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RESEARCH ARTICLE

Chipping ability, specific gravity and resistance to *Pectobacterium carotovorum* in advanced potato selections

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ABSTRACT
The availability of potato varieties combining good tuber quality and resistance traits is very important for both processors and supermarkets. In the present study, post-harvest quality traits as well as resistance to tuber bacterial soft rot were evaluated in 42 advanced potato clones belonging to seven hybrid families. Differences in specific gravity were found among families and among clones, with about 30% of clones showing a high specific gravity (>1.080). Clones and varieties were assayed also for a comprehensive chipping profile. Overall, 43% of clones presented an acceptable chipping score (≤4.5) at harvest. In addition, following cold storage with and without reconditioning, various clones performed acceptably. Five clones from three families (Bolestra X MC 329, Spunta X Victoria and Majestic X Alcmaria) were classified as resistant to bacterial soft rot. An evaluation index is proposed to help identify clones with interesting combinations of traits.

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Introduction

Potato (*Solanum tuberosum*) is a primary component of the human diet in several parts of the world, representing the third most important food crop worldwide after rice and wheat. Its global production was 364.8 million tons in 2012 (FAO 2012). The high nutritional value of tubers is associated to the significant content of carbohydrates, proteins, dietary fibres, minerals and vitamin C (Burlingame et al. 2009). Tubers are either used fresh or are processed by the food industry, obtaining several products such as French fries, mashed and canned potatoes. Although the direct consumption of potato represents an important part of the market, more than 50% of tuber yield is used by processing firms (Carputo et al. 2005). These require a supply of raw materials with specific internal attributes (e.g. no physiological defects, low reducing sugar content and starch attributes). Additional characteristics such as regular tuber size, high yield and resistance to stresses are considered important factors to increase profits and reduce tuber waste. For the production of French fries and chips, tubers must have a high specific gravity and the ability to be manufactured in light-golden products. The former is particularly important in that it

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guarantees greater efficiency, decreasing processing time and oil absorption (Storey & Davies 1992).

An important consideration with regard to the production of chips and French fries is that tubers are often stored at low temperatures before processing. Cold storage minimises losses due to respiration and sprouting. However, it causes the accumulation of reducing sugars that results in an undesirable browning of chips. Although reconditioning of stored tubers at higher temperature is used to decrease the level of reducing sugars, this process is time-consuming. Besides, not all potato varieties recondition sufficiently to produce acceptable brands (Hayes & Thill 2002). Since crop losses in potato stores can be high, in recent years tuber disease resistance has been considered a major varietal attribute (Keijbets 2008). Resistances are important not only because there are few effective post-harvest chemicals, but also because they meet consumer demand on the development of more sustainable food.

Among the various diseases of concern in potato storage, tuber soft rot caused by *Pectobacterium carotovorum* is potentially the most damaging. *P. carotovorum* can grow in a wide range of hosts and under different temperatures, making the disease difficult to control. In addition, the bacteria could propagate throughout the vascular system and lenticels in asymptomatic tubers. Nowadays, cultivated potatoes show only partial resistance to the bacteria and chemical control is not effective (Zimnoch-Guzowska et al. 2000, 2006).

Despite the large number of potato varieties available, there is the need for new varieties. Due to the stringent targets, it often takes 10–15 years to develop a new variety suitable for processing. This period reflects the wide range of traits to be improved as well as the germplasm available and the strategy employed. A typical breeding programme starts with sexual hybridisation between tetraploid varieties or advanced clones followed by tuber evaluation for quality traits and selection of the most interesting clones (Mackay 2006). Given the autotetraploid nature of *S. tuberosum* and its high level of heterozygosity, sexual generations usually present trait segregation. They are followed by a number of vegetative generations subjected to field evaluations and selection. At the end superior clones are identified (Haynes et al. 2012). Breeding efficiency increases intercrossing parents with complementary and interesting traits, and then selecting specific cross combinations showing useful agronomic, morphologic and industrial traits (Hayes & Thill 2002; Brown et al. 2003).

In this paper, we evaluated quality attributes of selected potato clones obtained through crosses between tetraploid varieties/clones. The main objective of this work was to identify clones with a useful combination of traits.

Methods

Plant material and sampling site

Forty-two advanced potato clones belonging to seven families (S04-1 to S04-7) from crosses involving nine cultivated varieties (Bolesta, Spunta, Victoria, Agria, Alcmaria, Carmine, Sandy, Majestic, Jenny) were used in this study, as well as two breeding clones (MC 329 and S87-24-20) (Table 1). These materials derived from a conventional programme of clonal selection which started in 2010 with 996 clones grown in a single-

Table 1. Description of the four-year breeding programme that produced the potato clones evaluated in this study. Details in Methods section.

Family	Code	No. of clones planted in 2010	No. of clones planted in 2011	No. of clones planted in 2012	No. of clones planted in 2013
Bolesta X MC 329	S04-1	180	55	20	9
Spunta X Victoria	S04-2	144	51	38	18
Agria X Alcmaria	S04-3	156	26	13	4
S87-24-20 X Carmine	S04-4	108	6	2	1
Agria X Sandy	S04-5	84	18	7	2
Majestic X Alcmaria	S04-6	108	24	9	4
Jenny X MC 329	S04-7	216	24	13	4
Total		996	204	102	42

hill plot. In 2011 and 2012, selected clones were cultivated and screened in larger unreplicated plots with spaced plants for further evaluation aimed at discarding clones with undesired characteristics (i.e. long stolons, deep tuber eyes and tuber defects) and increasing the number of seed tubers. The remaining 42 clones were evaluated in replicated trials in 2013 for tuber specific gravity, chipping ability and resistance to *P. carotovorum*. Trials were carried out in Celano, Piana del Fucino, an area particularly suited for potato production (Latitude: 42.023067, Longitude: 13.515709). The experiment was set up in three replications with randomised blocks. Ten tubers per clone were planted in a single row with spacing of 30 cm between genotypes in the row and 70 cm between rows. Cultivars Agria, Adora, Spunta and Desiree were also planted and used as controls. Standard agronomic and irrigation practices were followed during the growing season. Foliage earliness (from 1 = very late to 5 = very early) was visually assessed at 90 days of field growth by comparing foliage senescence of each clone to the control Spunta (earliness score = 3). Tubers were harvested 120 days after planting when materials had already started senescence. Immediately after harvest, tubers from each clone were enclosed in paper bags and were stored at 7°C and 85–90% relative humidity for further evaluation.

Tuber quality evaluation

The specific gravity of each clone under investigation was estimated at harvest as weight in air/weight in water of 1 kg of tubers for each clone according to Woolfe (1987). To test chipping ability, two tubers of each clone were used. Chips were produced by frying 10 longitudinally cut tuber slices from the centre of each tuber. Tuber slices were washed in water before frying in soybean oil. They were considered completely fried when oil ceased to bubble. For every clone, three chipping evaluations were performed: soon after harvest, and after three months of cold storage with and without reconditioning for two weeks at warmer temperature (20–24°C). Chip processing ability was determined based on a colorimetric scale from 1 (very light) to 10 (very dark). Clones with a score $\leq$ 4.5 were considered suitable for chipping (Carputo et al. 2002).

Resistance to P. carotovorum

P. carotovorum strain Ecc 009 from the International Potato Center, Lima (Peru) was used. Immediately after harvest, 7–10 medium-sized tubers per genotype were inoculated with 20 µl of bacterial suspension 10^7 colony-forming unit/mL as previously reported

(Carputo et al. 2007). In brief, each tuber was first surface-sterilised with sodium hypochlorite for 20 min; then seven holes (2 mm wide and 2 cm deep) were carved for each tuber. Six holes were used for bacteria inoculation while one was used to generate the control, inoculating sterile water. Following inoculation, tubers were incubated for 72 h at 24°C in a dew chamber. The diameter of the decay was measured after cutting the tuber vertically through the injection point. According to Carputo et al. (2007), the clones were considered resistant (R) when the diameter of the rotted area was <4 mm, and intermediate (I) when it was 4–6 mm and susceptible (S) when it was >6 mm.

Evaluation index

To select clones with a desirable combination of traits an evaluation index (EI) was elaborated by assigning the following arbitrary scale to each trait: specific gravity of tubers, 1 = ≤1.080 (not suitable for processing), 2 = 1.081–1.090, 3 = 1.091–1.100, 4 = >1.100; chipping colour, 1 = ≥4.5 at each test after cold storage, 2 = ≤4.5 at least in one test after cold storage, 3 = ≤4.5 at both tests after cold storage; earliness, 1 = earliness score 1; 2 = earliness score 2, 3 = earliness score 3, 4 = earliness score >4; resistance to *P. carotovorum*, 1 = Ø rotted area >8 mm, 2 = Ø rotted area 6–8 mm, 3 = Ø rotted area 4–6 mm, 4 = Ø rotted area <4 mm. The EI represents the sum of scores for each trait: the higher the index values, the more desirable the genotypes. The EI was calculated only for clones for which all the evaluation data were available.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using JMP 7 software (SAS Institute, Cary, NC, USA). When a significant F was found ($P < .05$), separation of means was accomplished by Tukey's post hoc multiple comparison test. For each trait, single degree of freedom contrasts were used to compare the mean values among clones to the mean of cultivars and to cultivars individually. Additionally, the linear relationship between TSG, chipping ability and resistance to *P. carotovorum* was calculated.

Results and discussion

Potato breeding requires a constant production of valuable new clones. One of the strategies is to sexually hybridise tetraploid varieties/advanced clones and then evaluate the advanced progeny based on phenotypic traits. An important aspect for a successful breeding programme is the selection of the parental genotypes. Indeed, crossing parents are picked with complementary features in order to generate genetic variation in the progeny as well as to maximise heteroallelic interactions required to guarantee heterosis (Carputo & Frusciante 2011).

In this study, following a three-year visual selection pressure to discard undesired materials (Table 1), 42 clones were characterised for traits that are important breeding targets, especially when tubers are produced for processing. Indeed, the potato processing industry requires raw materials with high specific gravity, no physiological defects and the ability to produce light-coloured chips. Most of our clones originated from Spunta X Victoria. Indeed, out of 144 clones planted in 2010, 18 (13%) were selected over the years for

2013 evaluations (Table 1). Meanwhile, the worst family was obtained from S87-24-20 X Carmine, where out of 108 clones of 2010, only 1 (<1%) was evaluated in 2013.

Tuber specific gravity, chipping ability and resistance to *P. carotovorum* of materials under evaluation are summarised in Table 2. Data are given on a family basis. Mean specific gravity among the seven families showed significant differences and ranged from 1.089 to 1.074. As for controls, Agria showed the highest specific gravity (1.088), Adora the lowest (1.058). The mean specific gravity of clones was significantly higher than the mean of controls (Table 3). Seventeen clones showed a higher specific gravity than the mean of the controls. The potato processing industry commonly uses specific gravity values for rapid estimation of the dry matter content of tubers (Kumar et al. 2005), and tubers with specific gravity higher than 1.080 are generally considered suitable for processing (Kabira & Berga 2003). In our study, 13 clones (31%) displayed a specific gravity higher than 1.080 (not shown).

Based on the requirements of the Italian processing market and industrial interests, the chip-processing ability of tubers was studied at three different times: at harvest and after 90 days of cold storage at 7°C, with and without reconditioning at room temperature. Potato tubers are consumed all year round. As a consequence, tubers are often cold stored to prevent diseases (such as bacterial soft rot), sprouting, loss of dry matter and to guarantee an uninterrupted supply of tubers throughout the year (Malone et al. 2006). On the other hand, cold storage is usually accompanied by accumulation of reducing sugars (glucose and fructose) from the degradation of starch. This process is known as 'cold-induced sweetening' and is recognised as a severe problem for the potato processing industry because it causes browning of chips (Dale & Bradshaw 2003). The high temperature reached during frying activates the Maillard reaction between sugars and amino acids present in tubers (Kumar et al. 2004). This reaction produces dark-coloured bitter-tasting (unmarketable) chips. Both sugar levels (basal and after storage) are hereditable traits (Kumar et al. 2004) and identification of clones with resistance to cold sweetening represents an important goal for potato breeding. ANOVA indicated the presence of significant differences for all chipping treatments among families (Table 2). We did not find a significant contrast between the mean chipping value of clones and that of control varieties fried at harvest and after three months of cold storage with reconditioning (Table 3). By contrast, this comparison was significant when chips were fried after cold storage without reconditioning. In this case, 10 clones had a significantly better chip score than the mean of the controls. Analysis of the ranges in chipping scores revealed that a number of clones were good chippers (not shown). Indeed, out of 42 clones tested, 18 (43%) had a chipping score ≤ 4.5 at harvest. As expected, this value decreased to 10 (24%) and to 8 (19%) following cold storage without and with reconditioning, respectively. A good chipping score at harvest was displayed by Desiree, Spunta, Adora and Agria (Table 2). However, following three months' storage at 7°C these controls did not chip acceptably. Only after reconditioning Desiree and Agria chip well. The reduction of chipping ability after cold storage has been extensively reported (Capo et al 2002; Hayes & Thill 2002; Otmans & Novy 2002; Rak et al. 2013; Zhao et al. 2013; Ali et al. 2016). Following reconditioning, reducing sugars are reconverted into starch, and this may decrease the number of dark chips (Hamernik 1998). However, we did not observe a general increase in potato with chipping score ≤ 4.5 in reconditioned vis-à-vis non-reconditioned clones. This could be related to the characteristic sugar content of each genotype that might induce a limited

Table 2. Results on the evaluation of 42 potato clones belonging to 7 families with different genetic background.

Family	No. of clones under evaluation	TSG[1]	Chip category colour[1]			Resistance to Pc (ø mm)[1]	EI
			Direct	Cold storage – Rec	Cold storage + Rec		
F-ratio		7.18	3.0	7.0	4.0	8.18	ns
P-value		<.0001	.0011	.0059	.0023	<.0001	
Bolesta X MC 329	9	1.081 (1.072–1.093)^{a}	4.9 (2.0–9.0)^{ab}	5.2 (3.0–9.0)^{ab}	6.0 (4.0–8.0)^{ab}	9.7 (2.0–24.0)^{bc}	8.4 (5.0–13.0)
Spunta X Victoria	18	1.074 (1.066–1.080)^{bc}	6.2 (3.0–10.0)^{a}	5.9 (4.0–9.0)^{ab}	6.0 (2.0–8.0)^{ab}	8.3 (2.0–20.0)^{cd}	7.6 (6.0–11.0)
Agria X Alcmaria	4	1.077 (1.073–1.083)^{abc}	4.3 (2.0–7.0)^{ab}	5.0 (2.0–7.0)^{ab}	4.0 (2.0–8.0)^{b}	8.8 (2.0–20.0)^{bc}	9.0 (7.0–11.0)
S87-24-20 X Carmine	1	1.076^{bc}	8.0^{a}	5.3^{ab}	6.7^{ab}	7.0^{a}	4.0
Agria X Sandy	2	1.076 (1.064–1.084)^{bc}	3.7 (3.0–4.0)^{ab}	6.0 (5.0–7.0)^{ab}	4.0 (2.0–6.0)^{b}	13.1 (5.0–20.0)^{ab}	5.5 (7.0–11.0)
Majestic X Alcmaria	4	1.079 (1.076–1.084)^{ab}	7.8 (7.0–9.0)^{a}	6.3 (4.0–7.0)^{ab}	9.0 (7.0–10.0)^{a}	11.3 (2.0–25.0)^{d}	8.8 (8.0–10.0)
Jenny X MC 329	4	1.089 (1.085–1.093)^{a}	4.0 (2.0–7.0)^{ab}	4.5 (3.0–6.0)^{b}	5.5 (3.0–7.0)^{ab}	6.6 (2.0–22.0)^{cd}	9.5 (6.0–13.0)
Desiree	1	1.070^{bc}	3^{ab}	7^{b}	4^{ab}	6.2^{cd}	10.0
Spunta	1	1.062^{bc}	4.3^{ab}	8.0^{ab}	6.7^{ab}	9.9^{abcd}	7.0
Adora	1	1.058^{c}	2.0^{b}	8.7^{a}	5.0^{ab}	9.32^{bcd}	7.0
Agria	1	1.088^{a}	3.0^{ab}	8.0^{b}	3.0^{b}	9.24^{bcd}	8.0

Notes: Reported here are the following traits: tuber specific gravity (TSG), chip category colour and resistance to *P. carotovorum* (Pc). Chip category colour was evaluated at harvest and after 90 days of cold storage at 7°C, with and without reconditioning at room temperature for two weeks (respectively – Rec + Rec) (see Methods section). In addition, based on the clone performance for the studied traits, an EI was calculated based on clone performances. For each parameter, the average family value (range) is reported. 'ns' indicates not statistically significant data.

[1]Means comparisons using Tukey's test. Levels not connected by same letter are significantly different ($P < .05$).

Table 3. Means, ranges and comparisons between clones and control varieties (Desiree, Spunta, Adora, Agria). Tuber specific gravity (TSG), chipping ability (direct, after cold storage ± Reconditioning, Rec) and resistance to *P. carotovorum* (Pc) were evaluated.

		Chip category colour[a]			
Family	TSG[a]	Direct	Cold storage – Rec	Cold storage + Rec	Resistance to Pc (ø mm)[a]
Selections	1.078 (1.064–1.093)	5.6 (2.0–10.0)	5.5 (2.0–9.0)	6.0 (2.0–10.0)	8.97 (2.0–25.0)
Cultivars	1.069 (1.058–1.088)	3.0 (2.0–4.0)	7.3 (7.0–8.0)	5.5 (3.0–7.0)	8.66 (6.0–10.0)
Comparisons					
Selection vs. Cultivar	17**	ns	10**	ns	ns
Selection vs. Desiree	ns	ns	ns	ns	4*
Selection vs. Spunta	40**	ns	22*	ns	ns
Selection vs. Adora	41**	0*	28**	ns	ns
Selection vs. Agria	2*	ns	22*	0*	ns

[a]Number of clones with a significantly better score compared to the control.
ns, *, and ** indicate that means are not different or statistically different at $P < .05$ and $P < .01$, respectively (LSD 0.05).

reconversion of reducing sugars into starch. Importantly, in almost every family we were able to identify at least one clone with a chipping ability score <4.5 after cold storage. Of particular importance is the availability of good chippers that do not need reconditioning. It is indicative that the controls used here never reached a good chip score when fried directly out of storage. During reconditioning diseases can occur and chemical treatment (i.e. sprout inhibitors) should be scheduled. This procedure is time-consuming and causes loss of tubers. The development of a cultivar resistant to cold sweetening would eliminate the reconditioning step. Tubers not requiring a treatment at higher temperature after cold storage would greatly simplify frying. Here we identified four very promising clones resistant to cold sweetening belonging to Bolestra X MC 329 (1), Agria X Alcmaria (1) and Jenny X MC 329 (2) (not shown). Quality attributes studied herein will be monitored during cold storage to check whether they are maintained.

Currently, emphasis is given to the development of sustainable potato varieties that combine good quality attributes with resistance to biotic stresses (Keijbets 2008). In this study, we focused on the resistance to tuber soft rot, considered a very important and damaging disease due to the lack of resistant varieties (Zimnoch-Guzowska et al. 2006). Mean resistance to *P. carotovorum* among the seven families showed significant differences (Table 2), whereas the mean resistance of selections did not significantly differ from the mean of cultivars (Table 3). Resistant clones were identified within the families Bolestra X MC 329 (1), Spunta X Victoria (2) and Majestic X Alcmaria (2), while intermediate resistance was found only in two clone families: Spunta X Victoria (2) and Majestic X Alcmaria (1) (not shown). None of the controls displayed resistance to *P. carotovorum*. Given that the resistance to *P. carotovorum* in potato is polygenic (Zimnoch-Guzwska et al. 2000), further experiments would be required to confirm the presence of stable resistance in the studied clones. However, it is promising that recent results suggest that resistance does not change during storage (Chung et al. 2013). The relationship between TSG, chipping ability and resistance to *P. carotovorum* was explored and no statistically significant correlation was found.

To facilitate the screening of genotypes with interesting combinations of traits, an arbitrary EI was calculated (Table 2). On a family basis, the average EI ranged from 4 (S7-24-20 X Carmine) to 9.5 (Jenny X MC 329). The best control was Desiree, with EI = 10.

Table 4. Characteristics of best performing clones selected for further evaluation. Data displayed are: pedigree, tuber specific gravity (TSG), chipping ability score (at harvest and after cold storage ± Reconditioning, Rec), response to *P. carotovorum* (Pc) inoculation (diameter of the lesion, mm), earliness (based on comparisons with Spunta as control), tuber skin and flesh colour and tuber form.

			Chipping ability							
Clone	Family	TSG	Harvest	Cold storage – Rec	Cold storage + Rec	Resistance to Pc (ø mm)	Earliness	Skin colour	Flesh colour	Form
S04-1-10	Bolesta X MC 329	1.089	2	5	5	3.9	5	Yellow	Yellow	Round
S04-1-11	Bolesta X MC 329	1.084	3	3	4	13.4	4	Yellow	Yellow	Oblong
S04-2-3	Spunta X Victoria	1.073	8	4	6	2.0	4	Yellow	Yellow	Oblong
S04-3-25	Agria X Alcmaria	1.083	2	2	2	13.9	5	Yellow	Yellow	Oblong
S04-3-40	Agria X Alcmaria	1.074	2	2	2	7.71	5	Yellow	Yellow	Oblong
S04-5-30	Agria X Sandy	1.084	4	5	2	6.7	5	Yellow	Yellow	Oblong
S04-7-8	Jenny X MC 329	1.093	4	3	3	7.9	5	Yellow	White	Oblong

Inspection of the ranges in EI revealed that in seven clones EI was higher than in the best control variety Desiree (Table 4). The capacity of these clones to chip well out of cold storage and their earliness is particularly attractive. This latter trait was expected since in previous years we applied negative selection, thus discarding materials with undesired traits (including late maturity). It should be pointed out that the index does not aim to rank clones under evaluation relative to a genetic base, but rather summarise evaluation for various parameters in a single number, thus providing a practical selection criterion. Clones with a low EI can be considered inferior, and hence subject to negative selection. Those with higher EI can be analysed in further detail to examine each single component that contributed to EI before being evaluated in costly replicated tests. Depending on the breeding targets, it may also be possible to attribute a different weight to each individual component contributing to EI, as already reported in tomato (Frusciante et al. 2007). The selection programme carried out here allowed identification of potato clones with a good combination of useful traits. Although we are aware that results come from a one-year experiment and that the number of clones evaluated here is not comparable with that of large breeding programmes carried out elsewhere, we focused on the goal to improve potato within a specific agro-ecological area rather than across areas. This has important implications for the strategies employed. Indeed, since genetic variation is exposed and available for selection mainly in the seedling and first clonal stages, we could start our (negative) selection from the first generations in the target environment. Superior clones are currently being tested for additional traits and in replicated field trials, with the aim of both developing a new variety suitable for our environmental conditions and identifying parental clones for further breeding.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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