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Yield Formation Of PVY-Resistant and Susceptible Potato Breeding Lines.

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ABSTRACT

We studied the resistance to potato virus *Y* (PV*Y*) in a potato hybrid population, and its effect on the yield formation under contrasting meteorological conditions. The virus resistance analysis was conducted after growing the plants under natural virus infection and after PV*Y* inoculation. The dominant Ry_{adg} gene allele in the potato breeding lines genotypes was revealed by detection of the PCR-based molecular marker RYSC3. Virus resistant lines were selected more often in the potato breeding program than susceptible forms due to higher productivity and larger number of tubers. PV*Y*-resistant samples demonstrated a higher yield than susceptible forms. Significant differences ($p < 0.05$) were detected by univariate ANOVA in three of the four years studied. The virus resistance effect value ranged from 4 to 22%. Resistant form yield was more stable than that of susceptible one, which was evident from the corresponding coefficients of variation. Resistant samples also had a greater number of tubers per plant. At the same time, univariate ANOVA revealed significant differences ($p < 0.05$) only in one of the four studied years. The virus resistance effect ranged from 2 to 22%.

Keywords: potato, breeding lines, yield, resistance, potato virus *Y*, ANOVA.

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INTRODUCTION

Potato is one of the major crops grown around the world [1]. Due to the vegetative propagation, viral diseases are particularly harmful for potato crop. Potato virus *Y* (PVY) is the most harmful among the potato viral diseases [2]. Yield losses can reach up to 85% when the plants are grown from PVY infected tubers [3]. PVY belongs to the non-persistent viruses group [4], which makes it difficult to control virus spread by insecticides (killing vectors) and seed certification [5].

Breeding and use of virus-resistant cultivars is the most effective way to control the potato virus disease. In addition, growing resistant cultivars is of great importance for the environment protection, since it reduces pesticides application [6].

Genetic resources analysis and use are the basis of resistance breeding. The virus resistance sources are wild and cultivated potato species. Their resistance genes are introduced in many cultivars and interspecific hybrids. The most frequent introduction was that from the Solanum tuberosum subsp. andigena and S. stoloniferum. The main value of these genotypes is the extreme resistance genes providing immunity to all known pathogen strains and having a dominant and monogenic inheritance [7].

The Middle Volga region climatic conditions are favorable for potato crop cultivation. There are large areas in this region occupied by seed and ware potato. PVY is the main biotic factor causing yield losses and tuber quality reduction [8]. In this regard, the study of plant resources that are resistant to this pathogen is highly important.

The aim of this study was to reveal the effect of PVY resistance on yield of resistant and susceptible samples of potato hybrid population, planted in Middle Volga Region conditions.

MATERIALS AND METHODS

We used a hybrid population obtained from the cross between hybrids 2-1-2 and 50-03. Both parents were characterized by medium late maturing type. The parent of 2-1-2 was obtained by crossing hybrids 575.010 × 128-6, performed in FGBNU "All-Russian Research Institute of Potato Farming named after A.G. Lorch", and previously it was described by us as PVY resistant [9]. Hybrid 50-03 was obtained by V.A. Kolobaev (FGBNU "All-Russian Institute of Plant Protection") as a result of interbreeding with S. polytrichon, S. verrucosum and S. simplicifolium [10] and is not resistant to PVY.

Studied samples were planted in field conditions and under natural virus infection. The crop was not treated with insecticides to encourage infestation with virus vectors (aphids) and subsequent natural virus transmission during the cropping period. The experiments were conducted at the Tatar Agriculture Research Institute experimental plots (Bolshye Kabany village, Laishevsky district, Tatarstan, Russia) using standard agricultural techniques [11]. Soil type of the experimental plot: gray forest, heavy loamy; humus content: 3,0–3,5%, alkali-hydrolysable nitrogen content: 100–122,5 mg/kg, P_2O_5 content: 290–295 mg/kg, K_2O content: 80–100 mg/kg, amount of absorbed bases: 20–21 mg–eq./100 g of soil. Potato planting time: third decade of May, harvesting time: first decade of September. Leaves were removed 10–14 days before harvesting.

The characteristic of the climate conditions during the growing period was determined using Selyaninov hydrothermal coefficient (HTC) as follows: $HTC = (R/T) \times 10$, where R is the rainfall for V–VIII months in millimeters, T is the sum of positive monthly temperatures for V–VIII months. Available soil moisture for plants (ASM) is presented in millimeters.

The region climate type is characterized as moderate continental at middle latitudes. Weather conditions during the described years were contrasting and had significant deviations from the average long-term rates. The vegetation period in the year 2012 (Fig. 1a) was characterized by sufficient available soil moisture during germination and flowering stages, which contributed to root development and tuber formation. In 2013 (Fig.1b), germination and plant growth period was accompanied by sufficient soil moisture. However, at the time of plants flowering, rains were uneven and insufficient, developing soil drought. This had a negative impact on the yield. The vegetation period in the year 2014 (Fig.1c) was the most arid. Rainfall was extremely uneven. Soil drought was observed throughout the growing season, affecting negatively plants growth and development. The year 2015 (Fig.1d) was the most favorable for plant growth and development and for yield formation. The rainfall amount and regularity met sufficient plant requirements of atmospheric and soil moisture in all phases of the growing season.

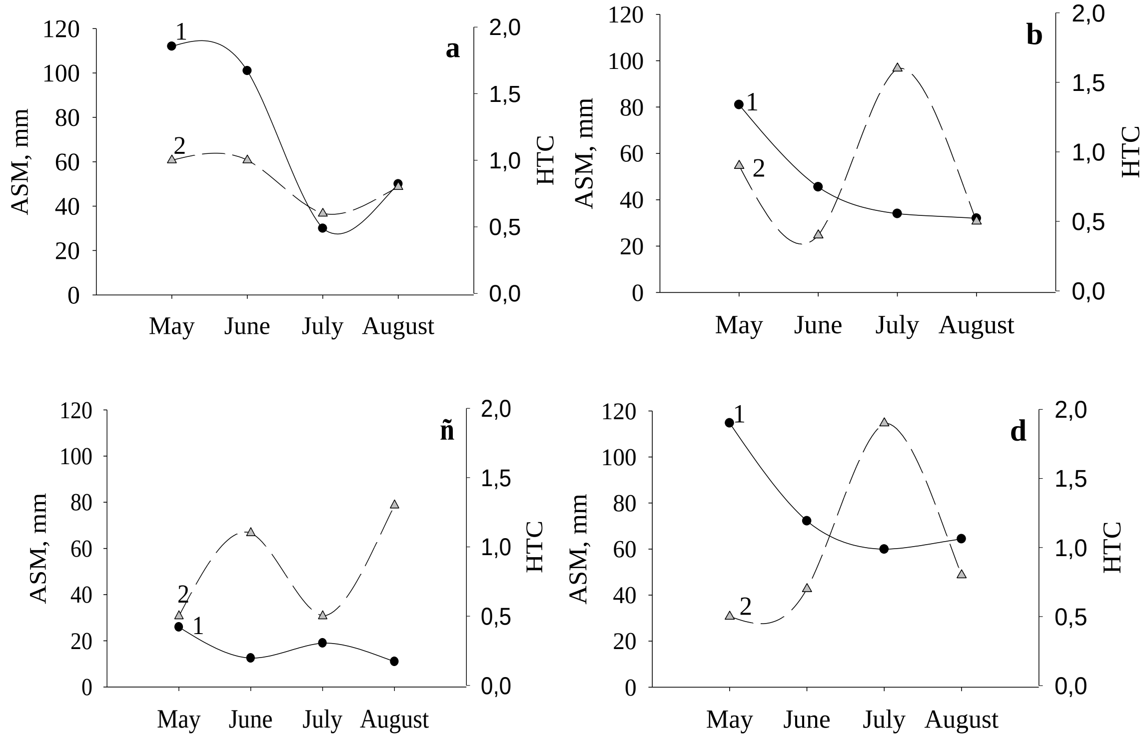

Figure 1. Weather conditions in growing seasons: a – 2012, b – 2013, c – 2014, d – 2015; 1 – resistant samples, 2 – susceptible samples; ASM – available soil moisture for plants, HTC – Selyaninov hydrothermal coefficient.

Samples evaluation for PVY resistance was carried out as follows. During cultivation under natural virus infection, the detection of PVY in the leaves of studied samples was carried out with enzyme immunoassay (ELISA), as described previously in [12]. For this purpose, we used a kit for detection of monoclonal antibodies against PVY (Neogen Europe, UK), in accordance with manufacturer's instructions.

Plants of the breeding lines that were PVY uninfected after four cultivation years were subjected to mechanical inoculation with inoculum containing a mixture of YBK^{O}, YBK^{N}, YBK^{NTN} and $YBK^{N\text{-}Wi}$ viral strains that had been identified previously [13]. After that, tuber sprouts were analyzed with the ELISA test. The uninfected samples were defined as PVY resistant.

The identification the molecular marker RYSC3 that is linked to the dominant Ry_{adg} gene allele was carried out with polymerase chain reaction (PCR). Genomic DNA was isolated using the "DNA-sorb-C" kit (InterLabService, Russia) in accordance with manufacturer's instructions. Amplification was carried out in the «Mastercycler gradient» (Eppendorf, Germany) as described in [14].

Potato yield structure assessment was performed as described in [15]. Statistical analysis was performed using STATISTICA software package. The analysis of variance (ANOVA) was performed according to [16], the construction of variational series was carried out according to [17]. The coefficient of association was calculated as described in [18].

RESULTS AND DISCUSSION

As a result of the cross between PVY resistant interspecific hybrid 2-1-2 and the susceptible hybrid 50-03, 338 samples were obtained. After the first growing season, the preliminary selection was done. Breeding lines with good tuber shape, resistance to late blight, Fusarium spp., common scab and silver scurf were selected. A population containing 100 samples was subjected to detailed examination.

A testing of breeding lines after second season growing under natural virus infection conditions revealed 51 PVY infected samples (50.5%). All PVY infected breeding lines showed specific symptoms: from severe to mild mosaic, leaf distortion. No PVY tolerant breeding lines were found. PVY infected breeding lines showing specific symptoms were defined as virus susceptible.

Examination of 49 uninfected breeding lines was continued during the third and fourth seasons of growing under natural conditions. All of above mentioned samples remained symptomless and PVY-free by the end of the 4th year. Further uninfected breeding lines were subjected to mechanical inoculation. Tuber sprouts of 49 mechanically inoculated breeding lines were tested, and PVY was not found. Breeding lines of this group were defined as PVY resistant.

The phenotype ratio was 1:1 (X^2=1.96; p<0.05) indicating the monogenic dominant inheritance of the resistance in the tested hybrid population. The molecular marker RYSC3 was detected in the PVY resistant parent 2-1-2 genotype and was not found in the susceptible hybrid 50-03. The molecular marker RYSC3 was detected in 47 resistant breeding lines of the progeny population and was not found in any susceptible sample. Two resistant breeding lines lacked the RYSC3 fragment. This was probably caused by an incomplete linkage between the marker and the resistance gene. The Phi-square association coefficient (0.96) between the resistance and the molecular marker inheritance in the hybrid population was high; this justifies the use of the molecular marker RYSC3 for preliminary screening of hybrid populations obtained with hybrid 2-1-2 as a PVY resistance donor. Thereby, the resistant samples selection on the seedling stage became possible.

Resistant and susceptible breeding lines were studied in order to determine the effect of Ry_{adg} gene on the potato yield. The potato tuber yield of examined samples was tested during four growing seasons after growing under natural infection conditions. During the all studied years, the tuber yield of the resistant samples group was higher than that of the susceptible group. That was evident from differences in arithmetic means, as well as in the variation range (Fig.2). The positive effect of the PVY resistance was first revealed on the second cultivation year, when the hybrid population differentiation on resistant and susceptible groups occurred. The greatest difference between the examined groups was observed in 2015, in the most favorable conditions for growth and development of potato plants. ANOVA confirmed the existence of statistically significant differences (p <0.05) between groups in all studied periods, except for 2014. The 2014 growing season was characterized by extremely stressful weather conditions. Therefore, in stressful environmental conditions, resistance to viruses does not have a critical effect on the potato tuber yield.

Yield stability is an important characteristic of the valuable potato genotypes. In contrasting weather conditions during the studied years, the variation coefficient of individual samples was in the range 18% ... 40%. This is an evidence of the presence of both stable and plastic genotypes in the hybrid population. The variation rate of PVY resistant samples yield was less than that of susceptible samples (Fig. 2), which indicates a greater yield stability for resistant breeding lines. The highest variation coefficients for both resistant and susceptible samples were observed in the 2014 growing season, during the most stressful environmental conditions (Fig. 2c).

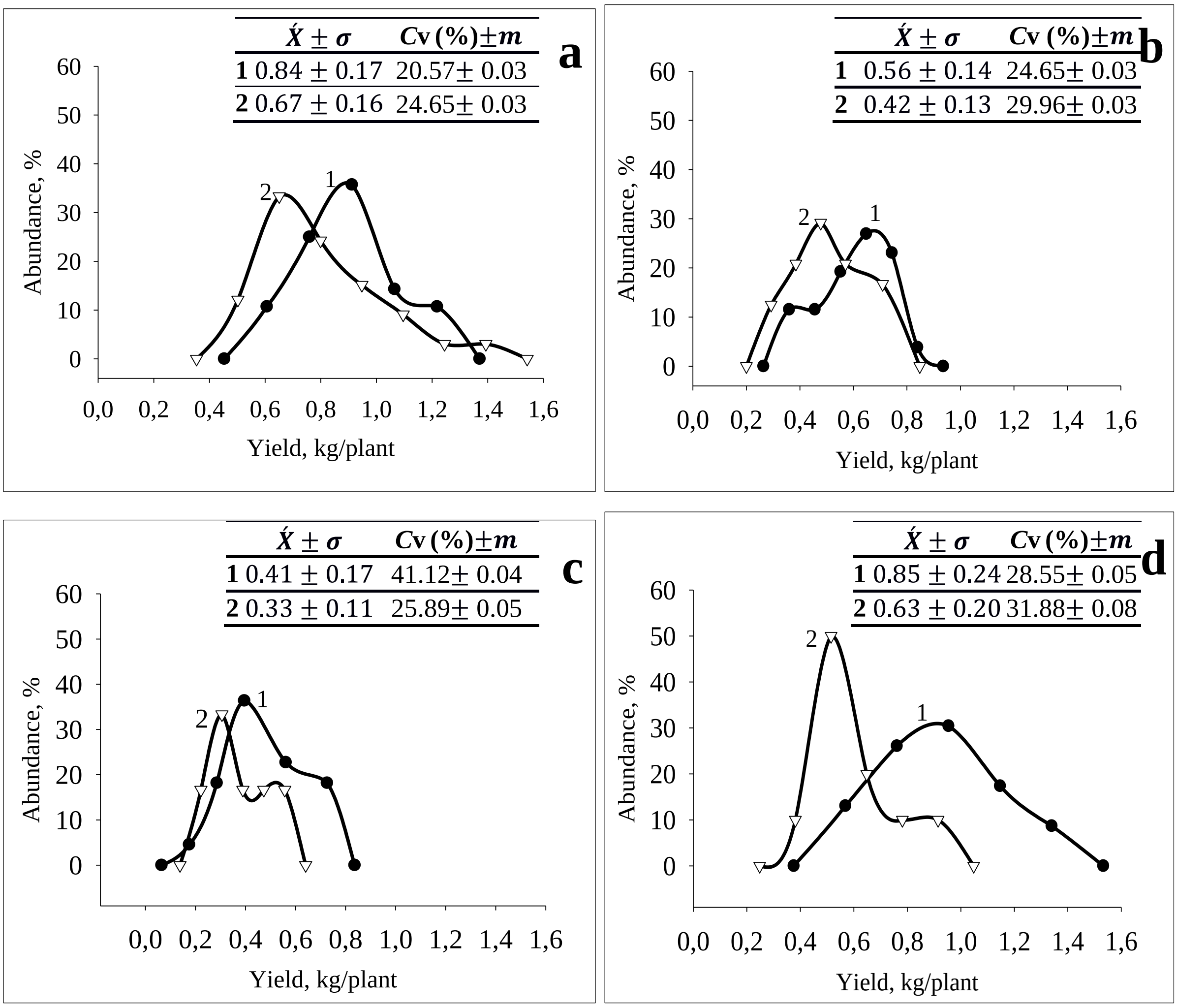

Figure 2. Yield formation variation series of the potato hybrid population: a – 2012, b – 2013, c – 2014, d – 2015; 1 – resistant samples, 2 – susceptible samples; $\acute{X} \pm \sigma$ – mean value and standard deviation, $Cv \pm m$– coefficient of variation and the corresponding error.

Univariate ANOVA was used to estimate the PVY resistance effect on the potato yield. The effect value was between 4 and 22% in the years of study (table 1). The largest effect was observed in the 2013 growing season, the minimal effect – in 2014.

Table 1. Univariate ANOVA of potato hybrid population yield

Year	Source of variation	mS	F	p	V, %
2012	Factor «PVY resistance»	0.28	7.64	0.008	13
	Error	0.04			
2013	Factor «PVY resistance»	0.21	11.76	0.001	22
	Error	0.02			
2014	Factor «PVY resistance»	0.03	1.10	0.304	4
	Error	0.03			
2015	Factor «PVY resistance»	0.24	4.27	0.049	14
	Error	0.06			

mS – mean square, F – Fisher's exact test, p – significance level, V – effect size.

Factorial ANOVA showed that the main effect on the yield formation had the weather conditions with a value 41%. Interaction between factors "Weather" and "PVY Resistance" was not found. The virus resistance effect value made up to 11%. The error expressed the individual genotype features of the breeding line and its interaction with the environment, and other factors uncontrolled in the experiment.

An important component of the yield is the number of tubers per plant. During the study period, PVY resistant samples formed a number of tubers greater than susceptible ones did (Table. 2). However, according to one-way ANOVA, significant differences ($p < 0.05$) between the compared hybrids groups were revealed only in 2013. The number of tubers was dependent mainly on the particular hybrid genotype and the weather conditions. During all the years studied, with the exception of the 2014 growing season, the trait variation coefficient was lower in the PVY resistant samples group compared to susceptible ones.

Table 2. Descriptive statistics of the number of tubers in potato hybrid population, pcs./plant

Year	Samples group	$\acute{X} \pm \sigma$	min…max	Cv (%)$\pm m$
2012	PVY resistant	11.34 ±3.48	5.80 … 21.00	30.69±0.67
	PVY susceptible	10.39 ±2.5	5.00 … 14.60	24.10±0.51
2013	PVY resistant	9.79 ±1.92	6.20 … 12.93	19.59±0.38
	PVY susceptible	7.6 ±1.22	5.71 … 10.27	16.09±0.30
2014	PVY resistant	7.97 ±2.06	3.80 … 12.21	25.89±0.44
	PVY susceptible	6.59 ±1.86	4.78 … 9.35	28.24±0.76
2015	PVY resistant	10.38 ±2.6	4.56 … 15.00	25.04±0.54
	PVY susceptible	9.05 ±2.28	6.20 … 13.00	25.17±0.93

$\acute{X} \pm \sigma$ – mean and standard deviation, min…max – variation range, $Cv \pm m$ – coefficient of variation and the corresponding error.

The overall PVY resistance effect on the number of tubers was calculated using univariate ANOVA. The effect values varied in the range 2 ... 22% (Table. 3). The greatest PVY resistance effect was identified in the year 2013.

Table 3. Univariate ANOVA of the number of tubers in potato hybrid population

Year	Source of variation	mS	F	p	V, %
2012	Factor «PVY resistance»	11.21	0.79	0.378	0.02
	Error	14.18			
2013	Factor «PVY resistance»	38.82	11.65	0.001	0.22
	Error	3.33			
2014	Factor «PVY resistance»	8.97	2.19	0.151	0.08
	Error	4.10			
2015	Factor «PVY resistance»	8.51	1.32	0.261	0.05
	Error	6.47			

mS – mean square, F – Fisher's exact test, p – significance level, V – effect size.

PVY resistant samples were selected more often than susceptible ones during the breeding cycles due to significantly higher tuber yield and larger number of tubers. In the first breeding cycle, 51 resistant and 49 susceptible breeding lines were selected. In the fourth breeding cycle, 22 resistant and 7 susceptible samples were selected. As a result, 6 breeding lines were selected in the fifth breeding cycle. All selected samples were resistant to PVY.

CONCLUSIONS

PVY resistant potato breeding lines had a higher yield and a larger number of tubers, compared to susceptible samples, during long-term vegetative reproduction under natural virus infection conditions. PVY resistant potato breeding lines also showed a more stable yield under contrasting weather conditions.

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