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Potential of Different *Actinidia* Genotypes as Resistant Rootstocks for Preventing Kiwifruit Vine Decline Syndrome

Giovanni Mian [1], Guido Cipriani [1], Simone Saro [2], Marta Martini [1] and Paolo Ermacora [1,*]

[1] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via delle Scienze 206, 33100 Udine, Italy; giovanni.mian@uniud.it (G.M.); guido.cipriani@uniud.it (G.C.); marta.martini@uniud.it (M.M.)

[2] ERSA, Regional Agency for Rural Development, Via Sabbatini, 5-3, 33050 Pozzuolo del Friuli, Italy; simone.saro@ersa.fvg.it

* Correspondence: paolo.ermacora@uniud.it; Tel.: +39-0432558540

Abstract: Kiwifruit Vine Decline Syndrome (KVDS) is currently affecting Italian kiwifruit cultivation, causing dramatic yield and economic losses. The syndrome's aetiology is due to soil-borne pathogens and waterlogging, leading to the decay of roots and then the canopy. Current knowledge about the disease is limited, and the techniques to control the syndrome are ineffective. The use of tolerant rootstocks is one of the most promising tools. Six genotypes of *Actinidia* were tested for two years at four infected experimental sites in Friuli Venezia Giulia (NE Italy). Plant evaluation and analysis were carried out on the root system and the vegetative parts. At all experimental sites, three genotypes, all belonging to the *A. macrosperma* group, grew normally. In contrast, plants of *A. polygama* died earlier and those of *A. chinensis* var. *deliciosa* 'Hayward' declined during the first year. *A. arguta* 'Miss Green' survived the first year but started to decline during the second year. After two years of study, we were able to identify three putative resistant genotypes: *A. macrosperma* accession numbers 176 and 183, and 'Bounty71', which will be a useful resource as rootstocks or as parents for breeding owing to their potential genetic resistance traits.

Keywords: canopy; germplasm; kiwifruit; Moria; root system; resistance/tolerance

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1. Introduction

Kiwifruit is a recently domesticated plant belonging to the family *Actinidiaceae* and the genus *Actinidia*, which contains ±54 species [1]. However, two taxa, *A. chinensis* var. *deliciosa* and *A. chinensis* var. *chinensis*, dominate the kiwifruit industry [2]. Breeders have shown an increasing interest in kiwifruit, and long-term breeding programs have recently resulted in the development of several cultivars [3]. Consumption of kiwifruit has been increasing steadily, with the fruit recognised as a superfood, owing to its high vitamin C content. Kiwifruit is a highly profitable crop with a long shelf life suited to global trade [4]. In Italy, the area planted with kiwifruit is currently 25,000 ha [5].

Recently, a new and complex disease called Kiwifruit Vine Decline Syndrome (KVDS), also known as "Moria del kiwi", has appeared in different kiwifruit growing regions, being reported initially in Italy and then worldwide [6,7]. This has led to significant yield and economic losses, around EUR 300,000,000 in 2020 [8]. KVDS was initially observed in poorly drained soils where waterlogging conditions occur most easily [9]. The abiotic factor of waterlogging is thought to favour the development of this syndrome [10], although KVDS can occur in many soil types. Several soil-borne pathogens have been implicated as aetiological agents (biotic factors) [10]. *Phytophthora* spp., *Pythium* spp., *Phytopythium* spp., *Fusarium* spp., *Cylindrocarpon* spp. and *Desarmillaria tabescens* were frequently isolated from affected plants [9,11–13]. Pathogenicity on kiwifruit cuttings has been demonstrated for some isolates: *Phytophthora cryptogea*, *Phytophthora citrophthora*,

Phytopythium vexans and *Phytopythium chamaehyphon* [7,14]. Only a few bacterial strains, belonging to *Clostridium* spp., have been identified as having a role in KVDS [15].

The most dramatic symptom is a rapid, sudden, and irreversible wilting of the plant [6]. The affected vines usually die within a few weeks, especially when evapotranspiration is the highest [9]. Canopy symptoms are accompanied by severe damage to the root system, which generally displays a reddish discoloration, giving the roots a "rat-tail" appearance and almost complete decay of the white roots. KVDS can affect both young and mature plants of the species, and both *A. chinensis* var. *deliciosa* and *A. chinensis* var. *chinensis* under different orchard management practices, and commonly plants do not recover [10].

Kiwifruit is usually not grafted in commercial orchards in Italy, but for its expansion into regions with heavy soils or other environmentally challenging characteristics, grafting of selected kiwifruit cultivars onto KVDS-resistant or -tolerant rootstocks might be essential for its future [16], as already occurs for others woody crops grafted for the purposes of resistance or tolerance to different soil stresses (both biotic and abiotic) [16,17]. Previous work on kiwifruit has considered the compatibility of the most widely grown cultivar 'Hayward' with other species [18] or has focused on micrografting methods and the evaluation of graft compatibility, shoot growth, and root formation and growth [19]. The effect of rootstocks in promoting scion vigour has also been assessed [20]. Other studies have evaluated rootstocks, focusing on abiotic stress, yield, vigour and yield components (production, quality, etc.) [21–23]. There are only a few reports of resistance to specific soil-borne pathogens [24], together with preliminary results concerning the resistance of *A. macrosperma* to waterlogging conditions [25].

In this study we investigated the main features of the root systems of six *Actinidia* genotypes cultivated in four KVDS affected sites in North-East Italy. The test plants were monitored over two years particularly the radical system, as the root development can be affected by several factors, with genetics strongly influencing root growth and distribution, and chemical parameters, thus leading to resistance to soil-borne pathogens [26]. For the aerial plant parts, only the total canopy biomass production was considered as these genotypes are intended for grafting or further breeding. The aims were to identify genotypes able to survive in KVDS-inducing soils and evaluate their agronomic performance in order to select the most promising rootstock candidates. This is the most desirable approach for establishing healthy and sustainable grafted kiwifruit plants.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The trial was conducted in the period 2020–2021 in the Udine and Pordenone provinces in the Friuli-Venezia Giulia Region (FVG) (Italy, NE), at four different sites including three orchards of *A. chinensis* var. *deliciosa* Hayward and one of *A. chinensis* var. *chinensis* Soreli. The locations of the sites are as follows: site-1 (45°59′55.4 N, 12°46′06.6 E), site-2 (46°00′31.6 N, 12°40′56.3 E), site-3 (45°50′43.6 N, 13°00′11.5 E) and site-4 (45°54′04.2 N, 12°56′45.4 E). The sites were chosen in accordance with previous research which recently found these sites are KVDS inducive due to the presence of the soil-borne pathogens already mentioned above [9]. The genotypes tested were *A. macrosperma*, accession numbers 176 (Ma176) (♂) and183 (Ma183) (♂), Bounty 71, which also belongs to the *A. macrosperma* group, *A. arguta* Miss Green (MG), and *A. polygama* (POL) (♂). *A. chinensis* var. *deliciosa* Hayward (HW) (♀) was used as a KVDS-susceptible control. The selected genotypes are represented in the University of Udine germplasm collection and were chosen according to putative tolerance to waterlogging and soil-borne pathogens [27,28] and upon preliminary results [29].

The cuttings were rooted in sterile Agriperlite during Spring 2019, then were grown in pots in a greenhouse for a year and planted in the field early in Spring 2020. Four plants at each experimental site were observed in our study; thus, there was a total of 16 replicates across the four experimental sites. The test plants were planted along the main plant

rows, replacing dead plants, and equally distributed at each experimental site, by adopting a randomised block design. Local standard practices were followed for pest management and fertilisation and were comparable at the different sites. The plants were irrigated according to the weather conditions to restabilize the field capacity, using a micro sprinkler system at each experimental site. Each year, at the end of the growing season, the plants were carefully removed from the soil and investigated. Measurements were taken during autumn (November 2020 and November 2021) when the plants were dormant. Root biometric indices were recorded, and samples of thick roots were kept and stored, as they are the most metabolically active [30], to carry out further chemical analyses. The whole developed canopy was also harvested to be further analyzed.

2.2. Pedological, Climatic and KVDS Characterisation of the Experimental Sites

Samples of soil were randomly collected at each site at 20 cm below ground and analysed according to the official Italian protocol (as indicated by Italian Law Decree n. 79/1992 and Law Decree n. 185/1999) [31]. Rainfall and temperature data were taken at a meteorological station located near the experimental sites. The presence of KVDS at each experimental site was initially assessed as incidence (% of symptomatic plants) and severity (% of attack per plant), using a previously used approach based on visual inspection of the aerial parts of the vines [32].

2.3. Survival of Genotypes at the Study Site

Each year, the percentage of plants of each genotype surviving was determined by a visual inspection at the end of growing season (November).

2.4. Root Analyses

2.4.1. KVDS Root Symptom Survey

For each plant, the percentage of roots with necrosis or a rat-tail appearance was assessed. The root systems were photographed with a digital camera. The photos were processed with ImageJ (National Institutes of Health, USA) to individuate the symptoms presence.

2.4.2. Root Biometric Indices: Volume, Width and Depth

The profile wall method of Böhm [33] was adopted to determine root indices. The width and depth (cm) of the root system were measured using a 35 cm high and 53 cm wide grid system positioned against the root system, keeping the vine trunk centred as a reference. The grid was divided internally into 1 cm × 1 cm squares. The volume (mL) of each below-ground part was recorded by dipping the whole sample into a graduated beaker partially filled with tap water and measuring the pre- and -post immersion difference in water volume.

2.4.3. Protein Content

The total protein content was measured in samples of thick roots because degradation of protein is known to be influenced by soil-borne pathogens [34]. The samples were dried in an oven at 65 °C for 1 day. The method of Fazeli et al. (2007) [35] was used: 1 g of root material was homogenised in 5 mL of 1M Tris-HCl buffer (pH 6.8). The homogenate was centrifuged in a refrigerated centrifuge at 10,000× *g* for 20 min, and the supernatant obtained was used for protein content determination. All the steps were carried out at 4 °C. The protein content of the extracts was determined according to Bradford [36] using bovine serum albumin (BSA) as a standard. A spectrophotometer (Dynatech MR5000, Dynex Technologies, Chantilly, VA, USA) was used to perform absorbance readings, at 560 nm. The values are expressed as mg g^{-1} Dry Weight (DW). Discontinuous sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in order to separate proteins and then determine their patterns.

2.4.4. Measurement of Total Non-Structural Carbohydrates (TNCs)

An assay of TNCs (sucrose, glucose, and fructose) on the thick roots was performed owing to the importance of TNCs in subsequent years for accumulation of starch reserves [37]. Briefly, TNCs were assessed using anthrone reagent (Merck, Darmstadt, Germany) and absorbance readings at 620 nm were performed using a Shimadzu UV Mini-1240 spectrophotometer (Kyoto, Japan) [38]. The data are expressed in mg g^{-1} DW.

2.4.5. Determination of Mineral Elements in Roots

Thick root (±50 g) samples were firstly washed with distilled water and then analyzed as described by Ari et al. [39] with the slight modifications of Mian et al. (2021) [40]. Briefly, after having obtained the dry material, 1 g DW was digested in nitric acid HNO_3 (65%) (Sigma Aldrich, St. Louis, MO, USA), then diluted in a solution of HNO_3 (1%). All the analytical solutions were prepared using ultrapure water (resistivity ≥ 18.2 MΩ cm) obtained from a Milli-Q® System (Millipore, Bedford, MA, USA). 15 mL of the resultant extract was filtered. The concentration of mineral elements was determined using an Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (5800 Agilent. Perkin Elmer Optima 3000) instrument, equipped with a crossflow nebulizer and an auto sampler. The detected elements in solution are expressed as mg g^{-1} DW.

2.5. Canopy Development

The total canopy biomass (stem plus leaves) produced each year was recorded during winter. The canopy biomass was collected from 10 cm above the root collar. This was dried in an oven at 65 °C for three days in order to record the total amount in g DW.

2.6. Statistical Analysis

One-way analysis of variance was performed by using "R" free software (version 4.0.3 2020-10-10) [41]. Statistical analysis to determine significant differences between treatment means was carried out using the Student–Newman–Keuls test ($p \leq 0.05$), as already used for these kind of parameters and types of studies [42]. Changes between study years in the measured parameters were calculated as mean values as this can provide a good indication of agronomic performance. The differential was calculated as follows: Δ2020–2021: #value2021-#value2020.

3. Results

3.1. Characterisation of the Study Sites

The incidence of KVDS at each site at the beginning of the experiment (2020) ranged from 30% (site-4) to 90% (site-2), whilst KVDS severity ranged from 30% (site-4) to 45% (site-2) (Table 1). KVDS incidence was similar at sites -1, -2 and -3, and was only relatively minor at site-4. Nevertheless, the KVDS severity was similar at all sites. The average values of the physical characteristics of the soils are listed in Table 1. Following the international nomenclature of the United States Department of Agriculture, the soils are classified as silt-loam, are similar at all the sites and are typical of this part of Italy, apart from the C content.

Table 1. Site soil features and KVDS incidence (%) in the existing orchards at the four test sites.

Parameter	Site-1	Site-2	Site-3	Site-4
Orchard area (ha)	0.72	2.14	0.95	0.30
KVDS incidence (%)	80	90	80	30
KVDS severity (%)	35	45	35	30
Sand (%)	32	28	32	26
Silt (%)	46	51	46	61
Clay (%)	22	21	22	13

Organic carbon (C)	1.66	3.23	1.66	1.13

The study areas have a typical Mediterranean climate (Table 2), with warm summers and cool winters. The annual rainfall was approximately 1150 mm in 2020, with a mean temperature of 14.10 °C. In 2021, the annual rainfall was about 1200 mm, whilst the average temperature was 13.50 °C. The two years were climatically similar. This was confirmed by the global radiation: 4926780 KJ m^{-2} in 2020 compared with 5,018,202 KJ m^{-2} in 2021 (data not shown).

Table 2. Rainfall (mm) and mean temperature (T°) in 2020 and 2021.

Month	Rainfall (mm)–2020	T°-2020	Rainfall (mm)–2021	T°-2021
1	7.10	3.67	163.30	3.81
2	11.60	6.87	102.10	7.38
3	132.00	9.09	21.40	8.16
4	23.20	14.24	158.60	11.00
5	66.00	17.75	219.50	14.80
6	205.50	20.56	28.60	22.94
7	84.00	23.55	79.40	24.40
8	132.20	24.14	114.70	22.85
9	104.60	19.88	92.30	19.82
10	103.00	14.91	43.50	13.19
11	14.40	6.27	122.50	9.82
12	272.60	5.60	61.40	3.80
	Total: 1156.2	Mean: 14.10	Total: 1207.3	Mean: 13.50

3.2. Genotype Survival in KVDS-Inducing Soils

The percentage of plants alive at the end of each vegetative season in the two study years is reported in Table 3. In 2020, all the plants of Ma176, Ma183, Bounty71 and MG survived, whereas only 16% of HW plants survived. None of the POL plants survived: they declined and died during the summer, 3–4 months after planting, so no further data were collected for these genotypes. Additionally, when the POL plants were removed, they had no roots, although at the time of planting in the field, all the genotypes showed similar levels of root growth in the pots (data not shown). Similar to POL in the first year, a serious decline in HW occurred during the second year of the study (the root system was heavily compromised and the plants were severely KVDS affected); hence, no measurements were taken for HW at this time. At the end of the two years, some MG plants also declined, with about 30% of the plants dying compared with none the first year.

Table 3. Plant survival of each genotype in soils affected by KVDS in 2020 and 2021. Within columns, values that are assigned different letters are significantly different at $\alpha \leq 0.05$, 0.01, 0.001. Data are the mean of 16 replicates (4 for each site). Statistical analysis (ANOVA) was carried out using the Student–Newman–Keuls test ($p \leq 0.05$). "-" = not measured.

Genotype	Percentage of Plants Alive (%)	
	2020	**2021**
Ma176	100 a	100 a
Ma183	100 a	100 a
Bounty71	100 a	100 a
MG	100 a	71.40 b
HW	16 b	0 c
POL	0 c	-

3.3. KVDS Root Symptom Survey

The root symptom survey data are listed in Table 4. Ma176 and Ma183, 'Bounty71' and MG did not show any symptoms in 2020, whilst for HW, about 90% of the root system was compromised. In 2021, Ma176, Ma183 and Bounty71 continued to show no KVDS symptoms, but all the HW plants died, and in MG, an average of 25% of roots were symptomatic.

Table 4. KVDS incidence in 2020 and 2021. Within columns, values that are assigned different letters are significantly different at $\alpha \leq 0.05$, 0.01, 0.001. Data are the mean of 16 replicates (4 for each site). Statistical analysis (ANOVA) was carried out using the Student–Newman–Keuls test ($p \leq 0.05$). "-" = not measured.

Genotype	KVDS Incidence (%)–2020	KVDS Incidence (%) -2021
Ma176	0 b	0 b
Ma183	0 b	0 b
Bounty71	0 b	0 b
MG	0 b	25 a
HW	90 a	-
POL	100	100

3.4. Key Elements of the Root System Relevant for Genotype Fitness

3.4.1. Biometric Indices of Roots

Table 5 reports all the root measurements from the two years of study and Figure S1 illustrates examples of the roots. In 2020, HW had the lowest mean root volume (56 mL), and Ma176 and MG had the highest volumes (407.33 and 389.33 mL, respectively). The differences between HW and the other two genotypes were statistically different. Ma183 and Bounty71 were similar to each other (187.50 and 226.75 mL, respectively), and also significantly different from the other genotypes. Regarding depth, only the low value measured in HW (16.10 cm) was significantly different from the other genotypes (29.80 to 31.34 cm). For the root system width, once again, Ma176 had the highest value (47.65 cm) and HW the lowest (18.25 cm), the difference being statistically significant. Values for the other genotypes were in the range 36.93 to 38.80 cm and thus significantly lower and higher than Ma176 and HW, respectively. In 2021, MG had the lowest root volume (123.33 mL) and was significantly different from Ma176 and Bounty71 (421 and 476.66 mL, respectively), which had the highest volumes. The root volume of Ma183 (295.83 mL) was significantly higher than that of MG and lower than the root volumes of both Ma176 and Bounty71. In terms of root depth, Ma176 and Bounty71 showed similarly high values (30.28 and 35.62 cm, respectively, no significant difference between them) and were both statistically different from Ma183 and MG (24.19 and 22.17 cm, respectively; no significant difference between them). For root system width, Ma176 (44.95 cm) and Bounty71 (45.11 cm) showed the highest values and were significantly different from Ma183 (34.48 cm), and the widths of all these genotypes were significantly greater than MG (27.45 cm).

Between 2020 and 2021, the root volume of Ma176 increased slightly (13.67 mL) whilst Ma183 and Bounty71 showed the greatest increases (108.78 and 249.91 mL, respectively). Interestingly, the volume of MG decreased significantly (−266 mL), which might be correlated with KVDS attack. Thus, all *A. macrosperma* showed increased root volumes. Regarding root depth, only Bounty 71 increased (+4.86 cm), whereas the other genotypes seemed to decrease in depth. There was a parallel outcome for width, which increased in Bounty71 (+6.76 cm) and decreased in the remaining genotypes. The most marked growth occurred in the *A. macrosperma* group, and disregarding HW, the greatest decline occurred in MG during the second year of the study.

Table 5. Genotypes root system indices in 2020 and 2021: volume, depth, width, and changes between the two years of study. Within columns, values that are assigned different letters are significantly different at $\alpha \leq 0.05$, 0.01, 0.001. Data are the mean of 16 replicates (4 for each site). Statistical analysis (ANOVA) was carried out using the Student–Newman–Keuls test ($p \leq 0.05$). "-" = not measured.

Genotype	2020			2021			Changes (2020 to 2021)		
	Volume (mL)	Depth (cm)	Width (cm)	Volume (mL)	Depth (cm)	Width (cm)	Volume (mL)	Depth (cm)	Width (cm)
Ma176	407.33 a	31.34 a	47.65 a	421.00 a	30.28 a	44.95 a	13.67	−1.06	−2.70
Ma183	187.05 b	27.36 a	35.93 b	295.83 b	24.19 b	34.48 b	108.78	−3.17	−1.45
Bounty71	226.75 b	30.76 a	38.35 b	476.66 a	35.62 Aa	45.11 a	249.91	4.86	6.76
MG	389.33 a	29.80 a	36.80 b	123.33 c	22.17 b	27.45 c	−266.00	−7.63	−9.35
HW	56 c	16.10 b	18.25 c	-	-	-	-	-	-
POL	-	-	-	-	-	-	-	-	-

3.4.2. Protein Content in the Root System

The total protein content in the root system of the tested genotypes in the two-year study is reported in Table 6. In 2020, Ma176 showed the highest value (5.60 mg g^{-1} DW) and HW the lowest (2.84 mg g^{-1} DW), with this difference being statistically significant. The other genotypes had higher values than HW (ranging from 4.80 to 5.10 mg g^{-1} DW), but no strong statistical significance was found apart from the difference from HW. Indeed, in 2021, Ma176 once more recorded the highest value (7.97 mg g^{-1} DW), but again there was no statistical significance among the other genotypes. Bounty71 showed 6.55 mg g^{-1} DW, and MG was similar (6.48 mg g^{-1} DW), whereas for Ma183, the value was 7.01 mg g^{-1} DW. Lastly, concerning the changes between years, the more marked increase occurred in Ma176 (2.37 mg g^{-1} DW). The other genotypes showed a smaller increase (± 1.7 mg g^{-1} DW). There were no differences between genotypes in protein patterns (Figure S2).

Table 6. Genotypes protein content in roots in 2020 and 2021 and the changes between the two years. Within columns, values that are assigned different letters are significantly different at $\alpha \leq 0.05$, 0.01, 0.001. Data are the mean of 16 replicates (4 for each site). Statistical analysis (ANOVA) was carried out using the Student–Newman–Keuls test ($p \leq 0.05$). "-" = not measured.

Genotype	Proteins–2020 (mg g^{-1} DW)	Proteins–2021 (mg g^{-1} DW)	Changes in Proteins (mg g^{-1} DW) (2020 to 2021)
Ma176	5.60 a	7.97 a	+2.37
Ma183	5.14 a	7.01 a	+1.87
Bounty71	4.80 a	6.55 a	+1.75
MG	4.75 a	6.48 a	+1.73
HW	2.84 b	-	-
POL	-	-	-

3.4.3. Accumulation of TNCs in the Root System

Concerning TNCs (Table 7), at the end of the 2020 season HW had the lowest value (13.33 mg g^{-1} DW) and was significantly different from the other genotypes. There were no differences among the other genotypes, where the values were around 22 mg g^{-1} DW. Again, in 2021, all the genotypes being tested showed similar values (around 30 mg g^{-1} DW). Unsurprisingly, the changes in TNCs in 2021 relative to 2020 were also similar among the genotypes tested (range: 7.82 to 9.98 mg g^{-1} DW).

Table 7. Genotypes TNCs content in roots in 2020 and 2021 and the changes between the two years. Within columns, values that are assigned different letters are significantly different at $\alpha \leq 0.05$, 0.01, 0.001. Data are the mean of 16 replicates (4 for each site). Statistical analysis (ANOVA) was carried out using the Student–Newman–Keuls test ($p \leq 0.05$). "-" = not measured.

Genotype	TNCs–2020 (mg g^{-1} DW)	TNCs–2021 (mg g^{-1} DW)	Changes in TNCs (mg g^{-1} DW) (2020 to 2021)
Ma176	23.76 a	33.74 a	+9.98
Ma183	21.30 a	29.19 a	+7.88
Bounty71	23.83 a	33.41 a	+9.58
MG	22.34 a	30.16 a	+7.82
HW	13.33 b	-	-
POL	-	-	-

3.4.4. Mineral Elements Determined in the Root System

Table 8 lists the mineral elements detected in the root system in 2020 and 2021. In 2020, HW had statistically lower values than the other genotypes for every element investigated. Apart from HW, no differences were found for nitrogen (N), carbon (C) or magnesium (Mg) between the tested genotypes. Nevertheless, Ma176 and Bounty71 had significantly higher phosphorus (P) contents (41.55 and 42.80 mg g^{-1} DW, respectively) than the other genotypes. At the same time, Ma176 also tended towards the highest calcium (Ca) value (7.56 mg g^{-1} DW), however, without statistical significance. In contrast, MG tended to have the lowest potassium (K) value (87.90 mg g^{-1} DW).

In 2021, no differences were found for the N, K and Mg contents. Significant differences were observed for P content with Ma183 having the highest value (39.79 mg g^{-1} DW). There were also significant differences in C content with Bounty71 and MG (666.42 and 662.96 mg g^{-1} DW, respectively) having lower values than Ma176 (738.11 mg g^{-1} DW) and Ma183 (719.24 mg g^{-1} DW). Lastly, Bounty71 had the lowest accumulation of Ca (5.13 mg g^{-1} DW).

Finally, regarding the changes between years (Table 9), every elemental content in each genotype was higher in 2021 than in 2020, with similar increases for each element across the genotypes. The most marked changes occurred in K and C accumulation.

Table 8. Mineral elements determined in roots of genotypes in 2020 and 2021 (except for HW in 2021). Within columns, values that are assigned different letters are significantly different at $\alpha \leq 0.05$, 0.01, 0.001. Data are the mean of 16 replicates (4 for each site). Statistical analysis (ANOVA) was carried out using the Student–Newman–Keuls test ($p \leq 0.05$). "-" = not measured.

	Mineral Element–s-2020 (mg g^{-1} DW)					
Genotype	**N**	**P**	**K**	**C**	**Ca**	**Mg**
Ma176	1.78 a	41.55 a	99.41 a	585.52 a	7.56 a	11.09 a
Ma183	1.81 a	32.30 b	104.61 a	568.62 a	6.19 a	11.53 a
Bounty71	2.05 a	42.80 a	108.41 a	559.32 a	4.36 b	9.57 a
MG	1.97 a	34.14 b	87.90 b	595.34 a	5.53 Aa	12.36 a
HW	1.30 b	1.57 c	1.48 c	40.09 c	2.74 c	2.08 b
POL	-	-	-	-	-	-
	Mineral Element–s-2021 (mg g^{-1} DW)					
Ma176	2.11 a	50.94 a	123.99 a	738.11 a	8.16 a	13.70 a
Ma183	2.14 a	39.79 b	123.17 a	719.27 a	7.28 a	14.25 a
Bounty71	2.41 a	51.24 a	122.98 a	666.42 b	5.13 b	11.83 a
MG	2.33 a	49.55 a	107.66 a	662.96 b	6.52 a	15.28 a
HW	-	-	-	-	-	-
POL	-	-	-	-	-	-

Table 9. Genotypes changes in determined mineral elements from 2020 to 2021.

Genotype	Changes in Mineral Elements (mg g^{-1} DW) (2020 to 2021)					
	N	P	K	C	Ca	Mg
Ma176	+0.33	+9.39	+24.58	+152.59	+0.60	+2.61
Ma183	+0.34	+7.49	+18.56	+150.65	+1.09	+2.72
Bounty71	+0.36	+8.44	+14.57	+107.09	+0.77	+2.26
MG	+0.37	+15.40	+19.76	+67.62	+0.99	+2.91
HW	-	-	-	-	-	-
POL	-	-	-	-	-	-

3.5. Canopy Development

Table 10 presents the DW of the canopy during 2020 and 2021. In 2020 there were significant differences among the varieties, with Ma176 along with Bounty71 possessing the greatest weights (23.53 and 22.22 g, respectively). Ma183 and MG had mid-range values (7.10 and 10.22 g, respectively), and HW had the lowest value (2.80 g). In 2021, once again, Ma176 and Bounty71 were similar and had the highest weight (27.08 and 25.90 g, respectively) and they were significantly different from Ma183 and MG, which had similar lower values (12.50 and 10.00 g, respectively). Between the study years, Ma176, Ma183 and Bounty71 showed similar small increases, whereas MG had a slight decrease in canopy DW, consistent with the other analyses, due to KVDS attack.

Table 10. Dry weight of canopy for genotypes in 2020 and 2021 and the change between the two years. Within columns, values that are assigned different letters are significantly different at $\alpha \leq 0.05$, 0.01, 0.001. Data are the mean of 16 replicates (4 for each site). Statistical analysis (ANOVA) was carried out using the Student–Newman–Keuls test ($p \leq 0.05$). "-" = not measured.

Genotype	Dry Weight (g–)-2020	Dry weight (g–)-2021	Changes in Dry Weight (g) (2020 to 2021)
Ma176	23.53 a	27.08 a	+3.55
Ma183	7.10 b	12.50 b	+5.40
Bounty71	22.22 a	25.90 a	+3.68
MG	10.22 b	10.00 b	−0.22
HW	2.80 c	-	-
POL	-	-	-

4. Discussion

Some KVDS epidemiological traits are still under investigation, and at present there are no effective methods of control. Kiwifruit growers have empirically tried many strategies to maintain crop yields and their incomes [43], but these strategies are not sustainable nor effective. The lack of effective control strategies results in continually increasing economic losses [8]. The most promising approach, as with any soil-borne disease, is the use of tolerant or resistant species as rootstocks [24,44]. Within the Italian kiwifruit sector, this practice has not been widely used before. We have, therefore, screened genotypes of different *Actinidia* species intended for use as rootstocks or for breeding programs for their putative resistance or tolerance to KVDS [27,29,45,46].

In the current research, six genotypes of *Actinidia* spp. were planted at four infected experimental sites and tested for two years: *A. macrosperma* (Ma176, Ma183 and Bounty71), *A. arguta* Miss Green (MG), and *A. polygama* (POL). *A. deliciosa* Hayward (HW) was chosen as the susceptible genotype. At all of the experimental sites, KVDS dated back to 2014, and the sites were similar in their levels of KVDS infection and general features (Tables 1 and 2). Consequently, the genotype behaviour recorded from each site was also similar. This allowed us to perform the experiment using four replicates per site, and this was

confirmed by data normalization. In the statistical analysis, a one-way ANOVA was performed as a two-way ANOVA was not applicable because young plants grow quickly, so the year × treatment interaction would obviously be statistically significant (data not shown). Instead, changes between study years in the measured parameters were calculated as mean values because this provides a good indication of agronomic performance.

All the three genotypes of *A. macrosperma*, Ma176, Ma183 and Bounty71, survived in KVDS-inducing soil, suggesting that they might be useful tools for providing resistance to the syndrome (Tables 3 and 4). Furthermore, a genotype of known sensitivity, HW, proved to be susceptible and did not survive in the test soils (all plants were dead by the second year). For this reason, HW was not included in the second year of the study. Moreover, the POL genotypes died in the spring of the first year, indicating that this species is highly susceptible to KVDS. This result indirectly emphasizes the importance of biotic factors relative to environmental factors in KVDS aetiology [6]. This is supported by the observation that the parents of the *A. polygama* plants, as well as Hayward, used in the experiment are present in the germplasm collection of the University of Udine where KVDS is not reported (a few kilometers away from the experimental sites), and show excellent vegetative development and strong vigour, without any KVDS symptoms. The *A. arguta* Miss Green plants showed no symptoms of KVDS in the first year, but in the second-year, root systems were affected (±25%), and consequently, many of the investigated root parameters were worse, with several plants dying. MG was affected at all experimental sites. This suggested that MG is not a resistant genotype but could be considered only tolerant. Consequently, where KVDS pressure was high, MG was not completely able to maintain a balanced root profile, nor was it fully capable of surviving.

A considerable range in kiwifruit root system agronomic parameters (biometric indices) was evident among the genotypes tested (Table 5). For *A. polygama* it was not possible to conduct any further analysis because all the plants died. Among the genotypes that survived the first year, HW had the worst performance. MG also performed poorly because by the second year it was affected by KVDS. In 2020, the best values were found in Ma176, and in the second year in Ma176 and Bounty71. Both Ma183 and Bounty71 showed strong increases in some indices (e.g., root volume) in the second year of the study, and Bounty71 in particular attained values that were similar to Ma176 during 2020. Bounty71 showed increases in all values. MG showed decreases in all the parameters due to KVDS. However, the small decreases in width and length of Ma176 and Ma183 were not significant.

The effect of soil-borne pathogens on protein degradation was clear (Table 6) when considering the protein content in the resistant genotypes compared with the sensitive genotype (HW), as has been reported previously [47]. Unsurprisingly, HW showed the greatest reduction in protein content, even when accounting for the data from the second year, with the remaining genotypes not noticeably affected. Once again, Ma176 tended towards the highest protein content; however, no statistical differences emerged among the tested genotypes. As previously discussed, there were no differences in protein patterns observed (Figure S2).

Concerning the TNCs content (Table 7), HW was the worst performing, further indicating its KVDS susceptibility. This parameter was clearly affected by KVDS and has known susceptibility to several biotic factors in many crops [48]. Although the other genotypes tested showed higher values than HW, there were no statistical differences between any of the genotypes, or for the change in TNCs between years among the surviving genotypes. Nevertheless, we may assume from this assay that the genotypes that performed well will be able to accumulate more reserves in subsequent years, which is important for plant budburst, and flowering, etc. [49,50].

The mineral elements detected present in the root system (Tables 8 and 9) showed some genotype specific effects. It is of great importance to understand their accumulation in the roots since they are further translocated to the aerial parts of the plant (i.e., leaves and fruits). At first glance, HW had significantly lower values for each element

investigated than the other genotypes. However, no great differences emerged between the resistant genotypes. Nonetheless, in 2020, Bounty71 recorded the lowest Ca accumulation value. Calcium is involved in determining fruit size, firmness and sugar translocation [51,52], so we can speculate that this rootstock might eventually result in fruit size and quality reductions. During 2021, the trend continued, with Bounty71 accumulating less Ca and C than most of the varieties (the C levels were similar to MG). Indeed, the fact that Bounty71 recorded the lowest C concentrations among the surviving genotypes may mean that this cultivar is not capable of further accumulation of reserve substances or of maintaining a well-balanced whole plant profile in the long term [53]. Regarding MG, despite it being affected by KVDS in the second year, it was surprising that the levels of mineral elements present in the root system tended to increase. We can assume that this was a concentration effect because the root volume of MG decreased in the second year of the study. Interestingly, the P content for MG in 2020 alone and for Ma183 in both 2020 and 2021 was significantly lower than that for the other genotypes. The P content should be carefully taken into consideration when selecting rootstock, because P is a component of essential molecules such as nucleic acids, phospholipids, and ATP (adenosine triphosphate), and is involved in controlling key enzyme reactions and the regulation of metabolic pathways [54]. Finally, Ma 176 seemed to have the highest elemental concentration of all the genotypes, as did Bounty71 for the majority of elements, but these values were not statistically significant. Similar results were just obtained in preliminary experiments [55,56].

Considering canopy development measured in terms of dry weight (Table 10), in both 2020 and 2021, Ma176 and Bounty71 had the highest values overall. In 2020, HW had a significantly smaller canopy than the other genotypes, and MG and Ma183 registered lower values than Ma176 and Bounty71 in both years. Perhaps owing to the effects of KVDS, canopy production in MG decreased in 2021. Finally, the changes in canopy dry weight between 2020 and 2021 were similar. We can report that the genotypes tested showed an interesting and different canopy development and appearance, which could be important for plant structure once grafting occurs, since the aptitude to develop canopy is an important factor for the whole aerial plant structure [57]. In fact, a different canopy structure not only affects the aerial plant structure, but the biodiversity of the cultivated land as well (e.g., light competition is one of the most important factors that limits both the growth of plant species and the biodiversity of the soil under kiwifruit canopy) [58].

As a final consideration, *A. macrosperma* and *A. arguta* showed better performance than HW and POL in every analysis carried out. Obviously, all the genotypes had different specific effects, but in the end, the majority demonstrated KVDS resistance.

5. Conclusions

In this paper we are reporting detailed evidence of resistance or tolerance to KVDS in *Actinidia* genotypes which can be used as rootstocks, once their affinity with the most important cultivars can be demonstrated, or as a source of resistance or tolerance in breeding programs. *A. macrosperma* genotypes are the most promising of those tested and, at present, they are the only way to overcome KVDS. Further studies will be required to understand how and why these genotypes are resistant or not, and experiments on these and other aspects are currently ongoing under controlled conditions (e.g., ability to modify the microbiota). In addition, we will further investigate the underlying genomics and the grafting long-term compatibility.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8070627/s1, Figure S1: Root system of tested genotypes. Figure S2. SDS PAGE analyses of the tested genotypes.

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References

1. Zhang, X.; Bao, W.; Zhang, A.; Pathirana, R.; Wang, Q.C.; Liu, Z.E. Cryopreservation of shoot tips, evaluations of vegetative growth, and assessments of genetic and epigenetic changes in cryo-derived plants of *Actinidia* spp. *Cryobiology* **2020**, *94*, 18–25. https://doi.org/10.1016/j.cryobiol.2020.05.004.
2. Mian, G.; Iseppi, L.; Traversari, G.; Ermacora, P.; Cipriani, G.; Nassivera, F. Consumers Perceptions and Motivations in the Choice of Kiwifruits: A Study-Case in Italy, North-East. *Qual. Access Success* **2022**, *23*, 163–175. https://doi.org/10.47750/QAS/23.188.23.
3. Testolin, R.; Cipriani, G. Markers, Maps, and Marker-Assisted Selection. *Kiwifruit Genome* **2016**, *1218*, 85–99. https://doi.org/10.1007/978-3-319-32274-2_7.
4. Ferguson, A.R. Kiwifruit: Evolution of a crop. *Acta Hortic.* **2011**, *913*, 31–42. https://doi.org/10.17660/ActaHortic.2011.913.1.
5. Costa, G.; Ferguson, R.; Huang, H.; Testolin, R. Main changes in the kiwifruit industry since its introduction: Present situation and future. *Acta Hortic.* **2018**, *1218*, 1–16. https://doi.org/10.17660/ActaHortic.2018.1218.1.
6. Bardi, L. Early Kiwifruit Decline: A Soil-Borne Disease Syndrome or a Climate Change Effect on Plant–Soil Relations?. *Frontiers in Agronomy*, **2020**, 2:3..
7. Tacconi, G.; Paltrinieri, S.; Mejia, J.F.; Fuentealba, S.P.; Bertaccini, A.; Tosi, L.; Giacopini, A.; Mazzucchi, U.; Favaron, F.; Sella, L.; et al. Vine decline in kiwifruit: Climate change and effect on waterlogging and *Phytophthora* in North Italy. *Acta Hortic.* **2015**, *1096*, 93–98. https://doi.org/10.17660/ActaHortic.2015.1096.7.
8. Tacconi, G.; Tosi, L.; Giacopini, A.; Nari, L.; Berra, L.; Spadaro, D.; Prencipe, S.; Rosati, M.; Savian, F.; Saro, S.; et al. La moria del kiwi al 2020: Lungi dalla soluzione! *Kiwi Inf.* **2020**, *4*, 6–17. ISNN: 2282-2224.
9. Savian, F.; Ginaldi, F.; Musetti, R.; Sandrin, N.; Tarquini, G.; Pagliari, L.; Firrao, G.; Martini, M.; Ermacora, P. Studies on the aetiology of kiwifruit decline: Interaction between soil-borne pathogens and waterlogging. *Plant Soil* **2020**, *456*, 113–128. https://doi.org/10.1007/s11104-020-04671-5.
10. Savian, F.; Martini, M.; Ermacora, P.; Paulus, S.; Mahlein, A.K. Prediction of the kiwifruit decline syndrome in diseased orchards by remote sensing. *Remote Sens.* **2020**, *12*, 2194. https://doi.org/10.3390/rs12142194.
11. Prencipe, S.; Savian, F.; Nari, L.; Ermacora, P.; Spadaro, D.; Martini, M. First report of *Phytopythium vexans* causing decline syndrome of *Actinidia deliciosa* "Hayward" in Italy. *Plant Dis.* **2020**, *104*, 2032. https://doi.org/10.1094/PDIS-10-19-2101-PDN.
12. D'Ippolito, I.; Mang, S.M.; Elshafie, H.S.; Camele, I.; Scillitani, G.; Mastrodonato, M.; Sofo, A.; Mininni, A.N.; Xylogiannis, E. Morpho-Anatomical and microbiological analysis of kiwifruit roots with KVDS symptoms. *Acta Hortic.* **2022**, *1332*, 131–136. https://doi.org/10.17660/ACTAHORTIC.2022.1332.18.
13. Donati, I.; Cellini, A.; Sangiorgio, D.; Caldera, E.; Sorrenti, G.; Spinelli, F. Pathogens associated to kiwifruit vine decline in Italy. *Agriculture* **2020**, *10*, 119. https://doi.org/10.3390/agriculture10040119.
14. Savian, F.; Prencipe, S.; Filippini, N.; Nari, L.; Martini, M.; Ermacora, P.; Spadaro, D. Pathogenicity of *Phytopythium chamaehyphon*: A new player in kiwifruit vine decline syndrome of *Actinidia chinensis* var. *deliciosa* 'Hayward' in Italy. *Plant Dis.* **2021**, *105*, 2781–2784. https://doi.org/10.1094/pdis-01-21-0143-sc.
15. Spigaglia, P.; Barbanti, F.; Marocchi, F.; Mastroleo, M.; Baretta, M.; Ferrante, P.; Caboni, E.; Lucioli, S.; Scortichini, M. *Clostridium bifermentans* and *C. subterminale* are associated with kiwifruit vine decline, known as *moria*, in Italy. *Plant Pathol.* **2020**, *69*, 765–774. https://doi.org/10.1111/ppa.13161.
16. Gandullo, J.; Ahmad, S.; Darwish, E.; Karlova, R.; Testerink, C. Phenotyping tomato root developmental plasticity in response to salinity in soil rhizotrons. *Plant Phenomics* **2021**, *2021*, 2760532. https://doi.org/10.34133/2021/2760532.
17. Friend, A.P.; Palmer, J.W.; Seymour, S.M.; Diack, R.N. Potential of clonal rootstocks for devigoration and enhanced fruit characteristics in kiwifruit orchards. *Acta Hortic.* **2014**, *1058*, 429–434. https://doi.org/10.17660/ActaHortic.2014.1058.52.
18. Chartier, J.; Blanchet, P. Reciprocal grafting compatibility of kiwifruit and frost hardy *Actinidia* species. *Acta Hortic.* **1997**, *444*, 149–154. https://doi.org/10.17660/ACTAHORTIC.1997.444.21.

19. Bao, W.W.; Zhang, X.C.; Zhang, A.L.; Zhao, L.; Wang, Q.C.; Liu, Z.D. Validation of micrografting to analyse compatibility, shoot growth, and root formation in micrografts of kiwifruit (*Actinidia* spp.). *Plant Cell Tissue Organ. Cult.* **2020**, *140*, 209–214. https://doi.org/10.1007/S11240-019-01723-2.
20. Clearwater, M.J.; Blattmann, P.; Luo, Z.; Lowe, R.G. Control of scion vigour by kiwifruit rootstocks is correlated with spring root pressure phenology. *J. Exp. Bot.* **2007**, *58*, 1741–1751. https://doi.org/10.1093/JXB/ERM029.
21. Abid, M.; Zhang, Y.J.; Li, Z.; Bai, D.F.; Zhong, Y.P.; Fang, J.B. Effect of salt stress on growth, physiological and biochemical characters of four kiwifruit genotypes. *Sci. Hortic.* **2020**, *271*, 109473. https://doi.org/10.1016/j.scienta.2020.109473.
22. Hettihewa, S.; Hemar, Y.; Rupasinghe, H. Flavonoid-Rich extract of *Actinidia macrosperma* (a wild Kiwifruit) inhibits angiotensin-converting enzyme *in vitro*. *Foods* **2018**, *7*, 146. https://doi.org/10.3390/foods7090146.
23. Matich, A.J.; Young, H.; Allen, J.M.; Wang, M.Y.; Fielder, S.; McNeilage, M.A.; MacRae, E.A. *Actinidia arguta*: Volatile compounds in fruit and flowers. *Phytochemistry* **2003**, *63*, 285–301. https://doi.org/10.1016/S0031-9422(03)00142-0.
24. Yano, T.; Shimizu, S.; Miyoshi, T.; Miyata, N., Immon; K., Shinozaki, T.; Sawada, H.; Kageyama, K. Tolerant *actinidia* species to *Pythium helicoides* and *P. vexans* causing root rot. *Acta Hortic.* **2010**, *913*, 517–523. https://doi.org/10.17660/ActaHortic.2011.913.69.
25. Kataoka, I.; Yamada, T.; Fukuda, T.; Ohtani, M.; Katsuhiko, S.; Beppu, K. Waterlogging tolerance of *Actinidia macrosperma* and its application to kiwifruit by grafting. *Hortic. Res.* **2021**, *20*, 265–271. https://doi.org/10.2503/hrj.20.265.
26. Hunter, J.J.; Archer, E.; Van Schalkwyk, D.; Strever, A.E.; Volschenk, C.G. Grapevine roots: Interaction with natural factors and agronomic practices. *Acta Hort.* **2016**, *1136*, 63–80. https://doi.org/10.17660/ActaHortic.2016.1136.10.
27. Kisaki, G.; Tanaka, S.; Ishihara, A.; Igarashi, C.; Morimoto, T.; Hamano, K.; Endo, A.; Sugita-Konishi, S.; Tabuchi, M.; Gomi, K.; et al. Evaluation of various cultivars of *Actinidia* species and breeding source *Actinidia rufa* for resistance to *Pseudomonas syringae* pv. *actinidiae* biovar 3. *J. Gen. Plant Pathol.* **2018**, *84*, 399–406. https://doi.org/10.1007/s10327-018-0804-5.
28. Mi, Y.; Ma, X.; Chen, S. Resistant evaluation of kiwifruit rootstocks to root zone hypoxia stress. *Am. J. Plant Sci.* **2013**, *4*, 945–954. https://doi.org/10.4236/ajps.2013.44117.
29. Mian, G.; Cipriani, G.; Saro, S.; Martini, M.; Ermacora, P. Evaluation of germplasm resources for resistance to kiwifruit vine decline syndrome (KVDS). *Acta Hortic.* **2022**, *1332*, 125–130. https://doi.org/10.17660/ACTAHORTIC.2022.1332.17.
30. Magalhães, P.; De Souza, T.C.; Cantão, F.R.O. Early evaluation of root morphology of maize genotypes under phosphorus deficiency. *Plant, Soil and Environment* **2011**, *57*, 135–138.31. https://doi.org/10.17221/360/2010-PSE.
31. Gaiotti, F.; Marcuzzo, P.; Belfiore, N.; Lovat, L.; Fornasier, F.; Tomasi, D. Influence of compost addition on soil properties, root growth and vine performances of *Vitis vinifera* cv Cabernet sauvignon. *Sci. Hortic.* **2017**, *225*, 88–95. https://doi.org/10.1016/j.scienta.2017.06.052.
32. Mian, G.; Cantone, P.; Golinelli, F. First evidence of the effect of a new biostimulant made by *Fabaceae* tissue on ripening dynamics and must technological main parameters in *Vitis vinifera* 'Ribolla Gialla.' *Acta Hortic.* **2022**, *1333*, 317–322. https://doi.org/10.17660/ACTAHORTIC.2022.1333.41.
33. Bohm, W. *Methods of Studying Root Systems*; Springer: Berlin/Heidelberg, Germany, 1979.
34. Palomares-Rius, J.; Castillo, P.; Navas-Cortes, J.A.; Jimenez_Diaz, R.M.; Tena, M. A proteomic study of in-root interactions between chickpea pathogens: The root-knot nematode *Meloidogyne artiellia* and the soil-borne fungus *Fusarium oxysporum* f. sp. *ciceris* race 5. *J. Proteom.* **2011**, *74*, 2034–2051. https://doi.org/10.1016/j.jprot.2011.05.026.
35. Fazeli, F.; Ghorbanli, M.; Niknam, V. Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. *Biol. Plant* **2007**, *51*, 98–103. https://doi.org/10.1007/S10535-007-0020-1.
36. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.
37. Martínez-Vilalta, J.; Sala, A.; Asensio, D.; Galiano, L.; Hoch, G.; Palacio, S.; Piper, F.I.; Lloret, F. Dynamics of non-structural carbohydrates in terrestrial plants: A global synthesis. *Ecol. Monogr.* **2016**, *86*, 495–516. https://doi.org/10.1002/ECM.1231.
38. Falginella, L.; Gaiotti, F.; Belfiore, N.; Mian, G.; Lovat, L.; Tomasi, D. Effect of early cane pruning on yield components, grape composition, carbohydrates storage and phenology in *Vitis vinifera* L. cv. Merlot. *OENO One* **2022**, *56*, 19–28. https://doi.org/10.20870/oeno-one.2022.56.3.5466.
39. Ari, H.; Erdemir, A. Effects of endodontic irrigation solutions on mineral content of root canal dentin using ICP-AES technique. *J. Endod.* **2005**, *31*, 187–189. https://doi.org/10.1097/01.don.0000137643.54109.81.
40. Mian, G.; Comuzzo, P.; Iacumin, L.; Zanzotti, R.; Celotti, E. Study to optimize the effectiveness of copper treatments for a low impact viticulture. *Internte J. Vitic. Enol.* **2021**. https://doi.org/10.53144/INFOWINE.EN.2021.06.05.4.
41. Tomasi, D.; Lonardi, A.; Boscaro, D.; Nardi, T.; Marangon, C.M.; De Rosso, M.; Flamini, R.; Lovat, L.; Mian, G. Effects of traditional and modern post-harvest withering processes on the composition of the *Vitis v*. Corvina grape and the sensory profile of Amarone wines. *Molecules* **2021**, *26*, 5198. https://doi.org/10.3390/MOLECULES26175198.
42. Tomasi, D.; Gaiotti, F.; Petoumenou, D.; Lovat, L.; Belfiore, N.; Mian, G. Winter pruning effect on root density, root distribution and root/canopy ratio in *Vitis vinifera* cv. Pinot gris. *Agronomy* **2020**, *10*, 1509. https://doi.org/10.3390/agronomy10101509.
43. Polat, Z.; Awan, Q.N.; Hussain, M.; Akgül, D.S. First report of *Phytopythium vexans* causing root and collar rot of kiwifruit in Turkey. *Plant Dis.* **2017**, *101*, 1058. https://doi.org/10.1094/PDIS-11-16-1554-PDN.
44. Panth, M.; Hassler, S.C.; Baysal-Gurel, F. Methods for management of soilborne diseases in crop production. *Agriculture* **2020**, *10*, 16. https://doi.org/10.3390/agriculture10010016.

45. Pistelli, L.; Iacona, C.; Miano, D.; Cirilli, M.; Colao, M.C.; Mensuali-Sodi, A.; Muleo, R. Novel *Prunus* rootstock somaclonal variants with divergent ability to tolerate waterlogging. *Tree Physiol.* **2012**, *32*, 355–368. https://doi.org/10.1093/treephys/tpr135.
46. Li, Z., Bai, D., Zhong, Y., Lin, M., Sun, L., Qi, X., Hu, C., Fang, J. Full-length transcriptome and RNA-seq analyses reveal the mechanisms underlying waterlogging tolerance in kiwifruit (*Actinidia valvata*). *Int. J. Mol. Sci.*, **2020**, 23(6), 3237. https://doi.org/10.3390/ijms23063237
47. Nguema-Ona, E.; Vicré-Gibouin, M.; Cannesan, M.A.; Driouich, A. Arabinogalactan proteins in root–microbe interactions. *Trends Plant Sci.* **2013**, *18*, 440–449. https://doi.org/10.1016/J.TPLANTS.2013.03.006.
48. Savi, T.; García González, A.; Herrera, J.C.; Forneck, A. Gas exchange, biomass and non-structural carbohydrates dynamics in vines under combined drought and biotic stress. *BMC Plant Biol.* **2019**, *19*, 408. https://doi.org/10.1186/S12870-019-2017-2.
49. Wang, Z.Y.; Gould, K.S.; Patterson, K.J. Comparative root anatomy of five Actinidia species in relation to rootstock effects on kiwifruit flowering. *Ann. Bot.* **1994**, *73*, 403–413. https://doi.org/10.1006/ANBO.1994.1050.
50. Buddhakulsomsiri, J.; Parthanadee, P.; Pannakkong, W. Prediction models of starch content in fresh cassava roots for a tapioca starch manufacturer in Thailand. *Comput. Electron. Agric.* **2018**, *154*, 296–303. https://doi.org/10.1016/j.compag.2018.09.016.
51. Gao, Q.; Xiong, T.; Li, X.; Chen, W. Calcium and calcium sensors in fruit development and ripening. *Sci. Hortic.* **2019**, *253*, 412–421. https://doi.org/10.1016/j.scienta.2019.04.069.
52. Michailidis, M.; Karagiannis, E.; Tanou, G.; Samiotaki, M.; Tsiolas, G.; Sarrou, E.; Stamatakis, G.; Ganopoulos, I.; Martens, S.; Argiriou, A.; et al. Novel insights into the calcium action in cherry fruit development revealed by high-throughput mapping. *Plant Mol. Biol.* **2020**, *104*, 597–614. https://doi.org/10.1007/S11103-020-01063-2.
53. Kong, D.; Wang, J.; Valverde-Barrantes, O.J.; Kardol, P. A framework to assess the carbon supply–consumption balance in plant roots. *New Phytol.* **2021**, *229*, 659–664. https://doi.org/10.1111/NPH.16807.
54. Schachtman, D.P.; Reid, R.J.; Ayling, S.M. Phosphorus uptake by plants: From soil to cell. *Plant Physiol.* **1998**, *116*, 447–453. https://doi.org/10.1104/PP.116.2.447.
55. Mian, G.; Cipriani, G.; Saro, S.; Bosetto, F.; Martini, M.; Ermacora, P. Effect of kiwifruit vine decline inducing soils on growth, mineral uptake, and protein content in different Actinidia genotypes radical systems. *Soil Plant Syst. Sci.* **2021**. https://doi.org/10.13140/RG.2.2.29542.34881.
56. Decorte, J.; Debersaques, F.; Mekers, O. Seasonal patterns of leaf mineral content of kiwiberry cultivars. *Acta Hortic.* **2015**, *1096*, 471–478. https://doi.org/10.17660/ACTAHORTIC.2015.1096.58.
57. Gullo, G.; Motisi, A.; Zappia, R.; Dattola, A.; Diamanti, J.; Mezzetti, B. Rootstock and fruit canopy position affect peach [*Prunus persica* (L.) Batsch] (cv. Rich May) plant productivity and fruit sensorial and nutritional quality. *Food Chem.* **2014**, *153*, 234–242. https://doi.org/10.1016/J.FOODCHEM.2013.12.056.
58. Triantafyllidis, V.; Zotos, A.; Kosma, C.; Kehayias, G.; Pittaras, A.; Drougas, P. Changes in plant species diversity as affected by shading conditions and continuous agricultural practices in kiwifruit orchards. *Emir. J. Food Agric.* **2021**, *33*, 11. https://doi.org/10.9755/ejfa.2021.v33.i11.2790.