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APPLICATION OF MULTIDIMENSIONAL METHODS TO SEPARATE VARIETIES ON THEIR RESPONSE TO ENVIRONMENT FACTORS

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Abstract

Till now, the areas under rice crops are mostly occupied with the limited number of varieties. For enriching genetic biodiversity, it is necessary to improve selection of unique rice genotypes, and provide ecologically-based location of each variety. Now the efficiency of breeding is decreasing because of incomplete characterization of potentially donor genotypes. Presently, the domestic standards for competitive state trail do not cover a detailed study of the samples, since the developed varieties are tested at a single level of mineral nutrients with no estimation of a response to stressful influences and yield production sustainability. That leads to rejection of those highly productive samples for which such conditions are not optimal. In the present work we firstly summarized methods to comprehensively characterize adaptive plasticity of rice plants under contrast conditions (i.e. different dates for planting, various levels of mineral nutrition and stressors). In a multifactorial experiment with 19 combinations of the factors tested, we investigated yield variability in 24 Russian rice (*Oryza sativa* L.) varieties. The samples were planted on April 15, May 15, or June 15 and grown at optimum ($N_{120}P_{60}K_{60}$) and excess ($N_{240}P_{120}K_{120}$) fertilizer rates, in thin and dense crops (200 or 300 plants per square meter, respectively), under artificial salinization (0.35 % NaCl added to the soil at tillering). The data were processed using cluster and discriminant analysis. The multidimensional statistical methods allow us to clasterize the varieties into four groups with the closest characteristics as influenced by the full set of studied factors, and then to allocate distinct factors for the most precise discrimination between the samples. A standard cultivation was found to be less effective for developing plant plasticity. It is more correct to compare samples when the conditions are favorable for plant performance and productivity potential. Stresses, in combination with favorable factors, contribute to an increase in trait variability and dispersion, resulting in more accurate dividing varieties into groups. In our case study, with the use of «step-by-step analysis back» module we reduced the number of discriminating factors to two ones adequate for 100 % reliable allocation of typical representatives of the groups. High mineral levels and water deficit were enough to truly classify 88 % of the samples. This is sufficient in genetic research where it is necessary to select the most typical representatives. Samples of the groups 1 and 3 have been classified correctly, and only three varieties of the group 2 have got to another cluster. The discriminant analysis also shows distance of each variety from the center of the group. Samples with the minimum distance are the most typical representatives which can be used as genetic sources of desired traits, as contrast parental forms in hybridization, or involved in marker-assisted selection and GTL mapping. Early planting, dense crops, high fertilizer rates, and lack of water were the factors which mostly influenced on the clear separation of the samples into clusters according to how the varieties responded to external environment. The virtual «ideal variety» (a model) and Kurchanka variety were grouped in the same cluster, and the varieties from the group 1 were close to the «ideal variety» on the response to environment. Despite high yield production, the dispersion in the group 3 which includes Kurchanka and the model variety was 3 times as much as in other groups. Therefore, stability of the varieties was lower in this cluster (group 3) as compared to the first and the second clusters (groups 1 and 2).

Keywords: rice, *Oryza sativa*, multidimensional methods, claster analysis, estimation of breeding material, discriminant analysis

Currently the areas under rice crops are mostly occupied with the limited number of varieties. For enriching their genetic diversity, it is necessary to improve effectiveness of evaluation and selection of unique genotypes, and provide ecologically based location of each variety [1-3]. Apart from that, the efficiency of breeding is decreasing because of incomplete characterization of parental material. Existing system does not provide for a detailed study of samples provided for competitive variety testing [4-5]. Productivity of the developed varieties is tested at a single level of mineral nutrition, which leads to rejection of those highly productive samples for which such nutrient status is not optimal. This approach prevents identification of potential yield production of a sample, norm of reaction of a variety to stressful influences and donors of high functional activity of genetic systems, determining productivity and adaptability [6-7], and does not provide for evaluation of stability of the developed forms [8-10]. As a result, the most valuable forms, which has taken years of selectionist efforts, does not find a use.

Potential productivity of rice, similar to many other crops, has been increasing very slowly since the latter half of the 20th century. Further increase in rice production may be achieved by intensification or promotion to regions with lower or higher temperatures, and areas with salinized or flooded soils [11-13]. Climatic changes will cause increase in average temperatures by the mid-21st century. Apart from that, short-term temperature variations, not common to the regions will be observed more often (climatic changes in tropical zone have already resulted in decreased rice yields). Temperature increase by 1 °C leads to decrease in yields by more than 10 % [14, 15]. Due to sea level rise expansion of territories with salinized soils is forecasted [16-18]. Thus, not only potential productivity shall be improved, but stability of yields and comprehensive stress resistance shall also be ensured in order to increase the crop production [19, 20]. Varieties with high potential productivity are more vulnerable to abiotic stress factors. That's why plant growing in many countries is focused on optimal and stable yield production, rather than on maximum yields [21-23].

Evaluation of plant stability is normally performed during growing in various environmental conditions or using contrast agrotechnical approaches [24-26]. However, stress levels (salinization, high or low temperatures) are hardly ever used in experiments [27-28] and contrast planting dates are rarely studied [29-31]. This significantly depletes the information obtained.

Mathematical processing is normally performed using analysis of variance and regression analysis [32-34]. Multivariate statistics methods for statistical evaluation of results have not been used till recent time [35-37]. Their introduction in breeding material and promising variety studies will ensure more effective identification of samples suitable for a broad cultivation area [38-40]. Multivariate statistics methods allow grouping of samples with the most similar responses to impact of various factors, determination of environmental conditions ensuring the most accurate differentiation of samples and requiring minimum expenditures for experiment conduct [41-43], exclusion of less informative variants and identification of minimum number of variants ensuring 100 % reliable assignment of a sample to the respective adaptability group [44-46].

In this work we have developed a breeding material evaluation system, including contrast planting dates, various mineral nutrition and stress levels, based on the fusion of previously proposed methods. Application of multivariate statistics methods for processing of results has significantly increased the informative value of the data.

The study was aimed at grouping of Russian rice varieties by the response to a complex of environmental factors and determination of conditions ensuring the most effective performance of such grouping.

Techniques. A total of 24 Russian rice (*Oryza sativa* L.) varieties were investigated in a multifactorial experiment (established at the growing site of

All-Russian Research Institute of Rice in 2004-2006). The plants were grown in lysimeter experiments at optimum ($N_{120}P_{60}K_{60}$) and increased ($N_{240}P_{120}K_{120}$) mineral nutrition levels; with spaced (200 plants per 1 m^2) and close (300 plants per 1 m^2) planting; with various planting dates (April 15, May 15, June 15); at salinization created artificially during the tillering phase by introduction of NaCl into the soil up to the concentration of 0.35 %; under lack of moisture (watering was ceased during the paniculation phase). The sample included 30 plants of a variety per the experiment variant. Planting was performed in single-row plots, 10 plants per row; the distance between the rows was 10 cm. The experiments were performed in triplicate, with randomized location of plots.

Productivity (grain weight per plant) was evaluated in all plants in the experiment. Growing of plants at optimum mineral nutrition level ($N_{120}P_{60}K_{60}$) with spaced (200 plants per 1 m^2) planting performed on May 15 served as a control in all variants. The seeding rate was 200 plants per 1 m^2 in all cases, except for close planting.

The data were processed using cluster and discriminant analysis and Statistica 6.0 software (StatSoft, Inc., USA) [36, 37, 39].

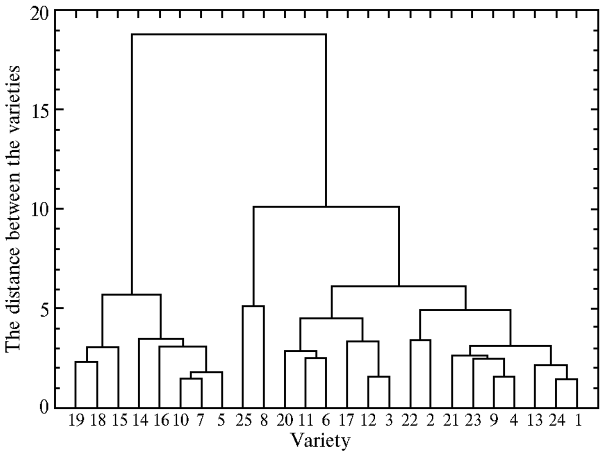

Fig. 1. Clusterization of Russian rice (*Oryza sativa* L.) varieties by the response to environmental factors based on productivity evaluation (Warda method)**:** 1 — Ametist, 2 — Boyarin, 3 — Dalnevostochnii, 4 — Druzhnii, 5 — Zhemchug, 6 — Izumrud, 7 — Kasun, 8 — Kurchanka, 9 — Lider, 10 — Liman, 11 — Pavlovskii, 12 — Primorskii, 13 — Rapan, 14 — Sadko, 15 — Serpantin, 16 — Snezhinka, 17 — Sprint, 18 — Strelets, 19 — Fakel, 20 — Fontan, 21 — Khazar, 22 — Jupiter, 23 — Yantar, 24 — group average, 25 — model (ideal) variety.

Results. Our experiment ensured analysis of organization of genetic systems, determining attraction of photosynthesis products from stem and leaves to panicle, their microdistribution between grain and chaff, effectiveness of soil nutrition, resistance to close planting, salinization and lack of moisture, variability of ontogenesis durations [1, 2]. Using cluster analysis of the data on productivity of varieties in all variants of the experience they were divided into 4 groups with different response to the studied environmental factors (Fig. 1).

During the next stage evaluation of significance of inter-group differences by the response to a complex of growing conditions was required, using discriminant analysis. Discriminant analysis is based on plotting of trait-function linear combinations, where each trait or experiment variant has its coefficient (contribution). We have plotted three discriminant functions using Statistica 6.0 software for separation of groups. The first two functions considered more than 97 % of initial dispersion of the experiment variants. Thus, the values of the remaining function could be omitted in solving of further problems, as it considered less than 3 % of dispersion.

Evaluation of significance of discrimination of variety groups, performed using χ-square test, has demonstrated that only the first discriminant function is effective for their separation. The possibility of absence of inter-group differences for this function was below the significance level permissible in biological studies ($p < 0.05$). However, even with the use of the first discrimination function, uniform grouping of varieties by stability of productivity in various environmental conditions was not achieved in all experiment variants.

Suitability of the experiment variants for identification of intervarietal differences was also evaluated using discriminant analysis (Table 1).

1. Standardized coefficients of discriminant functions during evaluation of differences between the studied rice (*Oryza sativa* L.) varieties by the response to environmental factors

Environmental conditions (experiment variant)	First discriminant function	Second discriminant function
Early planting, 2004	0.12	–0.48
Close planting, 2004	0.57	–1.21
Late planting, 2004	0.55	0.81
Optimum planting date, 2004	0.54	–1.56
Salinization, 2004	–0.27	3.82
High mineral nutrition level, 2004	–0.38	–1.12
Early planting, 2005	0.06	0.09
Salinization, 2005	0.88	–0.07
Late planting, 2005	0.54	–0.18
Close planting, 2005[a]	–2.63	–1.24
Lack of moisture, 2005[a]	–2.16	–1.09
High mineral nutrition level, 2005[a]	–3.03	0.41
Optimum planting date, 2005	0.26	0.11
Early planting, 2006[a]	1.98	2.93
Optimum planting date, 2006	0.35	1.45
Close planting, 2006	–0.40	–2.20
High mineral nutrition level, 2006[a]	–1.36	–0.48
Late planting, 2006	–0.89	–0.85
Salinization, 2006	0.18	1.98
Total percentage of considered dispersion	91	97

N o t e. [a] — the experiment variants with minimum contribution to inter-group differences.

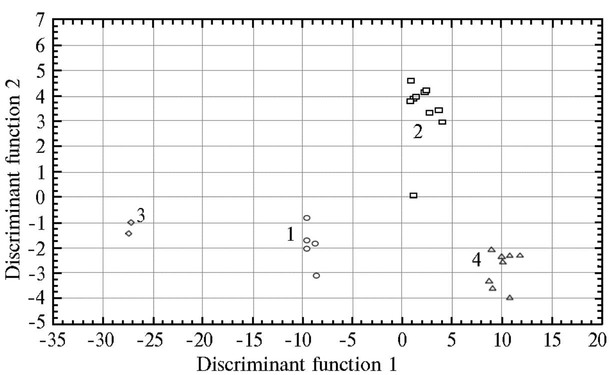

Fig. 2. Cluster distribution of Russian rice (*Oryza sativa* L.) varieties in two discriminant functions by the response to all studied environmental factors: 1 — first cluster (G_1:1), 2 — second cluster (G_2:2), 3 — third cluster (G_3:3), 4 — fourth cluster (G_4:4). Similarity measure is Mahalanobis distance (D^2).

Absolute values of standardized coefficients of discriminant functions allow determination of contribution of a certain variable or the experiment variant to the final function and their role in inter-group differences. In our case maximum absolute values were observed for five experiment variants with maximum contribution to separation of groups by the response to environmental conditions: early planting in 2006, high mineral nutrition level in 2005 and 2006, close planting and lack of moisture in 2005. It should be noted that standard growing conditions during all years of the study allowed differentiation of samples to a lesser extent, which was confirmed by relatively low absolute values of coefficients of discriminant functions.

Evaluation of similarity of the response to environmental conditions of the selected groups by means of calculation of distance between their centroids was one of the discriminant analysis results. Mahalanobis distance (D^2) acted as a similarity measure; it was the largest between the first, the second and the fourth groups, which indicates the most significant genetic differences between them (Fig. 2).

A variety model with maximum grain weight per plant in all studied experiment variants was also included in the analysis for identification of a group of varieties with maximum productivity. The model (ideal) variety and Kurchanka variety were grouped in the same cluster. Varieties of the first group were the most similar to the model variety in terms of productivity in all experiment variants, i.e. demonstrated the most stable high productivity in the studied conditions. The first group included varieties Dalnevostochnii, Izumrud, Pavlovskii, Primorskii, Fontan; the second — Ametist, Boyarin, Druzhnii, Lider, Rapan, Sprint, Khazar, Jupiter, Yantar; the third — Kurchanka variety and ideal variety model; the fourth — varieties Zhemchug, Kasun, Liman, Sadko, Serpantin, Snezhinka, Strelets, Fakel. Evaluation using F-test has demonstrated significance of differences between the third and the fourth groups (clusters) (null hypothesis probability 0.035), as well as between the first and the fourth groups (null hypothesis probability 0.048). The differences between the first and the second group of varieties were insignificant.

Absolute values of standardized coefficients of discriminant functions not only allow determination of contribution of a certain variable to this function, but also ensure reduction of the number of studies variants. For example, if an absolute value of standardized coefficients of discriminant functions of a variable (the experiment variant) is small, it can be excluded from the analysis. This procedure is referred to as determination of informative list of traits. We used it for determination of environmental conditions ensuring the most comprehensive identification of inter-group differences in case of reduction of the experiment variants.

The most significant reduction of the experiment variants ensures backward step-by-step analysis which we used to solve the problem. It has been established that even two variants of the experiment allow reliable grouping of varieties by the response to environmental conditions. Maximum high productivity of samples was obtained in 2005 in variants with high mineral nutrition level and lack of moisture, as compared with the value in 2004 and 2006. Thus, environmental conditions favorable for implementation of variety productivity potential allow correct comparison of samples. Stress development against the background of other favorable factors expands the range of trait variability and dispersion in the experiment, which results in more reliable grouping of varieties.

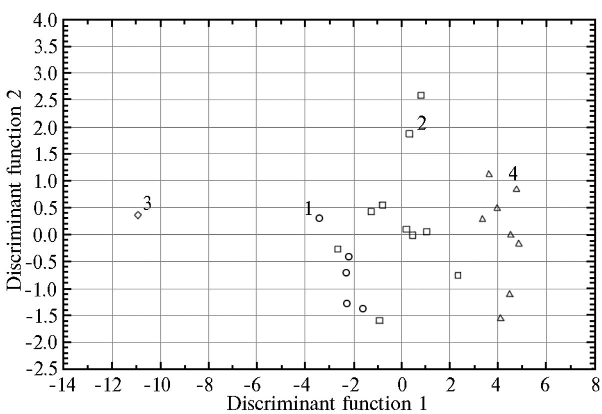

Fig. 3. Cluster distribution of Russian rice (*Oryza sativa* L.) varieties in two discriminant functions by the response to high mineral nutrition level and lack of moisture: 1 — first cluster (G_1:1), 2 — second cluster (G_2:2), 3 — third cluster (G_3:3), 4 — fourth cluster (G_4:4).

Genetic studies normally require identification of the most typical representatives of each cluster (group). In this case assignment of borderline samples to a wrong group does not impair the work effectiveness. Thus, our task is maximum reduction of the number of experiment variants and to preserve accuracy of identification of typical cluster representatives at the same time.

According to the results of clustering of the studied rice varieties using two selected experiment variants (high mineral nutrition level and lack of moisture), 88% of samples were classified correctly, which is acceptable for genetic studies. At that, complete match with the evaluation results for all experiment variants was observed in the first and the third groups; only three samples from the second group were assigned to another cluster (one to the fourth group, and two to the first group) (Fig. 3, Table 2).

Discriminant analysis also demonstrates the distance between each variety and a group centroid. Samples with minimum distance from centroid are the most typical group representatives, which can be used as the studied trait gene sources or contrast forms during selection of pairs for hybridization or molecular marking and quantitative trait gene localization.

2. Correctness of assignment of rice (*Oryza sativa* L.) varieties to clusters (groups) during discriminant analysis with reduced number of the experiment variants against the initial value

Group	Percentage of correct assignments	G_1:1	G_2:2	G_3:3	G_4:4
G_1:1	100.0000	5	0	0	0
G_2:2	70.0000	2	7	0	1
G_3:3	100.0000	0	0	2	0
G_4:4	100.0000	0	0	0	8
Total	88.0000	7	7	2	9

N o t e. G_ — group of samples, G_1:1 — the number of samples in the first group (during analysis of all experiment variants), assigned to the group in case of reduction of the number of analyzed variants during discriminant analysis.

3. The experiment variants identified during forward step-by-step analysis, joint application of which ensures reliable assignment of rice (*Oryza sativa* L.) variety to groups with different adaptability and stability of productivity

Variant	F	p
High mineral nutrition level, 2005	53.856	0.000
Lack of moisture, 2005	4.736	0.021
Early planting, 2004	3.855	0.038
Optimum planting date, 2005	3.089	0.068[a]
Late planting, 2006	2.069	0.158[a]
Close planting, 2005	4.271	0.029
High mineral nutrition level, 2006	2.198	0.141[a]
Optimum planting date, 2004	1.638	0.233[a]
Close planting, 2006	1.179	0.359[a]
Salinization, 2004	1.011	0.422[a]

N o t e . [a] — variants with unreliable discrimination of samples; F — *F*-test, p — possibility of absence of intergroup differences when using the experiment variant.

In order to obtain more accurate results, for example, for development of data bases on the contribution of genetic systems to productivity or adaptability, the number of the experiment variants shall be increased up to the values ensuring reliable separation and classification of samples. This task can be performed using forward step-by-step analysis, which reduces the number of experiment variants to a lesser extent. Application of this analysis allowed us to identify ten variants, ensuring 100 % effective assignment of samples to groups with different adaptability and stability of productivity (Table 3).

Thus, we have established that standard growing conditions allow differentiation of rice variants by the response to environmental factors to a lesser extent. Experiments ensuring more significant range of trait variability in case of external effects shall be established for determination of sample stability and plasticity. High mineral nutrition level, close planting, lack of moisture during the maturation phase and early planting acted as such conditions in our study. Varieties Dalnevostochnii, Izumrud, Pavlovskii, Primorskii, Fontan, Ametist, Boyarin, Druzhnii, Lider, Rapan, Sprint, Khazar, Jupiter, and Yantar (the first and the second clusters) have demonstrated the greatest stability of productivity during discriminant analysis, and we recommend to use them as sources for the trait “stable high productivity in various environmental conditions”. Varieties Kurchanka, Zhemchug, Kasun, Liman, Sadko, Serpantin, Snezhinka, Strelets, and Fakel (the third and the fourth clusters) were less stable, notwithstanding that a model ideal variety was assigned to one of the groups during clustering.

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