onset of symptoms, making them promising candidates for reducing fungicide applications during the growing season. Further molecular investigations are needed to explore the sensitivities of these genotypes, particularly in terms of gene expression. It is possible that resistance mechanisms vary depending on environmental stress or climatic factors, with certain genes activated at different stages of the infection. Resistance proteins, while enhancing plant immunity Jones, [45] may also trigger non-specific physiological responses, such as the accumulation of reactive oxygen species (ROS) and phytoalexins. Additionally, resistance may involve stomatal closure through the phosphorylation of mitogen-activated protein kinase (MAPK) pathways, activating transcription factors like WRKY22. The process could also involve specific proteins, such as coat proteins Gao et al. [46] or other effectors He et al. [47] In Turkey, viticulture plays a significant social, cultural, and economic role. Given the susceptibility of traditional grape varieties to powdery mildew, the development of resistant varieties is critical for both environmental and human health, as well as for reducing the cost of input such as fungicide use.

Author Contributions: A.B. and A.Y.; methodology, investigation, resources, data curation, writing original draft preparation, D.S.A.; writing—review and editing.

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