

R2-like Gene Contributes to Resistance to *Phytophthora infestans* in Polish Potato Cultivar Bzura

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Abstract Potato cultivar Bzura bred in 1986 is one of the few European cultivars outstanding in high level of durable resistance to late blight, but its resistance has not been characterized. The presented study was aimed at clarifying bases of durable resistance in Bzura by testing its resistance and resistance in progeny individuals. In ten-year field experiment Bzura was significantly more resistant than mid-resistant standards Escort and Robijn, and only slightly less resistant than highly resistant Sárpo Mira. Bzura expressed also high resistance in detached leaflet tests when inoculated with specific isolates of *Phytophthora infestans*. It was found in Bzura progeny that this race-specific resistance segregated in 1:1 ratio and is governed by a major resistance gene *R2-like*. However, the long-lasting field resistance observed in Bzura could be explained by combination of *R2-like* gene and specific genetic background of this cultivar rather than by this gene exclusively.

Resumen La variedad de papa Bzura, de 1986, es una de las pocas variedades europeas sobresalientes en alto nivel de resistencia durable al tizón tardío, pero no se ha caracterizado su resistencia. El presente estudio tuvo como propósito la clarificación de las bases de la resistencia durable en Bzura mediante la prueba de su resistencia y de la de individuos de su progenie. En experimentos de campo de diez años Bzura fue significativamente más resistente que las de resistencia media estándar Escort y Robijn, y solo ligeramente menos resistente que la altamente resistente Sárpo Mira. Bzura

expresó también alta resistencia en pruebas de hoja separada cuando se le inoculó con aislamientos específicos de *Phytophthora infestans*. Se encontró en la progenie de Bzura que esta resistencia específica a razas segregó en una proporción de 1:1 y está gobernada por un gene mayor de resistencia tipo R2. No obstante, la resistencia durable de campo observada en Bzura pudiera explicarse por la combinación de un gene tipo R2 y un respaldo genético específico de esta variedad, en vez de este gene exclusivamente.

Keywords Race-specific resistance · Durability · Detached leaflet assay · Late blight · Potato

Introduction

Polish potato cultivar Bzura was released in 1986, but it is still distinguished by durable resistance to late blight. This is a late maturing, high yielding, starch cultivar. In some regions of Poland Bzura is also grown as table cultivar due to its floury cooking type and good taste. This cultivar was bred in IHAR-PIB, Młochów Research Center, within the frame of program aimed at combination of multiple resistances to various potato diseases. Bzura is resistant to late blight (caused by *Phytophthora infestans* (Mont.) de Bary), potato viruses Y and X, and potato wart fungus (race D1 and some virulent pathotypes) (Chrzanowska 2000; Douches et al. 2002, 2004; Flis et al. 2005; Przetakiewicz 2010; Tatarowska et al. 2011; Zimnoch-Guzowska et al. 2013). Therefore, this cultivar was widely used in many research and breeding programs in Europe and in both Americas (Ahmadvand 2013; Bendahmane et al. 2000; Bisognin et al. 2002; Haynes et al. 1998; Polkowska-Kowalczyk et al. 2011; Querci et al. 1995). This cultivar is still cultivated in Poland, due to its high level of

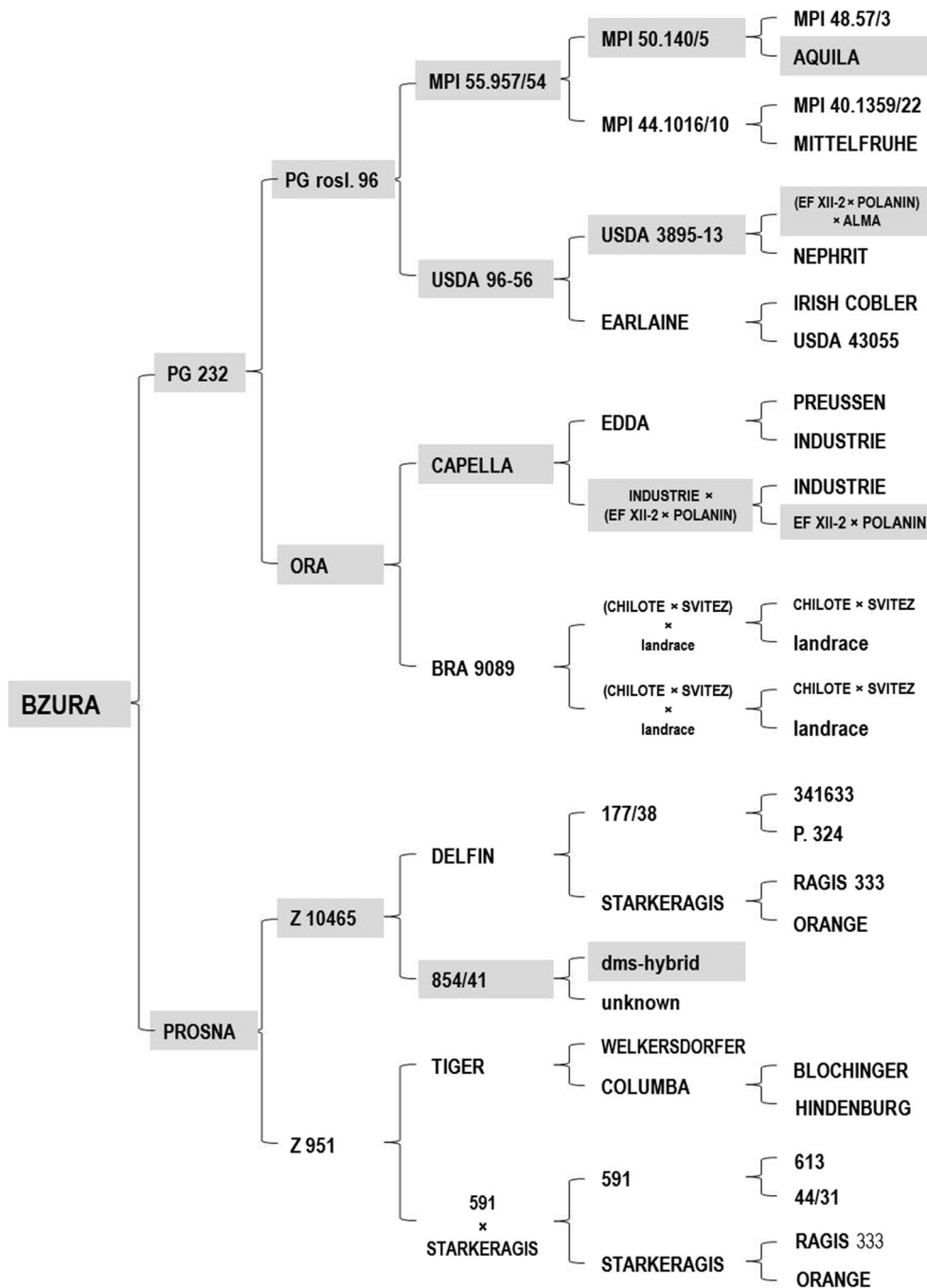
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field resistance to late blight. Resistance against *P. infestans* of cv. Bzura provides effective protection against late blight in various environments. Results of large-scale field experiments carried out in Poland (four locations) (Tatarowska et al. 2012) as well as in United States (eight locations) (Haynes et al. 1998) confirmed high and stable resistance of this cultivar. There are also reports about effectiveness of Bzura resistance under strong late blight pressure in Toluca Valley (GILB 2001).

Resistance of this cultivar originates probably from *S. demissum*, since in its pedigree multiple introductions of this species can be found (Swieżyński et al. 1997, Fig. 1).

The long lasting effective protection against late blight in field conditions and stable expression of resistance in various environments (parallel to noted susceptibility in the laboratory assay) may indicate an important role of horizontal resistance. The presence of *demissum*-derived R genes in Bzura is also expected (Dorrance and Inglis 1997; Lebecka et al. 2006; Świeżyński et al. 1991). So far, only the presence of the *R1* gene in Bzura was confirmed (Gebhardt et al. 2004). However, it is highly unlikely that the observed resistance is solely due to the presence of *R1* gene, since its effectiveness in field conditions is low (Vleeshouwers et al. 2011). In cv.

Fig. 1 Pedigree of cultivar Bzura - grey color marks *S. demissum* sources (According to Swieżyński et al. 1997)



Prosna, the female parent of Bzura, Siczka (1979) has found an unidentified, highly effective major resistance gene against late blight. This gene could be present also in Bzura, since after inoculation with some (incompatible) isolates leaflets of Bzura show typical hypersensitive reaction (HR). Analysis of virulence profiles of set of *P. infestans* isolates, which are avirulent to Bzura, indicate possible role of *R2* gene (R. Lebecka and A. Michalska - personal communication). These observations suggest presence of a major resistance gene in Bzura, which probably belongs to *R2*-gene-family and provides resistance to similar spectrum of *P. infestans* races like the *R2* gene.

Although host resistance is considered as effective and environmentally friendly way of restricting late blight infection, its practical use has had very limited success. There is still a scarcity of highly and durably resistant potato cultivars (Forbes 2012). Among a few Polish potato cultivars with a high level of resistance to late blight, Bzura stands out in terms of stability of this resistance in various environments (Haynes et al. 1998; Tatarowska et al. 2012). Despite of that, the genetic factors underlying the resistance against late blight in Bzura have been never characterized in detail. The objective of presented study was to clarify the bases of Bzura resistance by testing resistance of this cultivar in various conditions and by identification of genetic factors involved in Bzura race-specific resistance observed earlier in detached leaflet. The chromosomal localization and nucleotide sequence of identified gene were also determined.

Materials and Methods

Plant Materials

In field and laboratory experiments, the resistance of Bzura was assessed along with resistance standards: cvs Sárpo Mira (highly resistant), Escort (mid-resistant), Robijn (mid-resistant), and Bintje (susceptible). Set of eleven Black's differentials was also used to determine the virulence of *P. infestans*. The field trials were conducted with standard fertilization (120 kg N ha⁻¹, 80 kg P₂O₅ ha⁻¹, 120 kg K₂O ha⁻¹) and chemical plant protection excluding fungicide applications. For field experiments, Randomized Complete Block design was applied with 2 blocks and 6 hill plots. To study race-specific resistance, a tetraploid F1 progeny was developed by crossing of Bzura with susceptible male parent cv. Felka Bona. The unselected (Bzura × Felka Bona) F1 consisted of 140 individuals.

Resistance Tests

Field Resistance Tests Field resistance tests of cvs Bzura, Sárpo Mira, Escort, Robijn, and Bintje were performed during

ten consecutive years from 2004 to 2013 in Boguchwała (South-eastern part of Poland) under high, natural infection pressure. In this location the weather conditions are favorable for late blight infection and artificial inoculation was not applied. Each year six tubers per cultivar in two replications were planted. Spreader plants of a semi-susceptible cultivar Alicja or Gawin were used to separate plots and to ensure a reliable source of inoculum during the epidemic period. Disease severity was evaluated weekly from first days of July till the mid-August. The values of rAUDPC (relative Area Under Disease Progress Curve) were calculated from 6 or 7 readings, according to Fry (1978).

Laboratory Assessment In laboratory conditions, resistance was assessed in detached leaflet tests according to Zarzycka (2001) in three consecutive years 2007, 2008, and 2009. In all 3 years plants of (Bzura × Felka Bona) F1 were tested along with their parents (Bzura and Felka Bona) and standards. Five leaflets per genotype were tested in two replications twice each year. Leaflets were inoculated with a 30 μl droplet of sporangia/zoospore suspension (50 sporangia/μl). Resistance of leaflets was scored after 6 days of incubation, on a 1–9 scale, where 9 was the most resistant.

Isolates of *P. infestans* In detached leaflet tests, three isolates MP 725, MP 824, and MP 847 (race: 1, 3, 4, 7, 10, 11) from IHAR-PIB Młochów potato pathogen collection were used in years 2007, 2008, and 2009, respectively. These isolates were collected in various parts of Poland but all were avirulent to Bzura and exhibited the same virulence profiles, therefore in this study were considered as one group of isolates AvrBz (avirulent to Bzura). In all 3 years, a virulent to cv. Bzura isolate MP 324 (race 1, 2, 3, 4, 6, 7, 10, 11) was also used as a control. Isolate MP 324 collected in 1997 in Poland is highly aggressive, metalaxyl resistant and is of A1 mating type. This isolate has frequently been used in late blight resistance tests (Lebecka et al. 2006; Śliwka et al. 2006, 2007, 2013).

Statistical Analysis

To assess effects of genotype, year, and their interactions on the resistance scores, analysis of variance (ANOVA) was applied. Multiple comparisons of means were done by Tukey's test. The Pearson's correlation coefficients were calculated for results of detached leaflet tests between the individual years. The fit of segregation to the expected ratio was checked by the χ^2 test. Statistical analyses were performed with the use of MS Excel 2010 and STATISTICA'98 Edition.

DNA Extraction, Molecular Analysis and Gene Sequencing

Genomic DNA was extracted from 1 g of fresh, young leaves with the GenElute Plant Genomic DNA Miniprep kit (Sigma

Aldrich) according to the supplier's protocol. To verify hypotheses that the major resistance gene present in cv. Bzura is located on potato chromosome IV, the group of locus-specific DNA markers was used. Tested markers were previously used by Park et al. (2005) for mapping the gene *Rpi-blb3* (ortholog of *R2*) on potato chromosome IV. Primers sequences and annealing temperatures are listed in Table 1. A standard PCR conditions were applied: 5 min at 94 °C followed by 35 cycles of 94 °C for 30 s, appropriate T_m for 45 s, 72 °C for 45 s, then followed by 72 °C for 7 min. PCR analysis was performed in 20 µl reaction mix containing 1.5 ng/µl template DNA, 0.2 mM of each dNTP, 0.7 mM of primers and 1U Allegro Taq DNA polymerase in the reaction buffer provided by the manufacturer (Novazym, Poland). PCR products of B10L and 20D11R markers were digested respectively with *HinfI* and *TaqI* restriction enzymes and separated on agarose gels stained with ethidium bromide.

To confirm the presence of *R2* gene in cultivar Bzura, the PCR marker R2 was applied (Kim et al. 2012). Primers of this marker (Table 1.) are based on sequence of initial and terminal fragments of genes from *R2*-family, therefore product of its amplification is expected to be a full-length *R2* gene. For this marker modified PCR conditions were applied: 5 min at 94 °C followed by 35 cycles of 94 °C for 30 s, 60 °C for 150 s, 72 °C for 90 s, then followed by 72 °C for 7 min. PCR was performed in a total volume of 20 µl containing 0.2 mM dNTPs; 0.6 mM of primers and 1U Marathon Polymerase (A&A Biotechnology, Poland) in the provided reaction buffer. The obtained product of amplification (about 2,5 kb) was extracted from agarose gel (with GenElute Gel Extraction Kit, Sigma-Aldrich) and sequenced using four additional primers (Table 1). Sequencing of each fragment was performed at least two times in Genomed, Poland. The obtained sequences were analyzed using Chromas Lite 2.1.1 (Technelysium Pty Ltd) and SeaView 4.5.1 (Gouy et al. 2010). The search for sequence homology

with other potato *R* genes was carried out using the BLAST - Basic Local Alignment Search Tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results

Resistance Tests

Field Resistance The local populations of *P. infestans* in Boguchwała were virulent, and Black's differentials R1, R2, R3, R4, R6, R7, R10 and R11 were severely infected in each year of experiments. On differentials R5, R8 and R9, weak symptoms of infection were observed only occasionally. In each year, late blight symptoms were observed on plants of all tested cultivars with exception of Sárpo Mira. ANOVA for rAUDPC values revealed significant effects of cultivar, year and their interaction. The 'cultivar' source of variation accounted for more than 80 % of the total sum of squares, while 'year' and interaction 'cultivar × year' explained only 4.61 and 11.13 % of total variation, respectively.

During 10 years of field experiment we confirmed high and stable level of cv. Bzura resistance to late blight. The mean value of Bzura rAUDPC (0.084) did not differ significantly from mean rAUDPC of highly resistant standard Sárpo Mira (0.027), and it was significantly lower than values of susceptible standard Bintje or mid-resistant Robijn and Escort (Fig. 2, Table 2).

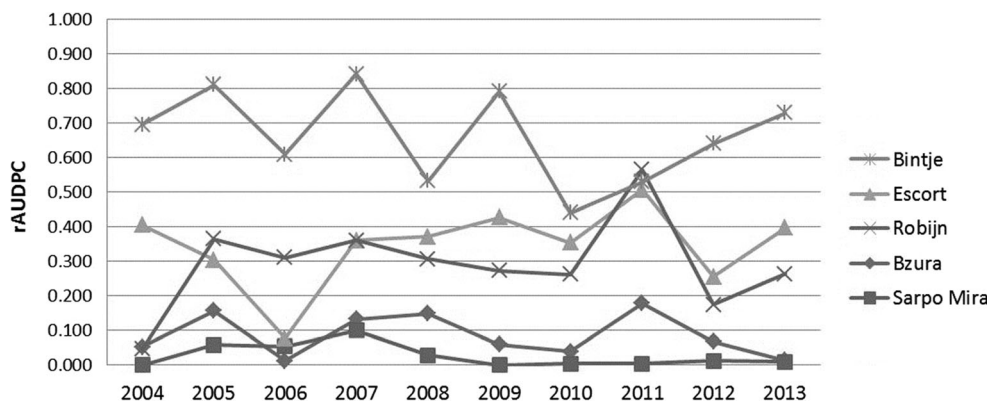
Detached Leaflet Tests – Parental and Standard Cultivars Susceptible parent Felka Bona proved to be highly susceptible after inoculation with isolates MP 324 and AvrBz. Bzura was susceptible after inoculation with MP 324, but highly resistant when AvrBz isolates were used. The standard cultivar Sárpo Mira was highly resistant, Robijn was rather susceptible and Bintje was highly susceptible, irrespectively to the isolate used. Standard cv. Escort, similarly to Bzura, was

Table 1 Sequences of primers used in this study

Marker	Sequence	T_m (°C)	Marker type	References
B10L F	TCAGAGGCATGACACCTGTG	58	CAPS/ <i>TaqI</i>	Park et al. 2005
B10L R	GTTGACCCAGTTGATTAAGTC			
20D11R F	AGCATCCGGAGGCAAATC	56	CAPS/ <i>HinfI</i>	Park et al. 2005
20D11R R	TAGGCTTAACTGTCAAATGG			
R2 F	ATGGCTGATGCCTTCTATCATTGTC	60	SCAR	Kim et al. 2012
R2 R	TCACAACATATAATTCCGCTTC			
R2 651 F	GATATGTGTCTCAAGAGTACAACA	58	SCAR	This study
R2 651 R	GATCACAAGCAACACCATCAACAAC			
BzR2 forw1 ^a	CCTGATTACTTGGCCCTTCTCTCT	–	SCAR	This study
BzR2 rev1 ^a	CAATCATTGCATCCCTTGAAT	–	SCAR	This study

^a markers used only in sequencing of initial and terminal fragment of potential *R2* gene

Fig. 2 Variation of rAUDPC values in 2004–2013 measured in Bzura and standards Sárpo Mira, Escort, Robijn, and Bintje



moderately susceptible to MP 324 but highly resistant to AvrBz isolates (Table 3).

Detached Leaflet Tests – (Bzura × Felka Bona) F1 Among clones of (Bzura × Felka Bona) F1 tested with isolate MP 324 resistant individuals were not found (mean score 2.9; range 1.8–4.6). However, the same clones inoculated with AvrBz isolates showed two clearly distinguishable types of reaction: no symptoms (or only small necrosis) or large lesions with visible sporulation, which cover large part of leaflet. Intermediate reaction was not observed (Fig. 3). The ratio of highly resistant clones to susceptible ones was 76:64, not significantly deviated from the ratio 1:1 ($\chi^2=1.03$, $P=0.311$) expected for segregation of a single gene. Results of 3 years tests were highly correlated ($r=0.857$ for 2007–2008, $r=0.794$ for 2007–2009 and $r=0.776$ for 2008–2009; $P<0.001$) and each year the same clones were classified in resistant or susceptible group.

According to ANOVA (data not shown), the genotype had the strongest effect on resistance and accounted for 93.7 % of variance in leaflets tests. Effect of year and interaction were also significant, but explained only 1.9 and 2.5 % of variation, respectively.

Table 2 Level of resistance to *P. infestans* of cvs Bzura, Sárpo Mira, Escort, Robijn, and Bintje in 10 years of field experiment 2004–2013 performed in Boguchwała

Cultivar	Mean rAUDPC	Minimum	Maximum	Standard deviation	Isolate	
					AvrBz	MP 324
Bzura	0.084	0.009	0.194	0.067	8.7 a	2.7 c d
Sárpo Mira	0.027	0.000	0.100	0.033	9.0 a	8.8 a
Escort	0.346	0.078	0.591	0.132	8.6 a	4.8 b
Robijn	0.292	0.032	0.595	0.138	3.8 b	3.7 c
Bintje	0.661	0.429	0.869	0.144	2.4 c	1.7 e

^a mean values followed by the same letter are not significantly different according to Tukey’s test

Molecular Analysis Resistance segregation in (Bzura × Felka Bona) F1 progeny suggests the presence of single dominant gene. To verify hypothesis, that this gene is located on potato chromosome IV, Bzura and Felka Bona, and all progeny clones were tested for the presence of DNA markers B10L and 20D11R. In our study these markers were polymorphic and associated with resistance. In (Bzura × Felka Bona) F1 progeny, the presence of markers was highly correlated with reaction observed in detached leaflet tests after inoculation with AvrBz isolates (Fig. 4). The percentage of recombination between markers and hypothetical gene R2 from Bzura was 4.3 and 5.0 % for B10L and 20D11R, respectively. Their presence explained respectively 82.9 and 79.6 % of variability observed in resistance.

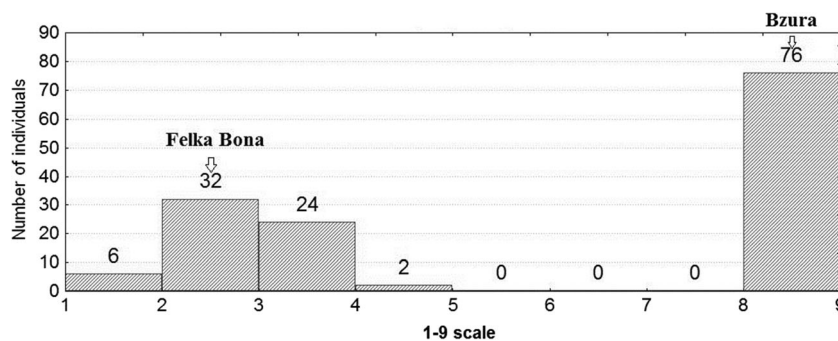
Bzura and Felka Bona, and all progeny clones were screened also for the presence of gene-specific marker R2. Product of amplification, sized ~2,5 kb, was present in Bzura and all resistant progeny clones, while absent in Felka Bona and all susceptible clones. This product was extracted from gel and sequenced. In result, 2544 bp long sequence was obtained. After comparison with other sequences in the

Table 3 Results of 3 years resistance assessment of parental cultivars Bzura and Felka Bona, and standard cultivars in detached leaflet tests in 2007–2009 with the use of avirulent and virulent isolates of *P. infestans*

Cultivar	Isolate			
	AvrBz		MP 324	
	mean ^a	range	mean ^a	range
Bzura	8.7 a	8.0–9.0	2.7 c d	1.3–4.3
Sárpo Mira	9.0 a	9.0–9.0	8.8 a	8.5–9.0
Escort	8.6 a	8.2–8.9	4.8 b	3.0–6.5
Robijn	3.8 b	3.0–5.0	3.7 c	2.6–4.8
Bintje	2.4 c	1.0–3.5	1.7 e	1.2–3.0
Felka Bona	2.7 c	2.0–4.0	2.5 d e	1.3–3.6

^a mean values followed by the same letter are not significantly different according to Tukey’s test

Fig. 3 The distribution of resistance mean scores from 2007 to 2009 obtained in detached leaflet tests for 140 individuals of (Bzura × Felka Bona) F1 inoculated with AvrBz isolates (scores of parents are also marked). All (Bzura × Felka Bona) F1 individuals were susceptible to isolate MP 324



databases using the BLAST algorithm, the sequence of Bzura gene has showed 97 % identity to *R2* gene (GeneBank: FJ536325.1), and has proved to be identical with the sequence of *R2-like* gene (GeneBank: FJ536323.1).

Discussion

Results of presented study showed effectiveness of late blight resistance of cv. Bzura in field conditions. In each of the 10 years of field experiment, Bzura was more resistant than mid-resistant standards Escort and Robijn, and only slightly less resistant than cv. Sárpo Mira. In local populations of *P. infestans*, races virulent to Bzura were present, since symptoms of infection on this cultivar were observed in each year of experiment. However, progress of late blight symptoms on this cultivar was restricted, and Bzura was always classified as highly resistant ($rAUDPC < 0.200$). The high level of Bzura field resistance is mainly based on low disease progress rate, probably related to its late maturity, and delay in disease onset (Plich, unpublished data). According to Andrivon et al. (2006) reduction of late blight apparent infection rate is typical for partial resistance, while delay in onset of the disease symptoms is usually attributed to the presence of R gene(s). It may suggest that effective resistance of Bzura results from combined effects of both horizontal and R-gene-based resistance. Until now only the presence of *R1* gene has been reported in Bzura (Gebhardt et al. 2004), but efficacy of this gene in protection against late blight is very limited. In this study, the presence of another major resistance gene was confirmed. This gene, present in Bzura and 76 progeny clones, provides resistance for *P. infestans* infection after inoculation with AvrBz isolates. This gene is located at major late blight resistance locus on potato chromosome IV and belongs to *R2*-gene-family. Its presence was confirmed with the use of markers B10L, 20D11R, and/or *R2*. The nucleotide sequence of this gene shares 97 % identity with *R2* gene and is identical with *R2-like* gene. Gene *R2-like* originally was found in potato clone AM3778-16 (dihaploid from AM78-3778) which, among other wild *Solanum* species, has *S. edinense* in its pedigree (Park et al. 2005). The same *S. edinense*, or rather

S. demissum source, clone EF II 13 (EF = Edinense Fraglich = *dms*-hybrid) can be found also in Bzura pedigree (Swieżyński et al. 1997). However, the presence of any R genes in this clones was not confirmed yet.

Several *R2* alleles and orthologs from different *Solanum* species have been already identified (Champouret 2010). They all recognize *PiAVR2* effector and confer resistance to similar spectrum of *P. infestans* races (Lokossou et al. 2009). Strains of *P. infestans* that are virulent on *R2* potatoes either do not express effector *Avr2* or express its distinct variant, that evades perception (Gilroy et al. 2011). Gene *R2* (or its homologs) has been widely exploited in agriculture and virulent races of *P. infestans* are present in populations of this pathogen. However, potatoes with *R2* gene have been reported to be still resistant to local *P. infestans* populations in China, Russia, France, and The Netherlands (Li 2012; Pilet et al. 2005; Vleeshouwers et al. 2011; Wang et al. 2012). Also in Poland, the *R2* is still fairly effective, since the corresponding virulence factor is only moderately frequent in local populations of *P. infestans* (Chmielarz et al. 2014). Members of *R2*-family genes are considered to be components of durable resistance, especially if combined with other elements that complement their efficacy (Gilroy et al. 2011; Jo 2013). In our experiment gene *R2-like* present in Bzura provide complete resistance for inoculation with AvrBz isolates in detached leaflet tests, but was insufficient to fully protect foliage of this cultivar in field conditions. Slight late blight symptoms were

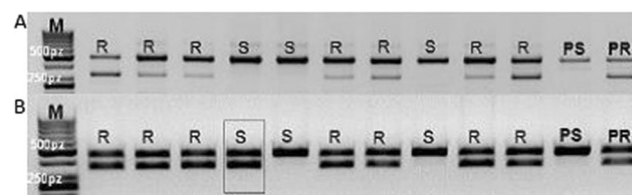


Fig. 4 Products of PCR amplification of CAPS markers: B10L (a) and 20D11R (b) after digestion with appropriate restriction enzymes for Bzura, Felka Bona, and individuals from (Bzura × Felka Bona) F1. M – DNA ladder; PR – resistant parent – cv. Bzura; PS – susceptible parent – cv. Felka Bona; R – resistant clones from (Bzura × Felka Bona) F1; S – susceptible clones from (Bzura × Felka Bona) F1. Rectangle marks susceptible clone from this progeny in which amplification product of 20D11R was found (recombinant)

observed on Bzura in each year of the field experiment in the middle or the end of epidemic periods. However, on foliage of other potatoes containing *R2* gene (Black's differential R2 and cv. Escort) the late blight symptoms were observed earlier (data not shown) and were more severe than on Bzura. According to Lokossou and coworkers (2009), the *R2-like* and *R2* genes provide resistance to the same spectrum of *P. infestans* isolates. Therefore, the observed differences between levels of resistance in Bzura and other *R2*-containing potatoes could be explained only by the influence of unique genetic background of this cultivar, which is responsible for high level of partial resistance against late blight. Probably such combination of *R2-like* gene and specific genetic background is also responsible for durability of Bzura field resistance observed in this and earlier studies (Haynes et al. 1998; Tatarowska et al. 2012).

In the case of potato, the achievement of durable resistance against late blight is very difficult, since *P. infestans* is highly adaptable pathogen (Fry 2008; Haas et al. 2009). Therefore various strategies have been developed to improve durability of potato resistance to *P. infestans*. Some breeders have focused on the development of cultivars with horizontal resistance (Thurston 1971; Van der Plank 1971), while others expect that stacking of R genes will bring durable resistance (Kim et al. 2012; Zhu 2014). Past experience showed that employment of single or even combined R genes did not bring durable resistance, while level of protection provided by horizontal resistance is most frequently insufficient (Colon 1994). Another strategy to achieve durable resistance is to bring together both types of resistance in one cultivar (Van der Plank 1966). Recently Palloix et al. (2009) and Brun et al. (2010) postulated usefulness of this strategy to facilitate disease control for other pathosystems. Expected benefits of such joint action of two resistance types arise from enhanced disease control provided by R gene(s), slower selection for virulent isolates and satisfactory level of protection (when the R gene is finally overcome) provided by a high level of quantitative resistance. As a consequence, quantitative resistance can increase the durability of R-gene mediated resistance and may enhance disease control and crop protection (Brun et al. 2010). Combining of selected R genes with genetic background, which provides rate reducing resistance, leads to improvement of durability and effectiveness of resistance also in case of potato late blight. The highly effective and durable genes *R8* and *R9* from *S. demissum* are background-dependent, and when transferred into susceptible cultivars could fail in providing high resistance (Kim et al. 2012). Also effectiveness of another highly promising resistance gene *Rpi-phu1* could be affected by influence of genetic background, since differences in transcript level in diploid and tetraploid breeding lines were found (Śliwka et al. 2013).

Recently, the number of potentially durable R genes, like *RB/Rpi-blb1*, *Rpi-blb3*, *Rpi-phu1*, *Rpi-vnt1.1*, *Rpi-rzc1*, *Rpi-*

sto1, and *Rpi-Smira2*, were found (Lokossou et al. 2009; Jo 2013; Pel et al. 2009; Song et al. 2003; Śliwka et al. 2006; Śliwka et al. 2012; Vleeshouwers et al. 2008). Potato resistance breeding will greatly benefit from the using of these promising R genes. However, to enhance durability of these new resistance sources they should be introduced into potato breeding pool carefully. Potato cultivars/clones, in which the durability of resistance has been already proved, are excellent breeding component for broad spectrum R genes. Example of cultivar Bzura confirms that proper combination of R gene(s) and genetic background could provide the benefits of improving the effectiveness of resistance and its durability.

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