

DNA-TECHNOLOGIES IN RICE BREEDING FOR RESISTANCE TO BIO- AND ABIOTIC STRESSORS

**Head of laboratory of information,
digital and biotechnologies
FSBSI «Federal Scientific
Rice Centre», Dr. of biology**

E.V. Dubina

Novosibirsk, June 14-17, 2021



DNA-TECHNOLOGIES IN RICE BREEDING FOR RESISTANCE TO BIO- AND ABIOTIC STRESSORS



RICE CROP AREA



Rice is susceptible to blast during all growing phases. The disease affects all above-ground plant organs – leaves, stem nodes, panicle. Losses from blast disease can range from 30-80%



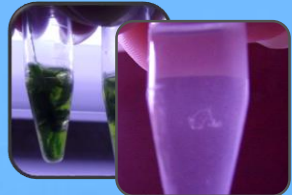
DNA-TECHNOLOGIES IN RICE BREEDING FOR RESISTANCE TO BIO- AND ABIOTIC STRESSORS

In program for developing rice genetic resources resistant to bio- and abiotic stressors we used domestic varieties Flagman, Snezhinka, early-ripening variety Novator and lines KP-163, KP-25-14, VNIIR9678, VNIIR5242

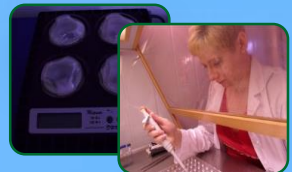


Donors of *Pi* genes: lines
C104 LAC (*Pi-1*);
C101-LAC (*Pi-1+Pi-33*);
C101-A-51- LAC (*Pi-2*);
IR 83260-2-10-5-2-1-B (*Pi-40*);
BL-1 (*Pi-b* ; IR-36 (*Pi-ta*))

Donors of *Sub1A* gene:
variety Khan Dan



When carrying out molecular genetic studies, genomic DNA from plant cells and fungal mycelium was isolated by the Murray and Thompson method, using cetyltrimethylammonium bromide as the main lysis buffer (CTAB, 1980)



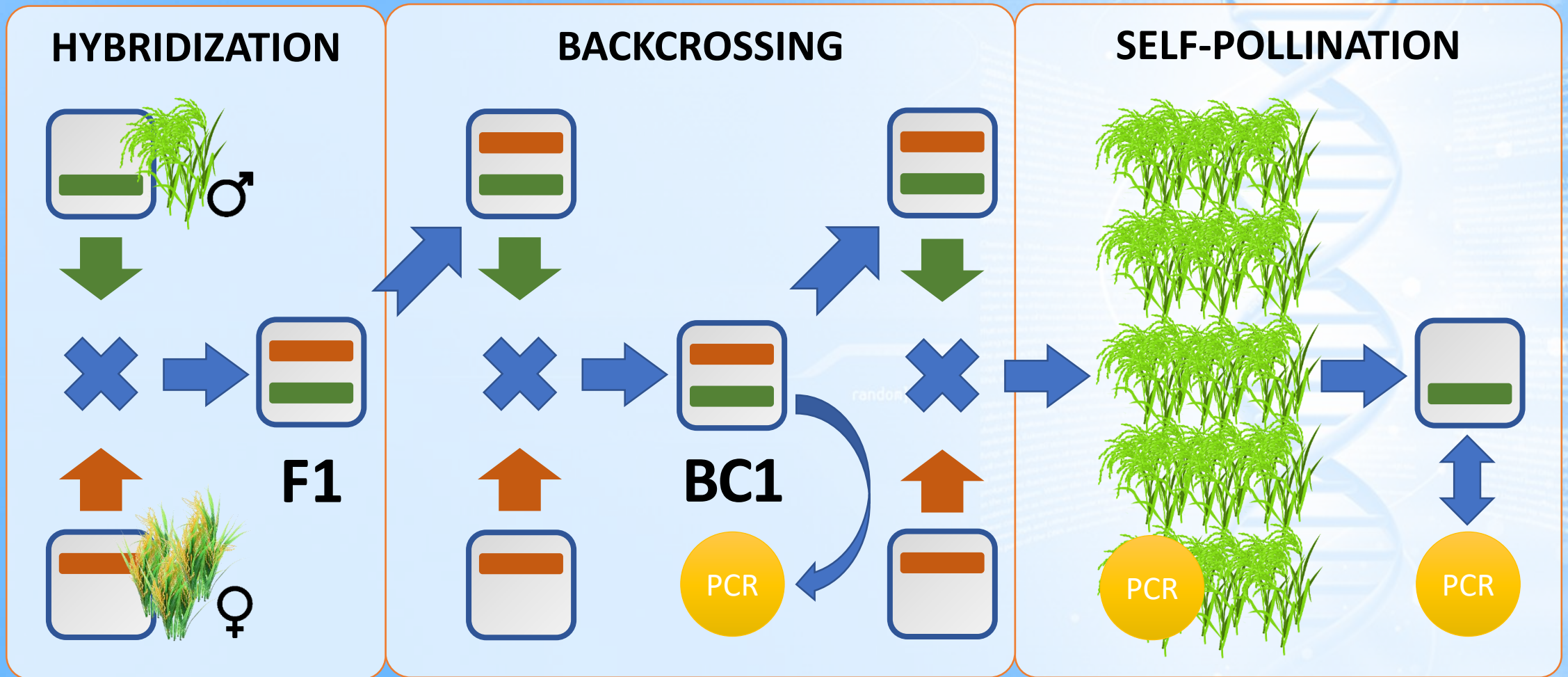
DNA amplification of rice plants and vegetable crops was carried out by PCR, while optimizing the conditions. DNA amplification of *Pyricularia oryzae* Cav. was carried out by the method of fragment analysis on the device "NANOFOR-05" in the center of collective use of the FSBSI "VNIISB"



Amplification products were separated by electrophoresis in 8% polyacrylamide and 2% agarose gels (Pomortsev et al., 2004).

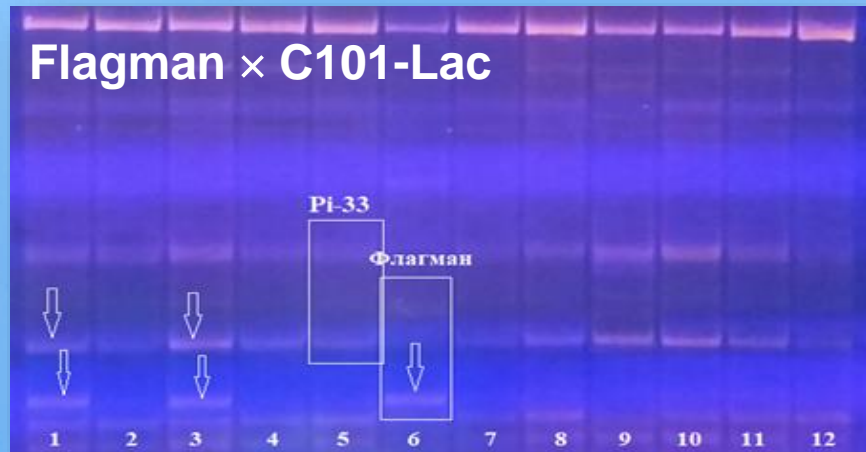


DNA-TECHNOLOGIES IN RICE BREEDING FOR RESISTANCE TO BIO- AND ABIOTIC STRESSORS

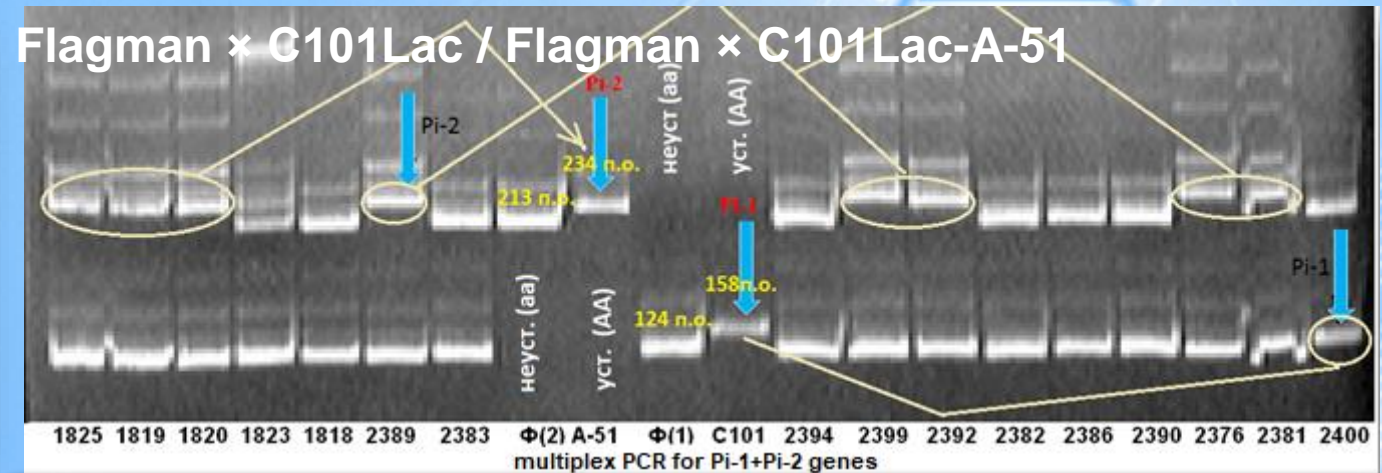
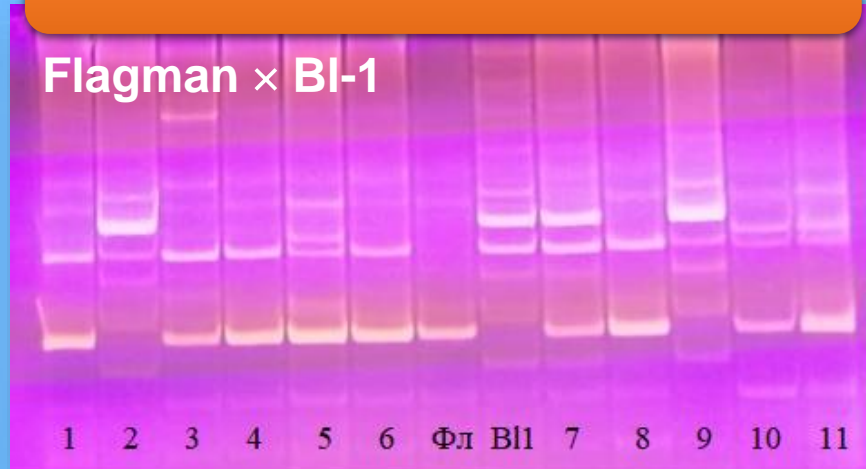




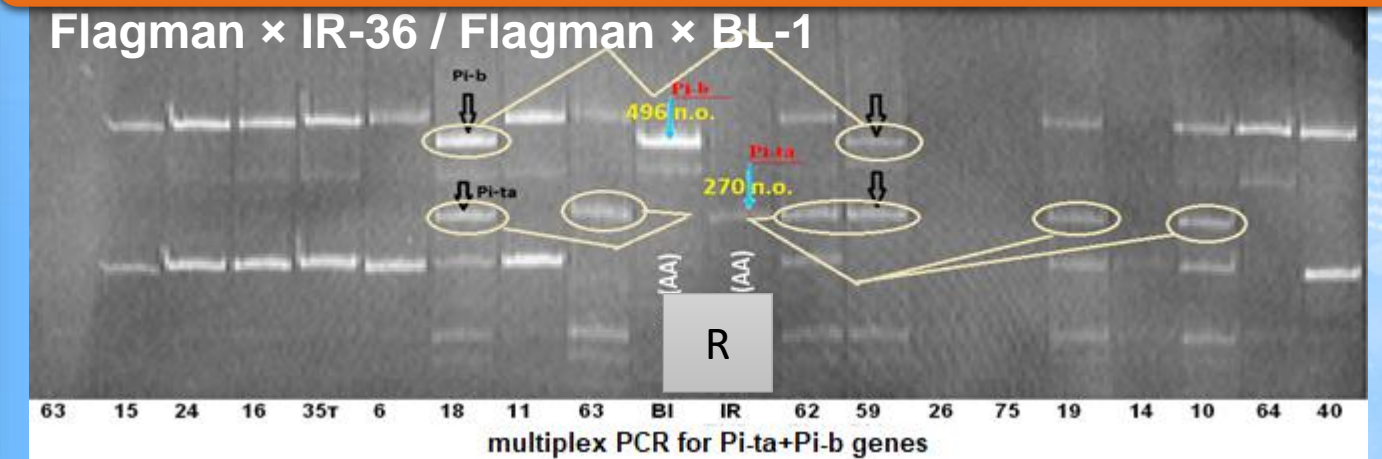
DNA-TECHNOLOGIES IN RICE BREEDING FOR RESISTANCE TO BIO- AND ABIOTIC STRESSORS



BREEDING FOR BLAST RESISTANCE



COMBINING BLAST RESISTANCE GENES





DNA-TECHNOLOGIES IN RICE BREEDING FOR RESISTANCE TO BIO- AND ABIOTIC STRESSORS

Characteristic of rice varieties with blast resistance genes CVT 2016-2018

Line/ variety	Yield, t/ha	Duration, days	Plant height, cm	Mass of 1000 grains, g	Grain l/b	Milling yield, %	IDD, %
KP-171-14 (Alliance)	9.1	120	85.3	29.1	2.6	73.2	32.3
KP-30 (Lenaris)	10.6	115	77.8	30.4	2.6	72.3	16.7
KP-23 (Captain)	9.1	115	75.6	30.2	2.4	71.2	21.3
Flagman (St)	8.1	116	91.0	26.7	1.9	71.6	59.1
LSD ₀₅	0.5	2.0	6.7	2.3	0.3	1.4	3.5



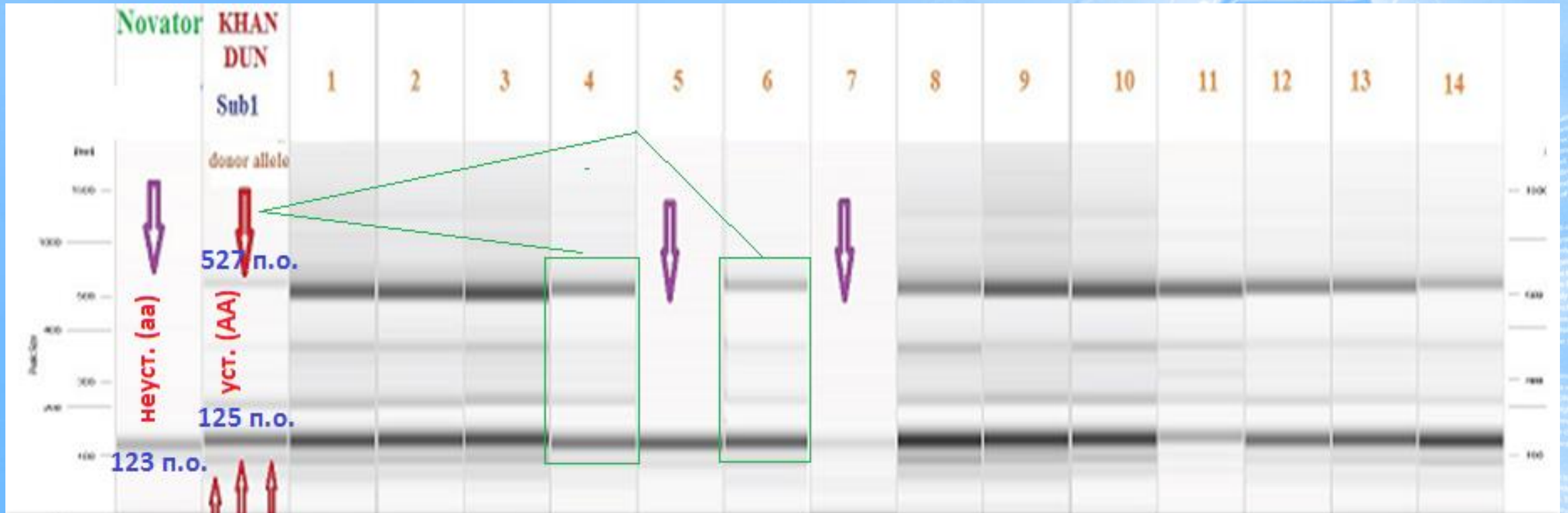
DNA-TECHNOLOGIES IN RICE BREEDING FOR RESISTANCE TO BIO- AND ABIOTIC STRESSORS



Indicator	Flagman (Standard)	Alliance	Lenaris
Average yield, t/ha	8,1	9,1	10,6
Costs, rub/ha	60000	60000	60000
Profit, rub/ha	69600	85600	109600
Profitability, %	89	142	182
Efficiency, rub/ha	-	182	40000



DNA-TECHNOLOGIES IN RICE BREEDING FOR RESISTANCE TO BIO- AND ABIOTIC STRESSORS

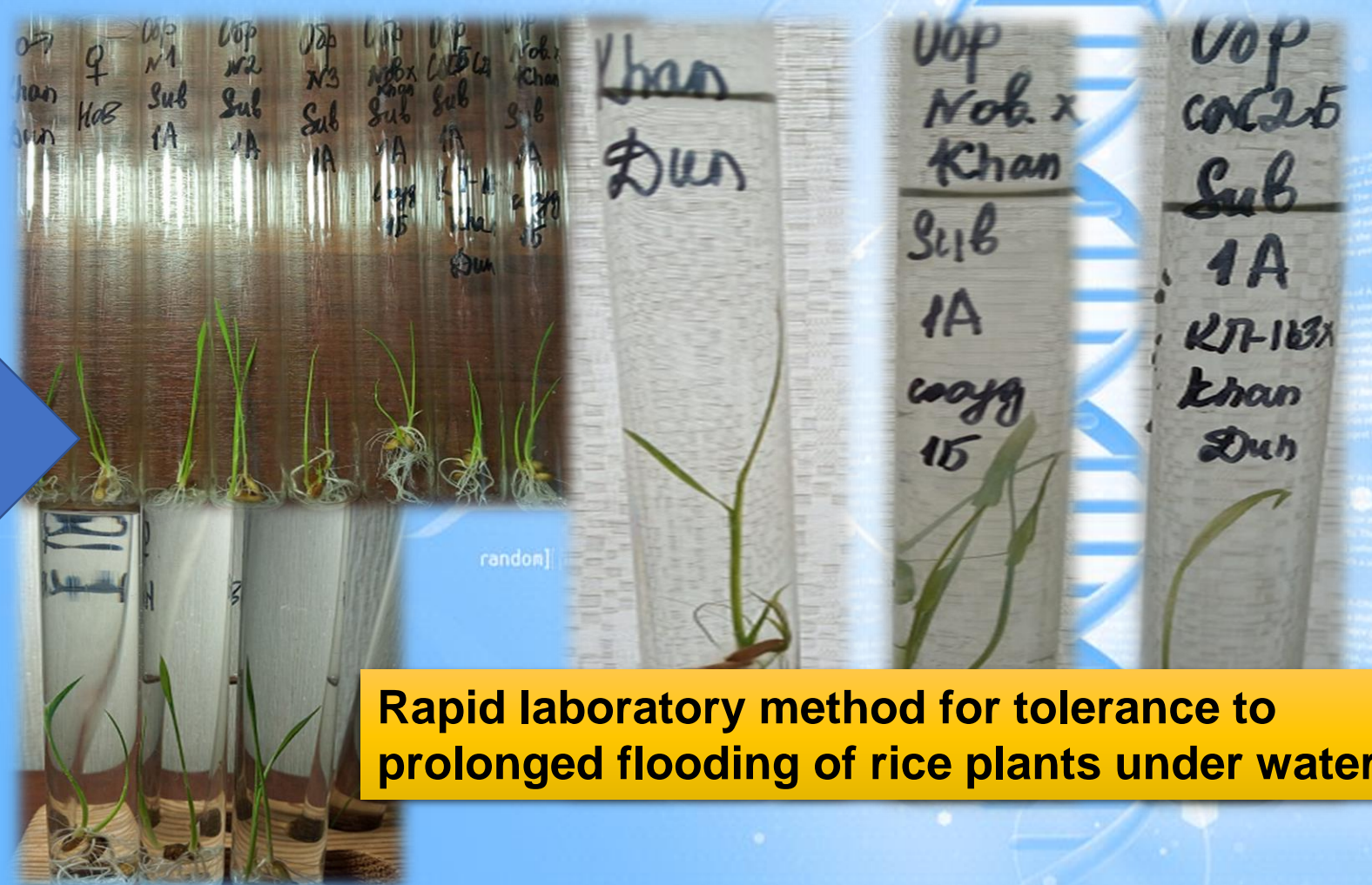


Results of PCR analysis by Sub1A203 locus

In a sample of 184 plants, the following ratio was obtained: 39 plants carry the dominant allele, 104 - heterozygotes, 41 plants - homozygotes for recessive, which corresponds to monogenic Mendel segregation 1: 2: 1.



DNA-TECHNOLOGIES IN RICE BREEDING FOR RESISTANCE TO BIO- AND ABIOTIC STRESSORS



Rapid laboratory method for tolerance to prolonged flooding of rice plants under water



DNA-TECHNOLOGIES IN RICE BREEDING FOR RESISTANCE TO BIO- AND ABIOTIC STRESSORS

1

- The introduction of blast resistance genes *Pi-1*, *Pi-2*, *Pi-33*, *Pi-ta*, *Pi-b*, *Pi-40* into highly productive domestic rice varieties to increase their immunity to the disease, as well as genes for tolerance to long-term flooding of rice plants under water *Sub1A*, as a factor in weed control, were performed. Based on the DNA analysis of hybrid plants using microsatellite molecular markers linked to these traits, forms with introgressed resistance genes in a homozygous state were selected. The irradiated pre-breeding material is introduced into the breeding process.

2

- The genes for blast resistance have been combined. This made it possible to obtain rice forms with two, three and five genes of resistance to the disease (*Pi-1 + Pi-2*, *Pi-ta + Pi-33*, *Pi-ta + Pi-b*, *Pi-1 + Pi-2 + Pi-33*, *Pi-1 + Pi-2 + Pi-33 + Pi-ta + Pi-b*). Hybridization of rice lines with Pi genes and lines with the *Sub1A* gene in the genotype was carried out. To increase the economic efficiency of marker selection, multi-primer identification systems have been developed for the simultaneous identification of two Pi genes (*Pi-1 + Pi-2*, *Pi-ta + Pi-33*, *Pi-ta + Pi-b*), as well as *Pi* and *Sub1A*. Optimal conditions for PCR have been selected. This made it possible to obtain highly reproducible results for DNA products. These marker systems are introduced into the breeding process. On their basis, backcross self-pollinated lines were obtained, which were introduced into the breeding process for studying by economically valuable traits.

3

- As a result of a field assessment in breeding and infectious nurseries, among the obtained source material with *Pi* genes for economically valuable traits and resistance to *Pyricularia oryzae Cav.* with strict rejection, promising lines were selected (KP-171-14, KP-23 and KP-30), on the basis of which varieties Alliance, Captain, Lenaris with the blast resistance gene *Pi-ta* and Piruet with three genes - *Pi-1*, *Pi-2*, *Pi-33*, were developed, which have successfully passed the state test and introduced into production.