Diploid Potato Hybrids as Sources of Resistance to Blackspot Bruising

Agnieszka Hara-Skrzypiec · Henryka Jakuczun

Published online: 10 May 2013

© The Author(s) 2013. This article is published with open access at Springerlink.com

Abstract Resistance to bruising is an important quality trait of potato depending on multiple genetic and environmental factors. Thirty one interspecific diploid hybrids and two tetraploid standards were evaluated for resistance to bruising in 2 years. Diploids originated from crosses between wild and primitive *Solanum* species and dihaploids of *S. tuberosum*. Two-year mean values of bruising resistance of tested genotypes indicated significant variability of the trait. Analysis of variance showed significant effects of genotype, year and interaction between them on the trait. Broad-sense heritability of bruising resistance was estimated as moderately high, $H_b=0.73$. Content of L-tyrosine, the main substrate of discoloration reaction, was not significantly correlated with bruising in tested diploid hybrids. The clones, that were resistant to blackspot bruise, also combined quality traits and resistances to many pathogens. All these traits could be transferred together into tetraploid level in $4x \times 2x$ crosses via male $2n$ gametes produced by diploid hybrids.

Resumen La Resistencia al daño mecánico es un carácter importante en la calidad de la papa que depende de múltiples factores genéticos y del ambiente. Se evaluaron 31 híbridos diploides interespecificos y dos estándares tetraploides para resistencia a la magulladura en dos años. Los diploides se originaron de las cruzas entre especies silvestres y primitivas de *Solanum* y dihaploides de *S. tuberosum*. Los valores de las medias de dos años de resistencia al daño de los genotipos probados indicaron variabilidad significativa del carácter. El análisis de varianza mostró efectos significativos de genotipo, año e interacción entre ellos en el carácter. Heredabilidad, en un amplio

sentido de resistencia al daño mecánico, se estimó como moderadamente alta, $H_b=0.73$. El contenido de L-tirosina, el principal sustrato de la reacción de oscurecimiento no estuvo correlacionado significativamente con el daño en los híbridos diploides probados. Los clones, que fueron resistentes a la mancha oscura, también combinaron los caracteres de calidad y resistencia a muchos patógenos. Todos estos caracteres pudieron transferirse juntos a nivel tetraploide en cruzas $4x \times 2x$ vía gametos macho $2n$ producidos en híbridos diploides.

Keywords Internal discolorations · L tyrosine · Interspecific diploid *Solanum* hybrids

Abbreviations

IHAR	PIB Plant Breeding and Acclimatization Institute - National Research Center
HPLC	High Performance Liquid Chromatography
RP	HPLC Reverse Phase High Performance Liquid Chromatography
PPO	Polyphenol Oxidase
PLRV	Potato Leafroll Virus
PVX	Potato Virus X
PVY	Potato Virus Y
PVM	Potato Virus M

Introduction

Blackspot bruising is an undesired formation of discolorations under the potato tuber skin initiated by mechanical impact. When cells are damaged, polyphenol oxidase (PPO) and phenols, normally separated by cellular compartmentation, come into contact. A series of oxidative reactions of phenols, primarily tyrosine, result in the formation of melanins (Cobb 1999). According to studies 42 % of internal

A. Hara-Skrzypiec (✉) · H. Jakuczun
Plant Breeding and Acclimatization Institute - National Research Center (IHAR-PIB), Młochów Research Center, Platanowa 19, 05-831 Młochów, Poland
e-mail: a.hara@ihar.edu.pl

bruising is formed after harvesting, and that value increases to 54 % after grading (Peters 1996). Internal discolorations drastically reduce commercial value of tubers, lead to rejection of the crop by consumers and processing industry and considerable economic losses (Storey 2007).

Blackspot bruising is a complex trait and depends on multiple factors: genotype, environment, anatomical, physical and physiological properties of tuber tissues such as starch content (Zgórska 2000; Urbany et al. 2011), tuber age, turgor pressure (Lærke et al. 2000, 2002a). According to many authors, blackspot bruise resistance depends essentially on the genetic factors which was indicated by the large share of this component in the analysis of variance (Zgórska 2000; Komorowska-Jędryś et al. 2002; Domkářová and Vokál 2005; Domański et al. 2007). Pavek et al. (1993) have showed very high heritability in narrow sense ($H_n=0.85$) of blackspot bruise susceptibility. Results of Komorowska-Jędryś et al. (2002), Domański et al. (2007) and Urbany et al. (2011) studies have indicated mid- high to high heritability in broad sense.

The utilization of wild potato species could be potential approach in prevention of bruising in the breeding programs. So far, one of wild species, *Solanum hjertingii*, has been identified as potential source of resistance to blackening (Woodwards and Jackson 1985). *S. hjertingii* has been an object of studies on PPO role in discoloration formation by several authors (Sim et al. 1997; Brown et al. 1999; Culley et al. 2002).

The level of tyrosine content and activity of PPO are considered to be the main factors involved in blackspot bruise formation (McGarry et al. 1996). The importance of PPO in discoloration process was confirmed by results in many studies (Coetzer et al. 2001; Arican and Gozukirmizi 2003; Urbany et al. 2011, 2012). Tyrosine is an essential substrate for discoloration reaction. The results of some earlier studies have indicated that tyrosine level is well correlated with enzymatic discoloration potential after tissue homogenization and after abrasive peeling (Dean et al. 1993). On the other hand, no correlation between bruising and tyrosine level has been found in studies using impact method (Mondy and Munshi 1993; Stevens and Davelaar 1997; Strehmel et al. 2010). Mondy and Munshi (1993) studied two potato cultivars, one resistant and one susceptible to bruising, have found that tyrosine level was not a limiting factor for bruising determination, since in the susceptible cultivar they observed a lower level of tyrosine. Stevens and Davelaar (1997) have demonstrated positive correlation between potential to discoloration formation and tyrosine content, but at the same time they have not found correlation between blackspot bruise susceptibility and level of tyrosine. Strehmel and coworkers (2010) have proved that the level of tyrosine did not change significantly after mechanical impact in two tested cultivars. Before impact, the content of tyrosine was higher in the cultivar

resistant to blackspot bruise. Other potential monophenolic substrates present in tubers, like caffeic and chlorogenic acid were not correlated with the blackspot formation (Lærke et al. 2002b).

Until now complex diploid *Solanum* hybrids obtained in IHAR-PIB Młochów, broadly tested and used for many purposes, were not evaluated for blackspot bruise resistance. They originated from a long-term program aimed at improving resistances to pathogens and quality traits by exploitation of new variation present in wild and primitively cultivated *Solanum* species (Zimnoch-Guzowska et al. 1998). The goal of the present study was to define variation in susceptibility to blackspot bruising among interspecific diploid hybrids. We would like to demonstrate usefulness of selected *Solanum* hybrids in potato breeding as sources of high resistance to blackspot bruise.

Material and Methods

Plant Material

From crosses between interspecific hybrids, 31 diploid clones were selected for this study (Table 1). They were resulted from long-term recombinant breeding process. In their pedigree there are following *Solanum* species: *Solanum acaule*, *S. chacoense*, *S. demissum*, *S. goniocalyx*, *S. gourlayi*, *S. microdontum*, *S. phureja*, *S. stenotomum*, *S. verrucosum*, *S. yungasense* and dihaploids of *S. tuberosum*. A detailed composition of the material is shown in Table 1. Resistances to PLRV, PVX, PVY, PVM, *Synchytrium endobioticum*, *Phytophthora infestans* and *Pectobacterium carotovorum* spp. *carotovorum* were identified in these clones along selection process (Table 2). Diploid clones, used in the experiment, possessed different leading traits: table usefulness, usefulness for chip processing, high starch content, resistance to soft rot and resistance to late blight. Non-bruising table cultivar (cv.) Vitara and bruising starch cv. Hinga were used as positive and negative standards.

Growing and Storage Conditions

All tested diploid clones and cultivars were planted in the end of April and harvested in the end of September in 2008 and 2009. They were propagated in experimental fields of IHAR-PIB Młochów. They were fertilized with 90, 90, 170 kg h⁻¹ of N, P and K in 2008 and 120, 90, 180 kg h⁻¹ of N, P and K in 2009, treated with pesticide whenever necessary. The total amounts of rainfall during the entire growing period were 187.7 mm and 348.2 mm in 2008 and 2009, respectively. The average daily temperatures during 5 months of growing season (from May to September) were similar in both years, 13.5 °C, 18.7 °C, 19.2 °C, 19.0 °C,

Table 1 Pedigree of tested diploid interspecific hybrids. Diploid clones are ordered according to increasing susceptibility to bruising

Clone	Parental forms	Mean surface of blackspot bruises (scale 1–9)	Theoretical contribution of <i>Solanum</i> species in %										
			<i>tbr</i>	<i>acl</i>	<i>chc</i>	<i>dms</i>	<i>grl</i>	<i>mcd</i>	<i>phu</i>	<i>ver</i>	<i>yun</i>	<i>stn-phu</i>	<i>gon</i>
DG 03-170	DG 97–266×DG 97-1805	9.0	71.8	0.6	5.0	0.6	1.2	0.8	16.4	1.6	2.0		
DG 03-232	DG 92–4862×CE-C 69	9.0	71.0	0.8	4.6	0.8	1.5	0.8	16.5	1.6	2.4		
DG 03-460	DG 97–1805×DG97-1506	9.0	68.6	1.0	5.0	1.0	3.9	0.8	13.6	1.6	3.0	1.5	
DG 97-1805	DG 97–1×DG 97-1805	9.0	70.8	0.8	6.8	0.8	2.4	1.2	11.8	2.4	3.0		
DG 03-131	DG 97–264×DG 97-1805	8.8	72.1	0.8	8.4	0.8	2.0	0.8	10.7	1.6	2.8		
DG 03-226	DG 97–1805×DG97-1506	8.7	68.6	1.0	5.0	1.0	3.9	0.8	13.6	1.6	3.0	1.5	
DW 96-667	DG 88–214×DW 92-3089	8.6	61.6	1.2	19.9	1.2	6.9	3.2	1.2	3.2	1.6		
DG 03-124	DG 97–264×DG 97-1506	8.5	70.3	1.0	9.7	0.8	4.3	0.8	7.4	1.6	2.6	1.5	
DW 96-1025	DW 92–2337×DW 87-4204	8.3	49.3		23.0		3.1	1.6	18.7		2.7		
DG 00-481	DW 92–2337×DW 87-4204	8.3	74.1	1.0	8.9	1.0	7.8		7.2				
DG 03-150	DG 97–264×DG 97-1805	8.2	72.1	0.8	8.4	0.8	2.0	0.8	10.7	1.6	2.8		
DG 97-943	DG 93–352×DG 92-227	7.8	72.7	0.4	8.6	0.4	4.6		6.6		3.6	3.1	
DG01-144	DG 91–28×DG 91-234	7.4	54.5	0.8	7.8	0.8	1.5	1.6	25.8	3.2	4.0		
DG 01-290	DG 92–227×DG 94-668	7.0	61.0	0.4	16.8	0.4	4.6	1.6	6.6	1.6	3.9	3.1	
DG 06-1072	DW 96–1025×DG 00-525	6.9	65.2	1.2	17.8	1.2	6.6	1.2	4.3	1.2	1.3		
DG 03-278	DG 97–836×DG 97-886	6.8	56.3	0.4	6.8	0.4	3.6		3.6		2.4	1.5	25
DG 00-270	DG 91–6×DG 94-1530	6.8	46.8		21.0		0.8	2.4	21.9	3.2	3.9		
DG 03-85	DG 97–14×DG 97-1805	6.7	70.8	0.8	6.8	0.8	2.4	1.2	11.8	2.4	3.0		
DG 00-349	DG D8330×DG 93-243	6.5	58.5	0.8	13.2	0.8	5.4	3.2	13.3	3.2	1.6		
DG 03-242	DG 97–836×DG 97-761	6.4	56.3	0.4	6.8	0.4	3.6		3.6		2.4	1.5	25
DG 94-15	DG 89–395×DG 90-402	5.4	64.8	1.6	13.9	1.6	7.7	4.0	1.6	4.0	0.8		
DG 06-348	DG 01–144×DG 00-525	5.1	56.4	0.8	17.0	0.8	3.6	2.0	13.3		2.8	3.3	
DG 92-227	DG 89–358×DG 88-596	5.0	67.2		3.9		6.2		12.5		4.0	6.2	
DG 06-345	DG 01–144×DG 00-525	4.2	56.4	0.8	17.0	0.8	3.6	2.0	13.3		2.8	3.3	
DG 01-139	DG 92–227×DG 91-234	4.0	52.4		6.2		3.0	0.8	28.1	1.6	4.8	3.1	
DG 01-3	DG 91–28×DG 91-234	3.9	54.5	0.8	7.8	0.8	1.5	1.6	25.8	3.2	4.0		
DG 06-5	DG 00–519×DW 96-1025	3.8	69.7	1.8	10.6	1.2	6.8	1.4	5.9	1.8	0.8		
DG 00-525	DG 94–15×DG 94-186	3.7	58.5	0.8	26.1	0.8	5.6	2.4	0.8	2.4	2.6		
DG 04-812	DG 94–15×DG 97-761	3.2	68.2	1.0	11.5	1.0	6.4	2.0	4.2	2.0	2.2	1.5	
DG 06-1081	DW 96–1025×DG 00-1017	2.8	66.0	1.2	18.0	1.2	5.4	0.6	4.3	1.0	2.3		
DG 06-1	DG 00–519×DW 96-1025	1.8	69.7	1.8	10.6	1.2	6.8	1.4	5.9	1.8	0.8		

S. acaule (*acl*): MPI.H. 08

S. chacoense (*chc*): MPI.H.408, PK 133 (Wyszobórz, Poland), CPC 3731, CPC3785, GLKS 66.51/6/6, CPC 3787, GLKS 66.8/26/4, GKLS 60.30/2R

S. demissum (*dms*): MPI.H.408

S. gourlai (*grl*): INTA 7356

S. microdontum (*mcd*): PI 26557

S. phureja (*phu*): MPI.H.408, CCC 1.3, PI 225696.1, 380051/7 (CIP)

S. verrucosum (*ver*): PI 195170, CPC 2644, PI 5256, PI 275256

S. yungasense (*yun*): GLKS 67.107/3R

S. stenotomum × *S. phureja* (*stn* × *phu*): 375186.3/48 (CIP)

S. tuberosum (*tbr*): own hybrids of tetraploid stock and cultivars; USW42; haploids of dH Black and dH Gineke from IVP (Holland); haploids of tetraploid stocks from Germany

S. goniocalyx (*gon*): KW 84-27-2571Hetteema- Zonen B.V., (Holland)

14.6 °C and 13.5 °C, 16.2 °C, 20.0 °C, 18.5 °C, 14.3 °C in 2008 and 2009, respectively. Diploid clones were hand-

harvested in September and stored up to a bruising test at 5 to 10 °C and 95 % relative humidity.

Table 2 Characteristics of diploid clones and standard cultivars: blackspot bruising, starch content and quality traits, resistance to pathogens, ability to produce 2n gametes and leading traits. Diploid clones are ordered according to increasing susceptibility to bruising

Clone	Mean surface of blackspot bruises ^a	SD (+/-)	Starch content (%) ^b	SD (+/-)	Quality traits ^c	Resistance to pathogens ^d	Ability to big pollen grains formation ^e	Leading trait ^f
DG 03-170	9.0	0.0	10.1	0.5	T,ED,ACD	L,Y,X,Sy	+ _x	A
DG 03-232	9.0	0.0	13.7	0.8	T,ED,ACD	Y	+ _x	A
DG 03-460	9.0	0.0	8.8	0.0	T,ED,ACD	Y,Sy	+ _x	A
DG 97-1805	9.0	0.1	9.5	1.7	T,ED,ACD	Y,Pca(6),Sy	+ _x	A
DG 03-131	8.8	0.2	11.5	1.1	T,ED,ACD	X	+ _x	A
DG 03-226	8.7	0.0	14.3	0.4	T,ED,ACD		+ _x	A
DW 96-667	8.6	0.6	16.2	1.2	CCh	L,X,Y,M, Phl(7.9),Sy	+ _x	C
DG 03-124	8.5	0.4	13.4	0.8	T,ED,ACD	PLRV,Y,X	+ _x	A
DW 96-1025	8.3	0.2	18.0	1.9	CCh	Y,M,Pca(6),Phl(7.4),Sy	+ _x	B
DG 00-481	8.3	0.0	19.6	0.0		M,Pca(7)	+ _x	E
DG 03-150	8.2	0.3	14.5	0.8	T,ED,ACD	L,Y,M,Pca(5)Sy	+ _x	A
DG 97-943	7.8	1.1	16.5	1.1	CCh	X, M,Pca(5),Phl(8.9),Pht(8.7),Sy	+ _x	B
DG 01-144	7.4	0.5	16.3	0.1	CCh,T,ED,ACD	Y,Phl(8.9),Pht(8.9),Sy	+ _x	C
DG 01-290	7.0	1.2	16.7	1.1	CCh,T,ED,ACD	Y,Phl(9),Pht(8.9),Sy	+	C
DG 06-1072	6.9	1.3	22.4	1.1				D
DG 03-278	6.8	0.3	13.2	1.4	CCh	Y, X	+ _x	B
DG 00-270	6.8	0.4	21.5	1.5		L,Y,Pca(7),Phl(6.5)	+	E
DG 03-85	6.7	2.5	13.3	0.3	T,ED,ACD	X,Sy	+	A
DG 00-349	6.5	2.4	22.0	1.3		L,Y,Pca(7),Phl(6.2)		E
DG 03-242	6.4	3.4	14.3	1.9	CCh	PLRV,Y,Sy		B
DG 94-15	5.4	2.3	24.7	0.0		L,Y,M,S,Pca(7),Phl(8)Pht(7.8)		D
DG 06-348	5.1	1.9	25.1	1.8		Sy		D
DG 92-227	5.0	1.4	13.4	0.7		L,X,Y,Phl(9)	+	C
DG 06-345	4.2	0.8	23.6	0.3		Sy		D
DG 01-139	4.0	0.3	16.2	1.0	CCh,T,ED,ACD	Phl(9),Pht(8.9),Sy	+	C
DG 01-3	3.9	1.3	15.3	0.2	CCh,T,ED,ACD	Y,Phl(8.3),Sy	+ _x	C
DG 06-5	3.8	1.3	24.5	0.4		Pca(7),Phl(8.3),Pht(8.7),Sy		D
DG 00-525	3.7	1.0	23.9	0.2		L,M,Y,Pca(5.5),Sy		D
DG 04-812	3.2	0.8	25.4	1.8		Y,X,Pca(6),Sy		D
DG 06-1081	2.8	0.4	21.6	1.4		Pca(6)		D
DG 06-1	1.8	0.4	27.9	0.7		Pca(6.7),Phl(7.9),Pht(8.6),Sy		D
Vitara	8.9	0.2	12.8	0.0	T,ED,ACD	L,Y		TC
Hinga	5.0	1.4	20.9	3.0		Y,Phl(7)		SC

^a evaluated in scale 1–9, where=80.1 to 100 % of tuber surface covered by bruises, 2=60.1 to 80 %, 3=40.1 to 60 %, 4=25.1 to 40 %, 5=15.1 to 25 %, 6=10.1 to 15 %, 7=5.1 to 10 %, 8=0.1 to 5 %, 9 - lack of bruises, mean value of 2- year evaluation. SD- standard deviation

^b mean value of two-year evaluation; SD - standard deviation

^c T - taste scored≥6; evaluated in scale 1–9, where 9=the best

ED - high level of resistance to enzymatic discoloration of tuber flesh (in scale 1–9, where 1 - dark, 9 - light, acceptable score≥8)

ACD - resistance to after- cooking darkening (in scale 1–9, acceptable score≥8)

CCh - chip color after 4–5 months of cold storage (4 °C) scores in scale 1–9, where 1 - dark, 9 - light, acceptable score≥6

^d L, Y, X, M resistance to PLRV, PVY, PVX, PVM; Phl, Pht, Pca, Sy resistance to *P. infestans* in leaves, to *P. infestans* in tuber slices, to *P. carotovorum* spp. *carotovorum*; to *S. endobioticum*

(values in brackets are mean resistance in scale 1–9, where 1- the most susceptible, 9 - the most resistant)

^e + - ability to form big pollen grains; +_x - seed set in 4x×2x crosses

^f A - table use; B - chip processing use; C - resistance to late blight; D - high starch content; E - resistance to soft rot

TC table cultivar, SC starch cultivar

Assessment of Blackspot Bruise Resistance

Experiment was conducted in mid - February in two consecutive years 2009 and 2010. To assess the susceptibility to blackspot bruising 20 undamaged, non-greening tubers with standardized weight, were selected for tests. Sample tubers were stored for 5 months at 5 to 10 °C. Blackspot bruise susceptibility was evaluated according to Domański et al. (2007). Just before test tubers were incubated for 12 h at 11 °C. After that tubers were placed in hexagon plywood drum and rotated ten times to produce simulated bruising (Douches et al. 2003). Subsequently, samples of tubers were stored for 72 h at 20 °C. Two-person team estimated visually a degree of bruising. Mean surface of blackspot bruises was evaluated for each tuber separately in scale 1 to 9, where 1=80.1 to 100 % of tuber surface covered by bruises, 2=60.1 to 80 %, 3=40.1 to 60 %, 4=25.1 to 40 %, 5=15.1 to 25 %, 6=10.1 to 15 %, 7=5.1 to 10 %, 8=0.1 to 5 %, 9 - lack of bruises. Evaluations were performed in two replications, in each 10 tubers were tested.

Assessment of Starch Content

Starch content was determined by underwater weight method. Starch content (%) was estimated from ratio of tuber weight in air (g)/tuber weight in water (g) according Lunden (1956).

Assessment of L-Tyrosine Content

L-tyrosine content was evaluated in tuber tissue extracts from 26 diploid hybrids collected from field in 2010.

Sample Preparation For analysis, five undamaged tubers from each diploid clone were taken. After skin removal, a cylindrical sample of tissue (ca 10 mm) from the stolon and bud ends was taken using cork borer. Samples were immediately frozen in liquid nitrogen, freeze-dried and grounded to powder. 0.5 g of sample was homogenized for 2 min at maximum speed in Ultra-Turrax homogenizer with 8 mL of mixture containing methanol and 0.5 % orthophosphoric acid (1 : 1) with BHT (butylated hydroxytoluene; 1 mg mL⁻¹). The extracts were centrifuged at 4,500×g for 25 min at 20 °C and 6 ml of supernatant was filtered and evaporated to dryness in a vacuum evaporator. Samples were diluted in 1.5 mL mixture of methanol and 0.5 % orthophosphoric acid (in ratio 1 : 1), filtered through a 25 mm, 0.2 µm syringe filter and 20 µL was injected for HPLC analysis. Each extract was injected in triplicate.

HPLC Analysis RP-HPLC was performed with LC Prominence HPLC system (Shimadzu, Japan) comprising a pump LC - 20 AD, a vacuum degasser (DGU - 20 A₃), UV - VIS detector (SPD-20 A) and using autosampler (SIL 20

ACHT). L - tyrosine standard was HPLC - grade purity from Roth (Switzerland). Methanol, acetonitrile, water and orthophosphoric acid were of HPLC-grade purity (POCh, Poland). Separation was performed using Vertex C-18 column (4.6×250 mm, 5 µm particle, Knauer) at 40 °C. The mobile phase consisted of a binary solvent - 0.5 % orthophosphoric acid (solvent A) and acetonitrile (solvent B) and gradient elution (0 to 2 min, 5 % B; 2 to 5 min, 5 to 10 % B; 5 to 7 min, 10 % B; 7 to 10 min, 10 to 15 % B; 10 to 13 min, 15 to 20 % B; 13 to 23 min, 20 % B; 23 to 25 min, 20 to 30 % B; 25 to 28 min, 30 % B; 28 to 30 min, 30 to 50 % B; 30 to 32 min, 50 % B; 32 to 33 min, 50 to 5 % B; 33 to 35 min, 5 % B) were used. Detection was at 275 nm. The flow rate was set to 1 mL min⁻¹. The external standard method of calibration was used with curve prepared from 5 different concentrations of the standard solution. The standard stock solution of L - tyrosine 1 mg mL⁻¹ was prepared in methanol. L - tyrosine peak was identified by a direct comparison of its retention time with the standard.

Statistical Analyses

Analysis of variance (ANOVA) was performed to assess the effects of genotype, year and their interaction on the results of the blackspot bruise resistance. Broad-sense heritability for diploid hybrids was estimated according following formula: $H_b = \sigma^2_g / \sigma^2_g + \sigma^2_{ge} + \sigma^2_e$ (where, $\sigma^2_g = M1 - M2/L$; $\sigma^2_{ge} = M2 - \sigma^2_e$; M1=mean square of genotype; M2=mean square of interaction year × genotype; L=number of year) (Domański et al. 2007). Homogenous groups of genotypes according to mean blackspot bruise resistance were distinguished by the Tukey's test. The reproducibility of the tests between years and the relationships of blackspot bruise resistance with starch content and L - tyrosine content were evaluated by calculating linear Pearson's correlation coefficients. All calculations were performed using computer program STATISTICA for Windows (Soft, Inc., Tulsa, OK, U.S.A.).

Results

Among tested diploid interspecific hybrids originated from wild *Solanum* species and dihaploids of *S. tuberosum*, 11 forms were highly resistant to bruising, scored above 8 (Table 2). *S. tuberosum*, *S. phureja* and *S. chacoense* were predominant in pedigrees of majority of the tested clones in comparison to remaining species. Additionally, in two clones, high contribution of *S. goniocalyx* in pedigree was observed (Table 1). Prevailing theoretical contribution of *S. tuberosum* (range 46.8 – 74.1 %) was noted both in susceptible and resistant forms. The associations between blackspot bruise resistance and content of any of predominant species in assessed 31 diploid clones were not proved.

Most clones resistant to bruising were also resistant to potato viruses, some were resistant to *S. endobioticum*, *P. infestans* or *P. carotovorum* (Table 2). All these resistant genotypes produced big pollen grains which effectively worked in 4x2x crosses in other studies.

Majority of clones classified as table stocks were highly resistant to bruising (Table 2). In other groups of usefulness, only single forms were resistant to blackspot bruise. All clones with high starch content were very susceptible to bruising. Starch content of all diploid hybrids varied from very low (8.8 %) to very high (26.4 %). Starch content of highly resistant clones ranged from 8.8 to 22.9 %, while starch content of diploids susceptible to bruising varied between 15.3 and 26.4 %. Starch content of cv. Vitara was 12.8 % and cv. Hinga 20.8 %. Correlation coefficient between blackspot bruise resistance and starch content for all diploid genotypes was significant and negative $r=-0.67$ ($p<0.05$). All tested diploid clones produced good yield. Average tuber weight of tested clones was ca. 30 g and the average yield was about 500 g/hill.

Two-year mean values of blackspot bruise resistance of diploid clones ranged from 1.8 to 9 and covered the whole scale. Significant variability in blackspot bruising among tested genotypes was confirmed by Tuckey's test at $p<0.05$ (Fig. 1, Table 3). Cv. Vitara was estimated as highly resistant to bruising (grade 8.9) and cv. Hinga as moderately susceptible (grade 5.0). The mean blackspot bruise

resistance of diploid hybrids was significantly correlated between 2 years and the correlation coefficient at $p<0.05$ was $r=0.73$. Generally, standard deviations of two-year mean values of bruise resistance (Table 2, Fig. 1) were lower for highly resistant and highly susceptible clones than for clones classified as intermediate.

Analysis of variance of diploid genotypes showed significant effect of genotype and year on blackspot bruising susceptibility (Table 4). Genotype had the largest influence on blackspot bruise formation, explaining 85.1 % of variance. The effect of year was weak, but significant and explained 5.0 % of variance. Interaction between genotype and year was significant and explained next 13.4 % of variance. Broad-sense heritability of blackspot bruise for diploid genotypes was estimated as moderately high $H_b=0.73$.

The endogenous amounts of tyrosine for 26 diploid clones varied between 0.23 and 0.77 mg per 1 g dry weight. In tubers of resistant and susceptible clones both high and low content of substrate was observed. For tested 26 genotypes correlation coefficient between blackspot bruise resistance and tyrosine content was not significant ($r=0.33$, $p>0.05$).

Discussion

Presented studies were the first effort to evaluate the blackspot bruise resistance variation of diploid interspecific

Fig. 1 Variability in blackspot bruise resistance among tested diploid potato clones with different leading traits and standard cultivars. Two-year mean values in 1–9 scale, where 9 = lack of bruises

Table 3 Mean blackspot bruise susceptibility of 31 diploid genotypes and their classification according to Tukey's test at $p < 0.05$

Diploid clones/standard cultivars	Mean surface of blackspot bruises (range) ^a		Homogenous groups according to Tukey's test
	min	max	
DG 06-1	1.8		a
DG 06-1081	2.8		b
DG 04-812	3.2		b c
DG 00-525, DG 06-5, DG 01-3, DG 01-139	3.7	4.0	c d
DG 06-345	4.2		d e
DG 92-227, cv. Hinga	5.0		e f
DG 06-348, DG 94-15	5.1	5.4	f
DG 03-242	6.4		g
DG 00-349, DG 03-85, DG 03-278, DG 00-270	6.5	6.8	g h
DG 06-1072, DG 01-290	6.9	7.0	g h i
DG 01-144	7.4		h i j
DG 97-943	7.8		i j k
DG 03-150, DW 96-1025	8.2	8.3	j k l
DG 00-481, DG 03-124, DW 96-667	8.4	8.7	k l
DG 03-226, DG 03-131, DG 03-460, DG 03-232, DG 97-1805, DG 03-170, cv. Vitara	8.8	9.0	l

^a scale 1–9, where 9=lack of bruising

hybrids obtained in IHAR-PIB Młochów. Diploid clones used in our study have in their pedigree various *Solanum* species, like *S. acaule*, *S. chacoense*, *S. demissum*, *S. goniocalyx*, *S. gourlayi*, *S. microdontum*, *S. phureja*, *S. verrucosum*, *S. yungasense* and dihaploids of *S. tuberosum*. None of these species was documented before as a source of resistance to blackspot bruise or enzymatic blackening, a trait significantly correlated with bruising. 11 of tested clones were highly resistant to bruising and scored above 8, in 1–9 scale. Analyzing theoretical composition of species in pedigree of majority of tested clones *S. tuberosum*, *S. phureja* and *S. chacoense* were found as predominant species (Table 1). In highly resistant hybrids higher contribution of *S. phureja* in origin was observed in comparison to the most susceptible to blackspot bruise clones. Nonetheless, we cannot indicate which of species are exactly donors of resistance to blackspot bruise, because no relationship

between blackspot bruise susceptibility and contribution of any predominant species was found (Table 1). Thus, we rather suspect that combination of specific alleles of genes determines decreasing discoloration formation after the impact. One of wild tetraploid 2 EBN species, *S. hjertingii*, has been recognized as a source of low enzymatic blackening (Woodwards and Jackson 1985). In this species the level of PPO was lower than in cultivated lines (Sim et al. 1997; Brown et al. 1999). There have been some attempts to transfer this trait to tetraploid level (Culley et al. 2002), however the use of sexual crosses of wild species directly to *S. tuberosum*, without prebreeding, means a laborious elimination of negative traits associated to the wild species. This option might be easier for selection when gene cloning and genetic engineering will be applied in breeding wider.

Tested diploid clones were obtained in long-term recombinant breeding process among above presented *Solanum*

Table 4 Analysis of variance for two-year mean values of blackspot bruise resistance for 31 diploid genotypes

Factor	^a Df	^b MS effect	^a Df error	^b MS error	<i>F</i>	^c R ² (%)	<i>p</i>
{1} Year	1	3.1616	62	0.1103	28.6579	5.0	0.000001
{2} Genotype	30	18.9609	62	0.1103	171.8678	85.1	0.000000
1*2	30	2.9978	62	0.1103	27.1729	13.4	0.000000

^a number of degrees of freedom

^b mean standard effect/error

^c percent of variance explained

species (Table 1). In the years of selection most of wild features were eliminated. Previously these diploids were selected for quality and resistance traits (Jakuczun and Wasilewicz - Flis 2004). Effectiveness of function of $2n$ pollen in $4x \times 2x$ crosses was also checked, and this character was frequent in tested set of diploids. In prior studies among tested diploid clones resistance to PLRV, PVX, PVY, PVM, *S. endobioticum*, *P. infestans* and *P. carotovorum* have been found (Table 2). High contribution of *S. tuberosum* in origin of tested hybrids determine good level of agronomic traits. Yield and average tuber weight of tested hybrids were on a good level, typical for interspecific hybrids selected for complex quality traits in IHAR - PIB Młochów.

Most of diploid clones highly resistant to bruising were useful for table use, and two of them were cold chippers (Table 2). It should be mentioned that some of presented clones have been successfully used as a source of “cold chipping” in $4x \times 2x$ crosses (Domański et al. 2006a, b, 2010). Authors have selected tetraploid progeny meeting desired agronomic and chipping quality traits on a better level than tetraploids obtained in traditional $4x \times 4x$ crosses.

The results of our studies indicated that blackspot bruise resistance was mainly influenced by genotype (85.1 %). Broad-sense heritability of bruising potential was estimated as moderately high ($H_b=0.73$). These results and conclusions from other studies (Pavek et al. 1993; Zgórska 2000; Komorowska-Jędryś et al. 2002; Domkářová and Vokál 2005; Domański et al. 2007; Urbany et al. 2011) have showed that the breeding for resistance to blackspot bruising could be very effective. Domański and coworkers (2007) from among 41 tested cultivars have identified several ones resistant to blackspot bruising. Authors have recommended them as source of this trait for potato breeding. Genetic pool of breeding potato needs new alleles of genes for broadening still narrowed variability. Thus, besides of cultivars the presented diploids could be the good donors of resistance to bruising. Diploid potatoes obtained in IHAR-PIB Młochów have been verified as good sources of other traits in $4x \times 2x$ program, like resistance to *P. infestans* (Śliwka et al. 2010), resistance to *Pectobacterium* spp. (Lebecka et al. 2004), cold chipping (Domański et al. 2006a, b), starch content and table traits (Domański et al. 2000, 2010).

It was found that a low level of tyrosine, the main substrate of discoloration reaction, is not related to resistance found in our diploid hybrids. The observed lack of correlation between low tyrosine content and resistance to bruising indicated that substrate level does not seem to be preliminary factor playing a main role in mechanism of bruising formation. This conclusion is in agreement with results of other studies (Mondy and Munshi 1993; Stevens and Davelaar 1997; Strehmel et al. 2010).

In this study we presented a chosen group of diploid potato hybrids and their value as potential donors of resistance to blackspot bruising. Although this trait is not the

main one in selection processing, but taking into consideration fact, that bruising is responsible for significant decreasing of tuber yield, it is worth to intensify introducing of this trait to breeding material. Diploid potato hybrids are excellent materials for clarification of genetic background of resistance to bruising, which has not been still explained.

Acknowledgments We gratefully thank Ewa Zimnoch-Guzowska and Jadwiga Śliwka for their comments helpful in preparing of the manuscript.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- Arıcan, E., and N. Gozukirmizi. 2003. Reduced polyphenol oxidase activity in transgenic potato plants associated with reduced wound- inducible browning phenotypes. *Biotechnology and Biotechnological Equipment* 17(2): 15–21.
- Brown, C.R., M. McNabney, and B. Dean. 1999. Genetic characterization of reduced melanin formation in tuber tissue of *Solanum hjertingii* and hybrids with cultivated diploids. *American Journal of Potato Research* 76: 37–43.
- Cobb, A.H. 1999. A review of the physiology of bruising in potatoes. The 14th Triennial Conference of the European Association for Potato Research, Sorrento, Italy: 198–199.
- Coetzer, C., D. Corsini, S. Love, J. Pavek, and N. Tumer. 2001. Control of enzymatic browning in potato (*Solanum tuberosum* L.) by sense and antisense RNA from tomato polyphenol oxidase. *Journal of Agricultural and Food Chemistry* 49: 652–657.
- Culley, D.E., B.B. Dean, and C.R. Brown. 2002. Introgression of the low browning trait from the wild Mexican species *Solanum hjertingii* into cultivated potato (*S. tuberosum* L.). *Euthytica* 125: 293–303.
- Dean, B.B., N. Jackowiak, M. Nagle, J. Pavek, and D. Corsini. 1993. Blackspot pigment development of resistant and susceptible *Solanum tuberosum* L. genotypes at harvest and during storage measured by three methods of evaluation. *American Potato Journal* 70: 201–217.
- Domański, L., M. Domańska, and H. Jakuczun. 2000. Evaluation of potato progenies and clones obtained from interploidy crosses. *Biuletyn IHAR* 216: 497–503.
- Domański, L., E. Zimnoch - Guzowska, M. Domańska, and H. Jakuczun. 2006a. The effect of heterosis of diploid potato clone DG 97–943 of cold chipping type and producing $2n$ gametes. *Biuletyn IHAR* 240(241): 307–313.
- Domański, L., H. Jakuczun, and E. Zimnoch - Guzowska. 2006b. Suitability of two diploid potato parental forms to developing the “cold chipping” tetraploid progenies. *Zeszyty Problemowe Postępów Nauk Rolniczych* 511: 115–121.
- Domański, L., K. Michałak, and E. Zimnoch - Guzowska. 2007. Variation of blackspot susceptibility of the selected potato cultivars. *Biuletyn IHAR* 246: 145–149.
- Domański, L., B. Flis, H. Jakuczun, and E. Zimnoch - Guzowska. 2010. Variation of processing quality and tuber morphology traits in potato progenies derived from interploidy matings $4x \times 2x$. *Biuletyn IHAR* 257–258: 57–69.
- Domkářová, J., and B. Vokál. 2005. The evaluation method of potato genotype resistance to blackspot bruise. *Plant Soil and Environment* 51(2): 74–81.

- Douches, D.D., R.W. Chase, J. Coombs, K. Flecher, L. Frank, J. Driscoll, E. Estellee, R. Hammerschmidt, and W. Kirk. 2003. Seventy-second annual report by Michigan State University. In: National Germplasm Evaluation and Enhancement Report, 2001. Ed. By K.G. Haynes. Seventy Second Annual Report by Cooperators. US Department of Agriculture, Agricultural Research Service, ARS-162: 171–199.
- Jakuczun, H., and I. Wasilewicz – Flis. 2004. New sources of potato resistance to *Phytophthora infestans* at diploid level. *Plant Breeding and Seed Science* 50: 137–145.
- Komorowska - Jędryś, J., T. Ohanowicz, and B. Szewczyk. 2002. Evaluation of potato tubers to after- wounding blackspot. *Biuletyn IHAR* 221: 147–152.
- Lærke, P.E., E.R. Brierley, and A.H. Cobb. 2000. Impact - induced blackspots and membrane deterioration in potato (*Solanum tuberosum* L.) tubers. *Journal of the Science of Food and Agriculture* 80: 1332–1338.
- Lærke, P.E., J. Christiansen, M.N. Andersen, and B. Veierskov. 2002a. Blackspot bruise susceptibility of potato tubers during growth and storage determined by two different test methods. *Potato Research* 45: 187–202.
- Lærke, P.E., J. Christiansen, and B. Veierskov. 2002b. Colour of blackspot bruises in potato tubers during growth and storage compared to their discoloration potential. *Postharvest Biology and Technology* 26: 99–111.
- Lebecka, R., E. Zimnoch - Guzowska, and Z. Kaczmarek. 2004. Resistance to soft rot (*Erwinia carotovora* subsp. *atroseptica*) in tetraploid potato families obtained from 4x-2x crosses. *American Journal of Potato Research* 82: 203–210.
- Lunden, A.P. 1956. Underlödokerd over forholdet mellom popetenes spesifikke vekt og deres torvstoff og Stivelsesinnhold Forlh. *Forso K Landbruket* 7: 81–107.
- McGarry, A., C.C. Hole, R.L.K. Drew, and N. Parsons. 1996. Internal damage in potato tubers: a critical review. *Postharvest Biology and Technology* 8: 239–258.
- Mondy, N.I., and C.B. Munshi. 1993. Effect of maturity and storage on ascorbic acid and tyrosine concentrations and enzymatic discoloration of potatoes. *Journal of Agricultural and Food Chemistry* 41: 1868–1871.
- Pavek, J., C.R. Brown, M.W. Martin, and D.L. Corsini. 1993. Inheritance of blackspot bruise resistance in potato. *American Potato Journal* 70: 43–48.
- Peters, R. 1996. Damage of potato tubers, a review. *Potato Research* 39: 479–484.
- Sim, S.K., S.M. Ohmann, and C.B.S. Tong. 1997. Comparison of polyphenol oxidase in tubers of *Solanum tuberosum* and the non browning tubers of *S. hjeringii*. *American Potato Journal* 74: 1–13.
- Śliwka, J., H. Jakuczun, P. Kamiński, and E. Zimnoch-Guzowska. 2010. Marker-assisted selection of diploid and tetraploid potatoes carrying Rpi-phu1, a major gene for resistance to *Phytophthora infestans*. *Journal of Applied Genetics* 51(2): 133–140.
- Stevens, L.H., and E. Davelaar. 1997. Biochemical potential of potato tubers to synthesize blackspot pigments in relation to their actual blackspot susceptibility. *Journal of Agricultural and Food Chemistry* 45: 4221–4226.
- Storey, R. M. J. 2007. The harvested crop. In: *Potato Biology and Biotechnology: Advances and Perspectives*, ed. Vreugdenhil D. et al.: 459–468. Amsterdam, The Netherlands: Elsevier.
- Strehmel, N., U. Praeger, C. Konig, I. Fehrle, A. Erban, M. Geyer, J. Kopka, and J.T. van Dongen. 2010. Time course effects on primary metabolism of potato (*Solanum tuberosum* L.) tuber tissue after mechanical impact. *Postharvest Biology and Technology* 56: 109–116.
- Urbany, C., B. Stich, L. Schmidt, L. Simon, H. Berding, H. Junghans, K.-H. Niehoff, A. Braun, T. Eckhard, H.-R. Hofferbert, J. Lübeck, J. Strahwald, and C. Gebhardt. 2011. Association genetics in *Solanum tuberosum* provides new insights into potato tuber bruising and enzymatic tissue discoloration. *BMC Genomics* 12: 7.
- Urbany, C., T. Colby, B. Stich, L. Schmidt, J. Schmidt, and C. Gebhardt. 2012. Analysis of natural variation of the potato tuber proteome reveals novel candidate genes for tuber bruising. *Journal of Proteome Research* 11(2): 703–716.
- Woodwards, L., and M.T. Jackson. 1985. The lack of enzymic browning in wild potato species, Series Longipedicellata, and their crossability with *Solanum tuberosum*. *Zeitschrift für Pflanzenzüchtung* 94: 278–287.
- Zgórska, K. 2000. Factors affecting the susceptibility of potato tubers to after- wounding blackspot. *Biuletyn IHAR* 213: 254–259.
- Zimnoch-Guzowska, E., M.T. Sieczka, and K.M. Świeżyński. 1998. Development of parental lines for breeding potatoes resistant to viruses. p. 217–224. In *Proceedings of the International Symposium: Breeding Research on Potatoes*.