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Breeding potatoes for resistance to bacterial wilt in Brazil: a quick review in face of a more effective screening protocol

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ABSTRACT

Bacterial wilt (BW), caused by *Ralstonia solanacearum*, is one of the most important diseases of potato (*Solanum tuberosum* subsp. *tuberosum*) in Brazil and the main cause of rejection of fields for tuber seed certification. Genetic resistance is not a feasible control currently since no commercially-appealing BW resistant cultivars are available. The development of resistant cultivars is challenging due to the genetic complexity of resistance, pathogen variability, lack of resistance sources in the species, and the tetraploid background of the crop. In addition, to date, only field selection has been effective in identifying stable resistance in progenies derived from crosses involving resistant wild relatives. Field selection is laborious and demands uniformly infested fields. After many years of germplasm breeding, we succeeded in developing two resistant clones, MB-03 and MB9846-01, both producing tubers with rather reasonable characteristics. These clones are being crossed with elite genotypes. To speed up progeny evaluation, we developed a straightforward screening protocol in greenhouse conditions, based on selection at the seedling stage. The methodology is presented and discussed here. Briefly, the early selection was very effective to screen a large number of seedlings in a rather short period of time. It considerably increased the rates of selection of resistant clones in the field when compared to selection directly in the field, without the prior greenhouse seedling stage. Nevertheless, field selection remains crucial for confirming resistance, testing for genotype-environment interaction and evaluating agronomic and tuber characteristics. Among the resistant clones previously identified in our program, progenies of clone MB9846-01 resulted in higher selection indexes in the field (BW resistance + tuber characteristics) than those of clone MB-03 when both clones were crossed with the susceptible cultivar Baraka. We adjusted the protocol to allow screening around 5,000 seedlings per year, counting with eight part-time workers, four in the laboratory/screenhouse and four in the field in critical periods.

Keywords: *Ralstonia solanacearum*, *Solanum tuberosum* ssp. *tuberosum*, greenhouse screening, early selection, selection rates, germplasm breeding.

RESUMO

Melhoramento de batata para resistência à murcha bacteriana no Brasil: um breve histórico vis-à-vis um protocolo de seleção mais efetivo

A murcha bacteriana (MB), causada por *Ralstonia solanacearum*, é uma das doenças da batata (*Solanum tuberosum* subsp. *tuberosum*) mais importantes no Brasil e a principal causa de rejeição dos campos de certificação de batata-semente. A resistência genética não é uma medida de controle viável atualmente, uma vez que não existem cultivares resistentes comercialmente aceitáveis. O desenvolvimento de cultivares resistentes é um desafio em vista da complexidade genética da resistência, variabilidade do patógeno, ausência de fontes de resistência na espécie e herança tetraploide da cultura. Além disso, até o momento, apenas a seleção de campo tem sido eficaz na identificação de resistência estável em progênies derivadas de cruzamentos com parentes silvestres resistentes. A seleção de campo é laboriosa e exige campos uniformemente infestados. Após muitos anos de melhoramento de germoplasma, identificamos dois clones resistentes, MB-03 e MB9846-01, que produzem tubérculos com características bastante razoáveis. Esses clones estão sendo utilizados em cruzamentos com genótipos elite. Para acelerar o processo de seleção nas progênies, desenvolvemos um protocolo simples de avaliação da doença em casa de vegetação a partir da inoculação de plântulas. A metodologia é apresentada e discutida aqui. Resumidamente, a seleção na fase de plântulas foi efetiva na avaliação de um grande número de genótipos em um período de tempo bastante curto. Seu emprego resultou em um aumento considerável nas taxas de seleção final das progênies no campo, quando comparado à seleção direta no campo, sem o estágio anterior em casa de vegetação. Entretanto, a seleção em campo permanece crucial para confirmar a resistência, estudar a interação genótipo-ambiente e avaliar características agronômicas e dos tubérculos. Entre os clones resistentes previamente identificados em nosso programa, as progênies do clone MB9846-01 apresentaram um índice mais alto de seleção final em campo (resistência a MB + características do tubérculo) que as progênies do clone MB-03, quando ambos foram cruzados com a cultivar suscetível Baraka. O protocolo de seleção precoce em casa de vegetação foi ajustado para permitir a avaliação de cerca de 5.000 plântulas por ano, contando com oito trabalhadores em meio período, quatro em laboratório/casa de vegetação e quatro em campo em períodos críticos.

Palavras-chave: *Solanum tuberosum* ssp. *tuberosum*, *Ralstonia solanacearum*, seleção em casa de vegetação, seleção precoce, taxas de seleção, melhoramento de germoplasma.

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POTATOES AND THE BACTERIAL WILT

Bacterial wilt (BW), caused by *Ralstonia solanacearum*, is one of the most important diseases of potato (*Solanum tuberosum* subsp. *tuberosum*) in Brazil, where the crop is grown in tropical and subtropical areas, in latitudes from 18°S to 32°S. Even in cooler regions, if warm spells are associated with high soil moisture, losses due to BW may reach up to 50%. Besides, BW is the main cause of rejection of fields for tuber seed certification (Lopes *et al*., 1990).

Integrated disease management has been successful in partially mitigating losses due to BW, combining practices such as adequate planting season, field selection, crop rotation, use of certified seeds, and resistant cultivars. The German cultivar Achat was the leading commercial material in Brazil during the 1990's to some extent because of its stable partial field resistance to BW (Lopes & Giordano, 1983; Lopes & Quezado-Soares, 1994). However, cultivar Achat is no longer used in Brazil. In spite of having only partial field resistance to BW, no similar level of resistance was identified in any other potato commercial genotype to date. Cultivar Achat was replaced by the Dutch cultivar Monalisa, due to its higher yield, shorter cycle, improved tuber external quality (bright and smooth skin, shallow eyes, and uniform oblong shape) and slightly better cooking quality. Cultivar Monalisa, in turn and for the very same reasons but cooking quality, was completely replaced by the Dutch cultivar Agata, currently covering more than 70% of the ware potato acreage in Brazil (about 100,000 ha). Cultivars Agata and Asterix, the latter the most important processing cultivar in Brazil, cannot be considered resistant to BW, but they are more tolerant (intermediate resistance) than cultivars Monalisa and Cupido. Cultivar Cupido has some importance in the country due to its partial resistance to late blight and extremely high tuber external quality.

The use of resistant cultivars is not currently a feasible alternative to control BW in potatoes. Even though resistance is an elegant and reliable measure to avoid disease losses, especially because it is easily adopted by growers and does not increase production costs, there are no commercially-appealing BW resistant potato cultivars available. The genetic complexity associated with BW resistance makes the development of resistant cultivars quite challenging: the BW resistance found in potato germplasm collections is low and unstable in time and space due to the pathogen high variability; while the quantitative resistance available does not withstand the high disease pressure caused by climatic conditions, especially under high temperature and high soil humidity (Nielsen & Haynes, 1960; Tung *et al*., 1990; French, 1994). In addition, resistance is usually available in very wild types. On the top of that, the vegetative propagation and the tetraploid nature of the crop make a long road even longer, complex and rather uncertain.

MINUTES OF HISTORY

A breeding initiative was started at Embrapa Hortaliças in 1987 by means of a cooperative project with the International Potato Center (CIP), Lima, Peru. The objective was advancing generations through successive selection cycles in infested fields to allow for the development of BW-resistant cultivars. TPS populations were received annually from CIP. These populations, segregating for resistance, were derived from accessions of *Solanum sparsipilum*, *S. chacoense*, *S. microdontum* and *S. phureja* (Schmiediche, 1986). *S. phureja* accessions were originally selected at the University of Wisconsin, in the first known systematic potato breeding program for resistance to BW (Rowe & Sequeira, 1972). More recently, crosses involving *S. commersonii*, selected at INIA, Uruguay, were incorporated to the Brazilian potato breeding program for BW resistance aiming to broaden its genetic basis (Siri *et al*., 2008).

Approximately 130,000 clones obtained from true-seeds were evaluated in the last 30 years of non-stop germplasm breeding activities. Two clones, MB-03 and MB9846-01, stood out for their highly stable resistance to BW, identified upon successive exposures to naturally infested fields in Brasilia-DF, and Caxias-RS, where different races of the pathogen prevail (Silveira *et al*., 2007). Although not commercially appealing in Brazil, since they do not match the high standards of the market concerning tuber shape uniformity and external characteristics, both clones are a precious source of resistance and have been widely used in Embrapa's breeding program in crosses with genitors that favor tuber appearance and quality.

Regardless the indisputable value of clones MB-03 and MB9846-01, the frequency of recovery of resistant clones out of the successive selection of TPS segregating populations directly in infested fields is low. An alternative selection method was needed to speed up the process, improve the success rate and, luckily, allow for the selection of clones possessing, in addition to resistance, other important traits, mainly marketable tuber appearance, high yield, and good cooking quality. About two decades ago, Embrapa Hortaliças and partners started developing and adjusting such protocol. Its description, utility and efficiency are summarized here, as well as some recent results.

A NEW PROTOCOL

All steps of the protocol are schematically shown in Figure 1. The breeding starts by carefully designing the crosses in which the potato clones with BW resistance will be involved. In general, Embrapa's resistant clones (especially MB-03 and MB9846-01, both derived from *S. phureja*) are crossed with cultivars, elite clones from Embrapa's cultivar development-oriented breeding program or other genotypes recorded as efficient in transferring good traits to their progenies. More recently, clones selected at INIA, Uruguay, were integrated into the program as a new source of BW resistance. INIA's clones derive from crosses between maternal progenitors with *S. commersonii* background and

cultivars, other INIA clones or clones from the International Potato Center. In Embrapa, the crosses are made in Southern Brazil (Embrapa Clima Temperado), where the environmental conditions are favorable for potato flowering and fruit and seed set. Then, the true potato seed (Figure 2A) is transferred to Embrapa Hortaliças, in Central Brazil.

TPS are sown in plastic trays containing sterile commercial substrate in screenhouse (Figure 2B). Two-leaf seedlings are transplanted to 250 mL plastic cups containing a balanced mixture of sterile substrate and local soil and kept in screenhouse (Figure 2C). Fifteen days after transplanting, seedlings are inoculated with 10 mL of bacterial suspension, applied at the plantlet base (Figure 2D). The bacterial suspension (c.a.10^7 cfu/mL) is prepared from *R. solanacearum* colonies grown in CPG medium (Kelman's medium without tetrazolium chloride), for 48 h at 38°C.

Inoculated plants are immediately transferred to a greenhouse with heating, to ensure temperatures do not fall below 20°C: low temperatures, especially at night, favor escapes, thus reducing the method efficiency (Figure 2E). Wilting seedlings usually start appearing from seven to 10 days after inoculation (DAI) and are removed daily (Figure 2F). Seedlings that survive inoculation for 15 days are transplanted to 3L pots containing sterile commercial substrate and moved back to a screenhouse (18-30°C), for tuber development. Plants that wilt in this phase are also discarded (Figure 2G).

Tubers of the putative resistant clones are harvested approximately 100 days after transplanting (Figure 2H) and placed in cold storage (6-10°C) for 3-5 months to sprout. Depending on the number and size of the tubers, it might be necessary to carry out an additional multiplication in a screenhouse for some clones, before bringing them to the selection field. Alternatively, these clones can also be multiplied in non-infested fields, where, in addition to tuber multiplication, a preliminary evaluation of tuber characteristics can be performed.

In May-June, when the dry and cool season starts in Central Brazil, five to 10 tubers (35 to 50 g) of each putative resistant clone are planted in plots, without replication, in a field naturally infested with *Ralstonia solanacearum*, race 1, biovar 1, phylotype II, at Embrapa Vegetables (Figure 2I). Five-plant plots of the resistant genotypes Cruza 148, MB-03, and MB9846-01 are randomly distributed in the field for comparison purposes. Three plants of cultivar Monalisa, the susceptible control, are planted in both ends of the row of each clone under test. This gives a clear picture of the uniformity of BW distribution in the field and prevents clones that eventually escaped the disease from being declared resistant.

Plants of cultivar Monalisa usually start wilting about 20 days after planting (DAP), with the disease progressing much faster after hilling at 25 DAP (Figure 2J). Disease progress is monitored every 10 days. Disease assessment takes place when at least 80% of cultivar Monalisa plants are wilted, usually around 65 DAP. Clones are considered resistant when they have the same proportion of wilting plants as at least one of the resistant controls. The haulm is destroyed 100 DAP and tubers are harvested 10 days later (Figure 2K). Tubers characteristics are described and tubers are also assessed according to scales of rotting.

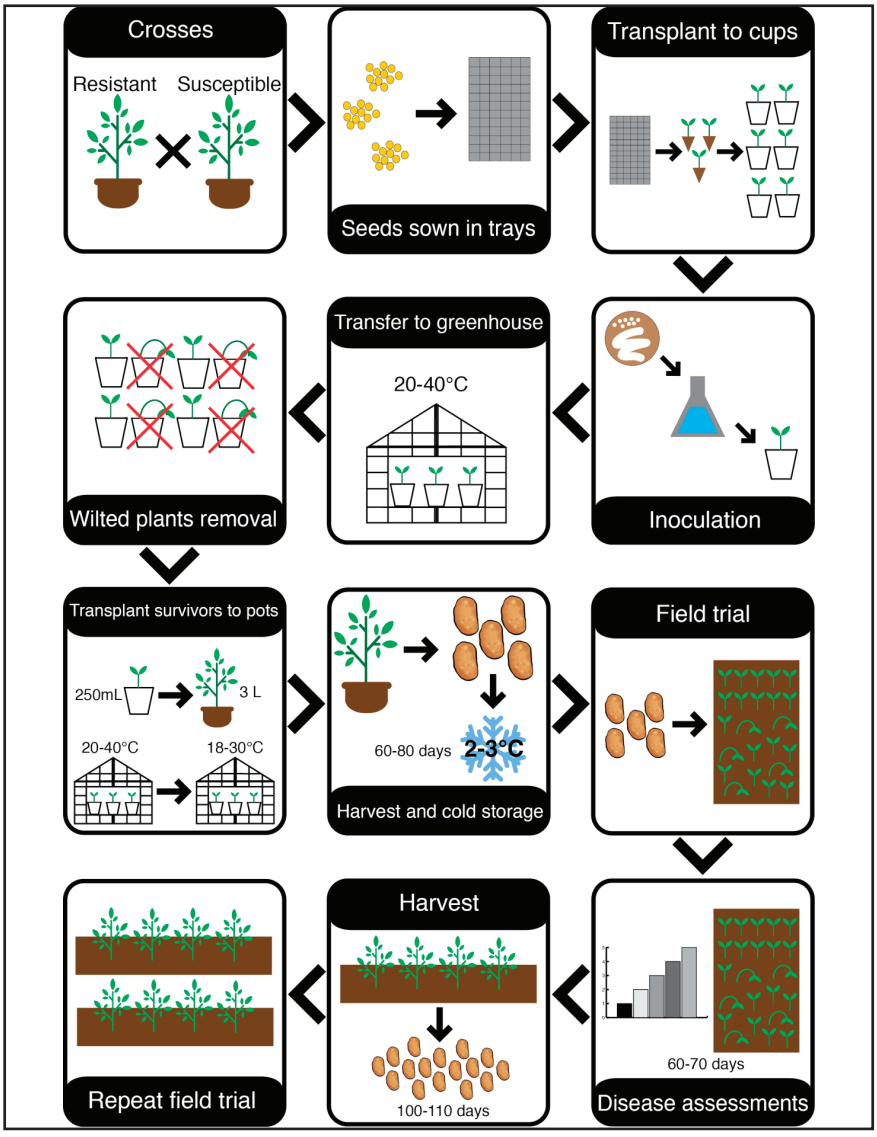

Figure 1. Schematic workflow of the protocol to screen potato genotypes for resistance to bacterial wilt under greenhouse conditions. Brasília, Embrapa Hortaliças, 2018.

Table 1. Survival of potato seedlings from crosses involving bacterial wilt resistant clones and susceptible cultivars after inoculation with *Rasltonia solanacearum* in greenhouse. Brasília, Embrapa Hortaliças, 2018.

Cross	Number of inoculated seedlings	Number of surviving seedlings	Seedling Survival (%)
MB-03 x Baraka	984	109	11.1
MB9846-01 x Baraka	1,800	149	8.3
MB9846-01 x Monalisa	960	46	4.8
MB9846-01 x MB-03	96	40	41.7
Cupido (open pollination)	192	2	1.0

Clones selected in the first year undergo an additional evaluation in the following year, in May-June, in the infested field. In this second round, with replications and 10-tuber plots, selection for tuber external appearance is carried out is more detailed and rigorous, using reference cultivars grown under the same conditions as standard (Figure 2L).

The unequivocal distinction between resistant and susceptible genotypes depends on many and often non-controlled factors. Two critical points must be specially taken care of to keep the selection efficiency high. The first crucial aspect is reducing escapes after inoculation in the greenhouse. To this end, (1) the correct inoculum dose (volume and concentration of the bacterial suspension) must be applied; (2) the isolate must be viable and virulent (reduced virulence is a common phenomenon in isolates kept *in vitro* for long periods); (3) temperatures should not fall below 20°C, especially at night, on the first three days after seedling inoculation; and (4) seedlings must not face shortage of water after inoculation. The second point is the contrary of the first: the failure in detecting interesting resistance levels due to disease overexpression. It might be caused by (1) too high inoculum doses (high volume and/or concentration of the bacterial suspension); (2) extremely virulent isolates; (3) too high temperatures after inoculation, especially on the first three days; and (4) inoculation of too young seedlings.

ASSESSING THE PROTOCOL

After a few years using and adjusting the protocol, in 2011 we decided to assess its effectiveness. To this purpose, we used a set of crosses involving two resistant clones (MB-03 and MB9846-01) and cultivars Baraka (susceptible) and Monalisa (very susceptible), as well as a cross between the resistant clones themselves (Table 1). TPS from open pollination of cultivar Cupido, very susceptible, were also included. Plastic cups with the inoculated plants were placed in plastic boxes (containing 24 cups each) and randomly distributed in greenhouse benches. Typical BW wilting started 10 days after inoculation and, for two weeks, we discarded the seedlings with irreversible wilting symptoms. Following, we transferred the surviving plants to a screenhouse and transplanted them to 3L plastic pots for tuber development.

The percentage of surviving seedlings was as expected (Table 1). Crosses involving the two resistant clones resulted in about 40% of survival; those involving cultivar Baraka stayed around 10%, independent of the BW resistant clone it was crossed with; and that of cultivar Monalisa did not reach 5%. Seedlings derived from the open pollination of cultivar Cupido presented 99% of irreversible wilting. The rate of surviving plantlets reflected the differential susceptibility level of cultivars Baraka, Monalisa and Cupido.

In the following season, 2012/2013, we carried out a new assessment. This time, we divided the TPS from two crosses involving the resistant clones MB-03 and MB9846-01 and cultivar Baraka in two subsets: in one, we followed our greenhouse protocol; in the other, we replaced the inoculum suspension by sterile water (mock-inoculation) (Table 2). Surviving seedlings of both inoculated and mock-inoculated subsets were transplanted to plastic pots in a screenhouse for tuber

Table 2. Survival and selection rates of potato progenies and clones after challenging with *Ralstonia solanacearum* under controlled and field conditions. Brasília, Embrapa Hortaliças, 2018.

Cross	Pre-selection (2012)			Field selection (2013)		
	Inoculated/total number of seedlings	Number of surviving seedlings	Seedling survival (%)	Number of clones planted*	Number of clones selected**	Selection (%)
MB-03 x Baraka	431/431	69	16.0	38	8	21.1
MB9846-01 x Baraka	639/639	106	16.1	52	29	55.8
MB-03 x Baraka	0/200	200	100	62	2	3.2
MB9846-01 x Baraka	0/200	200	100	64	2	3.1

*Clones that survived seedling inoculation were grown in 3L pots in a screenhouse. Some plants showed irreversible wilting after transplanting and were discarded. Clones of mock-inoculated seedlings were randomly selected for the field trial. **Clones that survived field infection and were free from severe tuber defects.

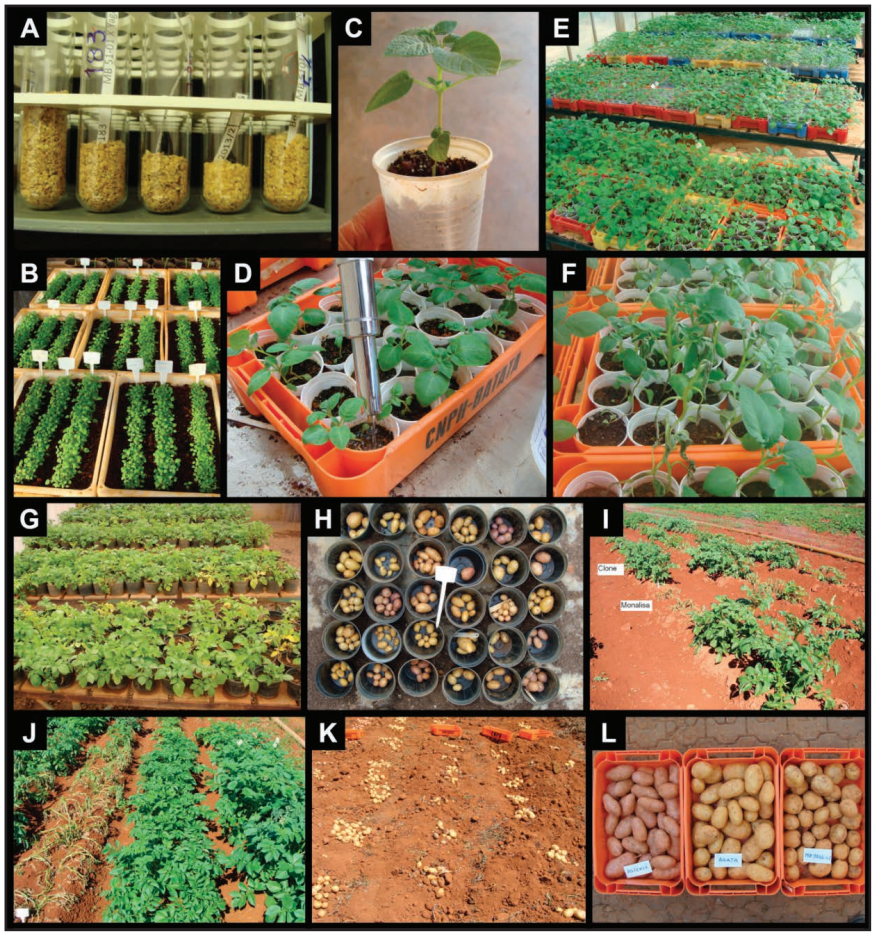

Figure 2. (A) Vial with true potato seeds from crosses involving bacterial wilt resistant clones; (B) Potato seedlings in plastic trays in the stage of transplanting to plastic cups; (C) A potato seedling in the stage of inoculation with *Ralstonia solanacearum*; (D) Inoculation of a potato seedling with *Ralstonia solanacearum*; (E) Potato seedlings after inoculation with *Ralstonia solanacearum* in greenhouse; (F) Putative resistance variability to wilting in potato seedlings after inoculation with *Ralstonia solanacearum*; (G) Pots in screenhouse with the potato seedlings that survived the inoculation with *Ralstonia solanacearum*; (H) Potato tubers from putative bacterial wilt resistant clones produced in pots; (I) Small plots planted with putative bacterial wilt resistant clones in a *Ralstonia solanacearum* infested field; (J) Second field exposition of a potato clone resistant to bacterial wilt (right) in comparison with a commercial susceptible genotype (left); (K) Tubers of clones resistant to bacterial wilt harvested in a field with high disease pressure; (L) Tubers of a bacterial wilt resistant clone compared with standard cultivars (tubers produced in a non-infested field).

development. Tubers were harvested, stored for six months in cold chamber and tested in the next season in a field naturally infested with *R. solanacearum*, in Brasília. Lines with three to five tubers of each clone were randomly distributed in the area. The resistant parents (MB-03 and MB9846-01) and the susceptible cultivars (Monalisa and Cupido) were also planted as a reference for disease reaction and uniformity of disease distribution in the field.

The surviving rates of the seedlings inoculated with *R. solanacearum* in plastic cups for both clonal families in 2012 (around 16%) were higher than in 2011 (around 10%) (Tables 1 and 2). The difference in the survival rate between the two years is very likely due to the cooler season in 2012 than in 2011, which stress the importance of a rigorous temperature control in the greenhouse after inoculation as we mentioned earlier. If we had not used a heated greenhouse, the season could have been useless for screening for BW resistance.

There were remarkable differences in the field selection rate between the two subsets of plantlets (Table 2). While greenhouse inoculated plantlets yielded selection rates of 21.1 and 55.8% for the crosses involving MB-03 and MB9846-01 respectively, the selection rate of mock-inoculated plants stayed around 3%, independent of the resistant parent. These figures speak for themselves when it comes to the effectiveness of the greenhouse inoculation protocol in screening for BW resistance.

Although it is not our objective here, it is worth commenting on the difference in the field selection rate between the two families. The cross MB9846-01 x Baraka resulted in a considerable higher selection rate than the cross MB-03 x Baraka (Table 2). The gap between the two families is related to tuber characteristics and not to BW resistance. Clones surviving the field challenge have their tubers further evaluated. Those whose tubers present severe deformations or widely diverge from commercial standards are discarded. Both situations are more frequent in crosses involving MB-03 than MB9846-01.

Both assessments elegantly indicate that the screening procedure is effective in the selection of potato progenies with quantitative resistance to BW, as we have been observing since we started implementing it. Our experience over the years using this procedure indicates that the recovering rate of surviving plants after the greenhouse phase stays around 10% in crosses involving a resistant parent, varying around it according to the susceptibility level of the other parent. The final selection rate, otherwise, is quite variable, as it goes beyond BW resistance and involves tuber selection criteria. Regarding the resistance clones we have been using, selection rates are higher when MB9846-02 is involved.

The procedure can be calibrated according to the priority given to resistance to BW within the breeding program, either for advancing germplasm or cultivar development. If resistance to BW is the main target, as in our case, keep the selection pressure we use. If resistance to BW is even more critical, enhance the selection pressure by increasing the inoculum concentration and/or the temperature in

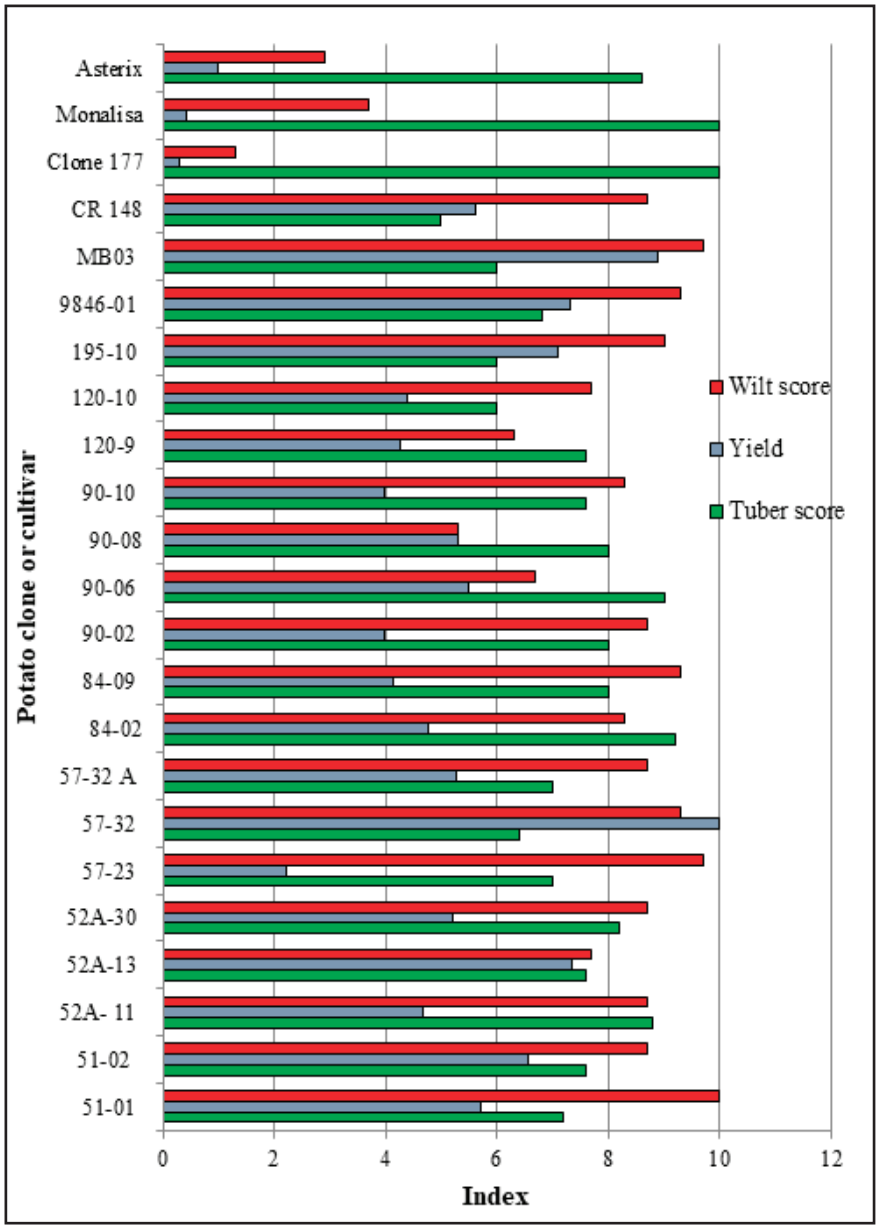

Figure 3. Bacterial wilt resistance index (red bars), yield in infested *Ralstonia solanacearum* field (blue bars) and tuber external appearance (green bars) of potato clones selected for bacterial wilt resistance in comparison with standard cultivars. Brasília, Embrapa Hortaliças, 2018. *Higher scores indicate better performance independent of the index.

the greenhouse after inoculation. On the other hand, if other traits have priority over resistance to BW, such as tuber shape, solid content or skin smoothness, decrease the selection pressure by lowering inoculum concentration and/or reducing the temperature in the greenhouse. When calibrating the selection pressure, one should keep in mind that the most stringent it is, the fewer plantlets will survive, which compromises the likelihood of identifying genotypes that combine resistance to BW and good agronomic and tuber traits and may require very large breeding populations.

GOING BEYOND BW RESISTANCE

We took a set of clones selected using the proposed protocol in different seasons, from 2005 to 2011, to be evaluated in an experiment in complete blocks at random, three replications, and 10-plant plots. The experiment was carried out from June to August 2014, in our naturally infested field (*R. solanacearum*, race 1, biovar 1, phylotype 2; Typic Hapludox) at Embrapa Hortaliças (15°56'S, 48°06'W, altitude 998 m), in Brasília, central Brazil. Cultivars Asterix and Monalisa and clones Cruza 148, MB-03 and MB9846-01 were added as standards. Clones and cultivars were multiplied in Brasília, in an *R. solanacearum*-free soil, in the previous year to ensure tuber size and sprouting uniformity. Soil preparation, crop fertilization, irrigation (sprinkling), and pests and other disease control were carried out following standard procedures for potato in the region. Bacterial wilt incidence was recorded on weekly basis, starting seven days after hilling-up, i.e., 25 days after planting. We developed a bacterial wilt index (BWI), ranging from 0 to 10, in which the highest the score, the more resistant the genotype (fewer wilting plants). Wilting plants are those with irreversible symptoms in at least half of the foliage. The BWI score 10 was first achieved 50 days after planting, when all plants of the susceptible control, cultivar Monalisa, wilted in all replications.

The experiment was carefully harvested manually, 107 days after planting, seven days after chemical haulm killing (herbicide Paraquat). At harvest, two evaluators assessed tuber characteristics, observing mainly tuber shape and uniformity, eye depth and skin smoothness. Tubers were classified according to the 1 to 7 standard scale used by the breeding program at Embrapa Hortaliças. The final score for each genotype was the average between evaluators. For establishing the tuber characteristic index (TCI), we converted the score 7 (based on the commercial cultivar Monalisa) into 10 and adjusted the other values proportionally. Tuber yield in each plot was recorded and transformed into a yield index (YI) by taking the highest yield (average of 9.2 kg 10 plants^{-1}, clone MB 57-32) and making it 10, with all other yields proportionally adjusted.

While plant wilting and yield indexes agreed to some extent, tuber index showed a distinct behavior (Figure 3). Such results are not surprising. In fact, it is expected that resistant clones overyield susceptible clones in the presence of disease pressure. It is also

expected that more resistant clones produce less marketable tubers, given that the original resistance sources are wild species. The cases that should be noticed are those that do not follow this logic. And fortunately, we have a few. Our resistant clone MB9846-01 and clone 52A-13, an MB9846-01 offspring, scored over 7 in all three indexes (Figure 3). But if we leave yield apart for a moment, accepting the quite reasonable license that the disease pressure in our selection field exceeds by far what ordinarily takes place in potato commercial fields in this country, we will have a handful of clones that combine high BW resistance to good tuber indexes, i.e., in addition to the resistance to bacterial wilt, such clones also produce rather commercially appealing tubers. Let us draw the attention to clones 52A-11, 52A-30, 84-02, 84-09, and 90-02, that present resistance levels similar to the standards, clones Cruza 148, MB-03 and MB9846-02, and tuber indexes above 8 (Figure 3). This set of clones is a large step forward in advancing potato germplasm for BW resistance.

ADVANCES, CHALLENGES, AND PERSPECTIVES

Many years of experience have shown that, in general, crosses between BW resistant clones and susceptible genotypes (cultivars or advanced clones) result in a low percentage (around 1%) of clones showing a resistance level similar to the resistant parent. Of these, less than 5% have market-acceptable tuber characteristics. Such low figures were also observed by Tung *et al*. (1990), who reported that resistant clones usually carry undesirable characteristics from their wild progenitors. This points to the need of (1) working with large breeding populations to increase the chances of recovering clones that combine promising BW resistance levels and good tuber quality/appearance and (2) continuous and progressively carrying out of germplasm breeding to improve the overall characteristics of resistant clones without compromising their resistance level. At Embrapa Vegetables, the proposed protocol was adjusted to allow for the screening of around 5,000 seedlings per year, counting on eight part-time workers, four in the laboratory/screenhouse and four in the field in the critical periods.

Some of the most advanced BW-resistant clones selected in Embrapa in the last decades have improved characteristics, such as good yield, virus resistance, reasonable dry matter content, and appealing tuber appearance (smooth skin, shallow eyes, uniform shape), which increases breeders' expectations of obtaining promising combinations of commercial characteristics and BW resistance in the cultivar-oriented program. The chances of obtaining such clones are higher for the potato processing segment than for the fresh potato market. In Brazil, tubers for the fresh market are washed before selling since consumers are very demanding in tuber external appearance: uniform oblong shapes, smooth and bright skin and shallow eyes. All these characteristics have quantitative nature and are complex to combine with BW resistance, especially in a tetraploid background. Molecular tools and the constant advances in gene mapping and editing techniques will certainly speed up the process and increase the likelihoods of obtaining good clones. Even though, combining traits that are mostly quantitative with the wild-origin BW resistance in single tetraploid genotypes will remain a fascinating challenge.

New sources of resistance derived from *S. commersonii* were recently included in our program to diversify the genetic background of resistance. First *S. commersonii* derived clones were evaluated in 2015. Some promising clones were selected, further evaluated and also used in crosses. The promising preliminary results indicate they are valuable assets to our program.

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