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INHERITANCE OF DETERMINANTS SPECIFIC FOR PHYSIOLOGICAL HETEROSIS BASIS IN RICE HYBRIDS

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S u m m a r y

The inheritance of determinants (growth speed of radical and stem, duration of leaf area photosynthetic activity, sensitiveness to level of mineral nutrition, content of a, b chlorophylls and carotenoids) specific for physiological heterosis basis in hybrids was investigated in six rice varieties and 30 hybrid combinations. It was shown, that resulting determinant manifestation is determined both intergenic and intraloci interaction, but the main role belong to additive gene effects. The polygenic character of inheritance and nondirectional domination among studied determinants were revealed. The superdominance is specific for stem growth speed, but the incomplete domination of major portion of parameters — for other determinants. The domination of majority parameters increased the adaptability permit to obtain the complex of useful genes already in F_1.

Keywords: heterozis hybrid, speed of growth, chlorophyll content, carotenoids, duration of photosynthetic activity, response, mineral nutrition, inheritance.

The use of heterotic hybrids significantly increases yields of many crops, and the potential of such hybrids grows along with creation of more productive varieties at conventional breeding. Thus, the recently created superhybrids of rice (China) show the potential productivity 12-15 t/ha, which exceeds earlier hybrids by 15-20% (1). Physiological basis of heterosis is still debated, but many morphological and physiological determinants responsible for its manifestation are known. In maize, the effect of heterosis is observed even in earliest ontogeny stages: the increased gene expression in hybrids (compared with parental forms) occurs immediately after fertilization, and the 6-day-old hybrid embryo is larger than non-hybrid one (2, 3). The increased level of expression was found in 15,3% of 13 999 studied genes, and in 8,7% of these genes, it more than twice exceeded that of parental forms (4). For about 4% genes expressed in roots, leaves and panicles, expression levels were different in hybrids and parental forms (5).

Functions of genes with increased expression are known: mostly, these are the genes involved in regulation of transcription, initiation of replication, synthesis of protein and RNA, cell division (6). In hybrids and parental forms, no significant differences in cell size was observed; therefore, the larger size of hybrid embryos can be explained by accelerated cell division (7). Under favorable growing conditions, heterosis is associated with additional accumulation of biomass per area unit and higher yield index compared with varieties of conventional selection (8). For example, the 4th chromosome of Arabidopsis includes a cluster of 23 genes, whose dominance, superdominance and an additive effect in different phases of development provided heterosis in biomass (dry weight of shoots) (9). Many studies explain the effect of heterosis by better reutilization of carbohydrates from stem to grain in ripening phase. In countries with temperate climate, heterosis was associated with high rates of growth and productive tillering resulting in earlier raise of leaf area index (10). Heterotic hybrids usually demonstrate smaller values of specific density of leaf surface and thinner leaves (11). The best conditions for cultivation of hybrids - medium and high backgrounds of mineral nutrition leading to higher yields and heterosis. In particular, the introduction of mineral nitrogen over 120 kg/ha caused the average yield equal to 6,5 t/ha, the standard heterosis 25,3% and yield gain 1,4 t/ha, and less than 120 kg/ha – respectively, 5,2 t/ha, 13,0% and 0,5 t/ha. The economic effect from cultivation hybrids increases at high doses of nitrogen (up to 180 kg/ha) as well.

Hybrids' productivity usually exceeds varieties in both high and low mineral background, but the high nutrition level causes significantly greater yield increase. Optimization of fertilizer timing and doses enhances heterosis effects. Thus, the use of nitrogen in flowering phase improved grain formation, and fractional introduction (twice - 60 kg/ha before sowing and 40 kg/ha in midst of tillering instead a single dose 100 kg/ha) resulted in 11,0% increase of standard heterosis, while no reliable differences were observed in varieties of conventional breeding. Reduce of nitrogen dosage from 200 to 125 kg/ha diminished the yield in hybrid by 13%, in the cultivated variety – only by 5% (12).

Heterosis is manifested in early ontogeny stages as faster mobilization and transformation of reserve substances – usually, heterotic hybrids have more intense metabolic processes. In heterotic hybrids *Vicia faba* L., about 9% of 5500 studied loci showed a modified expression including the genes that control metabolism of carbon and nitrogen, stress resistance, cell division rate, hormonal regulation and mitochondrial activity (13). Rapid development of root system in hybrids provides their advantage over varieties in higher intensity of mineral absorption and rates of formation of photosynthetic apparatus. Heterotic maize hybrids used to manifest greater number, length and branching of embryonic roots (14). It has been shown a close relationship between plant productivity and morphological parameters related to length and weight of roots and stems, photosynthesis efficiency and use of fertilizers (15-16).

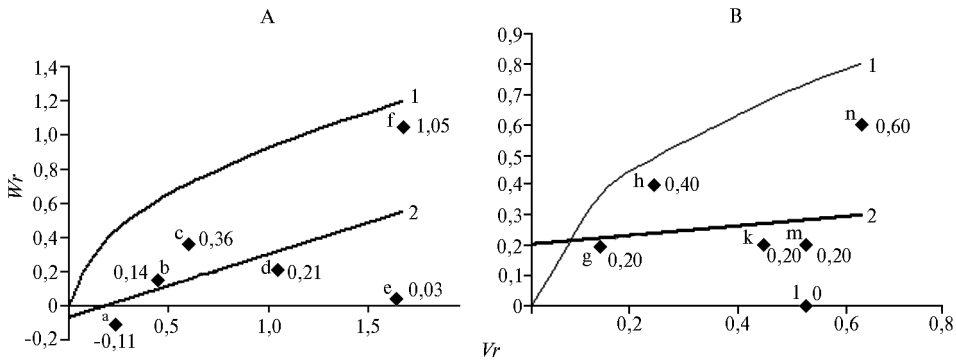
The purpose of this work - to study the inheritance of determinants affecting rates of growth, photosynthesis efficiency and use of fertilizers by rice seedlings

Technique. The objects of study were six rice varieties (Khazar, Nartsiss, Fontan, VNIIR 7718, VNIIR 7887, Liman) and 30 combinations obtained by hybridization of these varieties in full diallel scheme. The growth rates of stem and radicle were examined in 5-day-old seedlings grown in an incubator at 29 ^{o}C. The content of chlorophyll a, b and carotenoids were determined in flowering phase using the spectrophotometer Genesys 8 ("Minoltra", Japan) after their extraction with solvents, contents of pigments in leaves - in the ethanol extract of carvings taken from the middle part of 2 upper leaves (a cork drill of 0,5 cm diameter, 20 plants of each sample) using the Lichtenthaler's formula. The duration of photosynthetic activity in leaves was estimated by leaf tiers in flowering phase, which allowed to describe leaves' state in the period from early desiccation up to dying off. The state of leaves was estimated under the scale: 100% - green leaf with no signs of desiccation, 75% - yellowed areas occupy less than 1/4 surface area, 50% - about half yellowed leaf surface; 25% - green color remains at less than 1/4 leaf surface area and 0% - completely dry leaf.

Productivity and responsiveness to mineral nutrition were assessed in plants grown in containers (10 plants per container and 20 plants per each version of the experiment in 2 replicates) against an average ($N_{120}P_{60}K_{60}$) and rich ($N_{240}P_{120}K_{120}$) mineral backgrounds.

The results obtained in crosses were statistically treated by diallel analysis using Hayman's method (17): the diallel data tables were composed for each determinant, and the Hayman's charts were built to describe the genetical basics of determinants' inheritance and to identify donor varieties.

Results. On the Hayman's chart, the parabola $Wr_i = Vr_i \times Vp$ (Wr_i - covariance between parameter values in hybrids and their parental forms, Vr_i - variance of parameter values in hybrids, Vp - variance of parameter values in parental forms) has a very important biological sense: it intersects the regression line (Vr, Wr) in points corresponding to location of parents if they carried all dominant or all recessive genes. In the case of directed domination, these points indicate how much a selection work can move the value of parameter relative the level of parental form. If a parent carries many dominant genes, the variance will be small and such variety locates on the regression line close to point of origin. A cultivar with many recessive genes shifts right upwards the regression line, as its progeny shows high variance in crosses with cultivars carrying many dominant genes. For the directional dominance, the location of points of varieties as a compact group in the central region of regression line distant away from points of its intersection with the parabola indicates that selection from such population allows to create the genotype carrying complete set of dominant genes. A direction of dominance is defined by the parameter $r[(Wr + Vr)_i; x_i]$, which characterizes the correlation between average parameter values in parents and the sum $(Wr + Vr)$.



Hayman's charts built upon the data on diallel analysis of inheritance of determinants the rate of coleoptile growth (A) **and duration of photosynthetic activity in leaves** (B) **in rice varieties:** *Vr* and *Wr* — respectively, variance and covariance; a, b, c, d, e, f — respectively, the cultivated varieties VNIIR 7718, Liman, Khazar, Nartsiss, Fontan, VNIIR 7887, g, h, k, l, m, n — Khazar, VNIIR 7718, Liman, Fontan, VNIIR 7887, Nartsiss; 1 — $Wr_i = Vr_i \times Vp$, where Wr_i — covariance between parameter values in hybrids and parental forms, Vr_i and Vp — variance of parameter values, respectively, in hybrids and parental forms; 2 — regression line (Vr, Wr).

Determination of growth rates of radicle and embryonic stem revealed polygenic nature of inheritance of these parameters. The low correlation between average value of these parameters in parents and the sum *Vr* (variance) + *Wr* (covariance) indicated a directional dominance, and, therefore, the population carries both dominant and recessive genes enhancing manifestation of determinants. The average direction of dominance in the studied population has been found as targeted toward increased manifestation of determinants. For the growth rate of radicle, an incomplete dominance of higher parameter value has been detected. For the growth rate of embryonic stem, the location of regression line on Hayman's chart (Fig. A) indicated the overdominance of increased parameter value. The effect of interloci interaction (complementary epistasis) was typical for inheritance of both determinants, but it was significantly weaker for the growth rate of embryonic stem. The genetic variance for this parameter in population is mainly determined by additive effect of genes. High correlation between the number of grains per panicle and growth rate of radicle (r = 0,98), as well as between the number of grains per panicle and growth rate of embryonic stem (r = 0,99) suggests the selection of hybrid combinations with high growth rates in early ontogeny stages as the method for obtaining of high-yielding forms. The high heritability (87-90%) of determinants providing growth rate in early stages of development allows to recommend the selection of these characteristics during the creation of source material.

Heterotic hybrids demonstrate higher photosynthesis rates during the phase of grain formation; so, the selection of high-yielding varieties and hybrids requires the study of duration of photosynthetic activity in leaves (18). It has been established the polygenic control of this parameter, as well as the dominance at inheritance directed towards its increased value, while a low correlation between the average parameter value in parents and the sum *Vr* (variance) + *Wr* (covariance) - $r[(Wr + Vr)_i; x_i] = 0{,}16$. This fact indicates a non-directed dominance, and, therefore, the population carries both dominant and recessive genes which control the increased manifestation degree of the determinant. The dominance in population is directed towards the enhancing of determinant expression, and the location on Hayman's chart of regression line (see Fig. B) corresponds to incomplete dominance of longer duration of photosynthetic period in leaves, which reflects the significant impact from interloci interaction (complementary epistasis). For this parameter, maximum dominant genes has been revealed in the varieties Khazar, VNIIR 7718 and Liman, the highest total combining ability – in the varieties Fontan and VNIIR 7887, the highest specific combining ability – in the combinations VNIIR 7718 / Nartsiss, Nartsiss / Fontan, Fontan / Nartsiss. The varieties Fontan, VNIIR 7887 and Nartsiss were found to carry mostly the recessive genes for increased photosynthetic potential of leaf surface, which allows using them in targeted breeding programs.

This is a well-known fact, that poor background of mineral nutrition smoothes varietal differences in yields since nitrogen deficit is the limiting factor. At increased and high backgrounds, the varieties showed different coefficients of economic efficiency of photosynthesis – a complex indicator of donor-acceptor relations in plants reflecting a high correlation between their productivity and responsiveness to use of nitrogen. The hybrids demonstrated better responsiveness to the fertilizer: in all the studied combinations, an increase in heterosis against a high mineral background has been found. At medium nutrition background, the heterosis for number of

spikelets per panicle was: the hybrid combination Serpantin / Belozernyi – 43,0; Kurchanka / Liman - 32,3; VNIIR 100999/VNIIR 10132 - 21,7; Pervotsvet / Regul – 21,8; Lider / Belozernyi - 17,7; Khazar / Krasnodarskii 424 – 14,8 and Kuban' / Nartsiss - 13,6%. At rich background, the greater heterosis values were observed: Serpantin / Belozernyi – 72,4; Kurchanka / Liman – 75,2; Pervotsvet / Regul – 53,2; Lider / Belozernyi - 23,2; Khazar / Krasnodarskii 424 – 45,6 and Kuban' / Nartsiss - 64,3%. The maximum advantage of hybrids over varieties has been established in ripening phase by their responsiveness to mineral nutrition level. It has been reported, that many hybrids demonstrate heterosis as quantity and duration of nitrogen consumption after flowering (19).

The content of chlorophyll allows to assess the development of photosynthetic apparatus and physiological state of plants, assimilation processes and contribution of assimilating organs in yield formation, as well as the potential of its formation and possibility of yield accumulation (20-22). A.A. Nichiporovich (23) concluded, that the increased chlorophyll content builds a pool that can be used in conditions more favorable for photosynthesis than those at which the photosynthetic apparatus had been initially formed. In the author's opinion, the increased chlorophyll content is one of compensatory mechanisms for homeostasis under stressful conditions. The study of chlorophyll a, b and carotenoids contents revealed polygenic inheritance of these parameters, as well as the presence in population of both dominant and recessive genes providing the increased content of pigments. The dominance in population was directed toward the greater manifestation of these determinants. The intraloci interaction (incomplete dominance of higher value) and a significant impact of interloci interaction (complementary epistasis) have been observed. A high correlation between contents of chlorophyll a and b (r = 0,91), chlorophyll b and carotenoids (r = 0,81), chlorophyll a and carotenoids (r = 0,95) have been established, which allows using in large-scale studies the chlorophyll meter SPAD-502 ("Minoltra", Japan) for detection of samples with high contents of pigments (the device measures total chlorophyll content without damaging a leaf).

The high productivity in crosses, or heterosis, is manifested owing to integration in one genotype of many favorable genes increasing plant viability and leveling the effect of lethal and half-lethal alleles by their transition to heterozygous state in F_1 hybrids. Heterosis isn't a special genetic event, but the highest adaptability. The best viability is manifested by hybrids with powerful adaptive heterosis with sufficient full realization of their genotype, which leads to leveling individuals' variability by quantitative determinants and raise of average values in general for each hybrid (24).

Thus, the inheritance of several determinants providing physiological basis of heterosis in rice hybrids has been revealed. It was shown, that resulting manifestation of these determinants depends on both intergenic and intraloci interactions, while the main role belongs to additive effects of genes. Polygenic nature of inheritance and non-directional dominance over all the studied parameters has been shown. For growth rate of embryonic stem, the overdominance has been observed, for other determinants - incomplete dominance of higher values. Main directions of breeding work on rice are suggested - the development of methods minimizing lethal genes and combination of multiple positive genes in one genotype. In this regard, a set of favorable genes can be obtained already in F_1 owing to dominance of most determinants providing increased adaptability of plants.

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