



ARTICLES

SCAR marker for the selection of *Ry*-duplex potato clones immune to potato virus Y

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ABSTRACT - Vegetative propagation of potato allows the dissemination of viruses that can cause drastic drops in tuber yield. Potato virus Y (PVY) is one of the most important viruses of potato due to the non-persistent mode of transmission through vector insects. Genetic resistance is the recommended control measure since the chemical control of vector insects is not effective. This study aimed to identify PVY-immune clones through potato grafting onto virus-infected tobacco plants, check their genetic constitution for the *Ry* allele through PCR and evaluate the clones' field performance. Sixty-two clones were identified as PVY-immune through grafting. The SCAR marker allowed the identification of two duplex clones for the *Ry* allele (*RyRyryry*), which can be used as parents to originate progenies with about 80% PVY-immunity in crossings with susceptible clones. Besides, the clones were productive and presented high tuber dry matter content.

Key words: *Solanum tuberosum*, breeding, PVY.

INTRODUCTION

The quality of potato seed (*Solanum tuberosum* L.) is a factor of outmost importance for the crop yield, since potato is vegetatively propagated by means of tubers which are readily affected by fungal, bacterial and, mainly, viral diseases. Viruses are frequently related to the degeneration of potato seed in Brazil. Degeneration is caused mainly by the Potato Leaf Roll Virus (PLRV), the Potato Virus Y (PVY) and the Potato Virus X (PVX). According to several reports in literature PVY is one of the most important (Souza Dias et al. 1995, Figueira et al. 1985, Figueira et al. 1995, Slack 1995).

PVY control is difficult owing to its form of dissemination which occurs in a non-persistent mode through insect vectors, mainly the green peach aphid (*Myzus persicae*). The chemical control of insect

vectors has proved little effective, since insects can pick up the virus even in a short period of feeding and retain it for around one hour thereafter. During this period many plants could be infected through insect stings. Reports state that some aphids can retain the virus for up to 24 hours (De Bokx and Huttinga 1981).

Control measures for viral diseases are basically of preventive character. Genetic resistance is the most effective form since it is included as a trait of the cultivar itself. PVY resistance is controlled by the dominant allele *Ry* (Muñoz et al. 1975), which confers immunity (or extreme resistance) in the simplex form (*Ryryryry*) (Swiezynski 1994). One of the breeding strategies aiming at virus resistance is to increase the frequency of the resistance allele allowing the establishment of duplex parents (*RyRyryry*). These can bring forth over 80% of PVY-immune clones, making the breeding process easier (Pinto

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2003). Further selection of triplex (*RyRyRyry*) or even quadriplex clones (*RyRyRyRy*) that produce completely PVY-resistant descents is possible. The identification of clones with resistance alleles is hampered by the need of virus inoculations which can lead to escapes. The development of a SCAR marker by Kasai et al. (2000), localized within the proper *Ry* allele makes this identification a lot easier and eliminates all inoculation stages and symptom readings as well as the ELISA test.

The purpose of the present study was to (i) identify PVY-immune potato clones by means of grafting unto infected tobacco plants; (ii) identify potato clones with duplex genetic constitution for the *Ry* allele by PCR and (iii) evaluate the agronomic performance of PVY-immune clones.

MATERIAL AND METHODS

Two hundred potato clones (designated OAS and JUG) were evaluated. These had been obtained in the potato breeding program of the Universidade Federal de Lavras (UFLA) as PVX and PVY resistant by mechanical inoculation and diagnosed by the DAS-ELISA method (Silva et al. 2000, Gadum et al. 2003). The clones OAS and JUG were obtained through pair crossings performed at UFLA, with virus X and Y-immune clones in the simplex condition, introduced from the Centro Internacional de la Papa (CIP), Peru. The pedigrees of the clones OAS and JUG are available in Silva et al. (2000) and Gadum et al. (2003).

In the present study the PVY immunity of the clones was evaluated by grafting on tobacco plants (*Nicotiana tabacum*, cv. TNN) previously infected by mechanical inoculation. Virus Y-free cultivar Monalisa was used as control (susceptible) and grafted unto non-infected tobacco plants as well.

The inoculum was obtained from ground leaves of virus Y-infected plants (strain PVY^N-Br) from the collection of the Departamento de Fitopatologia (Department of Plant Diseases) of UFLA and established on the indicator plant *Nicotiana tabacum*, cv. TNN. This powder of infected leaves was mixed with phosphate solution buffer 0.01M, pH 7.0, containing sodium sulfite in the same molarity, in the proportion of 1g leaf/5ml solution. The tobacco plants were mechanically inoculated about five to ten days after the transplanting by rubbing the obtained extract onto the leaves previously dusted with Carborundum powder (400 mesh). Then the plants were rinsed under tap water and kept in a greenhouse throughout the experiment.

The tobacco plants were observed until the typical virus symptoms (mosaic) appeared and then grafted. The clones and control were cut at the base and grafted by top grafting onto the infected tobacco plants and tied up with a plastic string. The plants were labeled individually and kept in greenhouse until the final evaluation of the experiment. After the establishment of the graft (15 to 20 days), the clone's reaction to PVY was evaluated by the serological test DAS-ELISA.

To identify the clone's genotypes regarding gene *Ry* true seeds were obtained from the test cross involving approximately 75 OAS and 5 JUG clones with the susceptible cultivar Chiquita (*ryryryry*). The descents of these crossings were sown on trays with Plantimax® substrate and transferred to plastic pots approximately 30 days after planting. About 20 to 30 days after transplanting, approximately 2g young leaves per plant of 30 plants of each test cross were collected for DNA extraction to perform the PCR analysis. We further performed the PCR analysis of two susceptible controls (cvs. Monalisa and Chiquita) and of two clones (XY 7 and XY 9) originated from CIP and classified as immune for presenting allele *Ry* and a band of 321bp. The SCAR primer pair, designated RYSC3 according to Kasai et al. (2000), presented the sequence: 5' ATACACTCA TCTAAATTTGATGG 3' and 5' AGGATATACGGCATCATTTTTTCCA 3'. The genetic constitution of each clone was determined by the proportion of descent plants that did (resistant) or did not present the above mentioned band (susceptible).

For DNA extraction we used the procedure of Rogers and Bendich (1988). The DNA was diluted in TE to a concentration of 10 ng mL⁻¹ which was used in the PCR analyses. The amplification reactions were realized in an Eppendorf Mastercycle Gradient thermocycler. The PCR reaction included an initial denaturation at 94 °C for two minutes, followed by 30 cycles, each one represented by denaturation at 94 °C and annealing at 60 °C for 15 seconds and elongation at 72 °C for 30 seconds, followed by a final extension for two minutes at 72 °C.

The amplified DNA fragments were separated in 2.0% agarose gel in buffer TBE (TRIS, boric acid and EDTA) at a voltage of 100 V, during a variable period of two to three hours. The DNA fragments were stained with ethidium bromide for 30–50 minutes and the excess of dye was removed with distilled water, under agitation during 30–50 minutes. The gel was photographed by a digital camera Kodak DC290 Zoom under ultraviolet light.

For the evaluation of agronomic traits, five distinct

trials were conducted on the experimental area of the Departamento de Biologia of UFLA. All trials were carried out in the winter season (May through August). Two trials were conducted in 2001: The first in 9 x 9 simple lattice design to evaluate the JUG clones and the second in 8 x 8 triple lattice to evaluate the OAS clones, separately. Two trials were conducted in 2002: one in 8 x 8 triple lattice and the other in 12 x 12 simple lattice. In 2003, the design was a 12 x 12 triple lattice. In the trials of 2002 and 2003, the clones OAS and JUG were evaluated jointly.

In all trials, the plots consisted of five plants spaced 0.35 x 0.75m. The cultivars Monalisa (trials of 2001), Asterix, Atlantic, Monalisa and Achat (trials of 2002) and Asterix and Monalisa (trials of 2003) were used as controls.

Fertilization consisted in 3.0 t ha⁻¹ of the formula 4-14-8 (N, P₂O₅ and K₂O). Insecticide Aldicarb was applied at a rate of 10 kg ha⁻¹ along the planting furrow. Between 30 and 40 days after planting nitrogen fertilization was applied in topdressing with 300 kg ha⁻¹ of ammonium sulphate and 160 kg ha⁻¹ of potassium chloride, followed by the earthing up. Phytosanitary treatments were realized during the trials to rule out any competition through weeds or damages done by pests and diseases.

We evaluated tuber yield per plant, percentage of large tubers (transversal diameter > 45mm), the mean weight of large tubers, tuber specific gravity by the weight in air/weight in water method and the score for tuber appearance (1 = very poor to 5 = very good) assessed by two evaluators, taking into consideration tuber shape, skin and flesh color, eye depth, and the occurrence of external disorders. The clones were ranked by the index proposed by Mulamba and Mock (1978), considering all traits under study.

RESULTS AND DISCUSSION

Sixty-two PVY-immune clones were found in the grafting tests. These clones adapt better to the environmental conditions of the southern region of Minas Gerais than the clones originally introduced from CIP (Silva et al. 2000). According to Ross (1986), in a cultivar with the extreme form of resistance (immunity), the virus can not replicate in the plant cells, not even after grafting an immune onto an infected plant. The grafting test is therefore conclusive to establish the condition of immunity, since these clones had been considered PVX and PVY resistant in two consecutive mechanical inoculations performed by Silva et al. (2000). This allows their prompt use in genetic improvement programs as best parents, since besides being more

productive they also present better tuber quality traits such as a higher dry matter content.

The grafting test was effective for the identification of immune clones but the use of the SCAR marker made the identification far easier. The evaluation of the plants of the testcross with the SCAR marker allows the identification of allele *Ry*, so inferences could be drawn about the genetic constitution of the parental clone. The marker can be used in young plants (after few days of germination or emergency), speeding up the selection process of immune clones. Besides, no inoculum preparation is required, no planting of tobacco plants or even grafting. The detection of the band that identifies the immune clone is easy (Figures 1 to 3), leaving no doubt as to the presence of the allele *Ry* that confers immunity. It was however observed that some progenies presented a lower number of plants with the respective band. For example, simplex clones in testcrosses with cultivar Chiquita should present approximately 50% immune plants (with the band) and 50% susceptible plants (without the band) (Figure 3), but presented significant χ^2 tests for the segregation 1:1, due to the excess of plants without the band (Table 1). These results can be interpreted as possible flaws in the PCR reaction which hindered the development of the band, resulting in a lower number of plants in this class. The majority of the clones evaluated for the presence or absence of bands presented simplex genetic constitution (*Ryryryry*), similar to the original clones. However, two clones (OAS 3-30 and JUG 2-20) with duplex genotypes (*RyRyryry*) (Table 1 and Figures 1 and 2) were identified that can be used to produce progenies with approximately 80% of PVY-immune descents. It is worth mentioning that the identification of duplex clones and their intercrossing would allow the establishment of triplex (*RyRyRyry*) and quadriplex clones (*RyRyRyRy*). This result is encouraging, from the point of view of breeding, since from now on it will be possible to obtain large populations of immune clones that only have to be subjected to evaluations for agronomic traits. The clones whose testcrosses present a high proportion of immune plants (with bands) are certainly duplex, since in no case the presence of bands was observed in susceptible clones. Such high proportions of plants with the band would therefore not be found if the genetic constitution were different from the duplex.

One should bear in mind that the genetic constitutions of clones regarding locus *Ry* can only be efficiently discovered by the testcross with susceptible material (*ryryryry*). In this setting, the SCAR marker once more proved superior over the grafting test, since around 30 descents of each clone are necessary to attain a

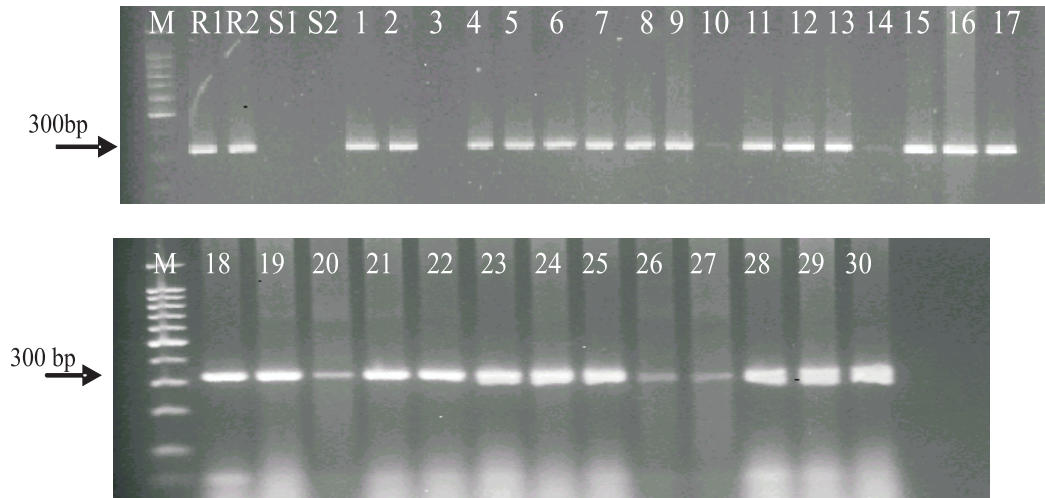


Figure 1. Presence or absence of bands identified by the primer pair RYSC3 in 30 potato plants (columns 1 to 30) from testcross progenies of clone OAS 3-30 and of the resistant R1 (XY9) and R2 (XY7) and susceptible controls S_1 (Chiquita) and S_2 (Monalisa). M is the band size marker. Note the proportion of 24 plants with presence of the band (immune) versus six plants without the band (susceptible)

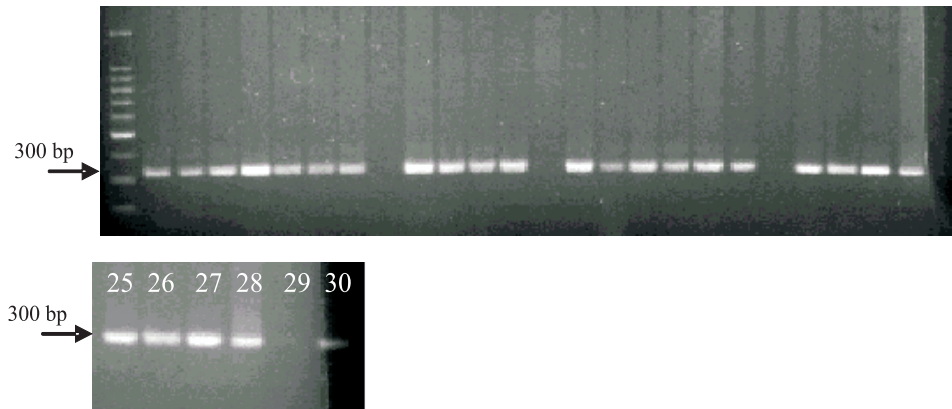


Figure 2. Presence or absence of bands identified by the primer pair RYSC3 in 30 potato plants (columns 1 to 30) from testcross progenies of clone JUG 2-20 and of the resistant R1 (XY9) and R2 (XY7) and susceptible controls S_1 (Chiquita) and S_2 (Monalisa). M is the band size marker. Note the proportion of 26 plants with presence of the band (immunes) versus four plants without the band (susceptible)

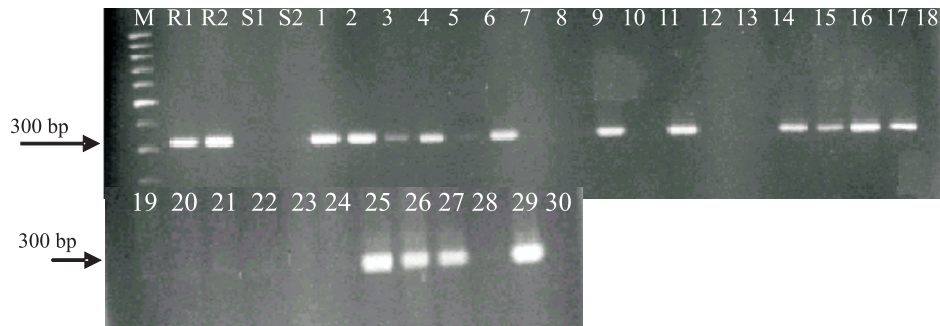


Figure 3. Presence or absence of bands identified by the primer pair RYSC3 in 30 potato plants (1 to 30) from testcross progenies of clone OAS 2-65 and of the resistant R1 (XY9) and R2 (XY7) and susceptible controls S_1 (Chiquita) and S_2 (Monalisa). M is the band size marker. Note the proportion of 16 plants with presence of the band (immune) versus 14 plants without the band (susceptible)

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Table 1. Number of evaluated plants and presence or absence of the 321 bp band of the SCAR marker and values of χ^2 for the simplex (segregation 1:1) or duplex genetic constitution (segregation 5:1) assuming chromosomal segregation in potato clones obtained by the testcrosses (clones x cv. Chiquita)

Clones	Number of plants	Presence of band		Genetic constitution	
		Yes	No	Simplex 1:1	Duplex 5:1
OAS 2-111	30	2	28	22.53	126.96
OAS 2-65	30	16	14	0.13 ns ¹	19.44
OAS 2-88	30	0	30	30.00	150.00
OAS 1-21	30	13	17	0.53 ns	34.56
OAS 1-66	30	15	15	0.00 ns	24.00
OAS 3-30	30	24	6	10.80	0.24 ns
OAS 3-48	30	5	25	13.33	96.00
JUG 2-20	30	26	4	16.13	0.24 ns
OAS 3-34	20	6	14	3.20 ns	40.96
OAS 1-91	20	3	17	9.80	67.24
OAS 3-27	20	5	15	5.00	49.00
OAS 2-22	20	5	15	5.00	49.00
OAS 3-45	20	3	17	9.80	67.24
OAS 2-74	20	4	16	7.20	57.76
OAS 1-28	20	2	18	12.80	77.44
OAS 4-40	20	2	18	12.80	77.44
OAS 1-56	20	4	16	7.20	57.76
OAS 6-75	20	6	14	3.20 ns	40.96
OAS 1-120	20	4	16	7.20	57.76
OAS 1-41	20	3	17	9.80	67.24
OAS 6-34	20	3	17	9.80	67.24
OAS 1-102	20	3	17	9.80	67.24

¹ not significant by the χ^2 test

Table 2. Summary of the joint analysis of variance for potato evaluated in the winter season of 2001, 2002 and 2003

Sources of variation	df	MS					
		Tuber yield (g plant ⁻¹)	% of large tubers	Mean weight of large tubers (g)	Tuber specific gravity (x10 ⁻⁴)	Score of tuber appearance	Mulamba and Mock index
Year	2	7432.464	379.977	322.269	34.731**	1.779*	5152.389
Clones	40	73844.505**	973.746**	1514.988*	1.743**	1.26**	4694.955**
Year x Clones	80	62259.864**	500.352**	1097.943	0.981	0.681*	2781.24 *
Pooled Error	240	36385.751	294.458	910.152	0.986	0.478	2062.389
CV(%)		26.62	19.16	15.84	0.53	21.30	20.36
h ² _a		50.73	69.76	39.92	43.43	62.15	56.07
General Mean		541.2	67.3	120.7	1.0822	2.2	149.5

** and *: significant at 1% and 5% probability, respectively, by the F test

Table 3. Means of the 40 best experimental PVY-immune potato clones and control Monalisa for some traits evaluated in five trials in the winter season of 2001, 2002 and 2003

Clones	Tuber yield (g plant ⁻¹)	% of large tubers	Mean weight of large tubers (g)	Tuber specific gravity	Tuber appearance	Mulamba and Mock index
OAS 2-74	697 a ^{1/}	83 a	152 a	1.0899 a	3 a	99 a
OAS 6-19	703 a	71 a	113 b	1.0940 a	2 b	100 a
OAS 4-67	644 a	80 a	128 a	1.0880 a	2 b	107 a
OAS 2-34	609 a	84 a	135 a	1.0838 a	2 b	108 a
OAS 2-42	607 a	71 a	139 a	1.0865 a	2 b	113 a
OAS 2-103	554 a	80 a	135 a	1.0857 a	3 a	117 a
OAS 6-34	676 a	81 a	129 a	1.0797 b	2 b	125 a
OAS 2-35	570 a	80 a	121 a	1.0833 a	2 b	130 a
OAS 2-88	594 a	66 a	114 b	1.0870 a	2 b	133 a
JUG 1-03	561 a	68 a	130 a	1.0841 a	2 b	137 a
JUG 1-09	579 a	73 a	131 a	1.0801 b	3 a	138 a
OAS 3-45	463 b	81 a	131 a	1.0810 b	2 b	144 b
OAS 7-11	470 b	79 a	131 a	1.0804 b	2 b	144 b
OAS 3-54	665 a	77 a	124 a	1.0782 b	2 b	147 b
OAS 1-02	552 a	61 b	117 b	1.0875 a	3 a	148 b
OAS 1-28	588 a	68 a	122 a	1.0792 b	3 a	151 b
OAS 1-64	554 a	77 a	112 b	1.0803 b	2 b	151 b
OAS 3-37	608 a	72 a	122 a	1.0827 a	3 a	151 b
OAS 2-83	450 b	61 b	137 a	1.0833 a	2 b	152 b
JUG 1-05	651 a	77 a	117 b	1.0761 b	2 b	153 b
OAS 1-21	626 a	66 a	112 b	1.0803 b	2 b	154 b
OAS 2-22	531 b	56 b	127 a	1.0820 b	2 b	154 b
OAS 2-108	572 a	64 b	125 a	1.0791 b	2 b	157 b
OAS 2-116	458 b	66 a	117 b	1.0831 a	2 b	157 b
OAS 3-30	593 a	76 a	134 a	1.0710 b	2 b	159 b
JUG 1-14	516 b	69 a	125 a	1.0799 b	2 b	160 b
OAS 2-123	383 b	52 b	104 b	1.0888 a	2 b	161 b
OAS 7-10	591 a	69 a	112 b	1.0786 b	2 b	162 b
OAS 1-66	520 b	66 a	106 b	1.0804 b	2 b	163 b
OAS 1-61	453 b	60 b	113 b	1.0840 a	2 b	164 b
OAS 2-30	522 b	55 b	116 b	1.0808 b	2 b	165 b
OAS 2-89	519 b	44 b	98 b	1.0876 a	2 b	169 b
OAS 4-24	395 b	63 b	120 b	1.0836 a	2 b	169 b
OAS 8-15	443 b	68 a	98 b	1.0798 b	2 b	170 b
OAS 1-44	402 b	55 b	95 b	1.0856 a	2 b	171 b
OAS 6-67	511 b	56 b	125 a	1.0784 b	2 b	172 b
OAS 7-40	406 b	52 b	118 b	1.0834 a	2 b	173 b
OAS 2-111	504 b	61 b	118 b	1.0764 b	2 b	179 b
OAS 1-15	441 b	45 b	96 b	1.0850 a	2 b	182 b
JUG 1-15	360 b	59 b	107 b	1.0775 b	3 a	198 b
Monalisa	648 a	67 a	143 a	1.0765 b	3 a	143 b

¹ Means followed by the same letter in a column did not differ by the test of Scott and Knott (P < 0.05)

confidence level of over 95% probability in relation to the genetic constitution. If one uses the grafting test, this evaluation is more troublesome, slower and more costly.

Significant differences were detected for all agronomic traits (Table 2), demonstrating the existence of genetic variability among the clones. The estimates of heritability varied from 0.40 for the mean weight of large tubers to 0.70 for the percentage of large tubers. The selection based on the index of Mulamba and Mock (1978) that allows gains for several traits simultaneously is promising, considering that the heritability was 0.56.

The means of the best clones for the agronomic traits are presented in Table 3. With exception of tuber specific gravity, the general means can be considered relatively low; this fact can partly be attributed to the high infestation

rate by the potato leafroll virus (PLRY). Still, the agronomic performance of the immune clones was similar to that of the control cultivar Monalisa for tuber yield, percentage of large tubers and mean weight of large tubers. On the other hand, many clones presented a higher tuber specific gravity (high dry matter content) than the control, underscoring their superior quality for frying. It is important to mention that cv. Monalisa does not have adequate qualities for frying so that the qualities of several clones presented could warrant their use in the industry, where a tuber specific gravity of over 1.080 is required (Gould 1988).

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Marcador SCAR para a seleção de clones duplex *Ry* de batata imunes ao vírus Y da batata

RESUMO - A propagação vegetativa da batata permite a disseminação de doenças, principalmente viróticas, que ocasionam queda drástica de produtividade. Dentre as principais viroses se destaca o vírus Y da batata (PVY), transmitido por afídeos de forma não persistente. Recomenda-se o controle genético dessa virose uma vez que o controle químico dos afídeos tem sido pouco efetivo. Os objetivos deste trabalho foram identificar clones de batata imunes ao PVY por meio da enxertia sobre plantas de fumo infectadas, determinar a sua constituição genética com relação ao alelo *Ry* por meio de PCR e avaliar agronomicamente os clones imunes ao PVY. Foram encontrados 62 clones imunes ao PVY. O marcador SCAR permitiu a identificação de dois clones duplex (*RyRyryry*) e que poderão ser empregados para gerar progênies com aproximadamente 80% de imunidade, quando cruzados com clones suscetíveis. Os clones imunes se destacaram pela produtividade e pelo alto teor de matéria seca nos tubérculos.

Palavras-chave: *Solanum tuberosum*, melhoramento genético, PVY.

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