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Search for genotypes resistant to Cercospora (*Cercospora beticola* Sacc.) in multigerm breeding materials of sugar beet

Poszukiwanie genotypów odpornych na *Cercospora beticola* Sacc. w wielonasiennych materiałach hodowlanych buraka cukrowego

Summary. In sugar beet cultivation, one of the main factors causing a significant drop in size and yield quality is the infection of plant leaves by the fungus *Cercospora beticola* Sacc. The main aim of the work was to identify inbred lines of sugar beet with genes of resistance to this pathogen. The research and selection works were carried out in multigerm breeding materials type 2xZN with a different degree of homozygosity. For the study, 36 genotypes of the S1 generation and 34 genotypes of the S2 generation were selected as well as 1 standard (Andante) variety with an increased level of fungal resistance. The sensitivity of materials to infection of *C. beticola* was assessed using a laboratory method (*in vitro* test). After twice selection a high stabilization of resistance was found within the examined progeny and a clear differentiation between the progeny of the S1 and S2 generation. The average number of spots on 10 leaf discs in the studied 9 progeny of S1 generation was from 10.50 to 31.04, and in 11 progeny of S2 generation from 6.30 to 28.49 using infection under optimal conditions for fungal growth. The wide range of variability that occurred in the tested materials made it possible to select 9 genotypes of the S1 generation and the 11 S2 generation with a high level of resistance to the *C. beticola* and high cultivation value.

Key words: beet, cercospora leaf spot, breeding

INTRODUCTION

In sugar beet cultivation, one of the main factors causing a significant drop in size (up to 50%) and yield quality is the infection of plant leaves by the fungus *Cercospora beticola* Sacc. – the perpetrator of leaf spot of beet [Shane and Teng 1992, Byford 1996,

Asher et al. 2000, Skarcis et al. 2010]. Since the beginning of the 1990s, a strong expansion of *C. beticola* has been observed in Poland [Nowakowska et al. 1997, 1999, 2002, Wójtowicz and Jakubowska 2000, Piszczek 2001]. This pathogen, under favorable weather conditions (hot and humid years), creates a huge problem on many industrial beet plantations. Due to the drastic increase in the number of fungicide-resistant fungicide strains [Piszczek and Czekalska 2006, Skarcis et al. 2010, Pieczul and Perek 2013], and in particular those that contain active substances from the strobilurin group [Kiniec et al. 2017], the best way to protect sugar beet crops against losses caused by leaf spot is to grow beet materials resistant to *C. beticola* infection [Asher et al. 2000, Panella and Frese 2000, Skarcis et al. 2010]. In the view of current European Union policy limiting the availability of plant protection products [EU 2009], this is particularly important, because despite the use of both classical and molecular breeding methods, new varieties meet the hopes placed in them only to a small extent.

The aim of the research was to isolate inbred lines of sugar beet with genes of resistance to leaf spot of beet with favorable economic traits, i.e. good root yield, high sugar content and low content of molasses-forming substances, which could be used in further creative breeding.

MATERIAL AND METHODS

Identification of sources of resistance to *C. beticola* was carried out using diploid multigerm sugar beet sugar-normal (2xZN) material with varying degrees of homozygosity. All evaluated lines originated from Polish beet breeding. The 36 genotypes of the S_1 generation and 34 genotypes of S_2 generation were selected for the five-year study along with 1 reference cultivar with increased resistance to the pathogen (Andante). The field experiments were conducted in Wielkopolska Sugar Beet Breeding Company, at Plant Breeding Station in Łagiewniki (WHBC SHR). Laboratory tests and statistical analysis of results by means of variance analysis (ANOVA) were carried out at the Institute of Plant Breeding and Acclimatization – National Research Institute in Bydgoszcz.

The basic method of growing the improved sugar beet material is intensive selection in terms of the tested traits carried out under provocative conditions [Dalke 2004]. Modified laboratory method developed by Stähle-Csech and Gisi [1991] was applied for testing of beetroot resistance to leaf spot, which allows rapid, weather-independent population assessment in an *in vitro* test. To assess the sensitivity to *C. beticola*, healthy beet leaves were taken from the central verticils from 50 plants of the tested genotype. From each leaf, 10 discs with a diameter of 10 mm were cut using a corkscrew and put on 5% agar-agar water solution. Then plates with cut discs were inoculated with the suspension of *C. beticola* at a concentration of 10^6 spores/ml and a mycelium homogenate of 10^2 hyphae/ml and placed in an air-conditioned chamber at 25°C with a 16-hour photoperiod. After 8 days, the number of appearing spots on each leaf disc was determined (Photo 1). The sum of spots from 10 discs was assumed as an indicator of infection for a single plant. Based on the results for individual plants, the average values for a given population were calculated. The choice of genotype was determined by the lowest average number of spots observed in the experiment in relation to the cultivar / material with increased resistance to the pathogen.

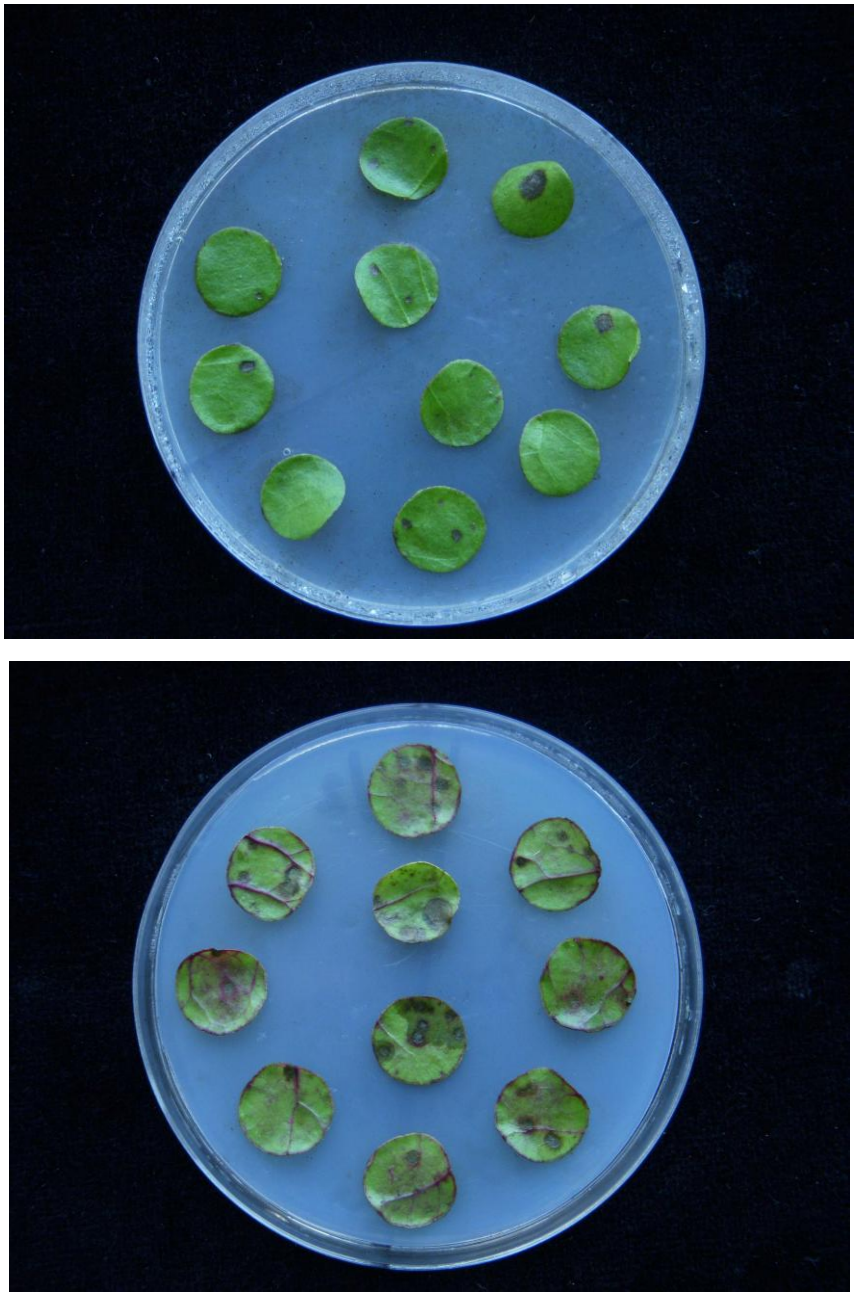

Photo 1. *In vitro* laboratory test for *C. beticola* resistance (after 8 day): A – genotype with an increased level of fungal resistance; B – genotype susceptible to pathogen (photo K. Kużdowicz)

After the first selection, progeny with higher than the standard resistance to *C. beticola* and percentage sugar content in the root ranging from 16.5% to 17.5%, taking the root weight, were selected for further research and selection. In order to multiply the obtained material, roots of selected offspring were planted in nursery of inbred growing in cannabis isolation. In the fourth year of research in individual nurseries, roots of the maternal line (CMS) were planted in the roots of the paternal line (2xZN). The utility value of selected 2xZN type S_1 and S_2 generation lines with genes of resistance to the leaf spot of beet, was estimated based on results of the field experiments.

RESULTS

In 2012–2016, 70 sugar beet lines of the 2xZN type were tested (Figs. 1, 2). After the first selection based on the *in vitro* test, 19 genotypes of the S1 generation and 19 genotypes of the S_2 generation with the lowest average number of spots in relation to the standard with increased resistance to *C. beticola* were selected for further research and selection. Wide range of variability occurring in tested components of the paternal component made it possible to select 9 genotypes of the S_1 generation and 11 genotypes of the S_2 generation with genes of the resistance to leaf spot (Figs. 3, 4).

Analysis of the obtained results showed that the reaction of tested sugar beet plants to infection by *C. beticola* varied and depended on the generation and genotype of a given line. As a result of the selection, there were clear differences in terms of the examined feature between 2xZN lines of generations S_1 and S_2 and hybrids produced on these lines.

After double selection for increasing the resistance to *C. beticola*, this feature was found to be highly stabilized within the tested offspring and a clear differentiation between the offspring of S_1 and S_2 generations. Variance analysis for the tested feature showed highly significant differences between particular lines of type 2xZN lines of S_1 and S_2 generations. The average number of spots on 10 leaf discs in the examined 9 genotypes of S_1 generation ranged from 10.50 to 31.04 and in 11 genotypes of S_2 generation from 6.30 to 28.49 applying infection under optimal conditions for fungal growth (Tabs. 1, 2). Significant differences also occurred between hybrids obtained on the S_2 generation lines at the significance levels $p = 0.01$ and $p = 0.05$. Selected line No. 69 of the S_2 generation of 2xZN type and hybrid obtained on this line were characterized by the lowest average number of spots on 10 discs, respectively 6.30 and 13.90 (Tab. 2).

Hybrids obtained on lines 2xZN of generation S_1 in terms of the examined feature did not significantly differ, which was probably related to the low degree of homozygosity of the paternal component.

With the participation of selected lines in the experiments, seed of 20 hybrids was obtained, of which 11 (S_2 generations) gave selected feature to the offspring, which was statistically confirmed at the significance levels $p = 0.01$ and $p = 0.05$ (Tab. 2).

Table 1. Testing of multigerm lines of S_1 generation 2xZN type and hybrids (F) produced on these lines for *C. beticola* resistance

Material Generation S_1		Laboratory test			
		Average number of spots of 50 plant		Range from – to	
		2xZN	F	2xZN	F
1.	ZN - 3	24.35	31.20	2–49	2–60
2.	ZN - 4	10.50	22.65	0–37	3–40
3.	ZN - 5	22.25	22.94	0–48	3–49
4.	ZN - 6	19.00	28.95	0–52	0–48
5.	ZN - 20	31.04	36.90	3–61	1–94
6.	ZN - 21	19.90	36.04	0–51	1–70
7.	ZN - 24	19.70	26.95	0–64	7–65
8.	ZN - 25	13.40	28.05	0–32	9–57
9.	ZN - 36	22.65	33.40	5–42	6–58
Mean total		20.31	29.67	–	–
LSD $\alpha = 0.05$		9.16	n.s.	–	–
LSD $\alpha = 0.01$		12.09	n.s.	–	–

n. s. – dependency not statistically significant

Table 2. Testing of multigerm lines of S_2 generation 2xZN type and hybrids (F) produced on these lines for *C. beticola* resistance

Material Generation S_2		Laboratory test			
		Average number of spots of 50 plants		Range from – to	
		2xZN	F	2xZN	F
1.	ZN - 37	15.30	33.05	0–61	7–63
2.	ZN - 43	23.75	35.10	3–51	0–60
3.	ZN - 47	13.85	23.75	0–30	1–32
4.	ZN - 48	18.20	28.70	4–65	8–48
5.	ZN - 49	14.60	27.45	6–36	3–55
6.	ZN - 50	27.25	35.95	4–51	17–55
7.	ZN - 54	28.49	26.45	11–67	2–56
8.	ZN - 55	19.65	32.59	0–37	7–49
9.	ZN - 57	20.00	22.94	0–45	7–41
10.	ZN - 67	11.55	16.45	0–40	1–37
11.	ZN - 69	6.30	13.90	0–17	4–29
Mean total		18.08	26.94	–	–
LSD $\alpha = 0.05$		8.12	7.98	–	–

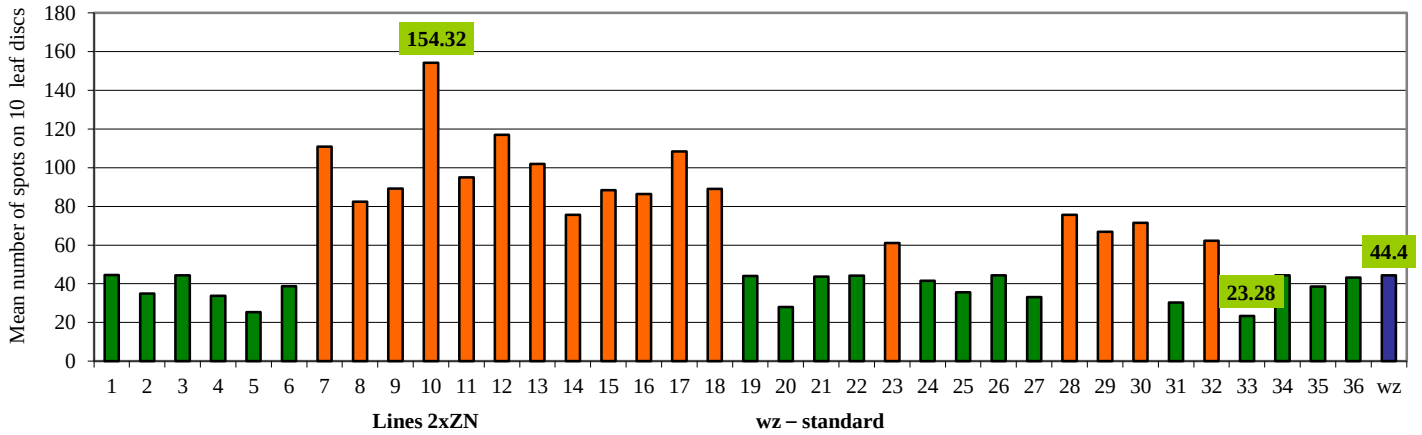

Fig. 1. Results of testing resistance of sugar beet S_1 generation materials to *C. beticola* after first selection

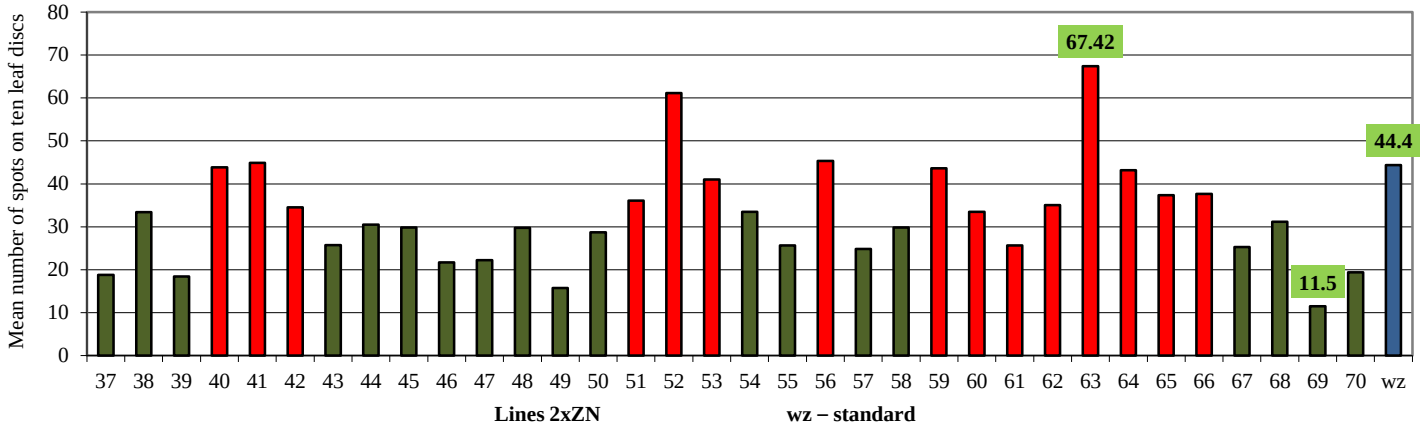

Fig. 2. Results of testing resistance of sugar beet S_2 generation materials to *C. beticola* after first selection

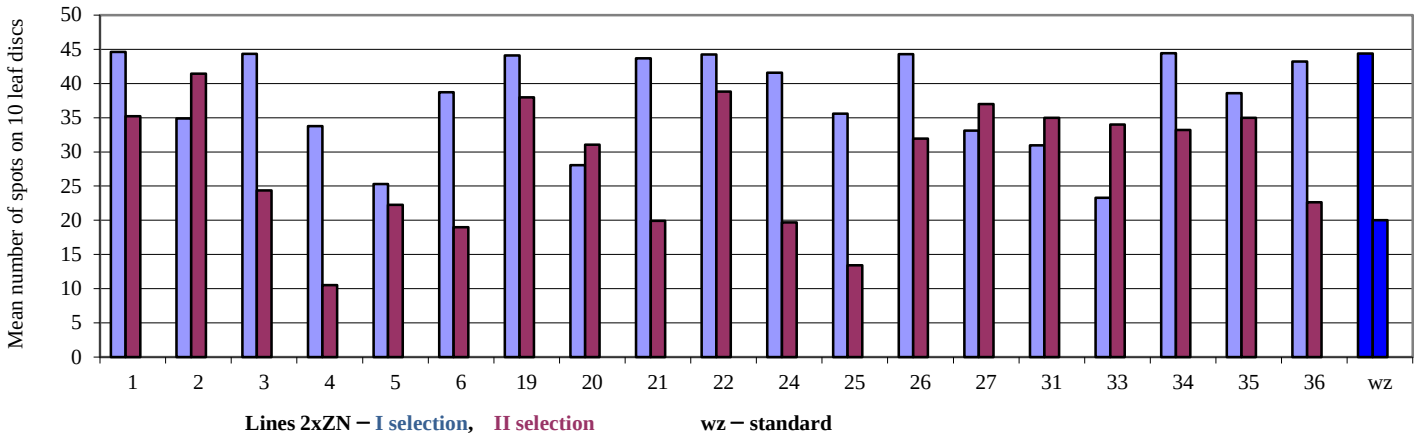

Fig. 3. Results of testing resistance of sugar beet S_1 generation materials to *C. beticola* after first and second selection

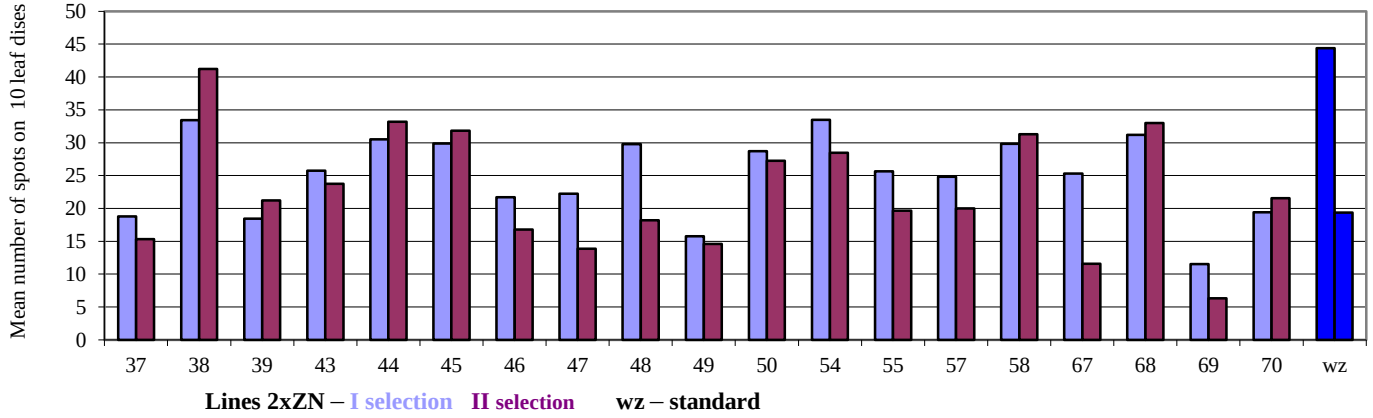

Fig. 4. Results of testing resistance of sugar beet S_2 generation materials to *C. beticola* after first and second selection

DISCUSSION

Conducted research gives a hope that appropriate selection and scrupulous selection of the research material possessed may result in starting lines for the creative breeding of new beet varieties with increased resistance to *C. beticola*, without losing favorable economic characteristics. Unfortunately, breeding work and related selection of genotypes is a long-term process, because resistance to this pathogen is conditioned by polygenicity [Asher et al. 2000, Panella and Frese 2000, Weiland and Koch 2004], and genetic basis of fungal resistance is protected by intellectual property rights even after registration of the variety. Therefore, degree of resistance of both sugar beet varieties and individual starting lines used in their breeding differs [Pfleinderer and Schäufele 2000, Kużdowicz 2010]. Practice indicates that varieties with medium resistance and proper chemical protection, during the time of the infection lack, give higher yields of sugar beet than those with high resistance [Windels et al. 1998]. Perhaps this is due to the fact that leaves of such varieties are easily infected by powdery mildew, which is not conducive to photosynthesis and other life processes of a plant [Piszczek and Mrówczyński 2012].

The use of varieties not susceptible to infection by *C. beticola* is an important element in the protection of beet in areas of its increased occurrence [Piszczek 2010, Piszczek and Mrówczyński 2012]. This fungus exhibits very high phenotypic and genotypic variation already present at the level of a population that colonizes one leaf [Moretti et al. 2004, Pieczul and Piszczek 2010]. Under favorable conditions, it can form several generations over a year, that are easily immunizing, as mentioned in the introduction of the paper, to fungicides frequently used in a given region. In this case, chemical protection may not be effective [Piszczek 2010, Pieczul and Perek 2013, Kiniec et al. 2017].

The obtained breeding materials are particularly desirable in creative breeding of sugar beet and fodder beet and are useful for genetic and biotechnological research. For this reason, seeds selected in the experiment having original genotypes with genes resistance to *C. beticola* were transferred to the beet collection of the National Center for Plant Genetic Resources in Radzików. In the near future, they will be made available for the use as a source of additional genetic variation originating from the natural gene pool of beet cultivars.

CONCLUSIONS

1. Based on a laboratory test, the 9 offspring plants were selected in the S_1 generation and in the S_2 generation – 11 offspring plants with a high degree of resistance to *C. beticola*.

2. With the participation of selected homozygous lines, seed of 20 hybrids was obtained, of which eleven (S_2) transferred the selected feature to the offspring, which was statistically confirmed at the significance levels $p = 0.01$ and $p = 0.05$.

3. Selection of 2xZN lines tested for susceptibility to infection by fungus *C. beticola*, aimed at obtaining the lines resistant to this pathogen, is possible but should also take into account the yield potential and sugar content.

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Streszczenie. W uprawie buraka cukrowego jednym z głównych czynników powodujących znaczący spadek wielkości i jakości plonu jest porażenie liści roślin przez grzyb *Cercospora beticola* Sacc. Celem prowadzonych prac było wyodrębnienie linii wsobnych buraka cukrowego z genami odporności na ten patogen. Prace badawczo-selekcyjne prowadzono w wielonasiennych materiałach hodowlanych buraka cukrowego typu 2xZN o zróżnicowanym stopniu homozygotyczności. Do badań wybrano 36 genotypów generacji S_1 i 34 genotypy generacji S_2 oraz 1 odmianę wzorcową (Andante) o podwyższonym stopniu odporności na grzyb *Cercospora beticola* Sacc. Wrażliwość materiałów na infekcję *C. beticola* oceniano metodą laboratoryjną (test *in vitro*). Po dwukrotnej selekcji stwierdzono wysoką stabilizację cechy odporności wewnątrz badanego potomstwa oraz wyraźne zróżnicowanie pomiędzy potomstwem generacji S_1 i S_2. Średnia liczba plamek na 10 krążkach liścia u badanych 9 potomstw generacji S_1 wynosiła od 10,50 do 31,04, a u 11 potomstw generacji S_2 od 6,30 do 28,49 przy zastosowaniu infekcji w optymalnych warunkach dla rozwoju grzyba. Szeroki zakres zmienności, jaki wystąpił w badanych materiałach, umożliwił wyselekcjonowanie 9 genotypów generacji S_1 i 11 generacji S_2 o wysokim stopniu odporności na *C. beticola* i o wysokiej wartości użytkowej.

Słowa kluczowe: burak, chwościk, hodowla

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