

Common scab performance of tetraploid potato genotypes from Argentina

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. Scab severity (%), type of damage and scab index (SI) 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 scab severity, type of damage and SI. 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.

Keywords: *streptomyces*, resistance, solanum, scab index

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Introduction

Potato (*Solanum 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. 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,¹ 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.^{2,3} *Streptomyces* is a filamentous heterotroph actinomyces that produces a thin pseudomicelium from which it can fragmentate or asexually subdivide and originate spores.⁴ *Streptomyces*scabies, *Streptomyces*acidi scabies and *Streptomyces*turgidiscabies have been identified in Argentina. These species not only produce the same symptoms, but they benefit from a similar pathogenic mechanism.⁵

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.⁶ Potato cultivars differ in their susceptibility to common scab and therefore the lesions can be superficial or very deep.⁷ For the moment, there are no commercial cultivars that show a complete resistance to common scab.⁷⁻⁹ The potato breeding program at Balcarce has a wide collection of genotypes coming from diverse sources in the world. They combine other characteristics as diverse resistances and nutritional factors under short or long day length backgrounds. Thus, it represents an opportunity to evaluate this material unknown for its performance against Common Scab.

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. (Annex 1) at the Balcarce Integrated Unit (EEA INTA-FCA, UNMdP) in July of 2014. The plants were watered according to the crop requirements. Weekly sprays of insecticides were performed to control white flies (*Bremiccia tabaci*) from November to senescence. 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 5ml of sterile distilled water in a mortar. One milliliter of the homogenate was placed in 9cm Petri dishes containing the Küster growing media.¹⁰ The plates were incubated during 10days at darkness and at 24°C.¹¹ Seven isolates were obtained. Pathogenicity tests in the lab and in the greenhouse were carried on these isolates.

Pathogenicity assessment

Laboratory pathogenicity test: A protocol developed by Loria et al.,¹² with some modifications was followed. 5ml 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 1x10⁷ conidia/ml by means of a Neubauer chamber. Flame and alcohol sterilized mini

tubers of cv. Spunta were cut into 1 mm thick pieces and inoculated with 20 µl of the bacterial suspension for each isolate. The mini tuber 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.

Greenhouse pathogenicity test: The protocol developed by Fischer et al.,^{4,8} 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 9cm diameter Petri plate was poured into each pot and mixed with the first 10cm 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 and inoculation: 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 (5ml) was added to each plate and the content was scrapped with a glass rod forming a bacterial suspension. An aliquot of 5ml from each of the two plates of a sample were mixed together and completed up to 15ml with sterile water. A 6x10⁷ conidia/ml concentration was determined in a Neubauer chamber. Inoculation was performed one day before planting, following the protocol of Bjor and Roer.¹³ 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), 9x10⁸ conidia were added per seven liter pot.

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 Food¹⁴ key. 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 (>3mm 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: $RSI = ((\% \text{ tubers with Type 1 damage} + \% \text{ tubers with Type 2 damage} + \% \text{ tubers with Type 3 damage}) \times \% \text{ of affected tuber surface}) / 300 \times \% \text{ of total affected tubers} / 10000$.

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 $\alpha=0,05$ 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.¹⁵ 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.¹⁵ Cultivars Snowden and Atlantic showed severities of 32% and 34%, respectively, although Navarro et al.,¹⁶ mentioned severities higher than 60%.

Lesion type

Superficial scab (type 1 lesion) was the lesion type found in higher frequency (Figure 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.¹⁵ Differences with that research were also observed in cvs. Achirana INTA, Pampeana INTA and Kennebec. Uruguayan cvs. INIA Yaguari and INIA Iporá showed type 2 lesions, coincident with Lapaz Eugui et al.¹⁷ Cvs. Snowden and Atlantic showed type 2 lesion instead of the type 3 lesion published by Navarro et al.¹⁶

Relative scab index

Significant differences ($p<0,001$) for RSI among genotypes were obtained (Tables 1) (Table 2) and (Figure 2). The RSI distribution curve was not normal and was shifted towards the low values. Cv. Bintje's RSI showed susceptibility coincidentally with Pasco et al.¹⁸ 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.,⁵ 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.¹⁵ According to Giménez et al.¹⁹ Yagana shows a good performance against the disease but here behaved as susceptible. INIA Yaguari showed an intermediate RSI similarly to what was informed by Giménez et al.¹⁹ and by Lapaz Eugui et al.,¹⁷ 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.²⁰ The same authors classify Snowden as susceptible coincidentally with the high SI obtained here. Nevertheless, Navarro et al.,¹⁶ classified Snowden as highly susceptible cultivar. The intermediate RSI obtained by Asterix is coincident with the moderately resistant classification by Canadian Food Inspection Agency.²⁰ Nicola also behaved similarly to what was reported by Peeten et al.,²¹ and by Pasco et al.,¹⁸ 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 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	0
304072.6LB	3.93E+08	Monalisa	1.667	0.002
B 03.602.4.	Frital	Santana	2.333	0.004
304152.5	393371.2	Desiree	5.25	0.009
Nicola	Olivia	6430/1011	7.6	0.01
B 03.04.573.1.	Ramos	Innovator	6.714	0.016
Innovator	Shepody	RZ 84-2580	10	0.017
B 92.10.1.	Quarta	Ariane	10.2	0.02
304056.4	392642,2	Mira	11.25	0.023
B 06.665.1.	Bonacord	Borden	12.75	0.035
Agata	BM 52,72	Sirco	12	0.037
Kantara	KA 77-133	AM 78-3704	12.5	0.042
Yaguari	92324,1	Daisy	20	0.042
Tacna	720087	386287.1	18.333	0.049
Chata Blanca	NN	NN	11,833	0.049
Newen	B 86.573.4	Amanda	20	0.052
Kennebec	(Chippewa x Kathadin)	(Earlaine x W-ras)	16.429	0.06
Purple Majesty	ND 2008-2	All blue	10.8	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	MPI 59.789/12	Huinkul	12.857	0.07
Baronesa	Loman	Loman	17.5	0.07
B 03.559.1.	Newen	Ramos	24.429	0.072
Araucana	Serrana	Huinkul	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	Serrana	Katahdin	16.875	0.079
B 79.571.1.	Achirana	St 48.1	14.75	0.081
Spunta	Bea	USDA 96.56	21	0.089
Ana	C-1750-15-95	Asterix	22.143	0.095
B 03.636.30 TT	92T.109.24	Robusta	22	0.095
Astarte	SVP RR 62 5 43	SVP VT 5 62 69 5	19.286	0.1
B 00.607.1.	393036,8	393381,4	22.5	0.1
Iporá	Achirana	7XY.1	30.833	0.103
Bintje	Munstersen	Fransen	21.818	0.115
304146.1	Achirana	391011,17	22.5	0.12
B 92.660.5.	B 86.626.3	Baraka	28.125	0.122
Achirana	MPI 61.375/23	B 25.65	25	0.125
Monalisa	Bierma A1-287	Colmo	30	0.133
Atlantic	Wauseon	B 5141-6	34	0.139
B 01.559.2.	Russet Burbank	Dorado	34.444	0.153
304013.2	391012,18	Kathadin	45	0.18
B 78.502.5.	Kennebec	Huinkul	25	0.185
B 87.605.1.	B 75.65	Vital	21.667	0.189
Arazati	386464,7	387660,1	26.667	0.193
393371.4	387170,16	389746,2	25.833	0.23
Snowden	B 5141-6	Wischip	31.923	0.246
B 84.617.4 LR	Achirana	P. Crown	40	0.333
B 07.516.1.	Gander	Calén	42.222	0.338

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

Source of variation	Degrees of freedom	Damage (%)		SI	
		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 $P > F$ ($p < 0,001$).

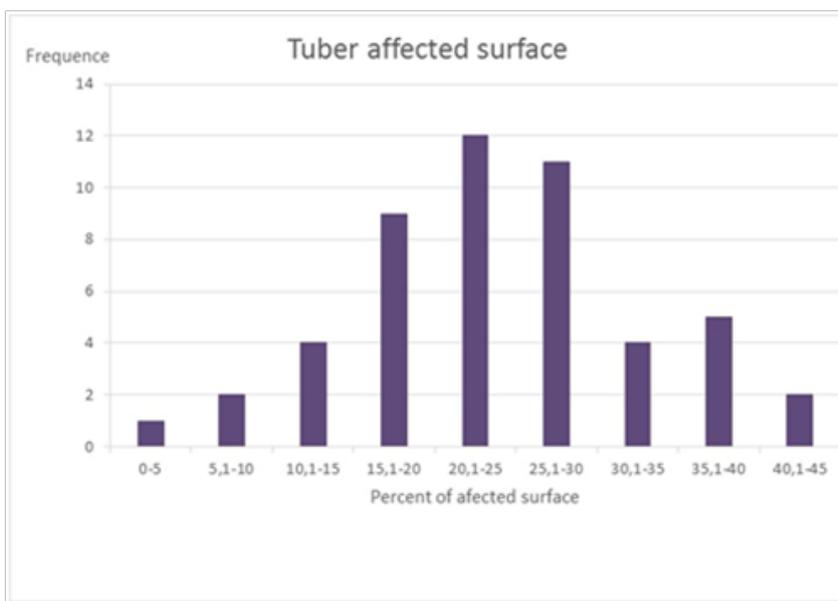


Figure 1 Superficial scab (type I lesion) was the lesion type found in higher frequency.

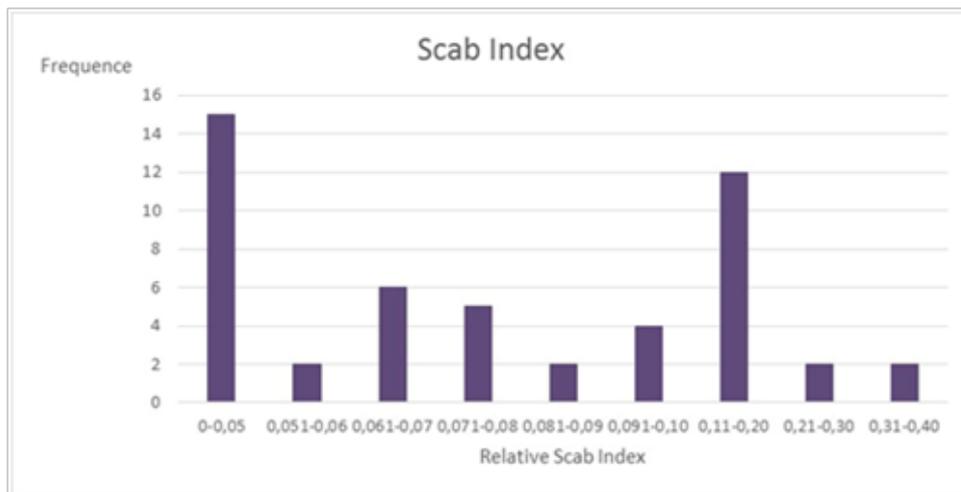


Figure 2 Significant differences ($p < 0,001$) for RSI among genotypes.

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
B 03.04.573.1	Kantara	Monalisa
	Yaguari	Atlantic
	Tacna	B 01.559.2
	Chata Blanca	304013.2
	Newen	B 78.502.5
	Kennebec	B 87.605.1
	Purple Majesty	Arazati
	Asterix	393371.4
	Yagana	Snowden
	B 85.616.3	B 84.617.4 LR
	Pampeana	B 07.516.1
	Baronesa	
	B 03.559.1	
	Araucana	
	B 03.574.2	
	395.192.1	
	Frital	
	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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None.

Conflict of interest

The author declares no conflict of interest.

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