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SEEDLING INOCULATION FOR SCREENING POTATOES FOR BACTERIAL WILT RESISTANCE

Lopes[1], Carlos A.; Souza[2], Zilmar S.; Melo[1], Paulo E.

[1]Embrapa Hortaliças, C.Postal 218, CEP 70359-970 Brasília, DF, clopes@cnph.embrapa.br
[2]Epagri, E.E. São Joaquim, C. Postal 81, CEP 88600-000 São Joaquim, SC

Bacterial wilt (BW), caused by *Ralstonia solanacearum*, is one of the most important diseases of potato (*Solanum tuberosum*) in Brazil. Even in cooler regions, if warm spells are associated to high soil moisture, losses may reach up to 50%; besides, BW is the main cause of rejection of fields for seed certification. Disease control has been partially achieved with integrated management measures such as planting season, field selection, crop rotation and certified seeds, but resistant cultivars are not available.

Genotype selection for BW resistance was started at Embrapa Hortaliças in 1980's through a cooperation project with the International Potato Center (CIP), Lima, Peru. The starting material was received from CIP and consisted of populations derived from the wild species *Solanum sparsipilum, S. chacoense, S. microdontum* and *S. phureja*. For a decade, the progress was slow, because selection rate was very low when we combined resistance with good tuber characteristics. We then decided to modify the screen procedure by selecting first for BW resistance in crosses involving the two resistant locally selected clones MB03 and MB9846-01 with susceptible commercial cultivars.

We here describe, in the periods of 2007-2010 and 2008-2011, the efficacy of the seedling inoculation method to select clones resistant to BW in a breeding program and the performance of the two clones in the inheritance of the BW resistance trait in crosses involving the cultivars Baraka and Monalisa.

The crosses were carried out in São Joaquim, SC. The cultivar Baraka was chosen due to its rusticity and good cooking quality, which made it withstand the quick cultivar change observed in the last two decades. The cross with 'Monalisa' was used as a control treatment because of its good acceptance as a commercial variety and its high susceptibility to BW.

True seeds from these crosses (Tables 1 and 2) were sown in trays containing sterile commercial substrate. When the seedlings had two leaves, they were transplanted to 250 mL plastic cups (water cups) and maintained in a screenhouse (18-30°C). Ten to 15 days after transplanting, each seedling was inoculated by pouring, in its base, 10 mL of a suspension (10^7 cfu/mL) of *R. solanacearum*, grown for 48 hours in Kelman's medium at 28°C. Two hundred seedlings of each family in Table 1 were left without inoculation.

Inoculated plants were transferred to a temperature-controlled greenhouse set to maintain above 20°C; low night temperatures favor BW escapes, what imposes difficulties in screening resistant clones. Wilting seedlings, which appeared from seven to 10 days after inoculation, were removed daily. Seedlings that survived for 15 d.a.i. were then transplanted to 3L pots containing sterile commercial substrate and moved back to the cooler screenhouse (18-30°C) for tuber production. Plants which wilted in this environment were also discarded. Tubers of putative resistant clones and the noninoculated ones were harvested approximately 100 d.a.t., let sprout in cold storage for 3-5 months and multiplied once in 3L pots with sterile substrate in a screenhouse.

In order to assess if seedling screening for BW was effective, a set of ten tubers of each clone (which yielded enough tubers) was planted in a field naturally infested with race 1, biovar 1 of R. *solanacearum* at Embrapa Hortaliças, Brasília, DF. Five sets of ten plants of susceptible cultivar Monalisa and the resistant clones MB03 and MB9846-01 were randomly distributed in the experiment for comparison purpose.

Plants of 'Monalisa' started wilting 20 dap, and disease progress was rather uniform and faster after hilling at 30 d.a.p. Field selection was performed 65 d.a.p., when all the plants of the 'Monalisa' plots were

wilt. Clones were selected as resistant when no more than two of the ten plants were wilt, what was the highest rate of wilting in the plots of the resistant clones.

For both families in the 2007-2010 experiment, seedling selection was about 16% (Table 1). However, when the seedling-selected clones were tested in the RS-infested field, the family MB9846-01 x Baraka had higher percentage (55.8%) of field selected clones in comparison with the cross MB03 x Baraka (21.1%). When seedlings were not inoculated, only about 3% of clones of both families were field selected (Table 1), thus indicating that seedling selection considerably increases the chances to select resistant clones under natural conditions.

For the 2008-2011 experiment, seedling selection was also similar, even though lower than in the first experiment for both families involving 'Baraka'. However, seedling survival was lower when MB9846-01 was crossed with the very susceptible cultivar Monalisa, and higher when MB9846-01 was crossed with the other resistant clone, MB03 (Table 2).

With the two experiments we concluded that: 1) high number of potato genotypes can be tested in a temperature-controlled greenhouse for bacterial wilt resistance by inoculating seedlings transplanted to plastic cups (250 mL) with sterile substrate; 2) the seedling selection was effective, since selected clones behaved much better in a field naturally-infested with *R. solanacearum*, as compared with nonselected clones of the same families; 3) field selection is essential, since it allows the elimination of escapes and the observation of plants and tubers; 4) the resistant clones MB03 and MB9846-01 are good genitors for bacterial wilt resistance and can be used in breeding programs that search bacterial wilt resistance and good tuber characteristics; 5) the selection rate for bacterial wilt resistance combined with good yield and tuber appearance is very low. Therefore, a good choice of genitors is essential for combining these characteristics in a single genotype.

Table 1. Clones of two families selected in the seedling stage for bacterial wilt resistance and then exposed to a field naturally infested *Ralstonia solanacearum*. Brasilia, 2007-2010.

	Seedling selection (2007-2008)			**Field selection (2010)**		
Family	**Seedlings total/inoc.**	**Clones survived**	**% Survival.**	**Clones planted**	**Clones selected**	**% selected**
MB9846-01 x Baraka	639/639	106	16.1	52	29	55.8
MB03 x Baraka	431/431	69	16.0	38	8	21.1
MB03 x Baraka	200/0	200	100	62	2	3.2
MB9846-01 x Baraka	200/0	200	100	64	2	3.1

Table 2. Clones of four families selected in the seedling stage for bacterial wilt resistance and then exposed to a field naturally infested *Ralstonia solanacearum*. Brasilia, 2008-2011.

	Seedling selection (2008-2009)			**Field selection (2011)**		
Family	**Seedlings inoculated**	**Clones survived**	**% Survival.**	**Clones planted**	**Clones selected**	**% selected**
MB9846-01 x Baraka	1.800	149	8.3	72	28	38.9
MB03 x Baraka	984	109	11.1	48	16	33.3
MB9846-01 x Monalisa	960	46	4.8	29	5	17.2
MB9846-01 x MB03	96	40	41.7	20	13	65.0
Cupido (open pollin.)	192	2	1.0	2	0	0.0

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[1]Bolsista do CNPq