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Preliminary screening of *Avena sterilis* L. for resistance to crown rust

Wstępna ocena *Avena sterilis* L. pod kątem odporności na rdzę koronową owsa

Summary. The best source of crown rust resistance genes (*Pc*) in genus *Avena* is a wild hexaploid *A. sterilis* L. In this study, accessions of *A. sterilis* gathered from European and North American gene banks, originated from 21 countries were evaluated at the seedling stage for crown rust reaction using the host–pathogen test and two *Puccinia coronata* f. sp. *avenae* isolates. Of the 45 oat accessions analyzed, 12 were resistant to one crown rust race (3.2). Resistance to both pathotypes used in the study was observed in two of the accessions, first of which was collected in Libya (AVE 2532) and second in Portugal (CN 26036). Further research is required to evaluate the genetic background of the discovered resistance, however, obtained results provide a valuable first step in the identification of new promising crown rust resistance sources.

Key words: *Avena sterilis* L., *Puccinia coronata* f. sp. *avenae*, host–pathogen test, reference lines

INTRODUCTION

Cultivated oat is affected by a number of fungal diseases, among which crown rust caused by *Puccinia coronata* f. sp. *avenae* is the most widespread. The use of race-specific, resistance genes is the primary mean of crown rust control. Growing resistant varieties is beneficial for both economic and phytosanitary reasons being an environmentally friendly alternative to chemical control [Jiráková and Hanzalová 2008, Carson 2011, Chong et al. 2011].

Crown rust resistance in oat is usually based on monogenic inheritance. So far, a number of potential *Pc* genes have been identified in oats cultivated (*A. sativa*, *A. byzantina*) and wild (*A. sterilis*, *A. strigosa*, *A occidentalis*, *A. barbata*) species. The best source of resistance genes to *P. coronata*, with about 45 described, and about 10 *Pc* introduced to common oat is *A. sterilis* L. Unfortunately, current sources of resistance to crown rust rapidly lose their effectiveness due to the fast evolution of pathogen virulence, therefore, recent efforts have focused on identifying new *Pc* genes.

The aim of the present study was preliminary screening of 45 genotypes belonging to wild oat *A. sterilis* species and identification of potential sources of resistance to *P. coronata*.

MATERIAL AND METHODS

Research was carried out on 45 accessions of the *A. sterilis* (Tab. 1) received from Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (IPK – 24 accessions), National Small Grains Collection, Aberdeen, Idaho, USA (NSGC – 12 accessions) and Plant Gene Resources of Canada, Agriculture and Agri-Food, Saskatoon, Canada (PGRC – 9 accessions). Examined plant material came originally from 21 countries located in Asia, Europe, and Africa (Fig. 1).

All accessions were assessed using two *P. coronata* races, 3.2 and 11.1 chosen from a wide collection of single-pustule pathotypes originated from Polish populations of the pathogen [Paczos-Grzęda and Sowa 2019]. The virulence of isolates was characterized based on susceptibility/resistance reaction of 35 differential oat lines with a single resistance gene (Tab. 2). Differentials were developed at either Iowa State University of Science and Technology, USA (Pc14, Pc36, Pc51, Pc52 Pc70, and Pc71) or Cereal Research Centre, Agriculture & Agri-Food, Winnipeg, Canada (Pc35, Pc38, Pc39, Pc40, Pc45, Pc46, Pc48, Pc50, Pc54, Pc55, Pc56, Pc57, Pc62, Pc63, Pc64, Pc67, Pc68, Pc91, Pc94, Pc96, Pc97, Pc98, Pc101, Pc103-1, Pc104) [Carson 2011, Chong et al. 2011, Menzies et al. 2015]. Additionally cultivars 'TAM-O-301' and 'TAM-O-312', developed at Texas Agricultural and Mechanical University, USA, were representatives for *Pc58* and *Pc59*, while 'Coker227' and 'Coker234' derived in the Coker's Pedigreed Seed Company, South Carolina, USA served as the *Pc60* and *Pc61* gene reference respectively [Simons et al. 1978] – Tab. 1.

Prior the inoculation, microcentrifuge tubes filled with frozen spores of 3.2 and 11.1 were heat-shocked for 5 min at 42°C and multiplied on leaf fragments of 10-day-old seedlings of the cultivar 'Kasztan' using the host–pathogen test procedure as described in Sowa et al. [2016].

To assess the susceptibility of the studied wild oat *A. sterilis* genotypes, grains obtained from genebanks and a control cultivar 'Kasztan' were seeded into plastic plug trays filled with universal substrate mixed with peat. After ten days, 3-cm-long leaf fragments were placed into 12-well culture plates filled with agar (0.6%) containing benzimidazole (3.4 mM). The same seedling of each accession was tested with both *P. coronate* pathotypes in three replicates. Inoculations were performed in a settling tower by spreading spores onto the plant material at a density of 500–700 spores · cm^{-2}. Plates were incubated for 10 days in a phytotron at 18°C with 70% humidity and a light intensity of approximately 4 kLx for

Table 1. Crown rust (*Puccinia coronata* f. sp. *avenae*) resistance phenotypes of the *Avena sterilis* accessions

Accession	Bank[a]	Origin		*Puccinia coronata* race[b]	
		Country	Region	3.2	11
PI 324816	NSGC	Algeria	Oran	S	S
PI 56503	NSGC	Crimea, Ukraine	–	S	S
AVE 2055	IPK	Belgium	–	S	S
AVE 1561	IPK	Cyprus	–	S	S
AVE 1983	IPK	Ethiopia	–	S	S
AVE 1510	IPK	France	–	S	S
CN 24394	PGRC	Georgia	Tbilisi	S	S
AVE 1562	IPK	Georgia	Tbilisi	S	MS
CN 24396	PGRC	Georgia	Tbilisi	S	S
CN 24398	PGRC	Georgia	Tbilisi	S	S
AVE 245	IPK	Greece	Megalopolis, Peloponnese	S	HR
AVE 446	IPK	Greece	Christofalaika, Peloponnese	S	S
AVE 5009	IPK	Iran	–	S	S
PI 379735	NSGC	Israel	–	S	R
PI 380008	NSGC	Israel	Haifa	S	R
PI 380114	NSGC	Israel	Haifa	S	R
PI 287211	NSGC	Israel	Haifa	S	HR
PI 378825	NSGC	Israel	Haifa	S	R
PI 378832	NSGC	Israel	Haifa	S	R
PI 378843	NSGC	Israel	Haifa	S	MR
PI 378859	NSGC	Israel	Haifa	S	HR
AVE 941	IPK	Israel	Ar'ara	S	S
PI 379677	NSGC	Israel	Jerusalem	S	HR
AVE 531	IPK	Italy	Roccella Ionica, Calabria	S	S
AVE 2631	IPK	Italy	Soveria Mannelli, Calabria	S	S
AVE 1373	IPK	Italy	Apenninen	S	S
AVE 2532	IPK	Libya	about 45 km south of Taknis towards Kharuba	R	HR
AVE 2116	IPK	Libya	about 45 km south of Taknis towards Kharuba	S	S
AVE 534	IPK	Morocco	–	S	S
CN 25677	PGRC	Portugal	10 km southeast of Lvora	S	S

Accession	Bank[a]	Origin		*Puccinia coronata* race[b]	
		Country	Region	3.2	11
CN 25717	PGRC	Portugal	20 km east of Costello Bronco	S	S
CN 25748	PGRC	Portugal	35 km southwest of Costello Bronco	S	S
CN 26036	PGRC	Portugal	São Martinho do Porto	HR	HR
AVE 2561	IPK	Portugal	–	S	R
AVE 2713	IPK	Russian Federation	Derbent	S	S
AVE 1283	IPK	Russian Federation	–	S	S
AVE 1895	IPK	Spain	Bonares, Huelva	S	S
AVE 2800	IPK	Spain	–	S	S
CN 20304 CAV 11060	PGRC	Syria	10 km south of Damascus	S	S
CN 20328	PGRC	Syria	Kalet-el-Madeik	S	S
AVE 2578	IPK	Tajikistan	30 km north of Dushanbe	S	S
AVE 2705	IPK	Tajikistan	20 km north of Dushanbe	S	S
AVE 2919	IPK	Tunisia	–	S	S
AVE 1935	IPK	Ukraine	Yalta	S	HR
PI 304556	NSGC	United Kingdom	Wales	S	S

[a] Gene banks: IPK – Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany; NSGC – National Small Grains Collection, Aberdeen, Idaho, USA; PGRC – Plant Gene Resources of Canada, Agriculture and Agri-Food Canada, Saskatoon, Canada

[b] Resistance phenotype: S – susceptible, large to moderately large pustules with little or no chlorosis; MS – moderately susceptible, moderately large pustules surrounded by extensive chlorosis; MR – moderately resistant, small pustule surrounded by chlorosis; R – resistant, chlorotic or necrotic flecking; HR – highly resistant, no visible reaction

Grey shading used to highlight genotypes presenting different levels of resistance

a 16-h photoperiod. After ten days of incubation in a growth chamber, crown rust disease symptoms were assessed using the numeric 0-to-4 scale of Murphy transformed as follows: 4 – susceptible (S), large to moderately large pustules with little or no chlorosis; 3 – moderately susceptible (MS), moderately large pustules surrounded by extensive chlorosis; 2 – moderately resistant (MR), small pustules surrounded by chlorosis; 1 – resistant (R), chlorotic or necrotic flecking; and 0 – highly resistant (HR), no visible reaction [Murphy 1935, Carson 2009, Sowa et al. 2016, Nazareno et al. 2018].

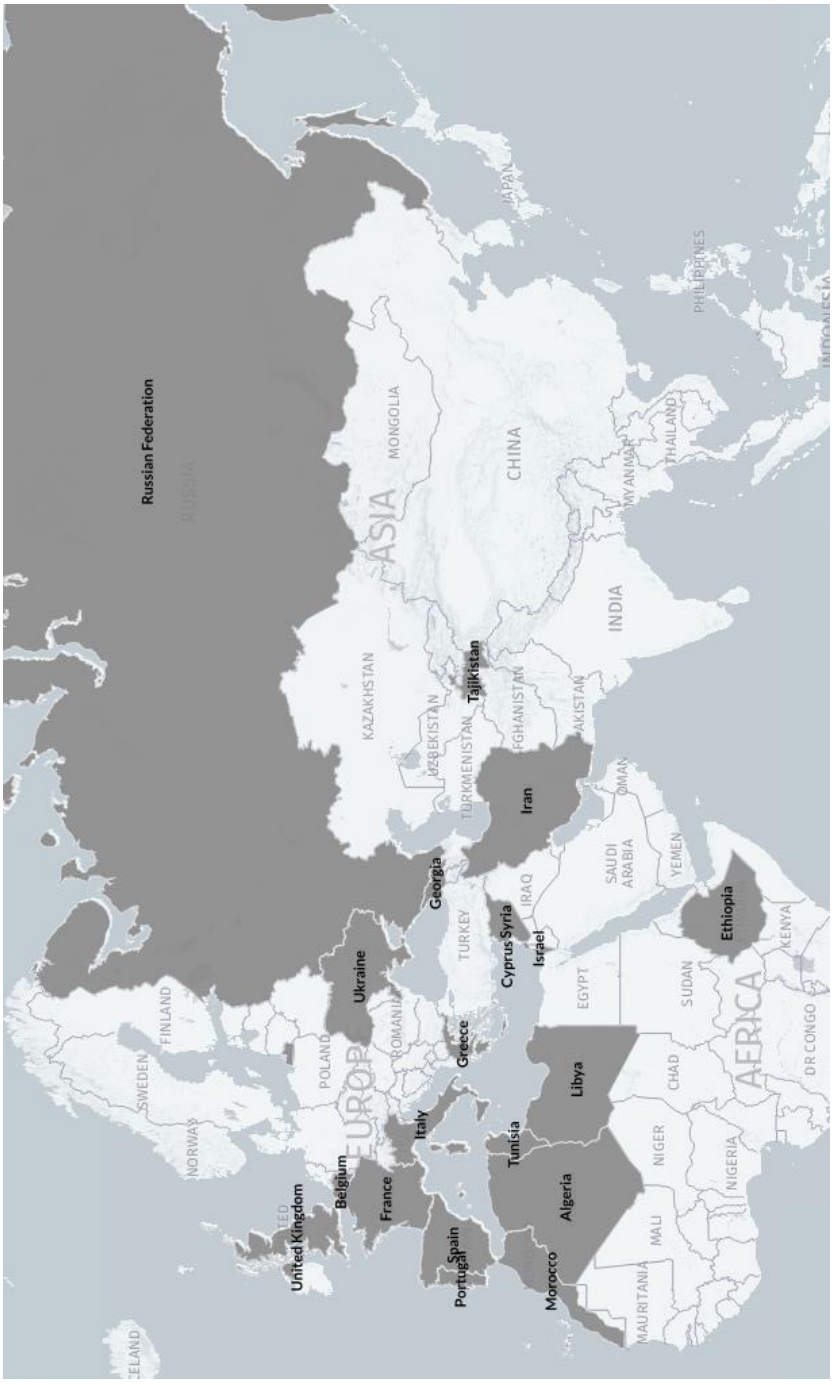

Fig. 1. Distribution of *Avena sterilis* L. genotypes used in the study for searching new sources of resistance to crown rust in oat; self-development, the map was generated on carto.com

Table 2. Virulence spectrum of *Puccinia coronata* f. sp. *avenae* isolates used for testing resistance of *Avena sterilis* L. genotypes

Puccinia coronata isolate	Virulence to crown rust differentials																																				
	Pc 14	Pc 35	Pc 36	Pc 38	Pc 39	Pc 40	Pc 45	Pc 46	Pc 48	Pc 50	Pc 51	Pc 52	Pc 54	Pc 55	Pc 56	Pc 57	Pc 58	Pc 59	Pc 60	Pc 61	Pc 62	Pc 63	Pc 64	Pc 67	Pc 68	Pc 70	Pc 71	Pc 91	Pc 94	Pc 96	Pc 97	Pc 98	Pc 101	Pc 103-1	Pc 104
3.2	+	+	+	+	+	+	+	+			+		+	+	+					+	+	+	+	+		+				+	+	+	+	+	
11.1			+		+	+	+						+	+	+					+	+		+	+		+								+	

RESULTS

Puccinia coronata isolates 3.2 and 11.1 used in the study displayed virulence towards 12 and 23 *Pc* differential oat lines respectively. None of the isolates broke the resistance of lines Pc48, Pc50, Pc52, Pc57, Pc58, Pc59, Pc60, Pc68, Pc71, Pc91, Pc94 and Pc104.

Most of the 45 tested *A. sterilis* accessions (31; 68.9%) displayed susceptible phenotype to *P. coronata* isolates used and crown rust response varied from susceptible (S) through moderately susceptible (MS) to highly susceptible (HS). Twelve accessions (26.7%) presented different levels of resistance to 3.2 *P. coronata* race ranging from moderately resistant (MR) to highly resistant (HR). Among this accessions, ten were collected in Israel, one was gathered in Megalopolis, Peloponnese, Greece (AVE 245), and one originated from Portugal (AVE 2561). Only two (4.4%) accessions, were resistant to both isolates (3.2 and 11.1) of which first displayed high resistance (HR) and was collected in São Martinho do Porto, Portugal (CN 26036), and second was gathered in Libya, about 45 km south of Taknis towards Kharuba (AVE 2532) and was resistant (R) to 3.2 and highly resistant (HR) to 11.1.

DISCUSSION

This study presents *P. coronata* resistance evaluation of 45 *A. sterilis* accessions gathered from European and North American gene banks. Genotypes were assessed at the seedling stage for crown rust reaction using the host–pathogen test and two *P. coronata* isolates. Twelve accessions presented resistance to less virulent 3.2 crown rust isolate. This pathotype displayed virulence towards 12 oat lines being *Pc* genes differentials. Ten of those twelve accessions were collected in Israel, one originated in Megalopolis, Peloponnese, Greece, and one in Portugal. *Avena sterilis* is a very common weed in Israel and is regularly infected by *P. coronata.* In Israel, we also observe the ubiquitous presence of an alternative crown rust host, *Rhamnus palaestina* on which sexual propagation of *P. coronata* takes place [Wahl et al. 1984]. Leonard et al. [2004] in their studies conducted from 1991 to 1996 confirmed extremely high virulence ('superraces') and polymorphism of crown rust population in Israel. Long process of rust – plant coevolution and selection pressures imposed by the host on the pathogen and by the pathogen on the host might contribute to genetic diversity of resistance in wild *A. sterilis* as well as virulence in *P. coronata* populations. Therefore, Israel and other Mediterranean countries, being the center of origin of oats and oat crown rust epidemiological zone, are the most significant origins of *Pc* resistance genes [Leonard et al. 2004, Cabral and Park 2014].

Only two accessions were resistant to both isolates used in the study (3.2 and 11.1) of which one was collected in Portugal (CN 26036), and one in Libya (AVE 2532). The use of pathotypes with defined infection profile allows us to speculate about the presence of particular *Pc* genes in the analyzed *Avena* materials. Most of the genes, whose presence in these two accessions cannot be excluded, because none of the isolates were virulent against them, came from *A. sterilis* accessions collected in Israel (*Pc48*, *Pc50*, *Pc52*, *Pc57*, *Pc58*, *Pc59*, *Pc60*, *Pc71*) [Leonard et al. 2004]. *Pc68* comes from an *A. sterilis* accession derived from Algeria [Wong et al. 1983] whereas *Pc104* from *A. sterilis* of unidentified

origin [Chong, unpublished]. The original source of *Pc91* is *A. magna* Murphy et Terrell (synonym: *A. maroccana* Gdgr.) [Rothman 1984], whereas *Pc94* was derived from *A. strigosa* Schreb. [Aung et al. 1996]. Distinct geographical origin, as well as different source species of the tested accessions and genotypes being original sources of resistance genes allow us to speculate that CN 26036 and AVE 2532 may contain new, previously unidentified resistance genes. Portugal is the origin of *A. sterilis* CI 8081, the donor of *Pc36* [Simons 1965], which was overcome by both of the crown rust isolates used in the study. Moreover, there is no information about *P. coronata* resistance genes identified in genotypes collected in Algeria.

CONCLUSIONS

Accessions identified in this research can be a valuable source of resistance to crown rust, and provide new germplasm for use in resistance breeding programmes. However, future work is required to clarify the genetic background and novelty of resistances observed.

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Streszczenie. Podstawowym źródłem genów odporności na rdzę koronową owsa (*Pc*) w rodzaju *Avena* są przede wszystkim gatunki heksaploidalne, a wśród nich *A. sterilis* L. będący donorem największej ilości genów *Pc*. W niniejszym badaniu oceniono na etapie siewki odporność na rdzę koronową pochodzących z 21 krajów genotypów *A. sterilis* pozyskanych z europejskiego i północnoamerykańskich banków genów. Testy przeprowadzono za pomocą testu żywiciel––patogen, z zastosowaniem dwóch izolatów *Puccinia coronata* f. sp. *avenae*. Spośród 45 analizowanych genotypów owsa, 12 było odpornych na jeden izolat rdzy koronowej (3.2). Odporność na oba patotypy prezentowały dwa genotypy *A. sterilis*, z których pierwszy pochodził z Libii (AVE 2532), a drugi z Portugalii (CN 26036). Konieczne są dalsze badania w celu oceny podłoża genetycznego odkrytej odporności, jednak uzyskane wyniki stanowią cenny pierwszy krok w identyfikacji obiecujących, nowych źródeł odporności na rdzę koronową owsa.

Słowa kluczowe: *Avena sterilis* L., *Puccinia coronata* f. sp. *avenae*, test żywiciel–patogen, linie referencyjne

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