All-Russian Rice Research Institute

CREATION VARIETIES BY FIXING HETEROSIS EFFECT IN RICE HYBRIDS

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Heterosis - property of hybrids to surpass the best parental forms - was discovered 230 years ago, but people start to use it commercially only in the 20th century

Hybrids not only exceed the best varieties of traditional breeding by yield on the average of 20-50 %, but they have increased protein in grain, they are highly adaptive to unfavourable environmental factors. At the same time productivity potential of released hybrids with increasing yield of new varieties of traditional breeding; thus, rice super hybrids, recently released in China, exceed hybrids of "first generation" to 15-20% by yield.

Why so impotent maintaining heterosis effect?

- The price for hybrid seeds is high because of labour-intensiveness of their obtaining.
- Developing countries do not have funds for creation their programmes on release of heterosis hybrids
- They also cannot purchase seeds of the most adaptive hybrids from created ones.
- Heterosis maintaining in next generations will help to use heterosis effect in other countries with unfavourable environmental and social condition.
- At the moment several approaches are offered for maintaining heterosis effect: creation of apomictic and vegetative propagated forms, transfer of genes, responsible for formation of the corresponding traits from other types of plants. But none of them is not fulfilled. Besides that, genotype of initial hybrid is not improved by use of the given methods.
- Unique germplasm with of compensation genes complex will be also obtained; it will be the basis for release hybrids of new generation.

- Despite of wide application of hybrids the genetic basis of heterosis is not completely discovered till now.
- Experimental data obtained for confirmation of different theory are controversial. In recent years a few experiments for expanding the obtained data have been carried out with application of molecular markers The data obtained by means of molecular marking also are quite controversial.

Last years a number of experiments were carried out with application of molecular markers to specify the data received earlier to clear up the nature of heterosis.

- Heterozygosity itself does not provide heterosis, but can give some advantage for separate traits above homozygous forms.
- All kinds of genetic effects take part in appearance of heterosis (partial, full or overdominance) both inside and between of various loci.
- In the heterosis manifestation the additive effects of genes are very important.
- Yuan L.P., G.S. Khush, Zhikang Li H., Li J.M. ,., Tanksley S.D., Xuezhi B., Raima R., J. Bennett ,Qifa Zhang, Jinping Hua, Lizhong Xiong, Caiguo Xu, Zhao M.F., Li X.H., Yang J.B., Xu C.G., Hu R.Y., Liu D.J., Gao Y.J., Yang S.H., Ragab R.A., Saghai Maroof M.A., Li Z.B.,Yu S.B., Li J.X., Xu C.G., Tan Y.F., Gao Y.J , Stuber C.W., Lincoln S.E., Wolff D.W., Helentjaris T., Lander E.

- There are still lots of arguments about heterosis nature, though Strunnikov offered in 1999 a theory which not only allows explaining the nature of heterosis, but also answering the main questions of heterosis breeding; how gene complexes, which provide heterosis effect are created, and how to maintain heterosis effect in the following generations

- According to Strunnikov V.A. heterosis effect develops because of inheritance from parents coordinated compensation complex of favorable genes emerging in the result of selection in conditions of bad genetic and ecological factors. This theory allows uniting all theories that were offered earlier. There is no big difference between heterosis hybrid and high productive variety. In both cases there are the same genetic mechanisms, and application of heterosis effect only allows finding more effectively nature created gene complexes.

Heterosis nature:

- Favorable influence of all allele types (in general) in heterozygous condition (over domination hypothesis).
- Heterozygosity of harmful recessive genes influence, favorable combination of non-allele, completely dominant genes (domination hypothesis).
- Correspondence of nuclear and cytoplasmtic genomes.
- Heredity from parents coordinated compensational favorable genes complex, appearing in result of screening on the basis of harmful genetic and ecological factors influence.

The idea of fixing of heterosis in a subsequent generations (without hybridization) has been prompted by the results of studying the heterosis nature.

Essence of experiment

- In order to understanding essence of genetic events, that led to heterosis effect, determined specific gravity each factor in their manifestation.
- Was formed group on the base of heterosis hybrid without addition of outside genetic material with different level of heterozygosity. Moreover in the second variant harmful gene was removed.

Plan of experiment

Group A

1. PK-29 (100%)
2. Aa (transformation in tetraploid)
   AAaa (meiotic parthenogenesis)
   1AA-4Aa-1aa (66.7%)
3. PK-29*F1(PK-29* gomozygote from PK-29) (50%)
4. Gomozygote from PK-29
   (meiotic parthenogenesis) (0%)

Group B

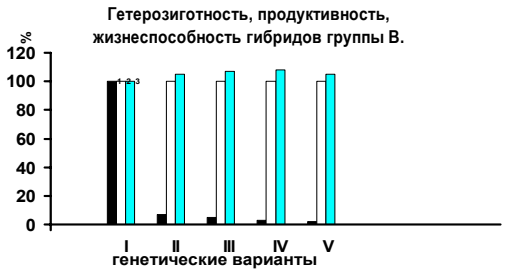
1. PK-29 (100%)

2-4. 1-4 back cross generations PK 29 and gomozygote from PK-29 (meiotic parthenogenesis)

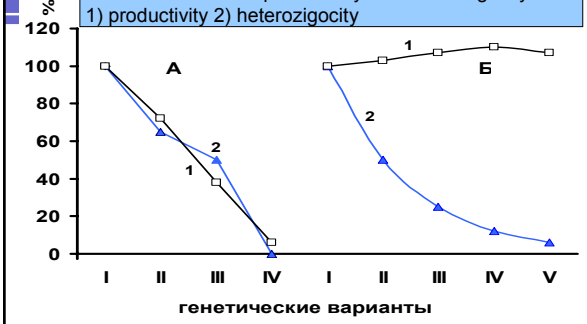
Genetic events in group B

- Heterozygosity neutral for adaptivity and favourable gene 50%, in the same time heterozygosity of harmful gene reduced from 50% - in first back cross generations, to 25% - in second back cross generations, to 12.5 in third back cross generations and to 6.25% in fourth back cross generations.
- Further was three inbred cross-breeding. In a result heterozygosity reduce accordingly to 25, 12.5 и 6.25%.
- Frequency of harmful recessive genes reduced consecutively to 3.1. 1.5 and 0.7%.

Interaction between productivity and heterozigocity
1) heterozigocity 2) productivity 3) viability



Interaction between productivity and heterozigocity
1) productivity 2) heterozigocity



Methods of heterosis fixing

- Hence, the problem of fixing heterosis can be solved by means of creation of hybrids without **lethal and half lethal gene**. Thus the basic genotype of initial hybrid should be not only kept, but also improved. This problem is solved also by method of back crossing. The number of harmful genes in everyone new back cross generation decreases twice in comparison with the previous generation.

Methods of heterosis fixing

- Presence of lethal gene in pollen grain, brings a plant (received from this pollen grain) to death on one of stages of development.
- Frequency of the survived individuals does not exceed 0.5 %, when we receive doubled h**aploid throw the anther culture** Quite clear, that back crossings the individuals, without harmful genes in homozygous and heterozygotic form, cannot led to occurrence in the genotype lethal gene in a homozygous condition.

Methods of heterosis fixing

- High viability back cross generations, since the first, represents the big interest as these generations occur as a result of self-fertilisation usually strongly decrease viability.
- High viability is explained by the fact that back-cross does not lead unlike simple bisexual propagation to appearance of bad genes in homozygous condition.

- Efficacy of the heterosis maintenance method in the successive generations was tested on the hybrids of silkworm and drosophila. But still its efficacy in case of plants has not been confirmed.
- Methods offered by academician V.A. Strunnikov, was tested at our Institute. This method is more progressive, then offered earlier, because they help not only to maintain heterosis effect in next generations, but to improve hybrid genotype by elimination of lethal and half-lethal genes decreasing productivity. It helps to release varieties with analogous yield on the basis of outstanding hybrid combinations even separate plants.

We are received back cross generation in 13 hybrid combinations

- The analysis of productivity of the received plants was carried out at comparison with:
- initial hybrid,
- doubled haploid, received of pollen of this hybrid,
- second generation of a hybrid,
- parental forms.
- It has been incorporated two variants of experience: reception back cross generations at crossing with a plant from the same hybrid combinations , and with an initial best plant in it selected for reception doubled haploid.

Results

- Segregation in the first variant of experience confirms multi linearity of varieties.
- The absence segregation in back cross generation in the second variant of experience, confirm opportunity of fixing heterosis effect by the offered method.
- In hybrid combinations (on which a plenty double haploid lines has been received, and there was an opportunity of selection highly productive double haploid lines for carrying out of back cross productivity of received plants was higher, than in initial hybrid.

Productivity of plants received at back crossing hybrid and doubled h**aploid line**, received from the pollen of this hybrid

Combination	Plant height, cm	Panicle length, cm	Grains panicle, no	Sterile spikelets panicle, no	Main panicle weight,g
Khazar/Liman					
F1/ double haploid	84,25	15,25*	135,5*	3,25	3,88*
F 1	77	14,25	106,25*	3,75	3,04*
F2	74	14	86,3	10,2	2,47
Pegasso/Viragh					
F1/ double haploid	76,5	15*	92*	8	2,94*
F 1	59	12	69*	2	2,21*
F2	58,1	11,7	56,9	12	1,82
Muiya / Sneghinka					
F1/ double haploid	79	17	112,3*	8,3	3,21*
F 1	**83**	**17**	**86***	**15**	**2,46***
F2	74	16,3	74	21	2,12
VNIIR 7718/ VNIIR 7887					
F1/ double haploid	88	15	181*	12	4,26*
F 1	77,67	13,00	120,7*	7	2,87*

Statistical indices (average among 13 back cross generation received in 2005 year

Statistical Indices	Back cross generation	F1	F2
Mean	2,22	2,39	1,79
Dispertion	0,06	0,06	0,16
Standart deviation	0,24	0,24	0,40
Coefficient of variation,%	11,49	10,17	22,40

Statistical indices (average among 13 back cross generation received in 2006

Traits	Mean	Standart deviation	Standart error of mean
F1			
Plant height (cm)	76,2	10,43	1,71
Panicle length (cm)	14,7	1,91	0,31
Grains panicle(no)	91,7	39,58	4,86
Sterile spikelets panicle(no)	9,3	8,83	1,45
Spikelets panicle(no)	100,9	39,91	4,91
F1/ double haploid			
Plant height (cm)	79,9	11,08	1,81
Panicle length (cm)	15,1	1,81	0,46
Grains panicle(no)	88,1	41,22	6,77
Sterile spikelets panicle(no)	8,6	5,61	0,92
Spikelets panicle(no)	96,7	41,47	6,81

Characteristics received double haploid **line**

Hybrid combination	Line № п/п	Plant height (cm)	Panicle length (cm)	Grains panicle (no)	Sterile spikelets panicle,(no)	Main panicle weight(g)	1,000 - grain weight (g)
Khazar/Liman	1	75	14,5	98	54	2,61	26,6
Khazar/Liman	2	83	15	89	27	2,9	32,6
Khazar/Liman	3	85	15	70	18	2,59	37,0
Khazar/Liman	4	65	12	71	5	2,08	29,3
Khazar/Liman	5	86	15	96	12	2,27	23,6
Liman/ Khazar	1	76	13	93	22	2,28	24,5
Liman/ Khazar	2	74	13	106	10	2,36	22,3
Liman/ Khazar	3	83	16	117	13	2,6	22,2
Liman/ Khazar	4	75	13	120	13	3	25,0
Liman/ Khazar	5	79	10	97	11	2,59	26,7
Liman/ Khazar	6	78	12	86	17	3,08	35,8
Liman/ Khazar	7	81	14	93	24	2,62	28,2

Productivity of the received after back crossing hybrid Khazar/Liman for doubled haploid line , hybrid and parental forms Khazar.

Chlorophyll mutation in population of double haploid line in seedling stage

Hybrid Combination	White Blade	Yellow Leaf	Striped leaf	Brown spot
VNIIR 7718/ VNIIR 7887	*	*		
Khazar/Phontan		*	*	
Serpantin/ Khazar	*	*	*	
Khazar/Izumrud	*	*	*	*

Chlorophyll mutation in population of double haploid line received from hybrid combination Khazar/Izumrud

Plant F1	Size of Population (no)	White blade (no)	Striped leaf (no)	Yellow leaf (no)	Brown spot (no)
c.39	59	13	2	10	
c.5, p.2	91	7	2		
c.27, p.5	102	12			
c.32, p.5	97		2	1	7
c.5, p 5	70	2		2	
c.5, p.3	131	10	4	2	
c.2, p.3	60	1		2	

Results

- Absence of segregation in back cross generation proved the theory about the nature of heterosis effect which is determined by the coordinated complex of favorably working genes, instead of their heterozygotic condition .
- Receiving of double haploid line allow cleaned initial hybrid from lethal and half lethal gene, brought by parental forms and causing decreasing of productivity in the subsequent generations,
- Back crossing hybrid for doubled haploid line allow to create the variety with productivity of initial hybrid