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Common scab performance of tetraploid potato genotypes from Argentina

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Abstract

Fifty potato genotypes from the breeding program were evaluated against common scab, caused by *Streptomyces sp.*, in a greenhouse of the Balcarce Integrated Unit (INTA-FCA, UNMdP). The isolation of *Streptomyces sp.* was done from tubers that exhibited symptoms of the disease. The "seed" tubers of each genotype were planted in plastic pots containing a mixture of sterilized soil and sand, previously inoculated with the bacteria. Cv. Bintje was included because of its known susceptibility to the disease. Plants naturally completed their cycle and tuber´s scab symptoms were evaluated. Affected tuber surface (%), type of damage and relative scab index (RSI) were calculated for each tuber of each genotype. A randomized complete block design with three replications was used. It was determined that there were significant differences among genotypes in affected tuber surface, type of damage and RSI. Six genotypes with a potentially resistant behavior to common scab were identified. This characterization of infection with common scab will allow resistance genes to be incorporated against this disease in breeding programs.

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Introduction

Potato (*Solanun tuberosum* ssp. *tuberosum* L.) is cultivated in Argentina mainly in the South East of the Buenos Aires Province (SEBAP) and in Córdoba, Mendoza and Tucumán provinces (Mosciaro, 2011). Common scab is caused by bacteria of the genus *Streptomyces* and is present in all regions of the country, but given that the SEBAP is the main producing region, it merits the concentration of studies concerning one of the main tuber commercial quality diseases. Although Common Scab does not affect yield, the quality reduction of the tubers is punished by both the fresh market and the processing industry. Besides, affected tubers are susceptible to dehydration during storage and the lesions can be the entrance of bacteria causing wet rots as well as insects (Melegari, 2009; Cancino Osorio, 2003).

Common scab is controlled by several methods for, e.g. chemical treatments, biological control, irrigation, soil pH management, crop rotation and resistant cultivars. The use of resistant cultivars is the most efficient method, which, besides giving a satisfactory control, is environmentally safe, economical and stable (Mishra; Srivastava, 2001). For the moment, there are no commercial cultivars that show a complete resistance to common scab (Fisher *et al*., 2009; Wanner; Haynes, 2009; Wanner; Dees, 2012).

In the potato breeding program at Balcarce, genotypes have been evaluated against diverse pathogens including common scab (Melegari, 1996) but this evaluation was not exhaustive. Therefore the aim of this research was to evaluate and determine the behavior of the genotypes against common scab under greenhouse conditions. Thus, it will be possible to contribute with the selection of genotypes resistant, providing a better strategy for the control of this disease.

Materials and methods

Twenty five potato clones and twenty five commercial varieties were assessed in a temperature regulated greenhouse for their performance against *Streptomyces sp*. (Supplementary materials) at the Balcarce Integrated Unit (EEA INTA-FCA, UNMdP) in July of 2014.

A healthy tuber of each genotype was planted in a pot of 7 liters. The plants were watered according to the crop requirements. Weekly sprays of insecticides and fungicides were performed. Plants were grown until natural senescence. Once the tubers were mature they were manually harvested by December 2014. The tubers were kept at room temperature until they were evaluated. A complete randomized block design with three replicates was used. The experimental unit was one potted plant of each genotype.

Isolates

Common scab isolates were multiplied in the lab ten days prior to inoculation. The isolates were obtained from diseased tubers which were washed carefully with distilled water. A piece of the surface of the tubers with scab symptoms was taken and subsequently scrapped off from the limit between the healthy and the diseased tissue. The tissue thus obtained was homogenized with 5 ml of sterile distilled water in a mortar. One milliliter of the homogenate was placed in 9 cm Petri dishes containing the Küster growing media (Küster; Williams, 1964). The plates were incubated during 10 days at darkness and at 24 °C (Lindholm *et al*., 1997). Seven isolates were obtained. Pathogenicity tests in the lab and in the greenhouse were carried on these isolates.

Pathogenicity assessment

a) Laboratory pathogenicity test

A protocol developed by Loria *et al*. (1995) with some modifications was followed. 5 ml of distilled water was added to each plate containing the growing media with the bacteria and then stirred. One milliliter of the resulting bacterial suspension was taken and the concentration was adjusted to $1x10^7$ conidia/ml by means of a Neubauer chamber. Flame and alcohol sterilized minitubers of cv. Spunta were cut into 1 mm thick pieces and inoculated with 20 µl of the bacterial suspension for each isolate. The minituber pieces were placed in trays inside an incubator during ten days at 24 °C. The presence of necrosis was evaluated in the inoculation spot in order to select the more pathogenic isolates.

b) Greenhouse pathogenicity test

The protocol developed by Fischer *et al.*, 2003 y 2009 was followed. Washed cv. Spunta minitubers were planted in one liter pots containing a mixture of sterile soil and sand. The bacterial colony grown on a 9 cm diameter Petri plate was poured into each pot and mixed with the first 10 cm of soil. Four replications per isolate were evaluated. Harvest of tubers produced was performed after 12 weeks and symptoms were evaluated, determining incidence and severity of scab and subsequently identifying the more pathogenic isolates.

Inoculum preparation

One isolate capable of producing symptoms in both pathogenicity tests was used. The isolate was multiplied and increased in Küster media ten days before inoculation. The 320 plates thus obtained were incubated for ten days at 24°C. Five random samples of two plates each were taken to estimate inoculum concentration. Sterile water (5 ml) was added to each plate and the content was scrapped with a glass rod forming a bacterial suspension. An aliquot of 5 ml from each of the two plates of a sample were mixed together and completed up to 15 ml with sterile water. A $6x10^7$ conidia/ml concentration was determined in a Neubauer chamber.

Inoculation

Inoculation was performed one day before planting, following the protocol of Bjor and Roer (1980). The inoculum content of two plates was placed in each pot and mixed manually with a sterile mixture of soil and sand (50:50 v/v).

Tuber scab evaluation

The affected surface and the lesion type were determined in January 2015 for each tuber from each genotype, according to the Ministry of Agriculture, Fisheries and Food key (1976). The lesion type is the visual assessment of severity in a scale of 1 to 3 [1, superficial scab; 2, intermediate scab (≈1–3 mm depth); 3, deep scab (>3 mm depth)]. For each genotype, a relative scab index that includes the percentage of tubers affected by type of damage, the percentage of affected area and the percentage of tubers affected was calculated as shown below:

RSI = (((% tubers with Type 1 damage + % tubers with Type 2 damage + % tubers with Type 3 damage) x % of affected tuber surface)/300) x % of total affected tubers)/10000.

Data analysis

The affected surface for each tuber and the percentage tubers affected for each genotype were normalized and an analysis of variance was performed. Means were compared with a Duncan test with α=0,05 using a SAS Institute Inc. (1990) statistical package.

Results and discussion

Affected Tuber Surface

Significant differences in the percentages of affected tuber surface among genotypes were obtained (Table 1). Clones 304013.18, B 07.516.1 and B 84.617.4 LR showed the highest affected tuber surfaces, 45 %, 42 % y 40 %, respectively. Cultivar (Cv.) Bintje showed a 22% of affected surface, significantly lower than those three highly susceptible clones. Bintje performance was similar to that shown by Melegari (1996). Clone B 07.577.3 presented no damage in its tubers. The genotypes with better performance were 304072.6LB and B and 03.602.4 1.67%, 2.33% respectively). Cultivars (cvs.) Frital INTA, Achirana INTA and Pampeana INTA behaved similarly as shown by Melegari (1996). Cultivars Snowden and Atlantic showed severities of 32% and 34%, respectively, although Navarro *et al.* (2011) mentioned severities higher than 60%.

Table 1. Common scab affected surface (%) and relative scab index in fifty potato genotypes evaluated in the greenhouse. Balcarce (2014-15).

Genotype	Female parent	Male parent	Tuber affected surface	Relative scab index
B 07.577.3	Innovator	Gander	0.000	0.000
304072.6LB	392657.171	Monalisa	1.667	0.002
B 03.602.4	Frital	Santana	2.333	0.004

Genotype	Female parent	Male parent	Tuber affected surface	Relative scab index
304152.5	393371.159	Desiree	5.250	0.009
Nicola	Olivia	6430/1011	7.600	0.010
B 03.04.573.1	Ramos	Innovator	6.714	0.016
Innovator	Shepody	RZ 84-2580	10.00	0.017
B 92.10.1	Quarta	Ariane	10.200	0.020
304056.4	392642,2	Mira	11.250	0.023
B 06.665.1	Bonacord	Borden	12.750	0.035
Agata	BM 52,72	Sirco	12.000	0.037
Kantara	KA 77-133	AM 78-3704	12.500	0.042
Yaguarí	92324,1	Daisy	20.000	0.042
Tacna	720087	386287.1	18.333	0.049
Chata Blanca	NN	NN	11,833	0.049
Newen INTA	B 86.573.4	Amanda	20.000	0.052
Kennebec	(Chippewa x Kathadin)	(Earlaine x W-ras)	16.429	0.060
Purple Majesty	ND 2008-2	All blue	10.800	0.062
Asterix	Cardinal	SVP Ve 70-9	17.769	0.063
Yagana	Hydra	904/61	19.375	0.065
B 85.616.3	B 71.74.177.5	Anosta	18.333	0.066
Pampeana INTA	MPI 59.789/12	Huinkul MAG	12.857	0.070
Baronesa	Loman	Loman	17.500	0.070
B 03.559.1	Newen INTA	Ramos	24.429	0.072
Araucana INTA	Serrana INTA	Huinkul MAG	17.167	0.076
B 03.574.2	Newen	Innovator	23.333	0.078
395.192.1	C 91.612	C 92.044	19.167	0.078
Frital INTA	Serrana	Katahdin	16.875	0.079
B 79.571.1	Achirana	St 48.1	14.750	0.081
Spunta	Bea	USDA 96.56	21.000	0.089
Ana	C-1750-15-95	Asterix	22.143	0.095
B 03.636.30 TT	92T.109.24	Robusta	22.000	0.095
Astarte	SVP RR 62 5 43	SVP VT 5 62 69 5	19.286	0.100
B 00.607.1	393036.8	393381.4	22.500	0.100
Iporá	Achirana	7XY.1	30.833	0.103
Bintje	Munstersen	Fransen	21.818	0.115
304146.1	Achirana INTA	391011.17	22.500	0.120
B 92.660.5	B 86.626.3	Baraka	28.125	0.122
Achirana INTA	MPI 61.375/23	B 25.65	25.000	0.125
Monalisa	Bierma A1-287	Colmo	30.000	0.133
Atlantic	Wauseon	B 5141-6	34.000	0.139
B 01.559.2	Russet Burbank	Dorado	34.444	0.153
304013.18	391012,18	Kathadin	45.000	0.180
B 78.502.5	Kennebec	Huinkul	25.000	0.185
B 87.605.1	B 75.65	Vital	21.667	0.189
Arazati	386464.7	387660.1	26.667	0.193
393371.35	387170,16	389746,2	25.833	0.230
Snowden	B 5141-6	Wischip	31.923	0.246
B 84.617.4 LR	Achirana	P. Crown	40.000	0.333
B 07.516.1	Gander	Calén	42.222	0.338

Lesion Type

Superficial scab (type 1 lesion) was the lesion type found in higher frequency (Fig. 1). Lesion types 2 (intermediate) and 3 (deep) obtained the same genotype frequency. Clones 304013.18, B 07.516.1 and B 84.617.4 LR, with the largest affected surface, also showed the deepest scab symptoms (lesion 3 type). On the other hand, genotypes with the lowest affected surface (304072.6LB, 304152.5, B 03.04.573.1, B 03.602.4 and Innovator) showed

type 1 lesion. Cv. Bintje showed type 2 lesions (intermediate) different from the deep scab symptoms reported by Melegari (1996). Differences with that research were also observed in cvs. Achirana INTA, Pampeana INTA and Kennebec. Uruguayan cvs. INIA Yaguarí and INIA Iporá showed type 2 lesions, coincident with Lapaz Eugui *et al.* (2014). Cvs. Snowden and Atlantic showed type 2 lesion instead of the type 3 lesion published by Navarro *et al.* (2011).

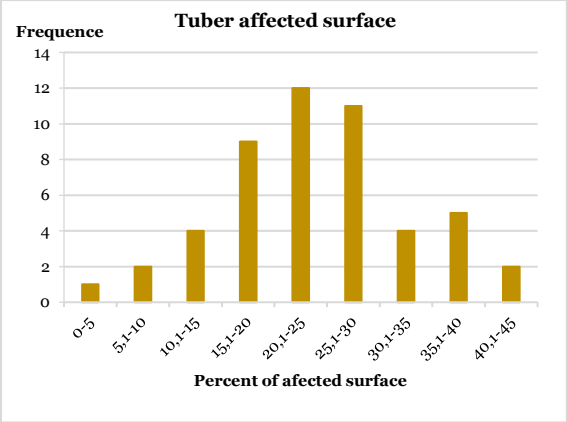

Fig. 1. Frequency histogram of genotypes according to percent of affected tuber surface.

Relative Scab Index

Significant differences (p<0,001) for RSI among genotypes were obtained (Tables 1 and 2 and Fig. 2). The RSI distribution curve was not normal and was shifted towards the low values. Cv. Bintje´s RSI showed susceptibility coincidently with Pasco *et al.* (2005). Genotypes with the higher RSI were clones 304013.18 (0.180), B 78.502.5 (0.185), B 87.605.1 (0.189), Arazati (0.193), 393371.35 (0.230), Snowden (0.246), B 84.617.4 LR (0.333) and B 07.516.1 (0.338), and therefore can be classified as highly susceptible.

B 07.577.3, 304072.6LB, B 03.602.4, 304152.5, Nicola, Alpha, B 03.04.573.1 and. Innovator showed the lowest RSI and were significantly lower (p<0,001) than the rest of the genotypes. Clone B 07.577.3 presented no damages in any of its tubers.

In this study, cv. Innovator behaved similarly as shown by Barrera *et al.* (2013), who classified this cv. as moderately resistant to common scab. Spunta, Frital INTA and Pampeana INTA performed as moderately susceptible in coincidence with the results obtained by Melegari (1996). According to Giménez *et al.* (2014), Yagana shows a good performance against the disease but here behaved as susceptible. INIA Yaguarí showed an intermediate RSI similarly to what was informed by Giménez *et al.* (2014) and by Lapaz Eugui *et al.* (2014) that classified it as susceptible. Agata showed in this work an intermediate RSI and it has been classified as susceptible by Canadian Food Inspection Agency (2015).

Table 2. Analysis of variance of scab affected tuber surface and relative scab index (SI) of 50 genotypes evaluated in the greenhouse for their performance against common scab. Balcarce, 2014-15.

		Damage (%)		RSI	
Source of variation	Degrees of freedom	Mean Squares	F	Mean Squares	F
Blocks	2	0,055		0.069	
Genotypes	49	0,245	6,74 **	0.178	7.30**
Error	349	0,036		0.024	
Total	400				

** indicates significant differences at Pr>F (p<0,001).

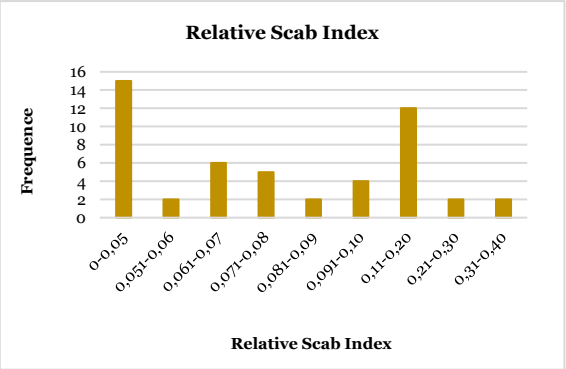

Fig. 2. Frequency histogram of genotypes according to relative scab index (RSI).

The same authors classify Snowden as susceptible coincidently with the high SI obtained here. Nevertheless, Navarro *et al.* (2011) classified Snowden as highly susceptible cultivar. The intermediate RSI obtained by Asterix is coincident with the moderately resistant classification by Canadian Food Inspection Agency (2015). Nicola also behaved similarly to what was reported by

Peeten *et al.* (2011) and by Pasco *et al.* (2005), who classified it as moderately resistant but Monalisa behaved as susceptible inversely to those authors reported. Table 3 shows the grouping (according to the national legislation) of genotypes based on their RSI in this study. Tuber affected surface was significantly correlated with RSI, r = 0.85 ($p<0.0001$). The index also considers the number of affected tubers and type of damage, therefore it has a more holistic approach to evaluate the disease.

Table 3. Grouping of fifty potato genotypes evaluated in the greenhouse according to their RSI. Balcarce (2014-15).

Low RSI	Medium RSI	High RSI
(< 0,016)	(0.017-0.10)	(> 0.10)
B 07.577.3	Innovator	Iporá
304072.6 LB	B 92.10.1	Bintje
B 03.602.4	304056.4	304146.1
304152.5	B 06.665.1	B 92.660.5
Nicola	Agata	Achirana INTA
B 03.04.573.1	Kantara	Monalisa
	Yaguarí	Atlantic
	Tacna	B 01.559.2
	Chata Blanca	304013.18
	Newen INTA	B 78.502.5
	Kennebec	B 87.605.1
	Purple Majesty	Arazati
	Asterix	393371.35
	Yagana	Snowden
	B 85.616.3	B 84.617.4 LR
	Pampeana INTA	B 07.516.1
	Baronesa	
	B 03.559.1	
	Araucana INTA	
	B 03.574.2	
	395.192.1	
	Frital INTA	
	B 79.571.1	
	Spunta	
	Ana	
	B 03.636.60 TT	
	Astarte	
	B 00.607.1	

Conclusion

Significant differences among the 50 genotypes studied were observed with respect to RSI, type of damage and tuber affected surface. The performance of the genotypes observed in this study was in most cases coincident with the data found in the literature, when available. A group of potentially resistant genotypes was identified. Among these, clone B 07.577.3 presented no scab blemishes. This low RSI group needs further evaluation in order to confirm resistance through progeny tests in the field, either with artificial or natural infection. This study resulted in the classification of a set of genotypes widely used as progenitors both in the Argentine breeding program as well as in programs in Latin America and Europe with respect to their performance against common scab.

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Supplementary materials

Potato genotypes evaluated for their performance against common scab in Balcarce (2014-15).

Genotype	Ploidy	Species
Achirana INTA	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Agata	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Ana	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Araucana INTA	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Arazati	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Astarte	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Asterix	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Atlantic	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Baronesa	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Bintje	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Chata Blanca	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Frital INTA	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Innovator	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Iporá	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Kantara	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Kennebec	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Monalisa	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Newen INTA	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Nicola	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Pampeana INTA	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Purple Majesty	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Snowden	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Spunta	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Tacna	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Yagana	2n=4x=48	*S. tuberosum* spp. *tuberosum*
Yaguarí	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 78.502.5	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 79.571.1	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 84.617.4 LR	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 85.616.3	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 87.605.1	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 92.10.1	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 92.660.5	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 00.607.1	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 01.559.2	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 03.559.1	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 03.574.2	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 03.602.4	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 03.636.30 TT	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 03.04.573.1	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 06.665.1	2n=4x=48	*S. tuberosum* spp. *tuberosum*
B 07.516.1	2n=4x=48	*S. tuberosum* spp. *tuberosum*

Genotype	Ploidy	Species
B 07.577.3	2n=4x=48	*S. tuberosum* spp. *tuberosum*
304013.18	2n=4x=48	*S. tuberosum* spp. *tuberosum*
304056.4	2n=4x=48	*S. tuberosum* spp. *tuberosum*
304072.6LB	2n=4x=48	*S. tuberosum* spp. *tuberosum*
304146.1	2n=4x=48	*S. tuberosum* spp. *tuberosum*
304152.5	2n=4x=48	*S. tuberosum* spp. *tuberosum*
393371.35	2n=4x=48	*S. tuberosum* spp. *tuberosum*
3951921	2n=4x=48	*S. tuberosum* spp. *tuberosum*