Rice breeding for blast resistance in Russia

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I – Blast as a harmful rice disease

Among rice diseases blast ifs the most noxious. It is caused by imperfect fungus *Pyricularia oryzae Cav.* Rice is susceptible to blast at all vegetation periods. Yield losses according to different assessments reach from 3% to 25% during normal years and during years with blast epiphytoty - up to 60%, and even 100% (Petrova, 1970).

Rice blast has shown a definite cycle of development in Krasnodar area over the years since rice introduction. The first outbreak of the disease was registered in 1937-1938. It was repeated in 1948-1949. In 1960 only in Slavyansk region over 3,000 ha of rice were completely damaged. In 1972-1973 another serious outbreak of the disease was observed in all rice growing areas of Krasnodar area. Then followed another epiphytoty in 1984-1985(Table 1).

High susceptibility of rice varieties Krasnodarsky 424 and Kuban 3 to blast resulted in quick reduction of sown areas. They were substituted by new short stem and less blast susceptible varieties: Spalchik, Start, Kulon, Liman. With timely fungicide treatments these varieties are not so seriously affected by blast. Nevertheless the 10-12 year cycle of *P. oryzae* allows forecasting of another epiphytoty, this time damaging new varieties at present.

In 1996 optimal weather conditions for blast disease were observed in August when main rice stands reached milky-waxy and waxy stages. Diseases caused harm to late maturing varieties. Climatic conditions of 1997 were extremely favourable for accumulation of infection and in July blast epiphytoty was registered in a number of farms. Majority of farms had no chemicals for blast control and by the end of vegetation period about 30% of rice fields were affected by blast. Some fields were not even harvested.

Favourable conditions for blast epiphytoty development were also in 1998, 1999 and 2000. Rice plant residues provided conditions for fungus wintering. Farms had no fungicides to control the disease. Weather conditions were favourable for blast development till tillering stage and the disease was registered in many farms. But dry and hot weather in the following months prevented spreading of the disease.

Calculations show that if the degree of blast infestation at milky stage reaches 55%, possible yield losses can be 72% (Bulavchenko et al., 1987). Situation is aggravated by the fact that many varieties grown in Russia are susceptible to blast to this or that degree.

Frequent blast epiphytoty in all rice growing areas of the world are explained by new agronomic approaches and mainly application of high mineral fertiliser rates, in particular nitrogen. High nitrogen rates stimulated blast development.

Chemicals are widely used to control blast. Aerial application is a forced method since there are no specialised ground equipment for rice treatment. It leads to growth of production costs and ecological damage. In Krasnodar territory over 40% of rice fields are situated close to built-up areas and water reservoirs and aerial application there is forbidden. List of allowed pesticides is also limited. Practice has shown that chemical crop protection is not always economically justifiable and cost effective. Permanent application of fungicides may result in mutant, fungicide resistant forms of *P. oryzae*. Thus introduction of high yielding and immune to pathogen rice varieties should be the main methods of blast control. This research started in 1983 was based on previous wide-scale study of *P. oryzae* by Lukyanchikov et al. (1972) from All-Russian Rice Research Institute (VNIIR). Nineteen races of *P. oryzae* represented by known races IA, IB, IC, ID, IE, IF, IG, IH, IJ were discovered in Krasnodar area during the period of 1969-1979. Scientists of the Research Institute of Phytopathology also studied and found 25 races *P. oryzae* in all rice growing areas of Russia: 20 of them were registered in the Far East (Primorye), 4 - in Krasnodar territory, 2 - in Astrakhan area, 1 - in Dagestan and Karakalpakia (Konovalova et al., 1978).

Successful rice breeding of blast resistant varieties is based on availability of initial material with wide range and high level of resistance. Donor selection is done using gene identification controlling of this trait.

Efficiency of resistance genes in different areas of the world varies. Data on resistance genes in rice varieties and virulence genes in pathogen population allow rice breeders to release and use varieties with effective resistance genes for definite area.

Observations in infection nursery confirm the scientific data that rice resistance to blast is a dominant trait. Resistance trait splitting in hybrid population F_2 was different (Table 2).

Resistance to *P. oryzae* in Miratelli 5A, Yerua P.A., VNIIR 87 and Dular is controlled by one dominant gene, it is confirmed by the ratio 3R : 1(S). In hybrid populations received after crossing VNIIR 7630 and Han Nam splitting was 9R : 7(S). This is evidence of two dominant genes of resistance to blast in these varieties. These data allowed reliable assessing of hybrid material and breeding required genotypes for breeding nursery.

Thus the most reliable factor controlling rice blast disease is timely substitution of old varieties with new ones having effective genes of resistance to pathogen.

II – Methods of assessing breeding material resistance to *P. oryzae*

For successful breeding of disease resistant varieties it is necessary to have good infection bases. Their availability allows discovering and using disease resistant and immune plant forms. P. P. Lukyanenko (1990) emphasised that breeding of pathogen resistant plants can not be assessed under weak infection levels. Plant forms resistant under weak infestation can be 100% damaged in the years of epiphytoties.

Infection nursery was created in order to evaluate breeding material resistant to blast. It increased assessment reliability of collection samples, varieties and hybrids under conditions of severe infection.

Nursery research, field preparation and rice growing are carried out according to accepted methods. Rice samples are sown in rows manually, with standard variety Spalchik (before 1990 - Krasnodarsky 424) after every 10 samples.

Plants are inoculated twice - at tillering and flowering stages. It is done in the evening after appearance of dew and with no wind. The most successful inoculation is observed when dew stays at least 8 - 10 hours with air temperature 18-25^{o}C. For inoculation water suspension of fungus spores or dry biological material of widely spread pathogen types and their mixtures are used.

Plant resistance is determined according to degrees of development of leaf, node or panicle form of disease. Leaf form is determined at tillering stage 10 and 20 days after inoculation. Type of response, intensity of infestation and spreading of diseases are assessed. 10-grade IRRI scale is used based on evaluation of infested surface. Grading is according to domineering type of response. Evaluation allows selecting samples with high degree of resistance or with weak infection of leaves (grade 1-6) and panicle (grade 3-6). At full maturity non-infected plants are screening taking into account their morphological and biological attributes. Seeds of selected plants are separated into two groups: one for multiplication in the breeding nursery, and the other - for repeated assessment of resistance to blast in infection nursery thus allowing reliability of breeding resistant varieties.

III – Initial stock for rice resistance breeding to blast disease

Research of rice varieties and samples resistance to blast disease was begun in VNIIR in the 60-ies. A. G. Lyakhovkin did thorough study of world collection of Research Institute of Plant Industry (VIR). In 1972 practically all samples of the collection (2,130) were assessed on their blast resistance in field trials and 1,008 samples were tested at the specialised plot under artificial inoculation.

Assessment of varieties and breeding samples was continued in the following years. Conclusion made as a result of these tests was that the majority of varieties grown at that period in Krasnodar territory and being under state trials had weak resistance to blast. In many of them even under natural inoculation *P. oryzae* infected up to 85-100% plants. This can be explained by the fact that practically all varieties were released from blast susceptible initial stock (Frolova, 1980).

Many years of research of population structure of *P. oryzae* showed that pathogen races had differed in virulence genes (Gorbunova et al., 1987). Thus in European part of Russia there were identified 6 virulence genes in Ukrainian and Dagestan pathogen populations: Av-ks+, Av-a+, Av-i+, Av-k+, Av-m+, Av-ta+, and 4 genes in Krasnodar population: Av-ks+, Av-i+, Av-k+, Av-m+. Thus the most efficient resistance genes for these populations are Pi-z, Pi-zt, Pi-ta2, Pi-b, and additional gene for Krasnodar - Pi-ta (Kolomiyetz, 1991). Phytopathologists have found out that rice varieties both commercially grown and under state trials, have efficient resistance genes Pi-ks, Pi-a or Pi-i and consequently are easily susceptible to blast.

It required search for effective donors of resistance and breeding of blast tolerant initial stock for consecutive breeding of immune varieties. Tests of 656 varieties of VIR collection from 8 ecological and geographical groups were carried out at greenhouse. Over 130 samples with high resistance to blast were selected. Genotype of resistance was determined in 123 samples (Kolomiyetz et al., 1986). The most valuable forms are those having 80-90 days before heading (Table 3).

Additional studies show that these are "old" type varieties. The plants are high, with low lodging resistance, large leaves, and few grains per panicle, easily shedding. Besides that the majority of them are late maturing and photosensitive. Under conditions of Krasnodar territory of 15-16 hour day they sharply increase vegetation period and do not mature in time. They can be used for hybridization growing them at 12-hour photoperiod or under artificial climatic conditions. All these varieties are valuable donors of race specific blast resistance.

Varieties of world collection with field resistance are of great interest for breeders. They show low level of disease development. These varieties can be divided into two groups: slightly susceptible to blast at tillering and panicle formation stage; highly susceptible at tillering and slightly susceptible at panicle formation. Varieties of the first group are very important for breeders. Varieties combining race specific and filed blast resistance are valuable initial stock. Among studied varieties such qualities have been found in samples Insen/Tremesino (Spain) and Maratelli 5A (France).

Testing of 2,544 sample of the world collection for varieties best suited for soil-climatic conditions of Russia showed that the majority is susceptible to *P. oryzae*. Only 69 samples were not infected with foliar form of disease, 20 samples were slightly susceptible to panicle form (grade 1-2) and 18 samples combined immunity to both forms of blast. The latter are the most valuable samples for rice breeding.

Testing of collection samples resistant or slightly susceptible to *P. oryzae* continued at Research Institute of Phytopathology resulted in discovery of samples with high resistance to local populations of blast due to resistance genes (Table 4).

Two original samples: breeding line 83-1-14-1 received by means of biotechnology and B3-600-436-85 from hybrid B3-600 are of great interest. They possess effective blast resistance genes. They were included into several crossing combinations. Selected sources of blast resistance are the base for production of hybrid material: in 10 years (1983-1992) there were created over 200 hybrid populations. Blast resistant plants were selected from many of them, thus forming bank of donors for blast resistant varieties.

IV – Breeding of race specific blast resistant rice varieties

Choice of breeding trends in rice blast resistance depends on soil and climatic conditions of rice growing area and genetic structure of *P. oryzae* population. The approach should be differentiated and determine initial stock, methods of its evaluation and selection. Durability of variety resistance depends on how these issues are solved.

Race specific breeding is advisable for zones where climatic conditions limit development of the pathogen. Many years of complex research have proved that these are European part of Russia, Ukraine and Karakalpakia. For the Far East it is recommended to work out breeding programmes for race specific and field resistance because of favourable climatic conditions for blast and high variability of pathogen.

Race specific, real or vertical resistance is connected with super sensitive reaction of host to pathogen and controlled by unique main gene. Thus initial stock should be genetically variable. Breeding based on one effective gene can result in appearance of races overcoming this resistance.

Effective blast resistance genes found for European part of Russia determined choice of donors for hybridization. The following varieties were included into crossings: with gene Pi-z: Zenith, VNIIR 7630, Yerua P.A. and samples F-G-84, BZ-600-436-85 as well as samples with gene Pi-z^t: 1-G-84 and 4-G-84. Breeding schemes have shown that twin simple crossings are not effective enough. Received breeding material in spite of its resistance to blast did not meet requirements of modern plant breeding. Necessity of back crosses with majority of donors was evident. Only among hybrids bred with French variety Maratelli 5A plants with promising results were found. Habitus of Maratelli 5A is close to that of Krasnodarsky 424, it is ripe only a few days later. But in hybrid population Krasnodarsky 424 / Maratelli 5A several dozens of plants with negative transgression were selected - they were earlier maturing and shorter than both parent forms. They combine resistance to blast with complex of valuable economic features. A number of samples were released after complete study and determination of resistance genes. After three-year evaluation variety VNIIR 92-98 under trade name Blastonik was released for state variety tests in 1992. It has been the first variety with race specific blast resistance released from local hybrid material. Later other varieties were released for state variety tests: Vityaz, Talisman, Snezhinka with similar race specific blast resistance and thus not requiring chemical protection.

V – Breeding of field blast resistant rice varieties

Evaluation of breeding material in infection nursery allows selecting not only immune samples, but also rice varieties and forms with high tolerance to diseases or having field resistance. The main advantage of this resistance is that it gives if not full, but at least complete protection and it is not ruined by the pathogen. Field or horizontal resistance is normally not race specific, more dependent on environmental factors and in majority of varieties it is polygene controlled.

Artificial inoculation in infection nursery is used to select immune and highly tolerant rice samples, varieties and forms. It guarantees trails of considerable amounts of breeding material on relatively small area of the nursery. Rice variety Slavyanetz is a good example: its study started in 1984-1985 as L-5-80 when it was chosen from other varieties due to its high blast tolerance. After artificial inoculation disease developed slowly, spore formation was not fast and secondary inoculation was practically not registered. This valuable feature of the variety is of particular importance in the years of epiphytoty. Since 1991 Slavyanetz has been in commercial use in Krasnodar territory as the most blast resistant variety.

Other samples with small damage caused by *P. oryzae* were selected from infection nursery during epiphytotic development. Possessing high field resistance they were used as parent forms for hybridisation. Among them there are local samples VNIIR 87, VNIIR 1619-90, Mutant 533, VNIIR 7630, Kr-3-84, and Shimokita and Reimei from Japan.

Medium maturing variety Pavlovsky characterised by high grain yield and quality, as well as good field resistance to blast, was released for state variety tests in 1991. New early maturing variety Sprint with complex resistance to blast and white tip was released for state variety tests in 1992. Salt resistant and blast tolerant variety Kurchanka was released for state variety tests in 1993.

These three varieties have had successful state variety tests and were included into State Registry of rice varieties allowed for commercial use in Krasnodar territory and Republic of Adygeya: Pavlovsky - from 1994, Sprint - from 1996 and Kurchanka - from 1996.

All these varieties have different origin and different methods have been used in their breeding. For example, Slavyanetz variety was obtained by individual screening from Spalchik variety. The rest ones have been a result of hybridization of different levels of complexity. Rice varieties Sprint and Kurchanka have been released from simple hybrid population, pedigree of Pavlovsky is more complicated (Fig. 1).

In 1997 a new rice variety Leader has been sent for state variety tests. It can be grown according to no-pesticide technology with low energy consumption. Blast epiphytoty has not reduced its yield in the Belozerny State Variety Station. Its high field resistance to blast has been confirmed. Leader was included into State Register in 2000.

Besides origin our varieties have a great difference in morphological and biological characters. But two indices unite them- it is a high grain quality and quick growth at germination period. It helps to get shoots from water layer and not to use herbicides at the period of cultivation. As a result of it the costs for rice growing decrease, the quality of production increases and ecological conditions in rice-growing zones improved.

Thus joint research of rice breeders and phytopathologists provides rice growers of Russia with genetically protected varieties from blast.

Table 1. Yield of rice varieties in Teuchezhsky State Test Field in 1983-1984 *(preceding crops - perennial grasses and rice)*

Variety	Preceding crop: Perennial grasses, Yield, t/ha		Perennial grasses, Blast development in 1984, %		Rice, Yield, t/ha		Rice, Blast development in 1984, %	
	1983	1984	node	panicle	1983	1984	node	Panicle
Kuban 3	5.62	1.88	60	24	4.76	1.49	66	16
Dubovsky 129	4.94	0.82	71	36	4.26	0.65	80	32
Liman	5.20	3.85	48	20	4.92	2.96	54	6
Mutant 210	6.12	1.49	70	49	5.25	1.36	74	12
Start	6.28	3.26	36	31	5.06	2.42	47	8
Spalchik	6.64	3.66	32	28	5.97	2.98	43	6
Salsky	5.50	0.87	60	42	5.22	0.87	71	37
Krasnodarsky 424	7.20	1.62	62	34	5.08	2.63	60	14
Zhemchuzhny	7.30	1.49	68	35	6.18	2.34	66	23
Kulon	7.66	1.02	64	38	5.53	1.65	66	34
Lenar	7.67	1.08	63	38	5.41	1.62	68	36

Table 2. Response of rice hybrids F_2 to P. oryzae (1986-1988)

Population number	Crossing combination	Ratio of resistance to susceptible plants, R:S
303	Krasnodarsky 424 / Maratelli 5A	3:1
369	VNIIR 8444 / VNIIR 87	3:1
394	VNIIR 7630 / NF-DZ-84	9:7
441a	VNIIR 8444 / Dular	3:1
540	Yerua P.A. /L-5-80	3:1
543	Ham Nam /VNIIR1588	9:7
585	Maratelli 5A / L-5-80	3:1
586	Kr-3-84 / Maratelli 5A	3:1

Table 3. World rice collection samples with effective blast resistance genes *(Yudina et al., 1988)*

№ in VIR catalogue	Sample	Origin	Plant height, cm	Days before flowering	Resistance genes
		European group			
6979	Insen/Tremi-sino	Spain	113	85	Pi-z
		Oriental group			
7265	Shimokita	Japan	97	85	Pi-ta
3805	PN 170	China	132	92	Pi-z^t
7233	Ham Nam	Korea	87	88	Pi-z^t
		Iranian group			
3787	Champa	Iran	138	80	Pi-z^t
		Central Asian group			
5065	Bir-me-fen	Afganistan	120	95	Pi-z^t

Table 4. VNIIR rice collection samples with effective blast resistance genes

№ in VNIIR catalogue	Sample origin	Plant height, cm	Days before flowering	Resistance genes
0590	O. glaberima/Souzny 244	106.9	87	Pi-z^{t}
01016	Korbeta/Souzny 244	55.5	89	Pi-z^{t}
01393	Taichung Native/DVROS 15	56.0	85	Pi-z
01717	Taichung Native/DVROS 15	97.8	84	Pi-z
01793	C.6063/Rialto	74.6	82	Pi-z
01905	VNIIR 3657/Rialto	102.2	85	Pi-z
01907	VNIIR 3657/Rialto	86.2	86	Pi-z
02268	VNIIR 7630	100.9	86	Pi-z
02360	Panoza sel. / VNIIR 5001	110.6	85	Pi-z
02518	Kashmir Basmati	-	-	Pi-ta
02890	Mutant 744-82	92.8	87	Pi-ta
02919	Breeding line 83-1-14-1	84.8	84	Pi-z^{t}
03186	B3-600-436-85	90.6	81	Pi-z

Figure 1. Pedigree of rice variety Pavlovsky