

SPRAWOZDANIE MERYTORYCZNE
z realizacji zadania na rzecz postępu biologicznego w produkcji roślinnej
w 2024 roku

Zadanie badawcze 25

Odporność roślin rzepaku na choroby powodowane przez grzyby i pierwotniaki

Kierownik:

Prof. Małgorzata Jędryczka, Instytut Genetyki Roślin PAN w Poznaniu
mjed@igr.poznan.pl

Wykonawcy:

dr Joanna Kaczmarek, mgr Witold Irzykowski – Instytut Genetyki Roślin PAN w Poznaniu
Piotr Stefański – Hodowla Roślin Strzelce
prof. Jan Piekarczyk, dr Jakub Ceglarek – Uniwersytet im. A. Mickiewicza w Poznaniu
prof. Agnieszka Pszczółkowska, dr hab. Adam Okorski, dr Łukasz Pauksto
– Uniwersytet Warmińsko-Mazurski w Olsztynie
Personel pomocniczy IGR PAN i HR Strzelce

Cele zadania badawczego PB25 w 2024 roku

1. Oznaczenie gatunków, patotypów bądź ras patogenów aktualnie wywołujących choroby rzepaku w Polsce.

V

Mierniki

<i>Plenodomus</i> 50 prób	<i>Verticillium</i> 25 prób
<i>Sclerotinia</i> 25 lokalizacji	<i>Plasmodiophora</i> 8 patotypów

2. Ocena odporności genotypów rzepaku o zróżnicowanym pochodzeniu na choroby.

V

Mierniki

<i>Plenodomus</i> 90 × 2 testy	<i>Plasmodiophora</i> 90	<i>Verticillium</i> 90
--------------------------------	--------------------------	------------------------

3. Uzyskanie form rzepaku ze zróżnicowaną odpornością na patotypy *Plasmodiophora brassicae* dla oznaczenia dziedziczenia odporności na kiłę kapusty oraz charakterystyka genomów patogenu.

V

Mierniki

20 form rzepaku	2 izolaty
-----------------	-----------

1. Oznaczenie powiązania pomiędzy fenomem roślin rzepaku a tolerancją na zgniliznę twardzikową w doświadczeniu polowym

V

Mierniki

16 odmian	2 czynniki	26 cech
-----------	------------	---------

Materiały i metody

1. Testy oceny odporności rzepaku na choroby :

- a) testy liścieniowe – odporność na suchą zgniliznę kapustnych (geny R)
- b) test Winkelmana – odporność na suchą zgniliznę kapustnych (typ APR)
- c) odporność na werciliozę
- d) odporność na kiłę kapusty

2. Przygotowanie agarowych i płynnych pożywek mikrobiologicznych.

3. Metody mykologiczne: izolacja patogenów z porażonych tkanek roślinnych, pasażowanie grzybów, identyfikacja morfologiczna i mikroskopowa.

4. Identyfikacja i charakterystyka molekularna izolatów grzybów chorobotwórczych wobec rzepaku.

5. Identyfikacja patotypów *Plasmodiophora brassicae* i sekwencjonowanie genotypów

1. Opis fenotypu roślin rzepaku z zastosowaniem kamer RGB i multispektralnych zamontowanych na dronie oraz na ciągniku z naczepą.

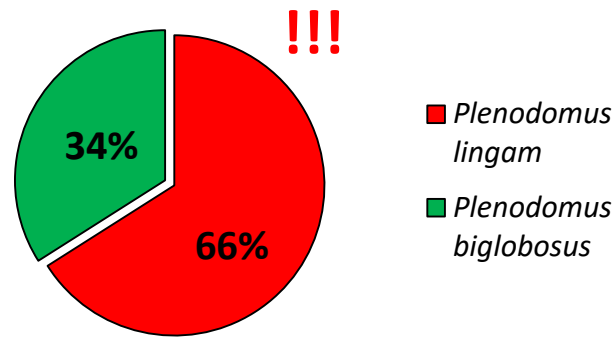


Temat 1: Identyfikacja i charakterystyka aktualnej populacji patogenów rzepaku w Polsce

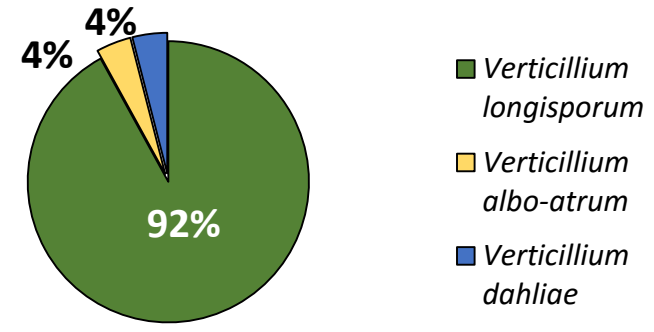
CEL: Oznaczenie gatunków, patotypów bądź ras patogenów aktualnie wywołujących choroby rzepaku w Polsce.

Plenodomus 50 prób

Latem 2024 roku, przed żniwami, na łądach rzepaku dominował grzyb *Plenodomus lingam* (66%)



Verticillium 25 prób



Proporcja gatunków *Verticillium* na łądach i resztkach poźniwnych rzepaku ozimego zebranych z pól na terenie Polski

Plasmodiophora 8 patotypów

Objawy kiły kapusty powodowanej przez *Plasmodiophora brassicae* w Polsce na kiło-odpornej odmianie rzepaku ozimego w północno-wschodniej Polsce w 2024 roku



Sclerotinia 25 lokalizacji

Porażenie roślin rzepaku ozimego grzybem *Sclerotinia sclerotiorum* w Polsce w 2024 roku na badanych plantacjach wynosiło poniżej 1% i rzadko znajdowano pola z objawami tej choroby na rzepaku



Temat 2: Poszukiwanie źródeł odporności na choroby rzepaku

CEL: Ocena odporności na choroby u genotypów rzepaku o zróżnicowanym pochodzeniu

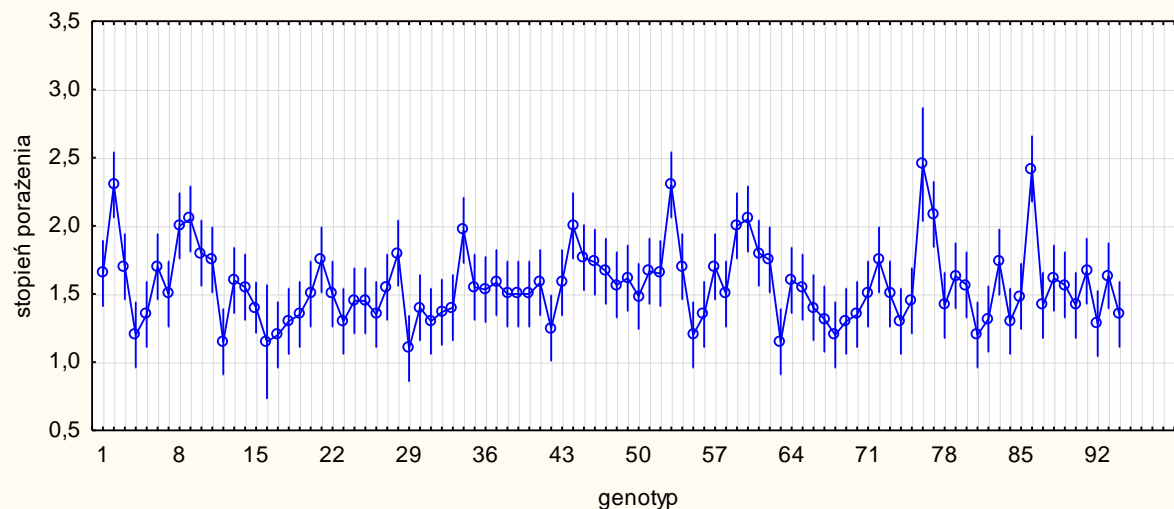
Test Winkelmana wiosna 2024



Ocenę objawów chorobowych wykonano na podstawie skali G2 (0-4), gdzie 0 oznacza rośliny zdrowe, 4 – całkowite wypróchnienie łodygi.

Wyniki

Pionowe słupki oznaczają 0,95 przedziały ufności



Kontrola 1	Kontrola 2	Kontrola 3	Inokulacja 1	Inokulacja 2	Inokulacja 3
------------	------------	------------	--------------	--------------	--------------

Badane genotypy rzepaku:

1-24 HR Smolice/Bąków (SMO)

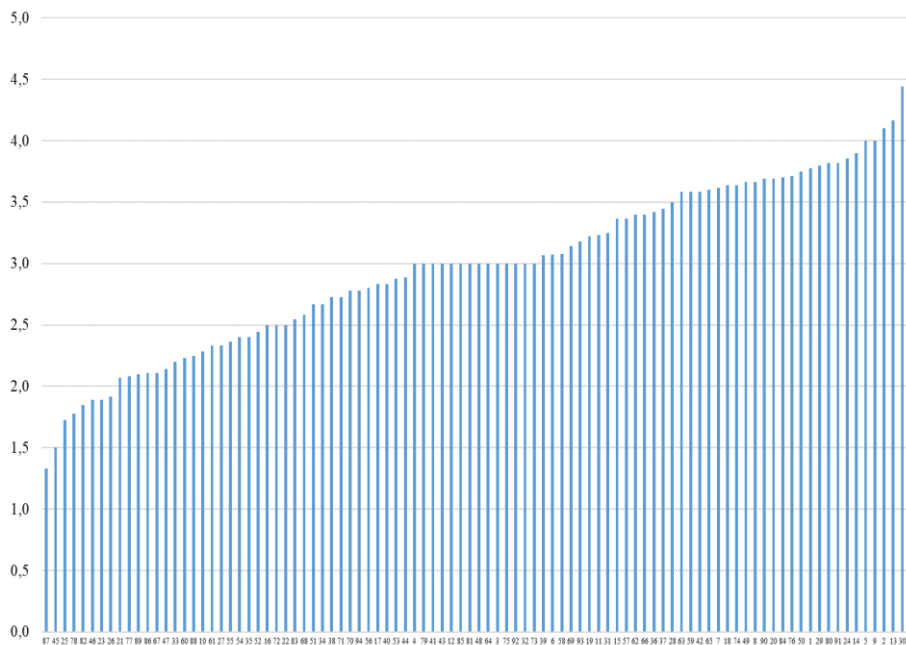
25-59 HR Strzelce, Oddz. Borowo (BOH)

60-94 HR Strzelce, Oddz. Matyszyn (MAH)

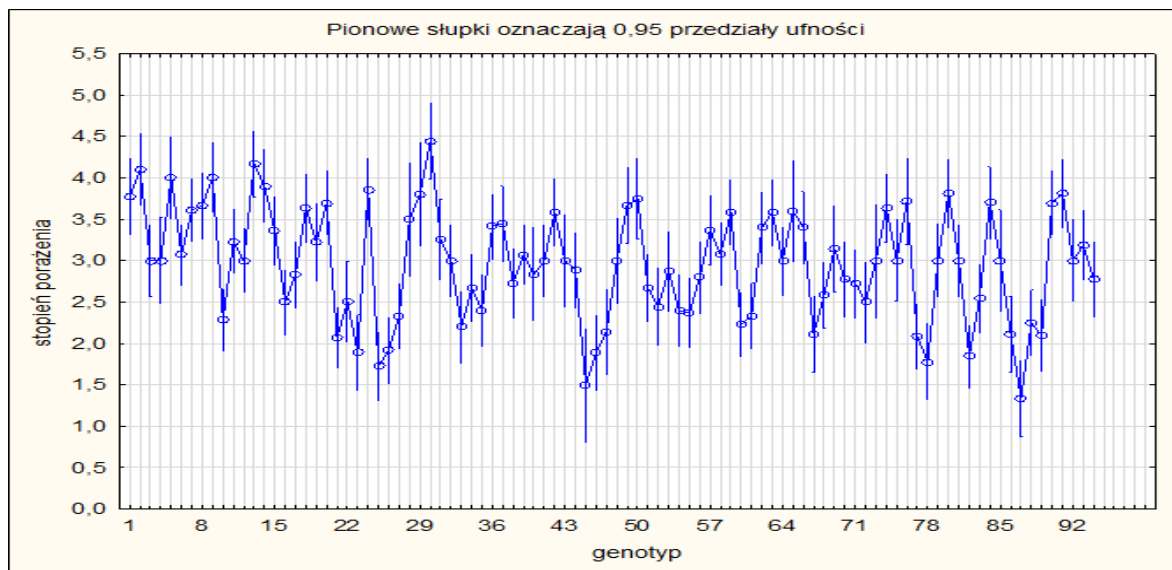
Temat 2: Poszukiwanie źródeł odporności na choroby rzepaku

Ocena odporności na choroby u genotypów rzepaku o zróżnicowanym pochodzeniu

Ocenę objawów chorobowych wykonano na podstawie skali 0-9, gdzie 0 oznacza rośliny zdrowe, 9 oznacza rośliny żółte i zaschnięte.



Korzenie umieszczano w zawiesinie zarodników *Verticillium longisporum* (1×10^6 zarodników/ml)



Temat 2: Poszukiwanie źródeł odporności na choroby rzepaku

Ocena odporności na choroby u genotypów rzepaku o zróżnicowanym pochodzeniu

L.P.	Kod	Odmiana/Ród	Wynik testu		L.P.	Kod	Odmiana/Ród	Wynik testu	
			Jeden izolat	Mix izolatów				Jeden izolat	Mix izolatów
1	SMO-1	BK 1/23	3,40	3,78	48	BO_24	BRH_21604	3,22	3,55
2	SMO-2	BK 2/23	3,42	4,00	49	BO_25	BRH_21605	3,87	3,67
3	SMO-3	BK 3/23	3,44	3,00	50	BO_26	BRH_21606	3,52	4,00
4	SMO-4	BK 4/23	3,50	3,00	51	BO_27	BRH_21610	3,11	4,00
5	SMO-5	BK 5/23	3,58	4,00	52	BO_28	BRH_21611	3,88	4,00
6	SMO-6	BK 6/23	3,58	3,07	53	BO_29	BRH_21613	3,98	3,87
7	SMO-7	BK 7/23	3,67	3,62	54	BO_30	BRH_21614	4,00	4,00
8	SMO-8	BK 8/23	4,00	3,10	55	BO_31	BRH_20112	4,00	3,00
9	SMO-9	BK 9/23	3,39	3,27	56	BO_32	BRH_21511	3,68	3,88
10	SMO-10	BK 10/23	3,23	3,08	57	BO_33	BO_26-9	3,98	3,67
11	SMO-11	BK 11/23	3,00	3,10	58	BO_34	BO_8-21	4,00	3,78
12	SMO-12	BK 12/23	4,00	3,10	59	BO_35	BO_1940	3,98	3,58
13	SMO-13	BK 13/23	3,90	3,17	60	MAH01		3,67	3,67
14	SMO-14	BK 14/23	3,36	3,17	61	MAH02		3,87	3,78
15	SMO-15	BK 15/23	3,66	3,27	62	MAH03		3,67	3,67
16	SMO-16	BK 16/23	3,83	3,33	63	MAH04		3,67	3,67
17	SMO-17	BK 17/23	3,64	3,38	64	MAH05		3,78	3,67
18	SMO-18	BK 18/23	3,22	3,50	65	MAH06		3,87	3,64
19	SMO-19	BK 19/23	3,69	3,78	66	MAH07		3,65	3,22
20	SMO-20	BK 20/23	3,69	4,00	67	MAH08		3,00	3,69
21	SMO-21	BAZALT	4,00	3,34	68	MAH09		4,00	3,07
22	SMO-22	BKH 6221	3,58	3,33	69	MAH10		3,07	3,78
23	SMO-23	BONO	3,60	3,60	70	MAH11		3,62	3,89
24	SMO-24	BRENDY	3,62	3,86	71	MAH12		3,67	3,86
25	BO_1	BRH_21128	3,64	3,33	72	MAH13		4,00	3,67
26	BO_2	BRH_21129	3,64	3,64	73	MAH14		3,87	3,92
27	BO_3	BRH_21132	3,67	3,54	74	MAH15		3,23	3,87
28	BO_4	BRH_21137	3,67	3,50	75	MAH16		3,00	3,50
29	BO_5	BRH_21139	3,69	3,80	76	MAH17		4,00	3,80
30	BO_6	BRH_21146	3,69	4,00	77	MAH18		3,90	4,00
31	BO_7	BRH_21148	3,70	4,00	78	MAH19		3,36	4,00
32	BO_8	BRH_21152	3,71	4,00	79	MAH20		3,32	3,67
33	BO_9	BRH_21246	3,71	3,85	80	MAH21		3,22	3,33
34	BO_10	BRH_21249	3,75	3,98	81	MAH22		3,89	3,67
35	BO_11	BRH_21251	3,78	3,87	82	MAH23		3,56	3,67
36	BO_12	BRH_21253	3,80	3,42	83	MAH24		3,87	3,42
37	BO_13	BRH_21301	3,82	3,44	84	MAH25		3,55	3,44
38	BO_14	BRH_21307	3,82	3,73	85	MAH26		3,87	3,67
39	BO_15	BRH_21309	3,86	3,85	86	MAH27		3,45	3,07
40	BO_16	BRH_21310	3,88	3,67	87	MAH28		3,86	3,83
41	BO_17	BRH_21312	3,90	3,00	88	MAH29		3,67	3,67
42	BO_18	BRH_21315	4,00	3,58	89	MAH30		4,00	3,58
43	BO_19	BRH_21317	4,00	4,00	90	MAH31		3,86	3,00
44	BO_20	BRH_21320	4,00	3,22	91	MAH32		3,56	3,89
45	BO_21	BRH_21564	4,00	3,68	92	MAH33		3,64	3,67
46	BO_22	BRH_21602	4,00	3,89	93	MAH34		3,87	3,89
47	BO_23	BRH_21603	3,82	4,00	94	MAH35		3,45	3,67

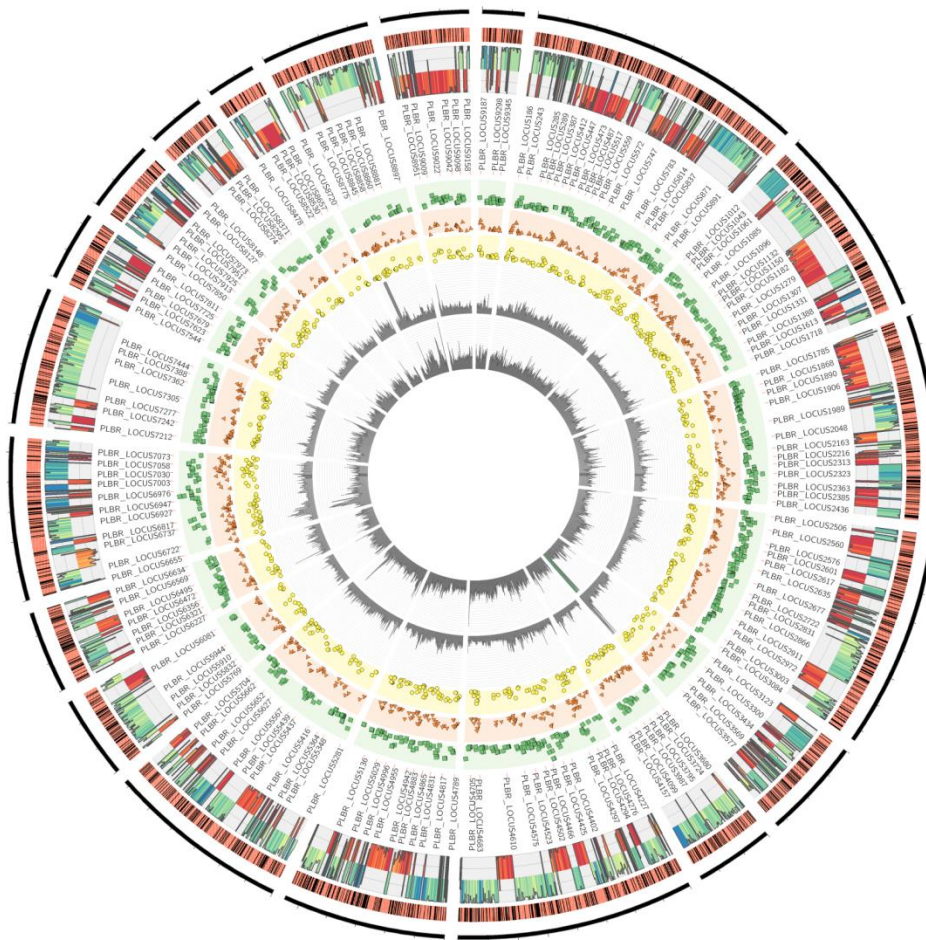


Silne objawy kiły kapusty
(*Plasmodiophora brassicae*)
na odmianie kiło-odpornej

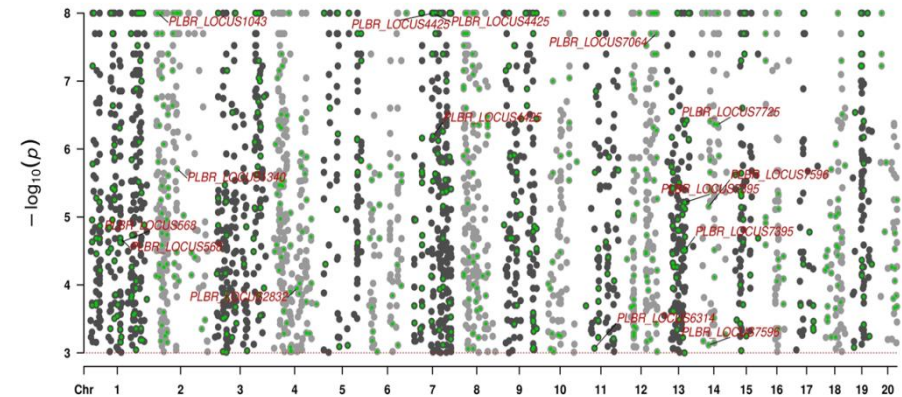
Wszystkie badane 94 rody hodowlane
rzepaku były podatne na kiłę kapusty
(*Plasmodiophora brassicae*)

Temat 3: Piramidyzacja odporności na patotypy *Plasmodiophora brassicae* w wysokoplennych formach *Brassica napus* typu canola

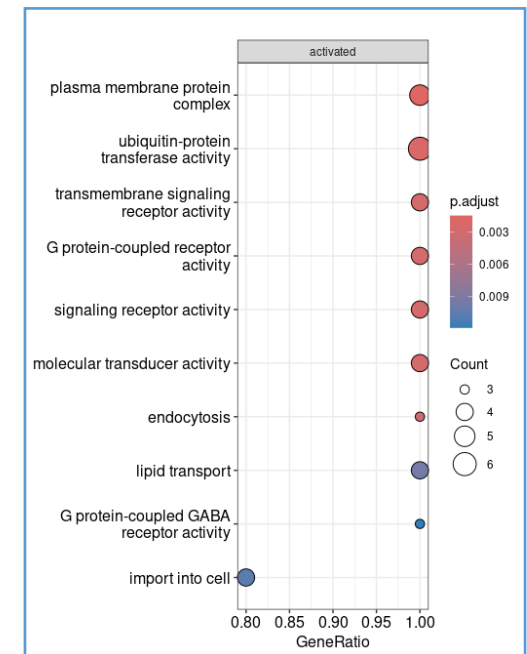
CEL: Uzyskanie form rzepaku ze zróżnicowaną odpornością na patotypy *Plasmodiophora brassicae* dla oznaczenia dziedziczenia odporności na kiłę kapusty oraz charakterystyka genomów patogenu



Wyniki analizy porównawczej dwóch izolatów *Plasmodiophora brassicae*
(wykres wykonany programem Circos)



Analiza porównawcza dystrybucji wariantów SNP dla dwóch izolatów *Plasmodiophora brassicae* (wykres typu Manhattan plot)



Annotacja funkcjonalna

Temat 4: Tolerancja rzepaku na zgniliznę twardzikową

CEL: Oznaczenie powiązania pomiędzy fenomem roślin rzepaku a tolerancją na zgniliznę twardzikową w doświadczeniu polowym

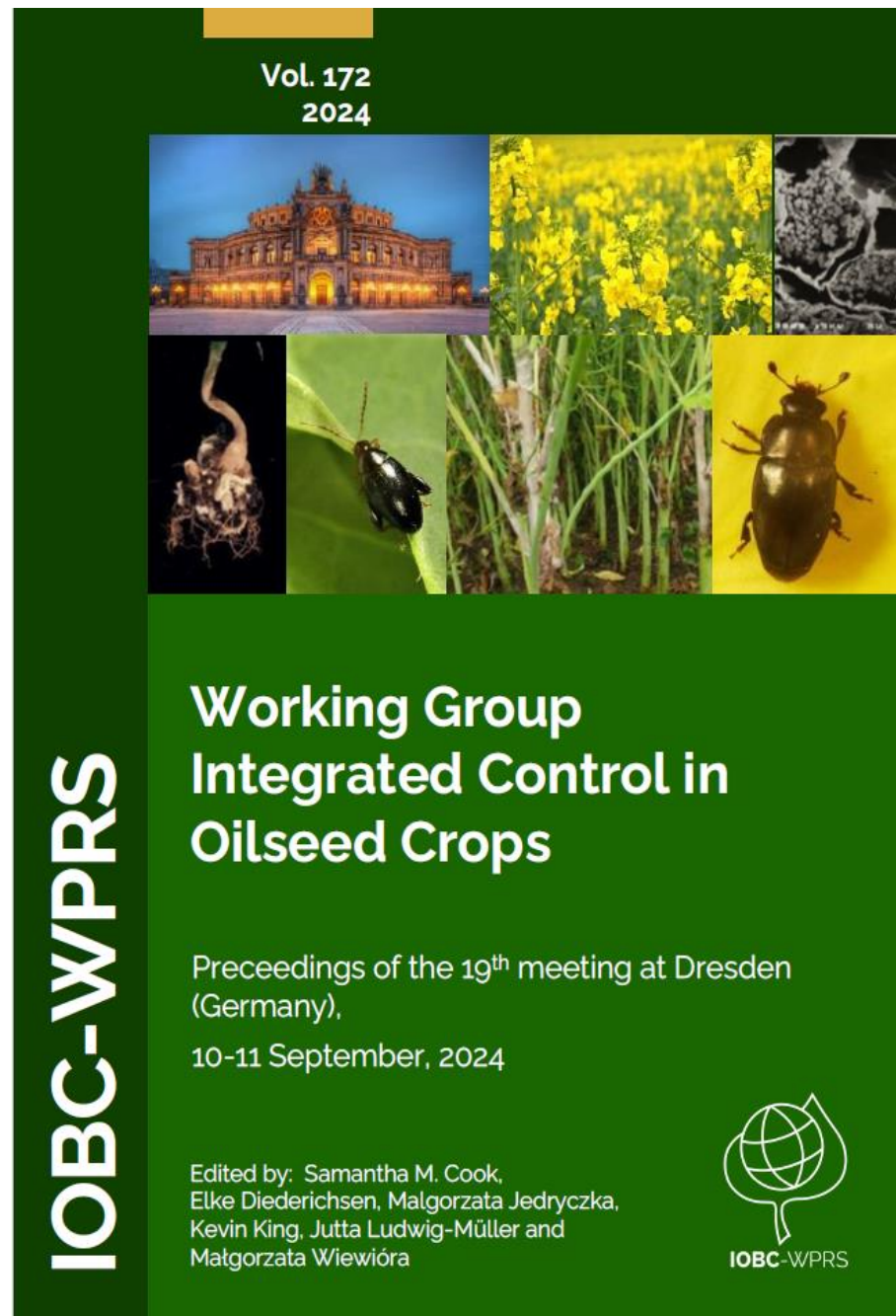
Układ doświadczeń polowych w latach 2023/2024 oraz 2024/2025

pas zakrzewień i drzew												
obsiew												
T R A W N I K	obsiew	nr polowy		nr polowy		nr polowy		nr polowy		nr polowy		obsiew
		16	16	11	32	13	48	16	64	11	80	
		15	15	2	31	3	47	15	63	2	79	
		14	14	8	30	9	46	14	62	8	78	
		13	13	4	29	16	45	13	61	4	77	
		12	12	7	28	1	44	12	60	7	76	
		11	11	5	27	4	43	11	59	5	75	
		10	10	6	26	8	42	10	58	6	74	
		9	9	14	25	12	41	9	57	14	73	
		8	8	15	24	5	40	8	56	15	72	
		7	7	16	23	2	39	7	55	16	71	
		6	6	3	22	10	38	6	54	3	70	
		5	5	13	21	15	37	5	53	13	69	
D R O G A	obsiew	4	4	1	20	7	36	4	52	1	68	obsiew
		3	3	9	19	6	35	3	51	9	67	
		2	2	12	18	14	34	2	50	12	66	
		1	1	10	17	11	33	1	49	10	65	



Operatorzy drona (UAM, Katedra Teledetekcji Gleb) oraz platformy HTTP do wysokoprzepustowego fenotypowania roślin (HR Strzelce) wykonujący obserwacje przy pomocy kamer multispektralnych w celu opisanja różnic między łanem rzepaku z roślinami zdrowymi oraz porażonymi grzybem *Sclerotinia sclerotiorum*, wywołującym zgniliznę twardzikową. Udoskonalono metodę opracowaną w 2023 roku.

Promocja wyników projektu



Załącznik 1. Poster na Konferencji Polskiego Towarzystwa Fitopatologicznego "Zdrowie roślin w dobie aktualnych wyzwań", Warszawa, 24-26.09.2024

Charakterystyka genomów *Plasmodiophora brassicae* z regionu północno-wschodniej Polski

Małgorzata Jedryczka¹, Łukasz Pauksto², Adam Okorski², Agnieszka Pszczołkowska³

¹Zakład Genetyki Patogenów i Odporności Roślin, Instytut Genetyki Roślin Polskiej Akademii Nauk w Poznaniu.

²Katedra Fitopatologii i Diagnostyki Molekularnej, Wydział Rolnictwa i Leśnictwa, Uniwersytet Warmińsko-Mazurski w Olsztynie

W STEP

Raniszczokowa Brzezina Wor. – teren będy szczytu Wyżyny Łąkowej stanowi zagęszczenie w uprawach rolni: warzywnych z rolnictwa Brzezina oraz łąk pastwisk i łąk podmokłych w kierunku południowym. Zagęszczenie rolni (Brzezina) posiada 1,2 ha. W tym podziale uprawy, a chłopi poddawają je pod uprawę pastwisk. W tym podziale uprawy, a chłopi poddawają je pod uprawę pastwisk. W tym podziale uprawy, a chłopi poddawają je pod uprawę pastwisk.

MATERIALS AND METHODS

[illegible]

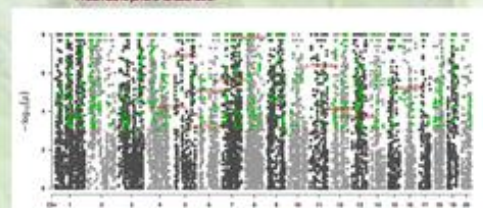
WYNIKI

- Sekwencjonowanie przy użyciu urządzenia MiSeq (Illumina) pozwoliło na uzyskanie 12 942 842 odczytów. Po kontroli jakości w programie Trimmomatic zapisano na genome nienajętych *R. brasiliensis* projektem SRA uzyskano odpowiednio 4 852 154 oraz 7 393 536 odczytów sekwencji nukleotydowych dla sekwencji reprezentujących różne pałotypy.
- Odczyty mapowały sięunkskądowo do genomu nienajętych na 46,93% do 55,47%.
- Największe liczby zmienników pojedynczych nukleotydów (SNV) zaobserwowano na chromosomach ChrC010.000.1, ChrC03.000.003.1, ChrC04.000.004.1 oraz ChrC05.000.005.1.
- W wyniku analizy statystycznej uzyskano 3 747 zmienników o zróżnicowaniu frekwencji allelu alternatywnego pomiędzy analizowanymi szczepami *R. brasiliensis* z obu stanowisk.
- Analiza porównawcza zmiennej liczby sekwencji *R. brasiliensis* określonej prymatami do pałotypu pozwoliła na wskazanie regionów genomu o sekwencjach unikalnych dla obu pałotypów (tab. 1, 2, Rys. 1, 2).

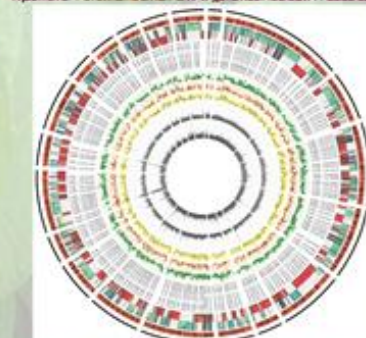
Tabela 1. Zmiana pojedynczych nukleotydów (SNV) zidentyfikowanych na genomie *R. brassicae* B i powiązanych z nimi wariantów w sekwencji kodującej genu *GLABRA1* (Czajka, Kępczyński)

[illegible]

Rysunek 1. Mapowanie zmiennych (SMM) wlotów różniących się pomiędzy dwoma zdaniami.
Reassembling phone bracelets.



Wykres 2. Porównanie zmian DMV w kategoriach urodzów R. brzojowej



Noting significant increases in supply, ending the year at 1.03 billion, with a record 1.05 billion in 1991. The country's low supply of many other commodities, including oil, is also noted.

PODSUMOWANIE

1. Genom *Plasmodium falciparum* składa się z 24 chromosomów o łącznej długości 283 Mbp. Jakiś składowany genom wynosił 1,22%.
2. W analizie oceniono najbardziej krytyczne iofelistej w strukturze genów, w tym sekwencje kodujące (CDS) i regiony niekodujące (UTR). Stwierdzono różnice pomiędzy badanymi izolatami.
3. Do analizy wykorzystano 1230 genów kodujących białka. Wykazało zmniejszony genetyczny błąd typu SNV (średnio 0,001) w porównaniu z innymi genami.
4. Analiza bioinformatyczna i sekwencjonowanie genomu izolatu reprezentatywnego linii petyjowej *Plasmodium falciparum* z wariantu 100% w tym celu.

Prof. Dr. C. Kornienko recebeu o seguinte presente da *Plasmidione brasileira*:



Fig. 2. Informacja MSig2 Burmna do wskazywania nowemu DNA.



Tabella 2. Lokalizacja zmian w DNA genetycznych u dwóch roślin *Plasmodiophora brassicae*.

Variant type	Utrav SNV	%	Variant type	Utrav SNV	%
5' prime UTR variants	910	0.075	splice donor variants	122	0.01
3' prime UTR variants	522	0.004	splice acceptor variants	10,241	0.07
ACGT codon gain variants	2191	0.16	ACGT loss	77	0.0006
downstream gene variants	179,260	29.827	stop gained	261	0.001
intronic codon variant	17	0.000	stop loss	20	0.000
intraexonic region	66,219	7.215	stop resolved variants	71	0.0004
intron variants	46,671	6.267	synonymous variants	65,004	2.561
missense variants	41,467	5.622	frameshift gene variants	160,120	29.827
splice acceptor variants	87	0.0006			

Badania wykonano w ramach programu Pospół Biologizacji 2020-2027 w ramach zadania badawczego 26 pt. „Odporność roślin rzepiku na choroby powodowane przez przynię i pierwotniaki”



KONFERENCJA NAUKOWA
Zdrowie roślin w dobie aktualnych wyzwań

STRESZCZENIA
ABSTRACTS

Warszawa 24 – 26 września 2024



Charakterystyka genomów *Plasmodiophora brassicae* z regionu północno-wschodniej Polski

Małgorzata Jędrzycka¹, Łukasz Paukszto², Adam Okorski³, Agnieszka Pszczółkowska³

¹ Zakład Genetyki Patogenów i Odporności Roślin, Instytut Genetyki Roślin Polskiej Akademii Nauk w Poznaniu

² Katedra Botaniki i Ekologii Ewolucyjnej, Wydział Biologii i Biotechnologii, Uniwersytet Warmińsko-Mazurski w Olsztynie

³ Katedra Entomologii, Fitopatologii i Diagnostyki Molekularnej, Wydział Rolnictwa i Leśnictwa, Uniwersytet Warmińsko-Mazurski w Olsztynie

mjed@igr.poznan.pl

Plasmodiophora brassicae Wor. – patogen będący sprawcą kily kapusty stanowi zagrożenie w uprawach roślin warzywnych z rodziny *Brassicaceae* oraz rzepaku jarego i ozimego w wielu krajach, także w Polsce. Rzepak ozimy (*Brassica napus* L.) jest ważną rośliną uprawną, a choroba powodowana przez ten patogen jest poważnym problemem dla plantatorów, ponieważ powoduje znaczne straty plonu nasion. Odporność na *P. brassicae* jest rasowo-specyficzna dlatego ważnym elementem diagnostycznym sprzyjającym prawidłowo prowadzonej ochronie roślin jest oznaczenie patotypu patogena. Do tego celu wykorzystywane są pracochłonne i czasochłonne testy biologiczne. Patotypy określone są na podstawie objawów chorobowych na zestawie żywicieli, na podstawie zdolności izolatu *P. brassicae* do infekowania różnych gatunków i genotypów roślin kapustowatych różniących się odpornością na kilę kapusty.

Badania nad sekwencjami genomów umożliwiają analizy porównawcze między różnymi izolatami patogenów należących do tego samego gatunku. Celem niniejszych badań było porównanie sekwencji genomów dwóch izolatów *P. brassicae* uzyskanych z różnych stanowisk położonych w regionie północno-wschodniej Polski, zidentyfikowanych jako patotypy P1 i P3. Sekwencjonowanie przy użyciu urządzenia MiSeq (Illumina) pozwoliło na uzyskanie 12 042 690 odczytów. Następnie odczyty poddano procesowi kontroli jakości w programie Trimmomatic a następnie zmapowano programem STAR do referencyjnego genomu *P. brassicae*. W wyniku sekwencjonowania DNA uzyskano odpowiednio 4 652 154 oraz 7 390 536 odczytów sekwencji nukleotydowych dla szczepów reprezentujących różne patotypy. Odczyty mapowały się unikatowo do genomu referencyjnego od 46.90% do 66.47% wszystkich zsekwencjonowanych odczytów.

Największa liczba zmienności pojedynczych nukleotydów (SNV) została zaobserwowana na chromosomach OVEO01000001.1, OVEO01000003.1, OVEO01000004.1 oraz OVEO01000008.1. W wyniku analizy statystycznej uzyskano 3 707 zmienności o zróżnicowanej frekwencji allelu alternatywnego pomiędzy analizowanymi szczepami z obu stanowisk. Przewidujemy, iż analiza porównawcza znacznej liczby izolatów *P. brassicae* o określonej przynależności do patotypu pozwoli na wskazanie regionów genomu o sekwencjach unikalnych dla tych patotypów.

Badania wykonano w ramach programu Postęp Biologiczny 2020-2027 w ramach zadania badawczego nr 25 pt. „Odporność roślin rzepaku na choroby powodowane przez grzyby i pierwotniaki”.

Characterization of the genome of *Plasmodiophora brassicae* from the region of north-eastern Poland

Małgorzata Jedryczka¹, Łukasz Pauksto², Adam Okorski³, Agnieszka Pszczółkowska³

¹ Pathogen Genetics and Plant Resistance Team, Institute of Plant Genetics, Polish Academy of Sciences, Poznań

² Department of Botany and Evolutionary Ecology, Faculty of Biology and Biotechnology, University of Warmia and Mazury, Olsztyn

³ Department of Entomology, Phytopathology and Molecular Diagnostics, Faculty of Agriculture and Forestry, University of Warmia and Mazury, Olsztyn

mjed@igr.poznan.pl

Plasmodiophora brassicae Wor. – the pathogen causing clubroot poses a threat to the cultivation of vegetable plants from the Brassicaceae family, including spring and winter oilseed rape in many countries, including Poland. Winter rapeseed (*Brassica napus* L.) is an important crop, and the disease caused by this pathogen is a serious problem for the growers. Resistance to *P. brassicae* is race-specific, therefore determining the pathotype of the pathogen is an important diagnostic element of plant protection. Laborious and time-consuming biological tests are used for this purpose. Pathotypes are determined based on disease symptoms on a set of hosts, identifying the ability of the *P. brassicae* isolate to infect various species and genotypes of Brassicaceae plants that differ in resistance to clubroot.

Genome sequence studies enable comparative analyses between different isolates of the pathogens belonging to the same species. The aim of this study was to compare the genome sequences of two *P. brassicae* isolates obtained from different sites located in the north-eastern part of Poland, identified as pathotypes P1 and P3. Sequencing using the MiSeq (Illumina) instrument resulted in 12,042,690 reads. Then, the readings were subjected to quality control using the Trimmomatic program and then mapped to the *P. brassicae* reference genome with the STAR program. As a result of DNA sequencing 4,652,154 and 7,390,536 nucleotide sequence reads were obtained for individual strains, representing different pathotypes. Reads mapped uniquely to the reference genome from 46.90% to 66.47% of all sequenced reads.

The highest number of single nucleotide variations (SNVs) were observed in chromosomes OVEO01000001.1, OVEO01000003.1, OVEO01000004.1 and OVEO01000008.1. As a result of the statistical analysis, 3,707 variations with different frequencies of the alternative allele were obtained between the analysed *P. brassicae* strains from both sites.

We anticipate that comparative analysis of a significant number of *P. brassicae* isolates with specific pathotype designation will help to identify the genomic regions with sequences unique to different pathotypes.

The research was carried out as a part of the Biological Progress 2020-2027 program, within the research task no. 25 entitled "Resistance of oilseed rape plants to diseases caused by fungi and protists".

Załącznik 2. Wystąpienie ustne na IOBCWPRS 19th Meeting
“Integrated Control in Oilseed Crops, Dresden, 10-11 September 2024



Invitation to attend

IOBC –WPRS MEETING

“Integrated Control in Oilseed Crops: Are we nearly there yet?”

Meeting announcement

IOBC-WPRS and clubroot workshop 2024 Dresden

Working Group “Integrated Control in Oilseed Crops”

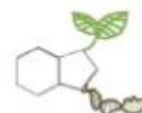
19th Meeting IOBC-WPRS 10 – 11 September 2024

Meeting ICW 12 September 2024

Location

Technische Universität Dresden, Faculty of Biology

Organizer: Jutta Ludwig-Müller



Convenor: Małgorzata Jędrzycka

SG Entomology Convenor: Sam Cook

SG Pathology Convenor: Małgorzata Jędrzycka



Vol. 172
2024



IOBC-WPRS

Working Group Integrated Control in Oilseed Crops

Preceedings of the 19th meeting at Dresden
(Germany),

10-11 September, 2024

Edited by: Samantha M. Cook,
Elke Diederichsen, Małgorzata Jedryczka,
Kevin King, Jutta Ludwig-Müller and
Małgorzata Wiewióra



IOBC-WPRS

Pathology Session 2: *Sclerotinia*

Assessment of environmental factors and pathogen virulence influencing the forecasting model for managing <i>Sclerotinia</i> stem rot in oilseed rape <i>Nazanin Zamani-Noor, Anto Raja Dominic, Vera Krause, Lena Müller, Kathleen Kohrs, Julianne Schmidt, Benno Kleinhenz</i>	108
Enhancing <i>Sclerotinia</i> risk forecasting model for winter oilseed rape in Germany: Integrating crop phenology and disease development into a Decision Support System <i>Vera Krause, Nazanin Zamani-Noor, Lena Müller, Kathleen Kohrs, Julianne Schmitt, Anto Raja Dominic</i>	109
Validation of a decision support system for predicting the carpogenic germination and infection risk of <i>Sclerotinia sclerotiorum</i> on winter oilseed rape (<i>Brassica napus</i> L.) <i>Lena Müller, Kathleen Kohrs, Anto Raja Dominic, Vera Krause, Nazanin Zamani-Noor, Juliane Schmitt, Benno Kleinhenz</i>	110-111
The pathogenic potential and fungicide response of <i>Sclerotinia</i> spp. isolates associated with <i>Sclerotinia</i> stem rot in oilseed rape cultivation in Germany <i>Nazanin Zamani-Noor, Beatrice Berger</i>	112
<i>Sclerotinia</i> disease severity and sclerotia formation in various cover crops, flower strips, and weed species inoculated with diverse isolates of <i>Sclerotinia sclerotiorum</i> <i>Nazanin Zamani-Noor, Hans-Peter Söchting</i>	113-117
Genome-wide association analysis identified loci and candidate genes linked to <i>Sclerotinia</i> stem rot resistance in turnip rape <i>Kibrom B. Abreha, Jagadeesh Sundaramoorthy, Mulatu Geleta, Ferdi Agil, Anders S. Carlsson</i>	118-123
Disease support for risk assessment of <i>Sclerotinia</i> stem rot in winter oilseed rape supported by DNA-analysis <i>Ann-Charlotte Wallenhammar, Maria Algerin</i>	124
Advancements in phenotyping of WOSR infected by <i>Sclerotinia</i> <i>Jan Piekarczyk, Jakub Ceglarek, Joanna Kaczmarek, Yuliia Kobayrenko, Małgorzata Jędryczka</i>	125-127

Advancements in phenotyping of WOSR infected by Sclerotinia

Jan Piekarczyk¹, Jakub Ceglarek¹, Joanna Kaczmarek², Małgorzata Jędrzycka²

¹Department of Environmental Remote Sensing and Soil Science, Collegium Geographicum, Adam Mickiewicz University, Poznań, Poland; ²Pathogen Genetics and Plant Resistance Team, Institute of Plant Genetics, Polish Academy of Sciences, 60-479 Poznań, Poland;

Abstract: Sclerotinia stem rot is a serious threat to oilseed rape worldwide. The aim of this study was to use the unmanned aerial vehicle to point the sites of the field that are heavily infected with *S. sclerotiorum*. To achieve this goal the field experiment was done over two years with 16 cultivars of winter oilseed rape artificially inoculated with the isolate Sc4 originating from oilseed rape in Poland. Several inoculation methods were used and the inoculum had a form of infected petals, confetti, wheat grains, toothpicks and homogenised mycelium. The biometrical measurements were done using inoculated and control plants, with the latter not treated with the pathogen. The Parrot Bluegrass drone equipped with a Parrot Sequoia multispectral camera with an RGB sensor and four spectral channels was used. The images were spectrally calibrated using gray plate calibration images with known spectral properties, and orthophotos were made in Pix4D software. The orthophotomaps were created for each date using photos from photogrammetric measurements, and the spectral indices NDVI and CHL RE were calculated for each plot. The results of the experiments showed that UAVs can successfully point out the sites at oilseed rape fields with the symptoms of Sclerotinia stem rot.

Key words: Sclerotinia stem rot, teledetection, orthophotomap, Normalised Difference Vegetation Index NDVI, spectral channels, spectral index, unmanned aerial vehicle UAV

Introduction

Sclerotinia sclerotiorum is an omnivorous fungus that causes Sclerotinia stem rot of oilseed rape, the devastating disease of this crop. The pathogen occurs in Poland and around the world, causing significant losses in the yield of oilseed rape as well as contamination of seed and soil with sclerotia, which can survive in the soil for as many as 20 years. Their elimination is reported only with long time or in the case of the use of its natural enemy – the hyperparasitic fungus *Coniothyrium minitans*. The presence of sclerotia in seed material is the reason for a negative assessment and low valuation of this material or the rejection of the entire batch of seeds obtained by the farmer, causing financial loss.

Numerous efforts of breeders and experts in plant cultivation are aimed at eliminating this dangerous pathogen. The infection of plants with *S. sclerotiorum* is associated with the occurrence of clear phenotypic symptoms in the form of whitening of the stem and a significant reduction of siliques. Cao et al. (2018) used an UAV simulation platform to observe rapeseed plants inoculated with *S. sclerotiorum* on thermal, multispectral and RGB images in indoor conditions. Zamani-Noor and Feistkorn (2022) used multispectral and RGB images recorded from a UAV to track the growth of winter oilseed rape in experimental plots with different seed densities and nitrogen rates. Spectral indices were calculated for each image pixel: NDVI derived from multispectral images and the NDYI (Normalized Difference Yellowness Index) from red–green–blue (RGB) images.

The aim of this research was to find the contactless and high throughput detection methods to evaluate the symptoms of Sclerotinia stem rot in real field situations. The

elaboration of such methods could help the breeders to find new resistance sources and it could also help the producers find the spots of the field with high incidence of *Sclerotinia* stem rot and avoid the collection of the contaminated material.

Materials and methods

The experiments were done in the seasons 2021/2022 to 2023/2024 in the field of IPG PAS located in Cerekwica (52°31'15"N 16°41'28"E), 20 km from Poznań, Poland using 16 varieties and breeding lines. The field test was designed in a randomized block scheme and it was divided to a non-inoculated part and a part used for the inoculation done with the *S. sclerotiorum* isolate Sc4 originating from naturally infected oilseed rape from Poland. Each individual plot was 3m × 7.5m in three replicates. Several inoculation methods were used including the inoculation with petals of oilseed rape or confetti infected with the pathogen and infected wheat grains attached to the stems with aluminium foil, medical plaster, or pins. The inoculum in the form of homogenized mycelium was also sprinkled on plants using a brush or the stems were pierced with infected toothpicks. Inoculation was carried out late at night, when air humidity is the highest. The control plots were not treated with *S. sclerotiorum*.

There were five and three field inspections in 2023 and 2024 respectively using the unmanned aerial vehicle (UAV): the Parrot Bluegrass drone equipped with a Parrot Sequoia multispectral camera with an RGB sensor and four spectral channels: Green (550 nm), Red (660 nm), Red Edge (735 nm), Near infrared (790 nm). The images were spectrally calibrated using gray plate calibration images with known spectral properties, and orthophotos were made in Pix4D software. The orthophotomaps were created for each date using photos from photogrammetric measurements, and the spectral indices NDVI and CHL RE were calculated for each plot according to the method elaborated by Piekarczyk et al. (2006). Test plots were marked on orthophotomaps, for the average reflectance values in individual spectral channels.

Results and Discussion

The greatest differences in the amount of solar radiation reflected from the plots in the beginning of study were observed in the near infrared (NIR) range. Lower reflectance from inoculated plots may indicate lower green biomass of plants in these plots. The decrease in NIR reflectance in both control and inoculated plots can be attributed to the beginning of plant drying. In the visible wavelength range (Green and Red channels), a slight increase in reflectance was visible with subsequent dates, and the greater reflectance in red wavelengths from the inoculated plots resulted from the weakening of the photosynthetic apparatus of the leaves caused by the disease, which resulted in faster drying off the plants. At all analyzed variants, the differences between inoculated and control varieties were statistically significant when using the NDVI index, in contrast to the use of the CHL RE index, where the differences were in most cases statistically insignificant. The results obtained in 2024 have not yet been analysed by the time of this abstract submission. However, the results of the experiments done in 2022 and 2023 already showed that UAVs can successfully point out the sites infected with *Sclerotinia* stem rot.

Acknowledgement

The study was funded by the Ministry of Agriculture and Rural Development by the project Biological Progress 2020-2027, scientific task 25 “Resistance of oilseed rape plants to diseases caused by fungi and protists”.

References

- Cao, F., Liu, F., Guo, H., Kong, W., Zhang, C., & He, Y. 2018. Fast detection of sclerotinia sclerotiorum on oilseed rape leaves using low-altitude remote sensing technology. *Sensors*, 18(12), 4464.
- Piekarczyk, J., Wójtowicz, M., Wójtowicz, A. 2006. Estimation of agronomic parameters of winter oilseed rape from field reflectance data. *Acta Agrophysica* 8 (1): 205-218.
- Zamani-Noor, N., & Feistkorn, D. 2022. Monitoring growth status of winter oilseed rape by NDVI and NDYI derived from UAV-based Red–Green–Blue imagery. *Agronomy*, 12(9), 2212.

The IOBC-WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC-WPRS).

Le Bulletin OILB-SROP est publié par l'Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Regionale Ouest Paléarctique (OILB-SROP).

Copyright: IOBC-WPRS 2024

The Publication Commission of the IOBC-WPRS:

Dr. Ute Koch
Schillerstrasse 13
69509 Moerlenbach, Germany
Tel +49-6209-1079
e-mail: u.koch_moerlenbach@t-online.de

Dr. Annette Herz
Julius Kühn-Institute (JKI)
Federal Research Center for Cultivated Plants Plant
Protection in Fruit Crops and Viticulture
Schwabenheimer Str. 101
69221 Dossenheim, Germany
Tel +49 3946 474965
e-mail: Annette.Herz@julius-kuehn.de

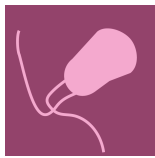
Address General Secretariat:

Paula Baptista
Polytechnic Institute of Bragança
School of Agriculture
Campus de Santa Apolónia
5300-253 Bragança, Portugal
Phone: +351 273 303 332
e-mail: pbaptista@ipb.pt

ISBN 978-92-9067-359-0

Web: <http://www.iobc-wprs.org>





Review


Pathotyping Systems and Pathotypes of *Plasmodiophora brassicae*—Navigating toward the Optimal Classification

Nazanin Zamani-Noor and Małgorzata Jędrzycka



Review

Pathotyping Systems and Pathotypes of *Plasmodiophora brassicae*—Navigating toward the Optimal Classification

Nazanin Zamani-Noor ^{1,*}  and Małgorzata Jędryczka ^{2,*} 

¹ Institute for Plant Protection in Field Crops and Grassland, Julius Kühn-Institute (JKI), D-38104 Braunschweig, Germany

² Pathogen Genetics and Plant Resistance Team, Institute of Plant Genetics, Polish Academy of Sciences, 60-479 Poznań, Poland

* Correspondence: nazanin.zamani-noor@julius-kuehn.de (N.Z.-N.); mjed@igr.poznan.pl (M.J.); Tel.: +49-(03946)-47-6460 (N.Z.-N.); +48-(061)-6550271 (M.J.)

Abstract: *Plasmodiophora brassicae* Woronin, an obligate biotrophic soil-borne pathogen, poses a significant threat to cruciferous crops worldwide by causing the devastating disease known as clubroot. Pathogenic variability in *P. brassicae* populations has been recognized since the 1930s based on its interactions with Brassica species. Over time, numerous sets of differential hosts have been developed and used worldwide to explore the pathogenic variability within *P. brassicae* populations. These sets encompass a range of systems, including the Williams system, the European Clubroot Differential set (ECD), the *Brassica napus* set, the Japanese Clubroot Differential Set, the Canadian Clubroot Differential Set (CCS), the Korean Clubroot Differential Set, and the Chinese Sinitic Clubroot Differential set (SCD). However, all existing systems possess both advantages as well as limitations regarding the detection of pathotypes from various Brassica species and their corresponding virulence pattern on Brassica genotypes. This comprehensive review aims to compare the main differential systems utilized in classifying *P. brassicae* pathotypes worldwide. Their strengths, limitations, and implications are evaluated, thereby enhancing our understanding of pathogenic variability.

Keywords: clubroot; physiological specialization; virulence and pathogenicity; genetic diversity; race differentiation; pathogen variability; disease management; host–pathogen interaction; Brassicaceae; crucifers



Citation: Zamani-Noor, N.; Jędryczka, M. Pathotyping Systems and Pathotypes of *Plasmodiophora brassicae*—Navigating toward the Optimal Classification. *Pathogens* **2024**, *13*, 313. <https://doi.org/10.3390/pathogens13040313>

Academic Editor: Giovanni Bubic

Received: 11 March 2024

Revised: 7 April 2024

Accepted: 8 April 2024

Published: 11 April 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Plasmodiophora brassicae Woronin, an obligate biotrophic soil-borne pathogen, poses a substantial menace to cruciferous crops worldwide by causing a devastating disease known as clubroot. The pathogen presents a significant threat to agriculturally important plants within the Brassicaceae family, including oilseed rape, cabbage, broccoli, cauliflower, and various mustard species [1–5]. First identified in the late 19th century [6], the pathogen has since accumulated attention for its ability to induce the formation of distinctive club-shaped galls on the roots of infected plants, leading to reduced nutrient uptake, stunted growth, and ultimately compromising crop yield [7–11].

The life cycle of *P. brassicae* begins with the activation of durable resting spores in the soil, germinating under favorable conditions and giving rise to motile zoospores [9,11–13]. These zoospores move toward susceptible plant roots, initiating infection. Within the root tissues, the pathogen undergoes a series of developmental stages, forming primary zoosporangia. These structures release secondary zoospores, facilitating the spread of the infection within the roots [14]. A distinctive life cycle feature is the induction of galls on the roots, creating a conducive environment for the pathogen's sustenance. As the galls mature, they release resting spores into the soil, completing the cycle. The longevity and resilience of these resting spores contribute to the persistence of *P. brassicae*, presenting an ongoing challenge in managing clubroot disease [15].

A critical aspect of *P. brassicae* lies in the existence of pathogenic variability [16], referred to as a pathotype [8]. According to the definition of a pathotype, sometimes also called a virulence phenotype, is any group of organisms of the same species that shows the same pathogenicity on a specified host. Pathotypes represent distinct variants of the pathogen that exhibit varying levels of infectivity on specific host cultivars, revealing a complex interplay between host resistance and pathogen diversity. Pathotypes highlight the ability of *P. brassicae* to diversify and adapt to given conditions [17,18]. Due to genetic variability, numerous isolates of *P. brassicae* with different pathogenicity can be found worldwide [19–26].

Developing clubroot-resistant cultivars is a predominant and sustainable strategy in clubroot management. To date, several clubroot-resistant cultivars of Chinese cabbage and oilseed rape/canola have been released across Europe, China, Japan, Korea, and Canada. However, the presence of pathotype-specific clubroot resistance genes, coupled with pathotype variability, contributes to the eventual loss of resistance in these cultivars within 3 to 4 years [19,27,28]. Therefore, classifying different pathotypes is essential for understanding the subtle interactions between the pathogen and its cruciferous hosts.

The molecular detection of *P. brassicae* in plants and soil samples is feasible [24,29–32] but it does not detect the pathotypes. The pathotypes of *P. brassicae* are differentiated based on their observable characteristics in bioassays using specific host differential sets. Host reactions are visually assessed within these assays by monitoring root gall development. To date, multiple sets of differential hosts, such as the Williams system, the European Clubroot Differential set, the *Brassica napus* set created by Somé et al., the Canadian Clubroot Differential set, and the Chinese Sinitic Clubroot Differential set, have been developed and utilized worldwide to investigate the pathogenic variability in *P. brassicae* populations [33–37]. The expansion of these differential host series makes it challenging, if not impossible, to interpret data from a global perspective [38].

In the present review, our objective is to compare the main differential systems for classifying *P. brassicae* pathotypes worldwide. We evaluate their strengths, limitations, and implications, thereby enhancing comprehension of the subtle variations in pathogenic variability.

1.1. American Differential Set

The differential set introduced by Williams in 1966 remains one of the primary systems worldwide for classifying *P. brassicae* pathotypes [33]. This system relies upon two distinct hosts of *Brassica napobrassica* (cv. Laurentian and cv. Wilhelmsburger) and two hosts of *B. oleracea* (cv. Badger shipper and cv. Jersey Queen), thereby facilitating the differentiation of 16 pathotypes or races (Table 1). The study involved 36 isolates of *P. brassicae*, primarily collected from cabbage *B. oleracea* (16 isolates) and cauliflower *B. oleracea* var. *botrytis* (6 isolates). Additionally, isolates were sourced from rutabaga *B. napus* var. *napobrassica* (4 isolates), turnip *B. rapa* subsp. *rapa* (4), kohlrabi *B. oleracea* var. *gongylodes* (2), Chinese cabbage *B. rapa* (2; the specific subspecies used was not specified), and oilseed rape *B. napus* (1). Moreover, one isolate of *P. brassicae* was obtained from candytuft *Iberis sempervirens*, which also belongs to the Brassicaceae family. In total, two-thirds of the tested isolates (24 out of 36) originated from various forms of cabbages belonging to *B. oleracea*, while only two isolates were derived from oilseed rape *B. napus*.

The tested isolates were sourced from various regions, including Australia (5), Canada (3), Czechoslovakia (1, presently located in the Czech Republic), England (2), Finland (2), Germany (8), Japan (3), New Zealand (4), Norway (3), USA (4), and Russia (1). This global collection represented significant diversity in isolates and encompassed geographically distant locations. All tested isolates fell into 9 categories, prompting the investigator to assign variant numbers 1–9 out of a possible 16 combinations. In the discussion, it was suggested by the author that some of the races found by other researchers would undoubtedly add to the nine races identified in his study. Despite the absence of known resistance genes, different combinations of plant reactions were classified as races, rather than pathotypes.

Table 1. *Plasmodiophora brassicae*-pathotype classification scheme on the hosts of the American Differential set proposed by Williams [33].

Differential Host	Resistance Genes	Pathotype Designation															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Brassica oleracea</i>																	
cv. Jersey Queen	n. i.	+	+	+	+	−	+	+	−	−	+	−	+	−	−	−	−
cv. Badger shipper	n. i.	−	+	−	+	−	−	+	−	−	+	+	−	+	+	+	−
<i>Brassica napobrassica</i>																	
cv. Laurentian	n. i.	+	+	+	+	−	−	−	+	+	−	+	−	+	−	−	−
cv. Wilhelmsburger	n. i.	+	−	−	+	−	−	−	−	+	+	+	+	−	+	−	+

n. i.: not identified; + indicates a susceptible host reaction; − indicates a resistant host reaction.

The decontaminated soil was evenly mixed with spore suspension in distilled water to obtain a concentration of 10⁸ *P. brassicae* spores/cc of soil. Seven-day-old seedlings of the differential hosts (Table 1) were transplanted from vermiculite to the infected soil, grown for 35 days, and then delicately pulled out and rated for disease symptoms. In the William system, the evaluation scale ranged from zero (indicating no galls) to three (signifying severe clubs on primary and secondary roots). The number of plants classified in class 0 was multiplied by 0, those in class 1 by 10, those in class 2 by 60, and those in class 3 by 100. The disease index (DI) was then determined by dividing the summed value by the total number of tested plants. If the DI fell below 90, the test was repeated to ensure that the observed resistance stemmed from the plant genotype rather than the low viability of spores. To attain dependable outcomes, Williams [3] recommended using high spore loads of a pure race. Additionally, his study revealed intermediate scores on certain host differentials, which were attributed to mixtures of races.

The Williams’ differential set comprises just two rutabagas and two cabbages. While this system is straightforward and involves only a small number of hosts, thereby reducing greenhouse space requirements for pathotype assessments, it was primarily designed to distinguish strains of *P. brassicae* from cabbage and rutabaga. Consequently, a pathotype designation based on the Williams’ system may not comprehensively reflect virulence patterns across all Brassica species. For example, in a study conducted by Kim et al. [39], pathotype 4, as per the Williams’ system and widely distributed in China, Japan, and Korea, exhibited varying disease severities in Chinese cabbage. This observation underscores the potential limitations of the Williams’ system for accurate pathotype classification and effective clubroot resistance breeding in the Asian context. An additional challenge linked to the utilization of Williams’ differential set is the presence of inconsistent host reactions, often falling between resistant and susceptible categories [19,20]. In certain instances, this inconsistency stems from heterogeneity within the differential host genotypes, which typically comprise older open-pollinated cultivars. Such intermediate and fluctuating reactions can complicate the pathotype classification of specific *P. brassicae* populations or potentially result in divergent outcomes across different countries.

1.2. European Clubroot Differential Set

The European Clubroot Differential or ECD set is the second most widely employed system worldwide for analyzing *P. brassicae* populations (Table 2). This differential set comprises 15 distinct plant species categorized into 3 genomic subsets. The initial set of five hosts corresponds to the *B. rapa* genotype, the second group involves the *B. napus* genotype, and the third subset encompasses hosts derived from the *B. oleracea* genotype. Within the *B. rapa* hosts, combinations of two to three monogenic resistances (A, B, and C) are present, while *B. napus* and *B. oleracea* are composed of a number of genes of small effect, contributing forms of field or polygenic resistance [1,40].

Table 2. European Clubroot Differential (ECD) set with associated host numbers and binary and denary values developed by Buczacki et al. [34] ^a.

Differential Number	Host	Binary Number	Denary Number
20-chromosome group (<i>Brassica rapa</i>)			
01	var. <i>rapifera</i> line aaBBCC	2 ⁰	1
02	var. <i>rapifera</i> line AAbbCC	2 ¹	2
03	var. <i>rapifera</i> line AABBcc	2 ²	4
04	var. <i>rapifera</i> line AABBCC	2 ³	8
05	var. <i>pekinensis</i> cv. Granaat	2 ⁴	16
38-chromosome group (<i>Brassica napus</i> var. <i>napus</i>)			
06	line Dc101–cv. Nevin	2 ⁰	1
07	line Dc119–cv. Giant Rape	2 ¹	2
08	line Dc128–cv. Giant Rape selection	2 ²	4
09	line Dc129–cv. New Zealand	2 ³	8
10	line Dc130–cv. Wilhemsburger	2 ⁴	16
18-chromosome group (<i>Brassica oleracea</i>)			
11	var. <i>capitata</i> cv. Badger Shipper	2 ⁰	1
12	var. <i>capitata</i> cv. Bindsachsener	2 ¹	2
13	var. <i>capitata</i> cv. Jersey Queen	2 ²	4
14	var. <i>capitata</i> cv. Septa	2 ³	8
15	var. <i>fimbriata</i> cv. Verheul	2 ⁴	16

^a In this system, the differential hosts are arranged in a fixed order and each is assigned a denary number, 1, 2, 4, 8, 16, etc.; these numbers correspond to the binary series 2⁰, 2¹, 2², 2³, and 2⁴.

In this system, each host receives a specific score in the event of an infection (Table 2). A numerical code, comprising three numbers representing the observed susceptibility of hosts within each of the three Brassica species, was then assigned to each *P. brassicae* isolate by adding the denary values of hosts within a species group showing a susceptible reaction with a disease severity index equal to or greater than 33 percent. Pathotype designations are systematically arranged in triplets and each genotype is counted independently. For example, ECD code 16/14/3 would represent a *P. brassicae* population that caused a susceptible reaction on *B. rapa* ECD 05, *B. napus* ECD 07, ECD 08, and ECD 09, as well as *B. oleracea* ECD 11 and ECD 12 (Table 2).

The ECD set theoretically has the capacity to identify 455 distinct pathotypes and has played an important role in resolving issues regarding the potential existence and importance or otherwise of physiological races or pathotypes of *P. brassicae*.

In the study conducted by Crute et al. [41], a comprehensive analysis was performed on 240 datasets obtained globally from ECD tests. Their findings indicated that the resistance to clubroot in *B. rapa* and *B. napus* was race-specific, while the resistance observed in the ECD hosts of *B. oleracea* appeared non-differential. However, the prevalence of *P. brassicae* populations capable of overcoming resistance in *B. rapa* hosts is significantly lower than isolates that can overcome resistance in *B. napus* and *B. oleracea* [24,26,28]. Interestingly, the observed pathogenicity towards resistant *B. rapa* hosts appears to be correlated with that exhibited toward resistant *B. napus* hosts. In contrast, resistant *B. oleracea* hosts tend to exhibit severe infections with *P. brassicae* isolates that lacked virulence on most resistant *B. rapa* and *B. napus* hosts [1,41]. This deviation in pathogenicity suggests the existence of distinct mechanisms for clubroot resistance that necessitate further exploration and understanding.

The ECD set has a sophisticated system of nomenclature, which has not been understood or accepted widely by farmers, breeders, or extension personnel, despite its ability to assist clubroot researchers in determining the resistance or susceptibility of differentials based on the ECD classification of each pathotype. Moreover, the large number of

hosts necessitates a significant amount of greenhouse space as well as materials when characterizing *P. brassicae* populations.

1.3. French Differential Set

In France, variation in virulence was investigated across 20 field collections of *P. brassicae*. In total, 7 of the 10 Brassica lines subjected to testing displayed distinct reactions upon inoculation. Notably, two oilseed rape cultivars exhibited previously unreported differential responses. It was observed that some of the differential lines previously used to classify *P. brassicae* pathotypes were susceptible to all collections, suggesting potential divergence in pathogen populations between France and other reported regions. Consequently, the Somé differential set was developed, comprising three genotypes of *B. napus* (ECD 06, ECD 10, and the spring-oilseed rape cultivar Brutor). In this system, the clubroot disease index varied from 0 (no galls) to 100 and a cut-off point of 25% was used to classify reactions as virulent or avirulent. Furthermore, in their study, Somé et al. [35] used a single-spore isolate, indicating the use of only one spore for host inoculation. The outcomes provided insights into a genetically uniform pathotype. This method demands a substantial investment of time and effort, given that single-spore inoculation achieves success in only 10% of cases. The French Differential Set developed by Somé is predominantly used in Europe and can distinguish eight pathotypes (Table 3).

Table 3. *Plasmodiophora brassicae*-pathotype classification scheme on French Differential Set, proposed by Somé et al. [35].

Differential Host	Resistance Genes	Pathotype Designation							
		P1	P2	P3	P4	P5	P6	P7	P8
ECD 06	n. i.	+	+	—	—	—	+	—	+
ECD 10	n. i.	+	—	—	—	+	—	+	+
cv. Brutor	n. i.	+	+	+	—	+	—	—	—

ECD 06: *Brassica napus* var. *napus* cv. Nevin; ECD 10: *B. napus* var. *napus* cv. Wilhemsburger; cv. Brutor: spring oilseed rape *B. napus*. n. i.: not identified; (+) indicates a susceptible host reaction for a cut-off point of 25%; (—) indicates a resistant host reaction for a cut-off point of 25%.

Similar to the differential set developed by Williams [33], some limitations have been identified within the differential set proposed by Somé et al. [35]. While this set comprises only three *B. napus* hosts that exhibit distinct reactions to the pathogen, its ability to differentiate pathotypes is relatively limited. Furthermore, this set exhibits even less capability for *P. brassicae* populations that have overcome the resistance in currently available clubroot-resistant oilseed rape cultivars. For instance, numerous *P. brassicae* populations have demonstrated moderate to high levels of virulence against the clubroot-resistant *B. napus* cv. Mendel [38,42–44]. These novel isolates have been provisionally designated as P1 (+), P2 (+), or P3 (+) as they correspond to P1, P2, or P3 on the differentials developed by Somé et al. [35] but unlike the original P1, P2, or P3 isolates, they exhibit high virulence against cv. Mendel.

1.4. Japanese Clubroot Differential Set

Due to the limitations of previous race differentiation systems, such as the Williams and ECD sets in distinguishing between pathogenic and nonpathogenic populations on clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) developed in Japan, Kuginuki et al. [19] introduced an innovative race-differential system. They aimed to elucidate the genetic diversity in the pathogenicity of *P. brassicae* in Japan using Japanese clubroot-resistant F1 hybrid cultivars and lines of *B. rapa* [19]. This system incorporated five clubroot-resistant F1 cultivars of Chinese cabbage (CR Kanko, CR Kukai 65, CR Ryutoku, CR Utage 70, and CR W-1116) along with a susceptible F1 cultivar of Chinese cabbage (Musu) as hosts. The incorporation of these cultivars enabled the identification of clear and distinguishable resistant or susceptible responses to *P. brassicae* populations from Japan.

Utilizing this system, 36 *P. brassicae* field populations from Japan were successfully classified into four groups or pathotypes.

However, Osaki et al. [45] modified the classification system proposed by Kuginuki et al. [19] by using six clubroot-resistant *B. rapa* and a susceptible Chinese cabbage cultivar (cv. Nozaki Nigo) as differential hosts. Four pathotypes (A–D) were identified after inoculation of seven cultivars of Chinese cabbage with 17 *P. brassicae* populations from cruciferous crops (Table 4).

Table 4. *Plasmodiophora brassicae*-pathotype classification scheme on the *Brassica rapa* hosts of the Japanese Clubroot Differential Set proposed by Osaki et al. [45].

Differential Host	Resistance Genes	Pathotype Designation			
		A	B	C	D
CR Kanki 100	n. i.	+	+	–	–
CR Kanko	n. i.	+	+	–	–
Utage 70	n. i.	+	–	+	–
Kukai 65	n. i.	+	+	–	–
Ryutoku	n. i.	+	+	–	–
Fukutakara 70	n. i.	+	+	–	–
Nozaki Nigo ^a	none	+	+	+	+

^a Susceptible *B. rapa* cultivar used as control; n. i.: not identified; (+) compatible; (–) incompatible.

Lastly, yet importantly, the Chinese cabbage cultivar Utage 70 possesses a distinct and important genetic background, as described by Kuginuki et al. [19], and plays a significant role in the pathotype classification system. However, this specific cultivar is not commercially available and cannot be procured anymore. Consequently, Hatakeyama et al. [46] have proposed a modified differential system, wherein the Chinese cabbage cultivar Super CR Hiroki is a substitute for CR Utage 70.

1.5. Canadian Clubroot Differential Set

The observed significant variability in the virulence of *P. brassicae* across all differential sets [33–35], as well as the increasing *P. brassicae* populations capable of overcoming the resistance of resistant canola cultivars, indicates that these differentials did not fully capture the range of pathogenic diversity present in *P. brassicae* populations from Canadian canola [21]. Consequently, Strelkov et al. [36] developed a new classification system called the Canadian Clubroot Differential, or CCD set, which included the differentials of Williams [33] and Somé et al. [35], eight hosts of the ECD Set [34], and the clubroot-resistant *B. napus* cultivar Mendel [47], the open-pollinated canola cv. Westar, and the clubroot-resistant hybrid canola cv. 45H29. *P. brassicae* isolates were inoculated onto each host within the CCD set and the disease development was evaluated six weeks post-inoculation. In this system, the clubroot disease severity index varied from 0 to 100 and a cut-off point of 50% was used to classify reactions as susceptible or resistant. Pathotypes were outlined by observing host reactions, characterized by distinct virulence patterns that differentiate individual pathotypes. Populations of *P. brassicae* that produced a unique virulence pattern on the CCD set were assigned a letter (A, B, C, etc.) to distinguish between different pathotypes [21]. Since the CCD set includes all of the differentials of Williams [33] and Somé et al. [35], each population also was assigned a pathotype classification based on each of those systems [21].

A proposed draft for the classification of *P. brassicae* pathotypes on the CCD set is presented in Table 5, wherein 106 isolates, which were collected in Alberta/Canada, were classified into 17 unique pathotypes. However, the CCD set has a greater differentiating capacity [36].

Table 5. *Plasmodiophora brassicae*-pathotype classification scheme on the hosts of the Canadian Clubroot Differential (CCD) set by Strelkov et al. [36].

CCD	Pathotype Designation ^a																
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	X
Williams	3	2	5	3	8	2	5	3	5	8	5	5	6	8	3	8	5
Somé	P2	P2	P2	P2	P2	P2	P3	P2	P2	P3	P3	P3	P2	P2	P3	P2	P3
Differential Host	Reaction ^b																
ECD 02	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ECD 05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ECD 06	+	+	+	+	+	+	—	+	+	—	—	—	+	+	—	+	—
ECD 08	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+
ECD 09	+	+	+	+	+	+	—	+	+	—	—	—	+	+	+	+	—
ECD 10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ECD 11	—	+	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—
ECD 13	+	+	—	+	—	+	—	+	—	—	—	—	+	—	—	—	—
Brutor	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Laurentian	+	+	—	+	+	+	—	+	—	+	—	—	—	+	+	+	—
Mendel	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
Westar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45H29	+	+	+	+	+	—	+	—	—	+	+	—	—	—	+	+	+

^a CCD pathotypes F, H, I, M, and N correspond to the original pathotypes 2, 3, 5, 6, and 8, as defined on the differentials of Williams [33], which cannot overcome resistance in any clubroot resistant *B. napus* host; CCD pathotype L corresponds to the *P. brassicae* population D-G3 reported by Strelkov et al. [27], while CCD pathotype X corresponds to the populations L-G1, L-G2, and L-G3 reported by Strelkov et al. [27]. ECD 02: *Brassica rapa* var. *rapifera* line aaBBCC; ECD 05: *B. rapa* var. *pekinensis* cv. Granaat; ECD 06: *B. napus* var. *napus* cv. Nevin; ECD 08: *B. napus* var. *napus* cv. Giant Rape selection; ECD 09: *B. napus* var. *napus* cv. New Zealand; ECD 10: *B. napus* var. *napus* cv. Wilhemsburger; cv. Brutor: spring oilseed rape *B. napus*; Laurentian: *B. napobrassica* cv. Laurentian; Mendel: first clubroot-resistant *B. napus* cultivar; Westar: the open-pollinated canola; 45H29: clubroot-resistant hybrid canola.

^b plus (+) sign denotes a susceptible host reaction, while a minus (—) sign denotes a resistant reaction. Host reactions were classified as + or — based on the index of disease (ID) that developed 6 weeks following inoculation; for a reaction to be considered resistant, the mean ID must be <50% and the 95% confidence interval must not exceed 50%.

Incorporating all differential hosts from Williams [33] and Somé et al. [35] into the CCD set allowed for determining pathotype designations based on these systems, facilitating direct comparisons. Each unique virulence pattern observed on the hosts within the CCD set was considered a distinct pathotype of *P. brassicae* and was denoted by a single uppercase letter for identification purposes. The authors mentioned that while several alternative pathotype nomenclature systems were evaluated, this set was chosen for its simplicity and ease of use in extension. Letters were preferred over numbers to prevent confusion with the numbering systems of Williams or Somé et al. If the total number of pathotypes identified using the CCD set surpasses the number of letters in the English alphabet, additional pathotypes could be designated using Greek letters or combinations thereof.

The inclusion of two clubroot-resistant *B. napus* cultivars, Mendel and 45H29, in the CCD set facilitated the identification of *P. brassicae* populations capable of overcoming clubroot resistance in the existing rapeseed cultivars resistant to clubroot. Segregation ratios from crosses with cv. Mendel demonstrated the involvement of at least one dominant and two recessive genes in this cultivar [47]. Moreover, the authors asserted that the spring oilseed rape cv. ‘Brutor’ was included in the CCD set because it was one of the differentials utilized by Somé et al. [35]. Nevertheless, ‘Brutor’ exhibited susceptibility to all of the tested populations of *P. brassicae*, thus failing to contribute to differentiation capacity. While excluding it from future studies could reduce the total number of differential hosts, it would be impossible to assign pathotype designations according to Somé et al. [35]. Similarly, the old canola cultivar ‘Westar’ showed susceptibility to all tested pathogen populations and given that ECD 05 already serves as a susceptible check, its removal from the CCD set appears justified. Eliminating ‘Brutor’ and ‘Westar’ would reduce the total number of CCD differentials to 11, thereby simplifying assessments of physiological specialization in the clubroot pathogen and conserving time and space.

1.6. Korean Clubroot Differential Set

The research team from Korea proposed the use of four distinct hosts of Chinese cabbage as an effective screening system for identifying resistant cultivars of *B. rapa* [39]. They specifically focused on Chinese cabbage as the primary source of *P. brassicae* isolates, using 11 isolates derived from Chinese cabbage and one isolate from cabbage (*B. oleracea*). These isolates were categorized into five pathotypes using the Williams differential set [33]. Subsequently, these isolates were inoculated onto clubroot-resistant cultivars of Chinese cabbage, revealing differential responses among isolates previously classified as the same pathotype by Williams. From an initial pool of 22 Chinese cabbage cultivars from Korea, China, and Japan, the authors identified four cultivars that seemed suitable for resistance breeding in clubroot-resistant Chinese cabbage (Table 6). Later on, populations of *P. brassicae* were inoculated into each host and the severity of club development on each plant was assessed using a scale ranging from 0 to 4, as outlined by Kuginuki et al. [19]. Hosts exhibiting disease severity (DS) ratings of ≤ 1 were classified as ‘resistant,’ whereas those with DS ratings of ≥ 2 were labeled as ‘susceptible’ (Table 6).

Table 6. *Plasmodiophora brassicae*-pathotype classification scheme on the Korean clubroot differential set developed by Kim et al. [39].

Cultivar	Pathotype Designation			
	Pathotype 1	Pathotype 2	Pathotype 3	Pathotype 4
Noranggimjang	S	S	S	S
CR-Cheongrok	R	R	S	S
DegaoCR1016	R	S	R	S
Akimeki	R	R	R	S

S: susceptible response; R: resistant response.

The investigation revealed that all field isolates examined in this study were classified into the four groups outlined in Table 6. However, the study encountered limitations due to the restricted number of *P. brassicae* isolates, with exclusive use of *B. rapa* as hosts. The ‘Korean’ system proposed by Kim et al. [39] is primarily adapted for Chinese cabbage, yet its applicability for diversifying isolates originating from winter oilseed rape or spring canola is constrained by the insufficiency of host differentials. To validate the efficacy of this system for Chinese cabbage breeding, it is imperative to assess a larger sample size of isolates from Asia, as well as those from Australia, Canada, and Europe. Given the prevalent infection patterns shared between cabbages in Europe and oilseed rape [32], the efficacy of ‘the Korean Differential Set’ may extend beyond Asia. However, its effectiveness might be limited to regions with extensive Chinese cabbage cultivation.

1.7. Chinese Sinitic Clubroot Differential Set

As more clubroot-resistance genes are being identified in *B. rapa*, several commercial clubroot-resistant hybrids of Chinese cabbage have been used for local pathogen classification in China, Korea, and Japan [19,39]. However, the availability of commercial F1 hybrids of Chinese cabbage suitable for clubroot differentiation is only sometimes assured, posing a potential limitation to their future utility [46]. Moreover, specific clubroot-resistant genes exhibit complete dominance in inheritance [48,49], while others follow a quantitative inheritance pattern [50]. In such instances, using F1 hybrids as hosts for differentiating pathotypes of *P. brassicae* is not recommended. Additionally, the genetic background and clubroot-resistant genes often need to be clarified for most hosts used in clubroot differentiation sets, with exceptions such as hosts ECD01 to ECD04 [51]. Hence, Pang et al. [37] have developed a set of differential hosts known as the Sinitic Clubroot Differential (SCD) set. This set utilizes eight clubroot-resistant inbred lines of Chinese cabbage with known resistance gene(s), along with a clubroot susceptible inbred line of Chinese cabbage, BJN3-1, to provide a stable platform for characterizing *P. brassicae* pathotypes (Table 7).

Table 7. *Plasmodiophora brassicae*-pathotype classification scheme on the *Brassica rapa* hosts of the Chinese Sinitic Clubroot Differential Set presented by Pang et al. [37].

Differential Host	Resistance Genes	Pathotype Designation															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
CR-096	Novel CR gene(s) ^a	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
CR-20	<i>Crr1</i> ^a , <i>Crr4</i> ^a	–	–	–	–	–	–	–	–	+	+	+	–	–	+	+	+
CR-26	<i>Crr1</i> ^a	–	–	–	–	–	–	+	+	+	+	+	–	–	–	+	–
CR-77	<i>CRA</i> ^b , <i>Crr1</i> ^a	–	–	–	–	–	+	–	+	+	+	+	–	+	–	+	+
222	<i>CRA</i> ^c	–	–	–	+	+	+	–	+	–	–	+	–	–	+	+	+
CR-75	<i>CRA</i> ^b , <i>Crr2</i> ^c	–	–	+	+	–	–	–	–	–	–	+	+	–	+	–	+
85–74	<i>CRd</i> ^b	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
CR-73	<i>Crr3</i> ^b , <i>Crr4</i> ^b	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
BJN3-1	None	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

^a Landraces; ^b Pang et al. [49]; ^c Chen et al. [52]; (+) sign denotes a susceptible host reaction, while a minus (–) sign denotes a resistant reaction. Host reactions were classified as + or – based on the disease index (DI) that developed 6 weeks following inoculation. The resistance was determined if the mean DI was lower than 25 and its associated 95% confidence interval did not overlap 50%.

The populations of *P. brassicae* were inoculated onto each host within the SCD set and disease development was assessed six weeks post-inoculation. Resistance was determined if the mean disease index was below 25 and its associated 95% confidence interval did not overlap 50%; otherwise, susceptibility was concluded. In theory, this system has the capability to distinguish 256 pathotypes of *P. brassicae*. However, Pang et al. [37] identified 16 distinct pathotypes among 132 field isolates.

The advantageous aspect of the SCD set is the presence of known resistance genes, offering a significant opportunity to develop differential host sets for pathogen differentiation and the breeding of resistant cultivars.

2. Limitations of the Current Host Differential Sets

All existing systems exhibit both advantages as well as limitations in identifying the pathotypes from each Brassica species and characterizing their virulence spectrum or pattern on Brassica genotypes. Table 8 presents a summary of the comparison of all classification systems listed in this review. While variations exist in the dates of inoculation, types and quantities of inoculum, and disease assessment data, determining an appropriate threshold for resistance and susceptibility of genotypes remains a challenging task.

Table 8. Summary of the comparison of all classification systems for the categorization of *Plasmodiophora brassicae* populations.

Classification Systems	Number of Plant Species	Brassica Species	Plant Age at the Time of Inoculation (Days)	Inoculum Type	Inoculum Concentration	Disease Assessment Date	Scale	Disease Index (DI)	Cut-Off Point
American set ^a	4	2 × <i>B. napobrassica</i> 2 × <i>B. oleracea</i>	7	infested soil	1 × 10 ⁸ spores/cc soil	35 dpi ⁱ	0–3	yes	not defined
European Clubroot Differentials ^b	15	5 × <i>B. rapa</i> 5 × <i>B. napus</i> 5 × <i>B. oleracea</i>	10	spore suspension	1 × 10 ⁷ spores/mL	35–56 dpi	0–3	yes	33%
French set ^c	3	3 × <i>B. napus</i>	6 to 10	spore suspension	1 × 10 ⁴ spores/mL	35–42 dpi	0–3	yes	25%
Japanese set ^d	6	6 × <i>B. rapa</i>	0 ^h	infested soil	5 × 10 ⁶ per g soil	42 das ^j	0–3	no	DS ≤ 1
Korean set ^e	4	4 × <i>B. rapa</i>	10	spore suspension	5 × 10 ⁷ spores/mL	30 dpi	0–4	no	DS ≤ 1
Canadian Clubroot Differentials ^f	13	2 × <i>B. rapa</i> 9 × <i>B. napus</i> 2 × <i>B. oleracea</i>	6	spore suspension	1 × 10 ⁷ spores/mL	42 dpi	0–3	yes	50%
Chinese Sinitic Clubroot Differentials ^g	8	8 × <i>B. rapa</i>	5	spore suspension	1 × 10 ⁷ spores/mL	42 dpi	0–3	yes	25%

^a Williams [33]; ^b Buczacki et al. [34]; ^c Somé et al. [35]; ^d Osaki et al. [45]; ^e Kim et al. [39]; ^f Strelkov et al. [36]; ^g Pang et al. [37]; ^h seed sown directly into infested soil; ⁱ days post inoculation (dpi); ^j days after sowing (das).

Moreover, the increase in *P. brassicae* populations capable of overcoming the resistance of Brassica cultivars presents an additional challenge in classifying pathotypes using current classification systems. The overcoming of clubroot-resistant cultivars may arise from the selective propagation of pathogenic genotypes on the Brassica cultivars. Each field population of *P. brassicae* is often heterogeneous and comprises multiple pathotypes, as evidenced by analyses of the pathogenicity of single-spore isolates of *P. brassicae* obtained from a single gall or field population [8,43,53–55]. Therefore, the optimal assessment of pathotypes should begin with isolating single spores to ensure that the study relies on pure genetic variants rather than mixtures of pathotypes. This effort is significantly challenging due to the potential presence of mixed strains within most *P. brassicae* populations found in individual root galls, as documented in canola roots in Alberta [56]. In this investigation, RNase H-dependent PCR (rhPCR) analyses revealed that 50 out of 79 galls contained *P. brassicae* populations including more than one strain. Consequently, researchers should aim to identify the prevalence of single spore isolates in the field and evaluate their relative proportions. The discovery that over 60% of galls contain ‘more than one’ isolate presents a formidable challenge, given the spatial and temporal limitations natural in pathotype classifications.

Moreover, international research teams should attempt to utilize assessments using near-isogenic lines with single resistance (R) genes, aiming to approach the detection of gene-for-gene relationships as closely as possible. This effort may prove challenging given the complexity of the *Brassica-Plasmodiophora* pathosystem, which encompasses numerous genes, gene variants, and types, along with a multitude of gene combinations. Additionally, discrepancies in the nomenclature of the same genes among various research teams and the identification of duplicates of the same gene [57] further complicate matters, potentially leading to significant variations in plant reactions.

Navigating towards standardized pathotyping tests should also involve considerations regarding the severity scales used, uniform formulas for calculating disease indices, and consistent thresholds. Broader scales, such as the 0–4 scale used by Kim et al. [39], straightforward calculations like the formula proposed by Horiuchi and Hori [58], which should be modified to the scale 0–4, and stringent cutoff thresholds e.g., 25%, as suggested by Somé et al. [35] appear to be the most suitable choices.

Recent study suggested that the evolution within a given pathotype is driven by changes in the aggressiveness of the pathogen [59]. The further intensive cultivation of oilseed rape and the popularity of cruciferous vegetables may accelerate the emergence of new pathotypes. The pathotype designation system will therefore also have to undergo evolutionary changes.

3. Concluding Remarks

Balancing regional or national priorities while simultaneously harmonizing or standardizing international systems presents a significant challenge. The designation of ‘custom-made’ pathotypes modified for nationwide screening or breeding purposes by specific companies offers numerous advantages. Users can precisely track the development of new pathotypes or races and compare current and previous pathogen populations, allowing conclusions to be drawn regarding the direction of their evolution. However, interpreting data internationally becomes challenging, if possible. The addition or deletion of a host differential, even among many, alters the pathotype designation. Frequently, new pathotyping systems utilize only a subset of existing differential sets and introduce their own new hosts, leading to considerable confusion. Nevertheless, the unique composition, diversity, and continual changes in local pathogen populations often necessitate such an approach. Furthermore, some users primarily focus on a specific Brassica host (e.g., *B. napus* or *B. rapa*), representing the unjustified use of host differentials from other Brassica species.

Using plant host differentials that remain consistently susceptible or resistant to the studied isolates not only expands the screening area unnecessarily but also entails excessive work and costs without yielding any novel or conclusive results. Consequently, adopting

the ‘Global Host Differential Set’ by breeders seems improbable despite the potential benefits it could offer for population studies of *P. brassicae*.

Due to the increasing cultivation of winter oilseed rape, spring canola, and the popularity of vegetable brassicas combined with short crop rotations, we are witnessing a rapid emergence of new pathotypes of *P. brassicae*. Furthermore, some pathogen populations, once confined to small areas, have now significantly expanded. Given the extensive breeding efforts to develop clubroot-resistant cultivars, numerous independent differential host series will likely continue to be utilized, with new ones being developed over time.

The promising prospect lies in genetic tools currently being used to sequence the entire genomes of the pathogen. This advancement is expected to facilitate comparisons between isolates of *P. brassicae* and pathotypes from various locations. However, genetic descriptions must be accompanied by screening test results using as extensive of a differential set as possible. It is also essential to use the seeds of host differentials from safe and reliable seed suppliers. Essentially, by navigating toward race-specific phenotyping and utilizing single spore isolates and near-isogenic lines that vary with R genes, we can significantly advance research on the current composition and shifts in *P. brassicae* populations. Harmonizing evaluation systems through the adoption of consistent symptom evaluation scales, disease index calculation formulas, and disease thresholds, as well as striving to match the Global Host Differential Set as closely as possible, are important steps in this advancement. Both basic and applied research stand to gain substantial benefits from this level of standardization.

Author Contributions: Both authors contributed substantially to the conception, design, and execution of the review paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Diederichsen, E.; Frauen, M.; Linders, E.G.; Hatakeyama, K.; Hirai, M. Status and perspectives of clubroot resistance breeding in crucifer crops. *J. Plant Growth Regul.* **2009**, *28*, 265–281. [\[CrossRef\]](#)
2. Jedryczka, M.; Kasprzyk, I.; Korbas, M.; Jajor, E.; Kaczmarek, J. Infestation of Polish agricultural soils by *Plasmodiophora brassicae* along the Polish-Ukrainian border. *J. Plant Prot. Res.* **2014**, *54*, 238–241. [\[CrossRef\]](#)
3. Strelkov, S.E.; Hwang, S.F.; Manolii, V.; Turnbull, G.D.; Fredua-Agyeman, R.; Hollman, K.; Kaus, S. Characterization of clubroot (*Plasmodiophora brassicae*) from canola (*Brassica napus*) in the Peace Country of Alberta, Canada. *Can. J. Plant Pathol.* **2020**, *43*, 155–161. [\[CrossRef\]](#)
4. Ludwig-Müller, J. What Can We Learn from -Omics Approaches to Understand Clubroot Disease? *Int. J. Mol. Sci.* **2022**, *23*, 6293. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Zamani-Noor, N.; Brand, S.; Söchting, H.P. Effect of Pathogen Virulence on Pathogenicity, Host Range, and Reproduction of *Plasmodiophora brassicae*, the Causal Agent of Clubroot Disease. *Plant Dis.* **2022**, *106*, 57–64. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Crute, I.; Gray, A.; Crisp, P.; Buczacki, S. Variation in *Plasmodiophora brassicae* and resistance to clubroot disease in brassicas and allied crops—a critical review. *Plant Breed. Abstr.* **1980**, *50*, 91–104.
7. Struck, C.; Rüsche, S.; Strehlow, B. Control Strategies of Clubroot Disease Caused by *Plasmodiophora brassicae*. *Microorganisms* **2022**, *10*, 620. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Xue, S.; Cao, T.; Howard, R.J.; Hwang, S.F.; Strelkov, S.E. Isolation and Variation in Virulence of Single-Spore Isolates of *Plasmodiophora brassicae* from Canada. *Plant Dis.* **2008**, *92*, 456–462. [\[CrossRef\]](#)
9. Strehlow, B.; de Mol, F.; Struck, C. Risk potential of clubroot disease on winter oilseed rape. *Plant Dis.* **2015**, *99*, 667–675. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Gossen, B.D.; Deora, A.; Peng, G.; Hwang, S.; McDonald, M.R. Effect of environmental parameters on clubroot development and the risk of pathogen spread. *Can. J. Plant. Pathol.* **2014**, *36*, 37–48. [\[CrossRef\]](#)
11. Javed, M.A.; Schwelm, A.; Zamani-Noor, N.; Salih, R.; Vañó, M.S.; Wu, J.; García, M.G.; Heick, T.M.; Luo, C.; Prakash, P.; et al. The clubroot pathogen *Plasmodiophora brassicae*—A profile update. *Mol. Plant Pathol.* **2022**, *24*, 89–106. [\[CrossRef\]](#) [\[PubMed\]](#)

12. Kageyama, K.; Asano, T. Life cycle of *Plasmodiophora brassicae*. *J. Plant Growth Regul.* **2009**, *28*, 203–211. [\[CrossRef\]](#)
13. Khalid, M.; Rahman, S.U.; Kayani, S.I.; Khan, A.A.; Gul, H.; Hui, N. *Plasmodiophora brassicae*—The Causal Agent of Clubroot and Its Biological Control/Suppression with Fungi—A Review. *S. Afr. J. Bot.* **2022**, *147*, 325–331. [\[CrossRef\]](#)
14. Feng, J.; Xiao, Q.; Hwang, S.F.; Strelkov, S.E.; Gossen, B.D. Infection of canola by secondary zoospores of *Plasmodiophora brassicae* produced on a nonhost. *Eur. J. Plant Pathol.* **2012**, *132*, 309–315. [\[CrossRef\]](#)
15. Wallenhammar, A.C. Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. *Plant Pathol.* **1996**, *45*, 710–719. [\[CrossRef\]](#)
16. Tinggal, S.H.; Webster, J. Technique for single spore infection by *Plasmodiophora brassicae*. *Trans. Br. Mycol. Soc.* **1981**, *76*, 187–190. [\[CrossRef\]](#)
17. Jones, D.R.; Igram, D.S.; Dixon, G.R. Characterization of isolates derived from single resting spores of *Plasmodiophora brassicae* and studies of their interaction. *Plant Pathol.* **1982**, *31*, 239–246. [\[CrossRef\]](#)
18. Scott, E.S. Production and characterization of single-spore isolates of *Plasmodiophora brassicae*. *Plant Pathol.* **1985**, *34*, 287–292. [\[CrossRef\]](#)
19. Kuginuki, Y.; Yoshikawa, H.; Hirai, M. Variation in virulence of *Plasmodiophora brassicae* in Japan tested with clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *Eur. J. Plant Pathol.* **1999**, *105*, 327–332. [\[CrossRef\]](#)
20. Strelkov, S.E.; Tewari, J.P.; Smith-Degenhardt, E. Characterization of *Plasmodiophora brassicae* populations from Alberta, Canada. *Can. J. Plant Pathol.* **2006**, *28*, 467–474. [\[CrossRef\]](#)
21. Hwang, S.-F.; Strelkov, S.E.; Feng, J.; Gossen, B.D.; Howard, R.J. *Plasmodiophora brassicae*: A review of an emerging pathogen of the Canadian canola (*Brassica napus*) crop. *Mol. Plant Pathol.* **2012**, *13*, 105–113. [\[CrossRef\]](#)
22. Zhao, Y.; Bai, Y.; Miao, Z.; Li, Y.; Zhao, K. Identification of physiological races of *Plasmodiophora brassicae* causing clubroot in Chinese cabbage from Northeast China. *J. Hunan Agric.* **2013**, *39*, 176–178.
23. Donald, E.C.; Porter, I.J. Clubroot in Australia: The history and impact of *Plasmodiophora brassicae* in Brassica crops and research efforts directed towards its control. *Can. J. Plant Pathol.* **2014**, *36*, 66–84. [\[CrossRef\]](#)
24. Řičařová, V.; Kaczmarek, J.; Strelkov, S.E.; Kazda, J.; Rysanek, P.; Lueders, W.; Manolii, V.; Jedryczka, M. Pathotypes of *Plasmodiophora brassicae* causing damage to oilseed rape in the Czech Republic and Poland. *Eur. J. Plant Pathol.* **2016**, *145*, 559–572. [\[CrossRef\]](#)
25. Yang, H.; Yuan, Y.; Wei, X.; Zhang, X.; Wang, H.; Song, J.; Li, X. A new identification method reveals the resistance of an extensive-source radish collection to *Plasmodiophora brassicae* race 4. *Agronomy* **2021**, *11*, 792. [\[CrossRef\]](#)
26. Zamani-Noor, N. Variation in pathotypes and virulence of *Plasmodiophora brassicae* populations in Germany. *Plant Pathol.* **2017**, *66*, 316–324. [\[CrossRef\]](#)
27. Strelkov, S.E.; Hwang, S.-F.; Manolii, V.P.; Cao, T.; Feindel, D. Emergence of new virulence phenotypes of *Plasmodiophora brassicae* on canola (*Brassica napus*) in Alberta, Canada. *Eur. J. Plant Pathol.* **2016**, *145*, 517–529. [\[CrossRef\]](#)
28. Zamani-Noor, N.; Wallenhammar, A.-C.; Kaczmarek, J.; Patar, U.R.; Zouhar, M.; Manasova, M.; Jedryczka, M. Pathotype Characterization of *Plasmodiophora brassicae*, the Cause of Clubroot in Central Europe and Sweden (2016–2020). *Pathogens* **2022**, *11*, 1440. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Wallenhammar, A.-C.; Almquist, C.; Söderström, M.; Jonsson, A. In-field distribution of *Plasmodiophora brassicae* measured using quantitative real-time PCR. *Plant Pathol.* **2012**, *61*, 16–28. [\[CrossRef\]](#)
30. Kaczmarek, J.; Irzykowski, W.; Burzyński, A.; Jedryczka, M. The detection of *Plasmodiophora brassicae* using loop-mediated isothermal DNA amplification. *Acta Agrobot.* **2014**, *67*, 59–66. [\[CrossRef\]](#)
31. Zhang, L.; Cai, X.; Wu, J.; Liu, M.; Grob, S.; Cheng, F.; Liang, J.; Cai, C.; Liu, Z.; Liu, B.; et al. Improved *Brassica rapa* reference genome by single-molecule sequencing and chromosome conformation capture technologies. *Hortic. Res.* **2018**, *5*, 50. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Czubatka-Bieńkowska, A.; Kaczmarek, J.; Marzec-Schmidt, K.; Nieróbca, A.; Czajka, A.; Jedryczka, M. Country-Wide qPCR based assessment of *Plasmodiophora brassicae* spread in agricultural soils and recommendations for the cultivation of Brassicaceae crops in Poland. *Pathogens* **2020**, *9*, 1070. [\[CrossRef\]](#)
33. Williams, P.H. A system for the determination of races of *Plasmodiophora brassicae* that infect Cabbage and Rutabaga. *Phytopathology* **1966**, *56*, 624–626.
34. Buczacki, S.T.; Toxopeus, H.; Mattusch, P.; Johnston, T.D.; Dixon, G.R.; Hobolth, L.A. Study of physiologic specialization in *Plasmodiophora brassicae*: Proposals for attempted rationalization through an international approach. *Trans. Br. Mycol. Soc.* **1975**, *65*, 295–303. [\[CrossRef\]](#)
35. Somé, A.; Manzanares, M.J.; Laurens, F.; Baron, F.; Thomas, G.; Rouxel, F. Variation for Virulence on *Brassica napus* L. amongst *Plasmodiophora brassicae*. Collections from France and Derived Single-Spore Isolates. *Plant Pathol.* **1996**, *45*, 432–439. [\[CrossRef\]](#)
36. Strelkov, S.E.; Hwang, S.-F.; Manolii, V.P.; Cao, T.; Fredua-Agyeman, R.; Harding, M.W.; Peng, G.; Gossen, B.D.; McDonald, M.R.; Feindel, D. Virulence and pathotype classification of *Plasmodiophora brassicae* populations collected from clubroot resistant canola (*Brassica napus*) in Canada. *Can. J. Plant Pathol.* **2018**, *40*, 284–298. [\[CrossRef\]](#)
37. Pang, W.; Liang, Y.; Zhan, Z.; Li, X.; Piao, Z. Development of a Sinitic Clubroot Differential Set for the Pathotype Classification of *Plasmodiophora brassicae*. *Front. Plant Sci.* **2020**, *11*, 568771. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Tso, H.H.; Galindo-González, L.; Strelkov, S.E. Current and future pathotyping platforms for *Plasmodiophora brassicae* in Canada. *Plants* **2021**, *10*, 1446. [\[CrossRef\]](#) [\[PubMed\]](#)

39. Kim, H.; Jo, E.J.; Choi, Y.H.; Jang, K.S.; Choi, G.J. Pathotype Classification of *Plasmodiophora brassicae* Isolates Using Clubroot-Resistant Cultivars of Chinese Cabbage. *Plant Pathol. J.* **2016**, *32*, 423–430. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Dixon, G.R. Clubroot (*Plasmodiophora brassicae* Woronin)—An agricultural and biological challenge worldwide. *Can. J. Plant Pathol.* **2014**, *36*, 5–18. [\[CrossRef\]](#)
41. Crute, I.; Phelps, K.; Barnes, A.; Buczacki, S.; Crisp, P. The relationship between genotypes of three Brassica species and collections of *Plasmodiophora brassicae*. *Plant Pathol.* **1983**, *32*, 405–420. [\[CrossRef\]](#)
42. Diederichsen, E.; Frauen, M.; Ludwig-Müller, J. Clubroot disease management challenges from a German perspective. *Can. J. Plant Pathol.* **2014**, *36*, 85–98. [\[CrossRef\]](#)
43. McDonald, M.R.; Al-Daoud, F.; Sedaghatkish, A.; Moran, M.; Cranmer, T.J.; Gossen, B.D. Changes in the Range and Virulence of *Plasmodiophora brassicae* across Canada. *Can. J. Plant Pathol.* **2020**, *43*, 304–310. [\[CrossRef\]](#)
44. Zamani-Noor, N.; Krohne, I.; Koopmann, B. Greenhouse Evaluation of Clubroot Resistant-*Brassica napus* cv. Mendel and Its Efficacy Concerning Virulence and Soil Inoculum Levels of *Plasmodiophora brassicae*. *Pathogens* **2021**, *10*, 151. [\[CrossRef\]](#)
45. Osaki, K.; Fujiyama, S.; Nakayama, A.; Shimizu, Y.; Ito, S.; Tanaka, S. Relation between pathogenicity and genetic variation within *Plasmodiophora brassicae*. *J. Gen. Plant Pathol.* **2008**, *74*, 281–288. [\[CrossRef\]](#)
46. Hatakeyama, K.; Fujimura, M.; Ishida, M.; Suzuki, T. New Classification Method for *Plasmodiophora brassicae* Field Isolates in Japan Based on Resistance of F1 Cultivars of Chinese Cabbage (*Brassica rapa* L.) to Clubroot. *Breed. Sci.* **2004**, *54*, 197–201. [\[CrossRef\]](#)
47. Diederichsen, E.; Beckmann, J.; Schondelmeier, J.; Dreyer, F. Genetics of clubroot resistance in *Brassica napus* ‘Mendel’. *Acta Hortic.* **2006**, 307–311. [\[CrossRef\]](#)
48. Hatakeyama, K.; Suwabe, K.; Tomita, R.N.; Kato, T.; Nunome, T.; Fukuoka, H.; Matsumoto, S. Identification and characterization of Crr1a, a gene for resistance to clubroot disease (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L. *PLoS ONE* **2013**, *8*, e54745. [\[CrossRef\]](#)
49. Pang, W.; Fu, P.; Li, X.; Zhan, Z.; Yu, S.; Piao, Z. Identification and mapping of the clubroot resistance gene CRd in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). *Front. Plant Sci.* **2018**, *9*, 653. [\[CrossRef\]](#)
50. Pang, W.; Liang, S.; Li, X.; Li, P.; Yu, S.; Lim, Y.P.; Piao, Z. Genetic detection of clubroot resistance loci in a new population of *Brassica rapa*. *Hortic. Environ. Biotechnol.* **2014**, *55*, 540–547. [\[CrossRef\]](#)
51. Hirani, A.; Gao, F.; Liu, J.; Fu, G.; Wu, C.; McVetty, P.B.E.; Duncan, R.W.; Li, G. Combinations of Independent Dominant Loci Conferring Clubroot Resistance in All Four Turnip Accessions (*Brassica rapa*) from the European Clubroot Differential Set. *Front. Plant Sci.* **2018**, *9*, 1628. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Chen, J.; Jing, J.; Zhan, Z.; Zhang, T.; Zhang, C.; Piao, Z. Identification of Novel QTLs for Isolate-Specific Partial Resistance to *Plasmodiophora brassicae* in *Brassica rapa*. *PLoS ONE* **2013**, *8*, e85307. [\[CrossRef\]](#)
53. Manzanares-Dauleux, M.J.; Divaret, I.; Baron, F.; Thomas, G. Assessment of Biological and Molecular Variability Between and Within Field Isolates of *Plasmodiophora brassicae*. *Plant Pathol.* **2001**, *50*, 165–173. [\[CrossRef\]](#)
54. Tanaka, S.; Ito, S. Pathogenic and genetic diversity in *Plasmodiophora brassicae* (clubroot) from Japan. *J. Gen. Plant Pathol.* **2013**, *79*, 297–306. [\[CrossRef\]](#)
55. Sedaghatkish, A.; Gossen, B.D.; Yu, F.; Torkamaneh, D.; McDonald, M.R. Whole-Genome DNA Similarity and Population Structure of *Plasmodiophora brassicae* Strains from Canada. *BMC Genom.* **2019**, *20*, 744. [\[CrossRef\]](#)
56. Fu, H.; Yang, Y.; Mishra, V.; Zhou, Q.; Zuzak, K.; Feindel, D.; Harding, M.W.; Feng, J. Most *Plasmodiophora brassicae* Populations in Single Canola Root Galls from Alberta Fields Are Mixtures of Multiple Strains. *Plant Dis.* **2019**, *104*, 116–120. [\[CrossRef\]](#)
57. Kopec, P.M.; Mikolajczyk, K.; Jajor, E.; Perek, A.; Nowakowska, J.; Obermeier, C.; Chawla, H.S.; Korbass, M.; Bartkowiak-Broda, I.; Karlowski, W.M. Local Duplication of TIR-NBS-LRR Gene Marks Clubroot Resistance in *Brassica napus* cv. Tosca. *Front Plant Sci.* **2021**, *12*, 639631. [\[CrossRef\]](#)
58. Horiuchi, S.; Hori, M. A simple greenhouse technique for obtaining high levels of clubroot incidence. *Bull. Chugoku Natl. Agric. Exp. Stn. Ser.* **1980**, *17*, 33–55.
59. Fontyn, C.; Meyer, K.J.G.; Boixel, A.-L.; Delestre, G.; Piaget, E.; Picard, C.; Suffert, F.; Marcel, T.C.; Goyeau, H. Evolution within a given virulence phenotype (pathotype) is driven by changes in aggressiveness: A case study of French wheat leaf rust populations. *Peer Community J.* **2023**, *3*, e39. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.