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RESEARCH PAPER

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COMPARISON OF THE COURSE OF PATHOGENESIS CAUSED BY *Verticillium dahliae* (Kleb.) AND *Phytophthora cactorum* (Lebert and Cohn) J. SCHRÖT IN THREE STRAWBERRY CULTIVARS UNDER *in vitro* SELECTION

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ABSTRACT

The strawberries are more or less susceptible to *Verticillium dahliae* and *Phytophthora cactorum*, that's why a constant need to expand knowledge about the mechanisms of resistance to diseases caused by these fungi is requied. In the present study, the course of pathogenesis caused by *V. dahliae* and *P. cactorum* in three strawberry cultivars, *i.e.*, Elsanta, Feltar and Teresa, was compared under *in vitro* selection. The results showed that the extent and rate of development of disease symptoms were mostly insignificantly differentiated on subsequent observation dates. The resemblance observed in Verticillium wilt and phytophthorosis course within each microclone suggested the occurrence of a similar genetic mechanism of resistance response to both fungi. The likeness of average susceptibility to both pathogenic fungi evaluated with McKinney Disease Index (DI%) corresponded with the genetic similarity on the DNA level, which was estimated between selected submicroclones resistant to pathogens within each microclone. The microclone most susceptible to *V. dahliae* and to *P. cactorum* was Teresa (DI = 34.02%; 43.53%, respectively), whereas the lowest susceptibility to *V. dahliae* was observed in Elsanta microclone (DI = 29.85%). The microclone Feltar was considered to be the least susceptible to *P. cactorum* (DI = 19.50%). Moreover, a strong positive correlation was observed for the extent as well as for the rate between the development of both diseases in each microclone. Heritability in a broad–sense (h^2_{bs}) of the extent and rate of development of both pathogeneses reached values above 70%, which revealed the strong genetic determination of the resistance response to both pathogens in the analysed cultivars.

Keywords: *Fragaria* × *ananassa*, Verticillium wilt, phytophthorosis, resistance, DNA analysis, heritability, correlation

INTRODUCTION

The strawberry (*Fragaria* × *ananassa* Duch.) remains one of the most important soft fruits cultivated worldwide. Due to its many essential nutrients, this fruit is a staple in the diets of millions, and it is cultivated in various regions of the world, from the Arctic to the tropics [Hummer and Hancock 2009, Mukherjee and Gantait 2024].

In 2022, the global strawberry production amounted to 9.5 million tonnes from approximately 397,000 ha. The largest fruit producers are China (3.35 million tonnes) and the United States (1.26 million tonnes), followed by Turkey (728 thousand tonnes), Egypt (637 thousand tonnes), Mexico (568 thousand tonnes), Spain (325 thousand tonnes) and Russia (254 thou-

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sand tonnes). Poland's strawberry production in 2022 amounted to 199,000 tonnes, on an area of 31,000 ha [FAOSTAT 2024].

The susceptibility of strawberry plants to common biotic stress factors causes significant yield reductions and considerable economic losses [Wijerathna-Yapa and Hiti-Bandaralage 2023]. The main phytopathogens found on European strawberry plantations are the following fungi: *Phytophthora cactorum*, which causes leather crown rot and leather fruit rot in strawberries; *Verticillium dahliae*, which causes Verticillium wilt; *Colletotrichum acutatum*, which causes anthracnose; *Sphaerotheca macularis*, which causes powdery mildew in strawberries; *Phytophthora fragariae* var. *fragariae*, which is responsible for red root rot in strawberries; *Botrytis cinerea*, which causes grey mould on fruits; and *Alternaria alternata f. sp. fragariae*, which causes leaf spot [Parikka 2004, Olbricht and Hanke 2008, Hu et al. 2023, Jiménez et al. 2023, Alam et al. 2024a].

Some of the most dangerous diseases found on strawberry plantations include crown rot of strawberry rhizome and leather rot of strawberry fruit [Hantula et al. 2000]. Both of these diseases are caused by the fungus of the genus *Phytophthora*, specifically by its specialised form of *Phytophthora cactorum*, which is a polyphagous organism commonly found in soils of the temperate climate zone. It causes a disease called phytophthorosis in 200 species of cultivated plants from 60 families [Ribeiro 1978, Hantula et al. 2000, Bielenin 2002, Horst 2008, Orlikowski et al. 2012, Garrido et al. 2016, Orlikowski et al. 2017]. Ellis et al. [1998] report that the average losses due to diseases on strawberry plantations in the USA amount to 20–30%, and during a particularly severe outbreak of the disease in 1981 in the state of Ohio, the losses were as high as 50%. As reported by Bielenin [1999], losses in strawberry seedlings due to crown rot, on a disease-affected plantation, reached 40% in 1994, whereas in the years 1995–1997, due to leather fruit rot on the Elsanta, Senga Sengana and Syriusz cultivars, losses in fruit reached from 20 to 80%.

Another dangerous soil-borne disease that affects strawberries is Verticillium wilt, which is caused by the fungus *Verticillium dahliae* (Kleb.) [Kurze et al. 2001, Masny and Żurawicz 2008, Żebrowska 2010, Sowik et al. 2016]. The fungus *Verticillium dahliae* is a polyphagous fungus widespread throughout the world, which is particularly dangerous in temperate and tropical climates [Sanei et al. 2008]. It causes diseases in more than 300 species of cultivated and ornamental plants [Profic-Alwasiak 2000, Meszka et al. 2005, Fradin and Thomma 2006, Żebrowska et al. 2006, Meszka and Bielenin 2009, Nouri et al. 2012, Masny et al. 2014, Özer and Bayraktar 2016]. Meszka and Bielenin [2009] report that losses on strawberry plantations, caused by the occurrence of *V. dahliae* under conditions favourable to its development, can account for up to 80%. Shaw et al. [2005, 2010] state that plant mortality caused by the presence of a pathogen in the soil on plantations of particularly susceptible strawberry cultivars reaches up to 75%.

One of the key resistance breeding goals for this species is to develop strawberry cultivars with increased resistance to naturally occurring pathogens. Developing cultivars that are less susceptible or resistant to pathogens contributes to a significant reduction in the use of plant protection products in cultivation, which is harmful to humans and the environment, and thus to a reduction in production costs [Zurn et al. 2020]. The fungicides currently approved for use are not highly effective, or their use is not economical. In some countries, they are being withdrawn from the market, and their continued use is prohibited due to their harmful effects on the environment [Antanaviciute et al. 2015]. One should also expect an increase in the pathogens' resistance to frequently used chemicals.

The breeding work to improve the genetic resistance of the strawberry is greatly facilitated by the use of modern biotechnological methods, *i.e. in vitro* selection, or a comparison of cultivars at the molecular level [Mukherjee and Gantait 2024]. One of the most useful methods is the selection process in tissue cultures. In a compact laboratory space, numerous plants can be selected based on biotic and abiotic factors. Selection conducted under *in vitro* conditions, performed under controlled environment, is highly effective [Rai et al. 2011, Wijerathna-Yapa and Hiti-Bandaralage 2023]. The plants with increased resistance to the selective factor, obtained in this way, can be used for further breeding research as a source of resistance genes.

The strawberry's mechanisms of resistance to *V. dahliae* and *P. cactorum* are not fully understood or explained. Little is known about the genetic factors that mediate resistance to these pathogens [Zurn et al. 2020]. Lynn et al. [2024] report that the strawberry's resistance to powdery mildew is polygenically controlled. To date, several genes associated with the strawberry's resistance to various pathogens have been discovered. Ma et al. [2023] write that the *FaMBL1* gene plays an important role in the strawberry's response to fungal diseases caused by *Colletotrichum fioriniae* and *Botrytis cinerea*. The loci *FaRMp1* and *FaRMp2*, *FaRMp3* are associated with partial resistance to *Macrophomina phaseolina* [Alam et al. 2024b]. Amil-Ruiz et al. [2011] report that, in response to biotic stress, strawberries exhibit responses similar to those of other plants. It is assumed that this plant recognises pathogens and responds accordingly, based on innate immunity, which comprises all cellular and molecular mechanisms that the plant has to defend itself against pathogens. According to recent research into the *Fragaria* × *ananassa* genome, 247 *WRKY* transcription factors have been identified. Genes from the *WRKY*TF family are known for their various roles in the resistance response of several plant species to biotic and abiotic stress [Garrido-Gala et al. 2022, Vondracek et al. 2024]. There is a great need for further research to identify the mechanisms of resistance to pathogens infecting strawberries to support further breeding work.

The present paper compared the susceptibility of three strawberry cultivars to infection by two pathogenic fungi, *i.e. Verticillium dahliae* and *Phytophthora cactorum*, and the course of pathogenesis under *in vitro* culture conditions. The study determined correlations between susceptibility to Verticillium wilt and phytophthorosis, and estimated the genetic determination of susceptibility of the cultivars tested to pathogens using the heritability coefficient.

MATERIALS AND METHODS

Preparation of starting material for *in vitro* selection

The strawberry cultivars used in the experiment included Elsanta (Gorella × Holiday), Feltar ((Senga Tigaiga × Merton Dawn) S_1) and Teresa (Redgauntlet S1 × Senga Sengana S1), originated from the collection of the Department of Genetic and Horticultural Plant Breeding at the University of Life Sciences in Lublin. The Dutch variety Elsanta is widely known to be susceptible to root diseases caused by soil-borne pathogens. The range, rate, and course of disease development in other varieties have not yet been investigated.

From these donor plants, 3–4-cm-long terminal sections of young stolons, along with a node containing meristematic tissue, were collected (approximately 50–60 units from each cultivar) to propagate the cultivars in an *in vitro* culture. The stolons were rinsed for 30 minutes under running water, and then in a disinfectant solution (200 mL sterile distilled water, 2 mL sodium hypochlorite, 2–3 drops of wetting agent, *i.e.* a liquid detergent) for 15 minutes.

For the *in vitro* regeneration of explants, Murashige and Skoog medium (MS medium) was used [Murashige and Skoog 1962]. The medium was supplemented with 1 mg × dm^{-3} IAA (indolyl-3-acetic acid), 1 mg × dm^{-3} BAP (6-benzylaminopurine), and 0.01 mg × dm^{-3} GA_3 (gibberellic acid, tertiary derivative), and solidified with 7.5 g × dm^{-3} agar, pH 5.7. The medium was decontaminated in an autoclave at 121 °C, under a pressure of 0.1 MPa, for 20 min.

The surface-decontaminated explants were individually placed into test tubes with MS medium enriched with growth regulators, under aseptic conditions, under a laminar horizontal air flow chamber (type: KL-21, Polon, Poznań, Poland). The culture was maintained for 5–6 weeks under controlled environmental conditions (phytotron – temperature of 20 °C, a photoperiod of 16 hours' day / 8 hours' night, light intensity of 30 μmol × m^{-2} × s^{-1}).

After that time, the obtained microshoots were transferred, under aseptic conditions, onto the proliferating MS medium by adding growth regulators (step II of proliferation). The proliferating cultures were divided and transferred onto a fresh MS medium without growth regulators so that the obtained microshoots could take root. The culture was maintained until the microshoots were well rooted and reached at least the 4-leaf stage. In this way, three microclones Elsanta, Feltar, and Teresa were obtained. Each microclone consisted of 300 microplants which served as starting material for *in vitro* selection.

Preparation of the selective factor

The experiment used two phytopathogenic soil-borne fungi, *i.e. Phytophthora cactorum* (Lebert and Cohn)

J. Schröt and *Verticillium dahliae* Klebahn. Pure phytopathogen cultures (*Phytophthora cactorum*, catalogue No 1559 and *Verticillium dahliae* catalogue No 1093) were acquired from the Bank of Pathogens in Poznań (Poland). The pathogens were cultured on the Potato Dextrose Agar (PDA) medium containing 300 g × dm^{-3} potatoes, 20 g × dm^{-3} agar and 20 g × dm^{-3} glucose in a distilled water solution. The medium was decontaminated in a pressure autoclave at 121 °C, under a pressure of 0.1 MPa for 20 minutes. Then, 100 mg × dm^{-3} streptomycin was added to the medium under sterile conditions, after which it was poured into aseptic Petri dishes and left to solidify. The pathogen was inoculated onto PDA medium under a laminar air flow chamber and cultured in the dark for 3 weeks at 18–20 °C. Three-week-old pathogen cultures were used to prepare a homogenate of live mycelium, serving as an inoculum for infecting the microplants. Under sterile conditions, the pathogen cultures were flooded with 50 ml of sterile distilled water. The suspension was then homogenised and diluted with sterile distilled water at a ratio of 1 : 10 V/V to obtain an appropriate spore concentration ($10^5 \times mL^{-1}$).

In vitro culture selection

Well-rooted microplants of each microclone, at least at the stage of four leaves, were the starting material for the selection. The plants were inoculated, under aseptic conditions, by immersing them in the inoculum for 1 minute, after damaging the roots by cutting them with a scalpel to a length of approximately 1.5 cm. The inoculated plants were placed into Petri dishes with a pre-prepared agar medium without minerals and sucrose (7.5 g agar dissolved in 1000 mL distilled water, pH 5.7). The experiment was set up in three repetitions for each microclone and for each pathogen. One hundred plants were used for a single repetition. Microplants within each microclone were divided into two groups. One group was infected by *Verticillium dahliae* creating ‘submicroclone V.d.’. The second group was infected by *Phytophthora cactorum* creating ‘submicroclone P.c.’ A control sample was also prepared for each microclone, where well-rooted plants at a stage of at least 4 leaves, after damaging the roots (by cutting them with a scalpel to a length of approximately 1.5 cm), were immersed in sterile distilled water for 1 minute (mock inoculation). The plants were then placed into the Petri dishes with agar medium. For each microclone, the control comprised 100 plants. The experiment used a total of 3,200 plants.

Observation of the degree of plant infection

According to the methodology provided by Żebrowska et al. [2006] and Żebrowska [2011], the development of disease symptoms was observed on five observation dates: date I – 15 days after inoculation; date II – 30 days after inoculation; date III – 45 days after inoculation; date IV – 60 days after inoculation; date V – 75 days after inoculation.

According to the methodology provided by Sowik et al. [2001], Żebrowska [2011], and Sowik et al. [2015], the degree of plant infection was assessed on a five-point valuation scale, where: 0 – plants without infection symptoms (100% resistance); 1 – infection affecting 1 leaf (25%); 2 – infection affecting 2 leaves (50%); 3 – infection affecting 3 leaves (75%); 4 – infection affecting 4 or more leaves, or totally affected plants (100% susceptibility).

The course of pathogenesis in the microclones tested after being infected *in vitro* by *V. dahliae* and *P. cactorum* was assessed at successive observation dates using disease indices (DI) for the range (DI%) [McKinney 1923] and rate of infection development (DIp) [Simmonds 1987]. Using the mean value of the infection range index (DI%), susceptibility of the microclones to pathogens was determined as well.

The disease index for the infection development range (DI%) was calculated using the following formula:

$$DI(\%) = (\Sigma vn)/(NV) \times 100$$

where:
DI(%) – disease index,
v – numerical value of the infection class,
n – number of plants at a particular observation date in a particular class,
N – total number of infected plants in a particular sample,
V – numerical value of the highest class.

‘Class’ is a disease rating scale of McKinney formula which is used for Disease Index (DI) evaluation (McKinney 1923). The values of this parameter are given in McKinney publication [1923].

The rate of infection development over time (DIp) was determined using the disease index calculated according to the following formula:

$$\mathrm{DIp} = p \times I\,(1 - I)$$

where:
DIp – rate of infection development,
I – proportion of plants (%) with symptoms of infection at a particular observation date in a particular class,
p – pathogen reproduction rate; for susceptible cultivars, it was assumed that $p = 1$.

In addition, within each microclone, the following were assessed for the range and rate of pathogenesis development:

1) correlations using Pearson's linear correlation coefficient (r);

2) heritability in a broad sense (h^2_{bs}) (according to the formula $h^2_{bs} = V_G/V_p$) [Falconer and Mackay 1996], where:
h^2_{bs} – heritability in a broad sense,
V_G – genetic variation (including effects due to dominance and epistasis),
V_p – phenotypic variation.

The heritability coefficient values were estimated based on genetic interpretation of statistical components of variance for the single classification system (one-factor analysis of variance (ANOVA)). The numerical data were statistically analysed using the Statistica 13.1 program [2020]. The significance of the differences between the values of the examined traits was estimated with the Student's t-test and Duncan's multiple range test at $P \leq 0.05$.

DNA isolation, ISSR PCR and electrophoretic separation of the obtained products

Once the observations were completed, within each microclone, DNA was isolated from ten randomly selected plants (submicroclones) resistant to pathogenic selective factors, using the modified CTAB method described by Gawel and Jarret [1991]. DNA purity and concentration were determined using a Thermo Scientific NanoDrop 2000 spectrophotometer.

Inter simple sequence repeat (ISSR) markers were used to examine the genetic similarity, at the DNA level, of forms resistant to the two pathogens. The experiment analysed 16 ISSR markers provided by Sigma-Aldrich.

DNA amplification was carried out in a thermocycler (TProfessional Basic Gradient Biometra GmbH) at a final reaction volume of 15 μL for each reaction, which contained 1.5 μL PCR buffer (Dream Taq Buffer, Thermo Scientific), 1.2 μL dNTP (10 mM dNTP MIX, Thermo Scientific), 0.7 μL oligonucleotide primer, 0.9 μL $MgCl_2$ (25 mM, Thermo Scientific), 0.15 μL Taq DNA polymerase (Dream Taq DNA polymerase 5 U/μL, Thermo Scientific) and 3 μL DNA template. Each of the 35 polymerase chain reaction (PCR) cycles comprised 3 steps: 45 seconds at 94 °C (DNA denaturation); 1 minute at the annealing temperature; 2 minutes at 72 °C (DNA elongation). After 35 cycles, the samples were maintained at 72 °C for 7 minutes to carry out the final elongation step. The annealing temperature was adjusted to the melting temperature (T_M) for the primers used in the reaction. In order to check repeatability, the primers used in the experiment were tested twice on the same sample.

The ISSR PCR reaction products obtained were separated by electrophoresis on 1.5% agarose gel containing 0.1% of ethidium bromide in 1 × TBE buffer. The electrophoresis was carried out for 90 minutes at a voltage of 100 V. DNA fragments stained with ethidium bromide were visualised under ultraviolet light (UV). Photographs of the gel were taken using GeneSnap Syngene. Further analysis of the images was carried out using the GeneTools Syngene program. Based on the results obtained, a dendrogram was generated in the Past5 program, using the UPGMA (Unweighted Pair Group with Arithmetic Mean) method, showing the genetic similarity of the resistant plants at the DNA level.

RESULTS

Course of pathogenesis

By analysing the course of pathogenesis in the microclones after being infected by *P. cactorum* or *V. dahliae*, it was found that the intensity of microplants' dieback varied, and the range and rate of disease development were impacted by the microclone tested. Gradual chlorosis of the leaves occurred on plants inoculated with mycelium homogenate, resulting in dieback of entire microplants. The first symptoms of phytophthorosis and Verticillium wilt on the microplants became visible 15 days after inoculation.

As for the Elsanta microclone (Fig. 1), no significant differences were noted between the mean range (DI%) of Verticillium wilt and phytophthorosis at successive observation dates. Significant differences between the mean range of Verticillium wilt and phytophthorosis were found for the Feltar microclone (Fig. 2) only at observation dates II and III. In contrast, in the Teresa microclone (Fig. 3), the differences between the range of pathogeneses were insignificant at observation date I, whereas at the subsequent observation dates (II–V), these differences proved to be significant.

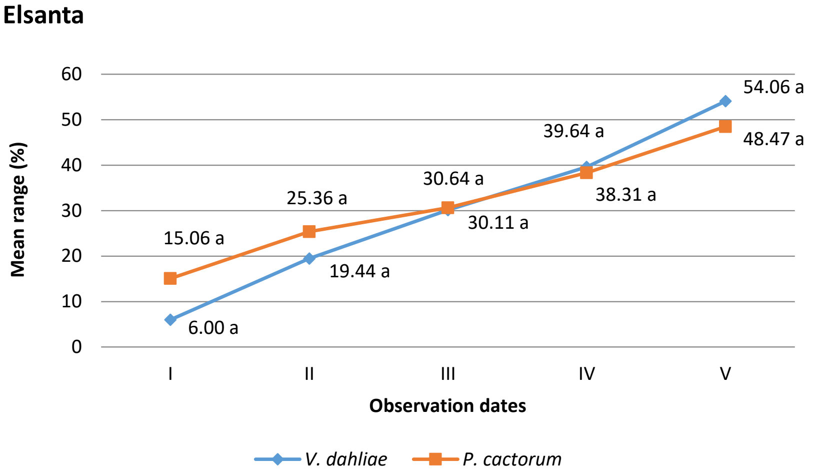

Fig. 1. Differences for the mean range of pathogenesis (%) in the Elsanta microclone after being infected by *P. cactorum* and *V. dahliae* at successive observation dates (I–V). The mean values marked with the same letter do not differ significantly at P ≤ 0.05

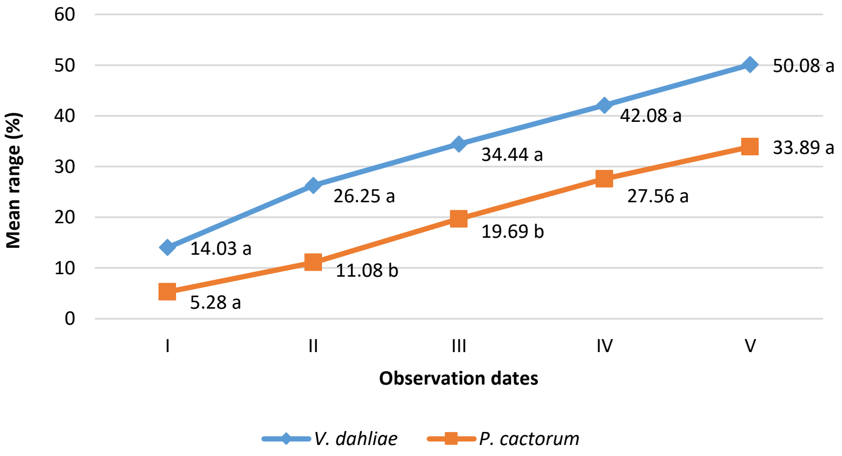

Fig. 2. Differences for the mean range of pathogenesis (%) in the Feltar microclone after being infected by *P. cactorum* and *V. dahliae* at successive observation dates (I–V). The mean values marked with the same letter do not differ significantly at P ≤ 0.05

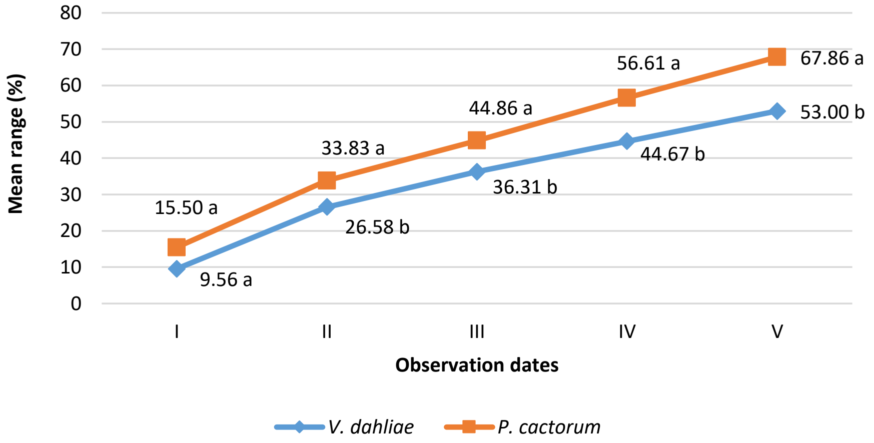

Fig. 3. Differences for the mean range of pathogenesis (%) in the Teresa microclone after being infected by *P. cactorum* and *V. dahliae* at successive observation dates (I–V). The mean values marked with the same letter do not differ significantly at $P \leq 0.05$

Table 1. Differences between the mean range (%) of Verticillium wilt and phytophthorosis in the microclones tested. The mean values marked with the same letter do not differ significantly at $P \leq 0.05$

	Elsanta	Feltar	Teresa
V. dahliae	29.85 a	33.38 a	34.02 a
P. cactorum	31.57 a	19.50 b	43.53 a

Statistical analysis (ANOVA) showed the occurrence of significant differences between the total mean range of both diseases only in the Feltar microclone. As for the Elsanta and Teresa microclones, these differences were insignificant (Tab. 1).

By analysing the differences in the rate of development (DIp) of Verticillium wilt and phytophthorosis at successive observation dates, it was concluded that they were only significant for the first observation date in the Elsanta microclone (Fig. 4). In the Feltar (Fig. 5) and Teresa (Fig. 6) microclones, no significant differences were noted in the rate of development of phytophthorosis and Verticillium wilt at successive observation dates.

No significant difference was noted between the total mean rate of development of Verticillium wilt and phytophthorosis in all the microclones tested (Tab. 2).

Correlations

The correlations for the range and rate of development of Verticillium wilt and phytophthorosis, estimated using correlation (r_{xy}) and regression (b_{yx}) coefficients, are provided in Tables 3 and 4 (resp.).

Heritability

The calculated heritability coefficients in a broad sense (h^2_{bs}) for the range of development of Verticillium wilt and phytophthorosis reached values of 85.04% and 97.97%, respectively, while for the rate of development, 75.04% and 77.90%, respectively.

Molecular analyses

Molecular analyses involved determining genetic similarity assessed by cluster analysis (UPGMA), based on DNA polymorphism identified in selected

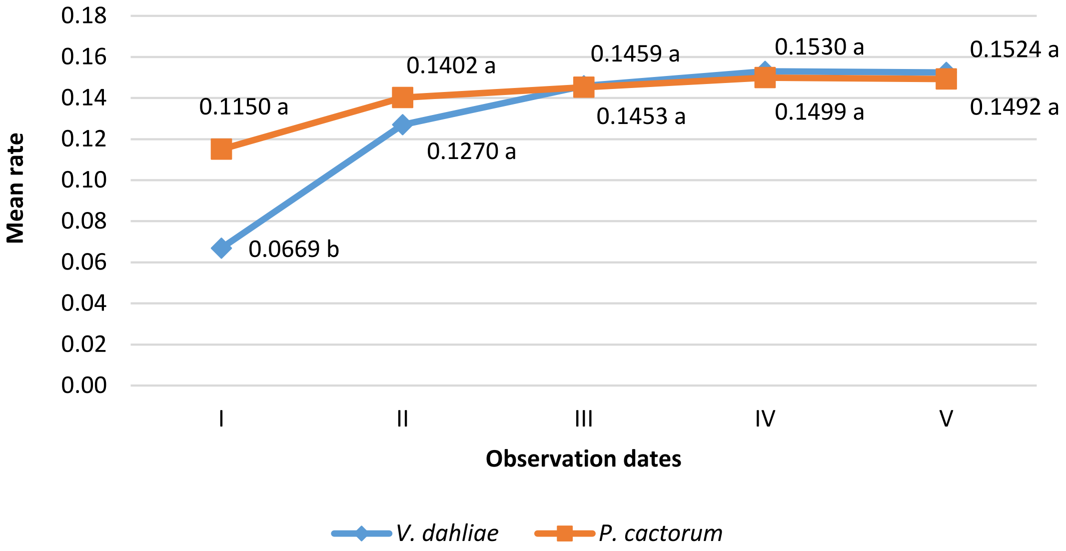

Fig. 4. Differences for the mean rate of infection development in the Elsanta microclone after being infected by *P. cactorum* and *V. dahliae* at successive observation dates (I–V). The mean values marked with the same letter do not differ significantly at P ≤ 0.05

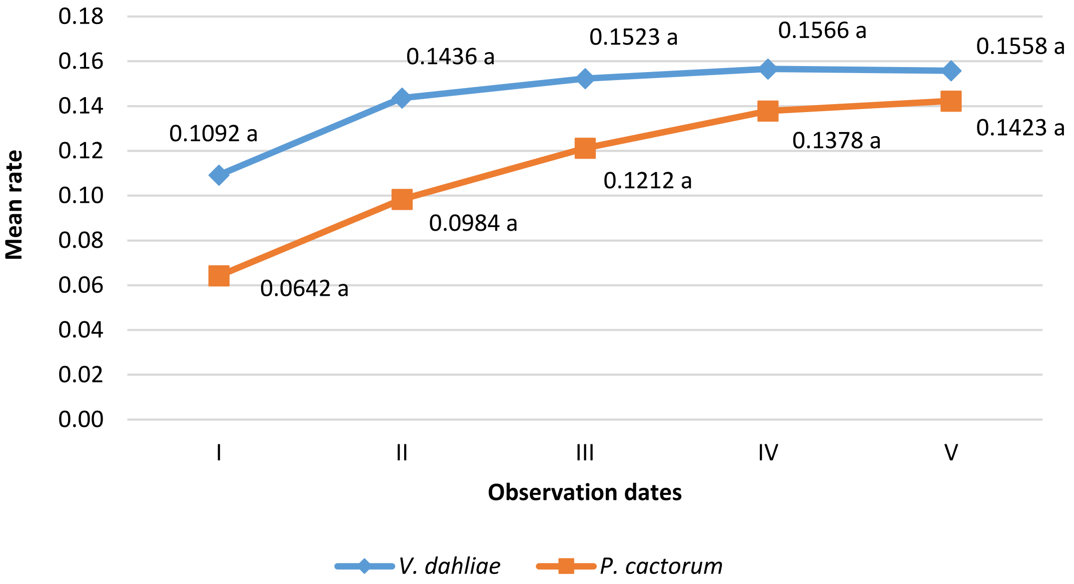

Fig. 5. Differences for the mean rate of infection development in the Feltar microclone after being infected by *P. cactorum* and *V. dahliae* at successive observation dates (I–V). The mean values marked with the same letter do not differ significantly at P ≤ 0.05

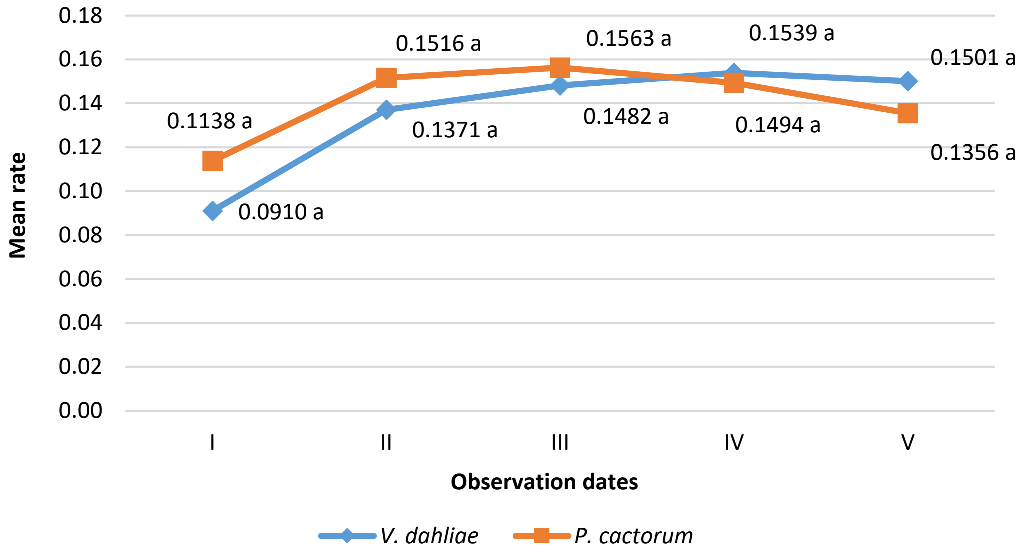

Fig. 6. Differences for the mean rate of infection development in the Teresa microclone after being infected by *P. cactorum* and *V. dahliae* at successive observation dates (I–V). The mean values marked with the same letter do not differ significantly at $P \leq 0.05$

Table 2. Differences between the total mean rate of development of Verticillium wilt and phytophthorosis in the microclones tested. The mean values marked with the same letter do not differ significantly at $P \leq 0.05$

	Elsanta	Feltar	Teresa
V. dahliae	0.1290 a	0.1435 a	0.1360 a
P. cactorum	0.1399 a	0.1128 a	0.1414 a

Table 3. Correlations between the range of development of Verticillium wilt and phytophthorosis in the strawberry microclones tested

	Elsanta	Feltar	Teresa
r_{xy}	0.7503*	0.7748*	0.9876*
R^2	0.5630	0.6000	0.9753
1) b_{yx}	0.6295	0.7353	0.8088
2) b_{yx}	0.8943	0.8164	1.2060

r_{xy} – Pearson's linear correlation coefficient; * – correlation significant at $P \leq 0.05$; R^2 – determination coefficient; b_{yx} – linear regression coefficient, where: 1) x – development of Verticillium wilt, y – development of phytophthorosis; 2) x – development of phytophthorosis, y – development of Verticillium wilt plant material using ISSR markers. The material consisted of microplants which withstood the pressure of selective factors by forming pathogen-resistant submicroclones within each microclone. For 8 out of the 16 ISSR primers tested, amplification products were obtained (Tab. 5). A single primer was involved in the synthesis of three (primer 11) to eight (primer 8) – an average of 5.9 – polymorphic products (Tab. 5).

For eight primers analysed, 52 DNA fragments were obtained, of which 47 (89.58%) were polymorphic. The size of the sequences obtained for individual ISSR primers ranged from 300 to 3,500 base pairs (Tab. 5). Primer 14 allowed all the cultivars (microclones) analysed to be distinguished from each other.

Analysis of the UPGMA dendrogram, plotted on the basis of the similarity matrix, enabled the determination of genetic similarity between the analysed submicroclones resistant to Verticillium wilt and phytophthorosis (Fig. 7). The presence of two main cluster groups was identified. The first group (I) and the second group (II) were 57% similar to each other.

In the first (I) group, submicroclones Elsanta, resistant to *V. dahliae* and *P. cactorum*, which were characterised by 88% genetic similarity, were most similar to each other. A comparable high similarity was demonstrated between the Teresa submicroclones (84%). The

Table 4. Correlations between the rate of development of Verticillium wilt and phytophthorosis in the strawberry microclones tested

	Elsanta	Feltar	Teresa
r_{xy}	0.9981*	0.9511*	0.8373
R^2	0.9962	0.9046	0.7011
1) b_{yx}	0.3970	1.5408	0.5559
2) b_{yx}	2.5086	0.5871	1.2612

Explanation as in Table 3

Table 5. Assessment of the polymorphism of the strawberry genome tested using ISSR primers

Number of primer	Sequence (5'–3')	Number of loci			
		Total	Polimorphic	%P	Size range (bp)
1	VBVACACACACACACAC	7	7	100.0	400–2000
7	HVHTGTTGTTGTTGTTGT	6	5	83.3	600–3000
8	BDBCACCACCACCACCAC	8	8	100.0	300–1500
10	GAAGAAGAAGAAGAAGAA	5	5	100.0	1000–1500
11	ATGATGATGATGATGATG	6	3	50.0	300–3000
13	GATAGATAGATAGATAGATA	7	7	100.0	700–3500
14	GACAGACAGACAGACAGACA	6	5	83.3	300–2500
16	AGTGAGTGAGTGAGTG	7	7	100.0	300–2500
	Mean	6.5	5.9	–	–
	Totality	52	47	89.58	300–3500

Explanation of symbols: H = A + T + C, B = G + T + C, D = G + A + T, V = G + A + C
%P – percentage of polymorphism

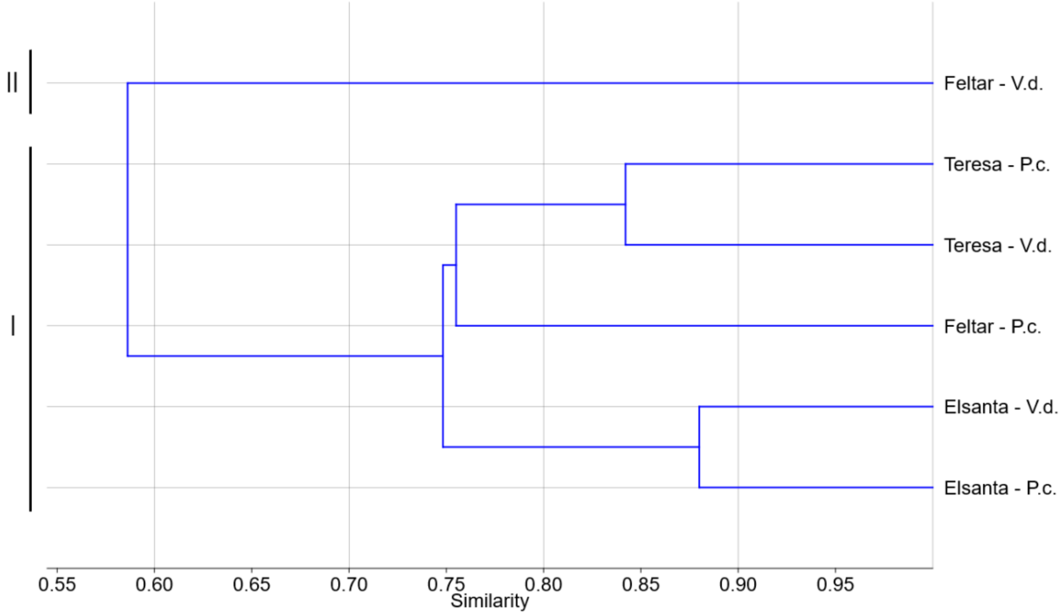

Fig. 7. UPGMA dendrogram generated in the Past5 program, showing the genetic similarity between the analysed strawberry submicroclones resistant to phytophthorosis and Verticillium wilt

Legend for the dendrogram:
Elsanta; Feltar; Teresa – P.c. – submicroclone resistant after being infected by *Phytophthora cactorum*
Elsanta; Feltar; Teresa – V.d. – submicroclone resistant after being infected by *Verticillium dahliae*

Feltar submicroclone, resistant to *P. cactorum*, exhibited 76% similarity to the analysed Teresa submicroclones.

DISCUSSION

Verticillium wilt and phytophthorosis caused by soil-borne pathogenic fungi, *i.e.*, *Verticillium dahliae* and *Phytophthora cactorum*, are very serious, non-chemically controlled infectious diseases of many plant species, including strawberries. An important factor limiting the occurrence of these diseases on strawberry plantations is the cultivation of cultivars with genetically determined resistance to these diseases.

Selection aimed at obtaining new strawberry cultivars, resistant or tolerant to *Verticillium dahliae* or *Phytophthora cactorum*, is currently one of the major breeding directions for this species in Poland and worldwide. Sowik et al. [2008] and Żebrowska [2010], thanks to the application of selection on the pathogenic fungus *Verticillium dahliae* in an *in vitro* culture, examined the resistance of certain strawberry cultivars and breeding clones to this pathogen. Żebrowska [2010] concluded that the susceptibility of the tested strawberry cultivars under *in vitro* conditions was similar to the susceptibility of these cultivars under field conditions, and that the selection in an *in vitro* culture can be successfully used in breeding programmes. It should be emphasised that the defensive response of plants, observed during *in vitro* selection under constant and controlled environmental conditions, reflects the genetically determined host-pathogen interaction. The use of *in vitro* eliminates variable environmental conditions that can affect a plant's genetically determined resistance to stress factors. This makes *in vitro* selection a highly valuable method for studying the genetic mechanisms behind plant resistance. The results obtained under these conditions, regarding the course of pathogenesis, are completely reliable and provide accurate information on the genetic resistance of plants to pathogens.

In the present study, each microclone responded differently to two pathogens, which was due to the genetic differences between the cultivars from which the microclones were derived. In contrast, the similar course of phytophthorosis and Verticillium wilt observed in each microclone, along with the slight and mostly insignificant differences in the range and rate of development of both diseases at successive observation dates, suggests that there may be similar mechanisms of genetic resistance to these pathogens. Thirty days after infection, there was a noticeable slowdown in the rate of infection development. In addition, the high genetic similarity of over 80% existing between submicroclones with a similar susceptibility to both pathogens (*e.g.* Elsanta and Teresa) also suggests that similar genetic defence mechanisms are operating in these cultivars. However, the significant difference found in the Feltar microclone in the susceptibility to both pathogens was justified by the lower genetic similarity at the DNA level (75%) between the submicroclones resistant to phytophthorosis and Verticillium wilt. The high and positive correlations, estimated for both the range and rate of development of pathogenesis in the analysed microclones, and the high heritability coefficient of these traits further confirm the activation of similar genetic defence mechanisms in plants in response to infection by *V. dahliae* and *P. cactorum*.

The results obtained in the present study suggest that there is no single defined resistance mechanism within the *Fragaria* × *ananassa* species, and that the plant-pathogen response is controlled by numerous factors, including genetic factors. Parikka [2003], when testing strawberry cultivars cultivated in Finland in terms of their susceptibility to phytophthorosis, noted that the susceptibility degree in particular cultivars can vary depending on the season in which the infection occurred. Resistance to *P. cactorum* was high in the plants during winter, but decreased during summer. Eikemo et al. [2003] report that the degree of resistance can also be affected by the plant's age and physiological condition.

The existence of differences in the susceptibility of a cultivar to different pathogens is confirmed by a study by Pérez-Jiménez et al. [2012]. The researchers tested the resistance/susceptibility of several strawberry cultivars cultivated in Spain to *Phytophthora cactorum*, *Verticillium dahliae* and two *Xanthomonas fragariae* strains (IVIA 349.94a and NCPPB 1469). Based on the results obtained, they concluded that none of the tested cultivars was, to a comparable degree, resistant to all selective factors. The Sieger cultivar, tolerant to both *P. cactorum* and *V. dahliae*, was resistant to the two tested *X. fragariae* strains. The Aguedilla cultivar, resistant to the IVIA 349.94a strain of *X. fragariae*, and tolerant to the NCPPB 1469 strain of *X. fragariae* as well as to *P. cactorum*, appeared to be susceptible to *V. dahliae*.

Shokaeva et al. [2011], when assessing the susceptibility of several strawberry somaclones to *Botrytis cinerea*, *Phytophthora cactorum* and salination, found that there were differences in susceptibility to the selective factor among the somaclones tested. Similar conclusions were reached by Eikemo et al. [2003], who examined the susceptibility of 26 strawberry cultivars to phytophthorosis. The results of their study show that resistance to *P. cactorum* varies greatly between cultivars. Zurn et al. [2020], when assessing different strawberry genotypes for their resistance to *Verticillium dahliae*, *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *fragariae*, found different levels of resistance to the pathogens tested within the cultivars and breeding clones. Based on the above results, it can be concluded that within the *Fragaria* × *ananassa* species, there is no single pathogen resistance mechanism, rather it largely depends on the cultivar's origin. Eikemo et al. [2003] and Schafleitner et al. [2013] report that resistance to *P. cactorum* appears to be polygenic, but not all factors affecting it are known. The results obtained in the present study, and the results obtained by other authors, suggest that each cultivar responds differently to a particular selective factor. They also suggest the lack of uniform resistance to many selective factors within the cultivar, *i.e.* polygenicity of resistance.

The correlations found in the present study for the range and rate of development of Verticillium wilt and phytophthorosis, estimated using correlation and regression coefficients, indicated the possibility of mutual prediction of the course of these diseases in the strawberry. The greatest predictable intensity of phytophthora symptoms, as the Verticillium wilt symptoms were expanding, and with the reverse relationship, was noted in the Teresa microclone, whereas the least predictable intensity was in the Elsanta microclone. By analysing the interrelationships between the disease development rate, it was found that the greatest predictable increase in the rate of phytophthorosis development, with an increase in the rate of

Verticillium wilt development, occurred in the Feltar microclone. The greatest predictable increase in the rate of Verticillium wilt development with an increase in the phytophthorosis rate occurred in the Elsanta microclone, and the least predictable increase was in the Feltar microclone.

The available domestic and foreign literature provides no information on correlations between the susceptibility of the strawberry to *P. cactorum* and *V. dahliae*. Few research papers in the field of resistance breeding of this species mention several other important correlations. Shaw et al. [1996] reported a correlation between resistance and fruit firmness in strawberries, while no correlation was found between resistance and productivity. Shaw et al. [2005], in the case of Verticillium wilt, observed no correlation between the percentage of infected parent plants and the percentage of infection in plants formed from the stolons. Fang et al. [2011] noted significant correlations between strawberry crown diseases and plant dieback, as well as between root diseases and plant dieback. The increased incidence of strawberry mortality, as well as root diseases, was negatively correlated with the dry weight of the plants. The authors found no significant correlation between strawberry crown diseases and root diseases. Liang and Lin [2014] observed a significant correlation between susceptibility of the leaves and susceptibility of the fruit to mildew, whereas the coefficients of correlation between susceptibility and the fruit weight, length and width were not significant. As reported by Eikemo and Stensvand [2015], no correlation was found between the resistance to strawberry crown rot and resistance to leather rot of the fruit. Lynn et al. [2024] noted that leaf disease phenotypes were not genetically correlated with fruit disease phenotypes, which suggests that two different genetic mechanisms may control the disease resistance of leaves and fruit. Ukalska et al. [2006] reported that there is a phenotypic correlation between strawberry leaf spot and the density of the plants, as well as between the flowering time and the plants' susceptibility to powdery mildew.

The heritability of resistance for the strawberry has not been well documented [Pincot et al. 2020]. High heritability results, ranging from 75% to 97%, obtained in the present experiment, suggest that in the strawberry microclones tested, susceptibility to Verticillium wilt and phytophthorosis are traits highly heritable, *i.e.* strongly determined genetically, and readily passed on to offspring. The results indicated that only genotypes that are least susceptible to Verticillium wilt and phytophthorosis should be selected for breeding work aimed at increasing strawberry's resistance to these diseases. A similarly high heritability result for Verticillium wilt was obtained by Shaw et al. [1996]. In their experiment, the heritability coefficient value fell within the range of h^2_{bs} = 84–88%. Shaw et al. [2008] estimated that the heritability coefficient for phytophthorosis was 63%. This coefficient proved similar for strawberry resistance to mildew, as observed by Lifshitz et al. [2007] and Liang and Lin [2014]. Lifshitz et al. [2007] report that heritability with respect to mildew was 50%, whereas in an experiment by Liang and Lin [2014], it ranged from 66% to 68%.

The results obtained in the present study demonstrated the usefulness of *in vitro* culture selection for conducting more detailed research into genetic resistance mechanisms in strawberries. This approach supports the continued development of breeding efforts within this species.

CONCLUSION

The susceptibility of strawberry microplants to infection by *Verticillium dahliae* and *Phytophthora cactorum* varied, and the range and rate of disease development depended on the microclone studied. The similar course of phytophthorosis and Verticillium wilt observed in each microclone, confirmed by slight and mostly insignificant differences in the range and rate of development of both diseases, suggested the occurrence of similar mechanisms of genetic resistance to these pathogens. Moreover, the large genetic similarity (reaching over 80%) observed between submicroclones with similar susceptibility to both pathogens (*e.g.*, Elsanta and Teresa) also indicated the operation of similar genetic defense mechanisms in these varieties. High and positive correlations estimated for both the scope and the rate of development of pathogens in the analyzed microclones and the high value of the heritability coefficient of these traits additionally confirmed the activation of similar genetic defense mechanisms in plants in response to infection caused by *V. dahliae* and *P. cactorum*.

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