14

"We do not phase out breeding as an art, but to ensure confidence, quick response and consistency of operations, we need well-developed consistent specific breeding theory. The team cannot work intuitively, using strikes of luck "

N.I. Vavilov

Teor. osn.selektsii, 1935. Vol. 1. P. 5. (in Russian).

# Epigenetics and constructing of breakthrough plant varieties with maximal yields

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Based on the discovery of a new epigenetic phenomenon (during the implementation of the DIAS program) — a change in the spectrum (set) of gene products under the trait of productivity when the limiting environmental factor changes - a theory of ecological-genetic organization of a quantitative trait was created and developed, from which 20 new biological, quantitatively predictable, consequences, 10 know-how and 16 innovative technologies for designing breakthrough plant varieties. With the use of these technologies, 4 varieties of grain crops have been created, giving a high economic effect. The physical instruments and equipment necessary for more efficient creation of new productive varieties are described. Key words: plant breeding, epigenetics, theory of ecological-genetic organization of plant traits, phytotron for breeding.

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### Introduction

During the period from 1972 to 1982, on the initiative of acad. D.K. Belyaev (Director of the Institute of Cytology and Genetics (ITsiG), Siberian Branch of the Russian Academy of Sciences (SB RAS), Novosibirsk Akademgorodok) with very strong support by acad. M.A. Lavrentiev, Chairman of the Siberian Branch of the Russian Academy of Sciences, the DIAS (diallel crossing) Interagency Cooperative Program was established to investigate production trait genetics of spring varieties of wheat selected by the Siberian plant breeders from the global list which are the most promising for yield gain in Krasnoufimsk (Cis-Ural region)-Ivolginsks (Transbaikal, Buryatia) - from west to east, and Tyumen (North. Trans-Urals)-Ust-Kamenogorsk (North. Kazakhstan) — from north to south. The program was ful'lled by more than 100 researchers from two SB RAS research institutes (ITsiG and Computation Center) and 8 breeding centers of the All-Union Academy of Agricultural It was the first wide study of production Sciences. trait genetics implemented in the USSR using a set of 15 thoroughly selected parental varieties of spring wheat and the world's first application of genetic diallel analysis methods in such geographically huge area. V.A. Dragintsev,

Head of Laboratory of Genetic Basis of Plant Breeding, ITsiG SB RAS, was appointed as the DIAS Program Director by the Executive Committee of SB RAS.

A database of measured production traits was collected and contained about 5 million values of 15 parental varieties and 210 first-generation hybrids. For each plant, 13 production traits were measured (during two years). Behavior of the main yield limiting weather factors during vegetation were analyzed in each point. Genetic analysis was carried out using computers of the SB RAS Computation Center (CC) using customized priority software developed by the SB RAS CC personnel.

## 1. About epigenetics in plant breeding

The genetic diallel analysis carried out from 1982-1984using the SB RAS CC computers made it possible to discover a new phenomenon — a change in the spectra of gene products determining the production traits when the limiting environmental factor changes [1,2]. Two categories of control mechanisms have been known before gene expression regulation and protein secretion regulation systems. Now there are three groups of mechanisms which possibly completely determine epigenetic shifts of intracellular reactions and adjustment of gene product spectra to the new limiting environmental factor. Based on this discovery from 1984–2014 our scientific school (35 Candidates of Sciences and 12 Doctors of Sciences by 2017) developed a theory ecological-genetic organization of quantitative traits (TEGO) — plant breeding theory for yield gain which was dreamt of by N.I. Vavilov [1–3]. The main fundamental result of TEGO is: "For a production trait (PT) susceptible to "genotype-environment interaction", no stable ("passport") characteristics can be obtained for different environments". When the limiting environmental factor is changed, the set (spectrum) of gene products determining the PT value is changed so that any PT has, figuratively speaking, "floating genetics" in its

basis" during its development. TEGO has defined the existing global ecological plant genetics level in all countries for further 10-15 years. It can be seen that the Russian top-priority scientific and technological framework has been provided to transfer the "Plant breeding for yield gain" industry from the third wave of innovation to the sixth wave of innovation.

Traditional genetics - Mendelism - describes only such inheritance phenomena when a major Mendelian gene (oligogene) has a definitive (railway) "gene-trait" pathway. In early 20th century, genetic scientists in all countries were sure that Mendelism was able to describe all 100% inheritance phenomena. However, evolvement and rapid development of a new general biology branch epigenetics [4] ("epi" means "above", i.e. epigenetics — in Russian is "above genetics") have shown that Mendelism can describe only about 10% of inheritance events, epigenetics describes 40%, and 50% are described by biosymmetrical inheritance — development of symmetrical structures controlled by biofields postulated by prof. Gurvich (their nature is still unclear). But since no people with two left hands or two right legs are observed in the human heterozygous population (while such combinatorics is required by Mendel's laws), this means that biosymmetrical inheritance is fundamentally different from traditional Mendelism.

Discovery of "floating" genetics behind production traits has proved that PT determination is of epigenetic nature rather than of genetic nature, there is no definitive (railway) "gene-trait" pathway between genes and PT values, change in gene product spectrum behind the trait takes place at the "body-environment" level rather than at the molecular level, and the reason of this phenomenon is a change in the previous limiting environmental factor to a new one. It is evident that genome editing methods which are quite suitable for microorganisms (very short "gene-end gene product" pathway) or warm-blooded animals and humans having tissue temperature and moisture homeostasis are not suitable for plant PT control. It is evident that prospects of marker support (DNA markers) for selection are quite doubtful, since no any stable set of DNA markers can be assigned to "floating" genetics behind any PT. It is evident that specific PT genes which a sought for by hundreds of genetic scientists (and strange as it may seem - they

find them) — QTL — do not exist at all [3]. For drought, PT are "supported" by drought-resistance genetic physiological systems (GPS), for cold — by cold-resistance GPS, for heat — by heat-resistance GPS, and for acid soil — by GPS of soil pH resistance. This causes change in production ranks in any set of varieties grown on the same field in different years or in different zones in one year. Phenomenon of change in production ranks in a set of varieties is referred to as "genotype-environment interaction" (GEI). It was discovered and described long ago, but there has been no any hypothesis on the nature of GEI in three genetics branches (Mendelism, biometrical and molecular) until recently. Recently, we succeeded to prove experimentally that the GEI phenomenon is of epigenetic nature --sets (spectra) of gene products "behind" PT change with a change in the limiting environmental factor [5].

TEGO gave 24 new biological consequences with precise quantitative forecasts [3], 10 powerful breeding know-how and 16 innovative technologies for designing breakthrough plant varieties in terms of yield gain and quality [6].

# 2. Innovative design technologies for breakthrough varieties derived from TEGO

1) Reliable identification technology for the best genotypes in terms of production in case of individual selection in cleavable hybrid generations beginning from  $F_2$  (or in wild populations). Before TEGO, fundamental opportunity of quick (without generation change) identification (recognition) of genetically best plants by their phenotypes in cleavable generations was strongly denied by the following statements.

- Professor N.P. Krenke [7, p. 167] noted: "Beginning from the first development stages, there is no constantly specific phenogenetic expression for modifications and inherited traits".

- Professor N.A. Plokhinsky [8, p. 5] emphisized: "For a single speimen, it is useless to define which part of its phenotype is caused by heredity and which part is caused by living conditions. Genetic information obtained by an individual is implemented in such interaction with living conditions when both causes are inseparable from each other".

– Professor W. Williams [9, p. 350] stated: "In production of one organism, it is impossible to separate genetic and external effects on traits with poor heritability and selection in  $F_2$  is unreliable".

– Z.S. Nikoro, Head of laboratory of ITsiG, SB RAS, et al. [10, p. 300] expressed their regret: "To assess the genotype, we need to know a genotypic value of the trait, however, there is no method that allows separation of a genotypic value from environmental one for each individual specimen".

- Academic P.F. Rokitsky [11, p. 200] wrote: "Specimen phenotype — is a single integral organism. We cannot

estimate a specimen genotype directly without progeny testing".

- Professor V.K. Savchenko [12, p. 19] supposed: "It does not seem possible to isolate the effect on the genotype and environment trait development directly for each organism ".

Experimental assessment by our scientific school of traditional visual selections by phenotypes has shown that only one plant from 1000 selected plants with the best phenotypes (for selections in light drought conditions) was genetically valuable, i.e. genotype "recognition" accuracy by phenotype was about 0.001 [13]. In such situations, random selection may be more effective than selection by the best phenotypes. It seems that the statements of the six leading phenogenetic and genetic scientists are true.

However, the first TEGO consequence — background trait principle (BTP) —has theoretically and experimentally denied these statements [14,15] and created quick (without generation change) genotype identification methods for any production trait of an individual specimen in a segregating population with an accuracy up to 1000 times higher than the traditional visual "recognition" of a genetically valuable specimen [16].

Further development of BTP and discovery of seven genetic physiological systems actually exceeding the new variety yields gave rise to a bidirectional ("orthogonal") identification principle (BIP) [17], which allowed, first, to identify quickly (without generation change) positive genetic contributions of each of seven genetic physiological systems (GPS) into production of any specimen, second, to phase out traditional low-efficient visual selections by phenotypic values of traits (this has been and is being done by all breeders globally for centuries), and, third, to use production traits as special two-dimensional coordinates where all "noises" (environmental, competitive genotypic and competitive environmental) shift the variety point on the positive regression line, and a valuable positive GPS shift, e.g. attractions - on the negative line ("orthogonality" effect). In this case, the valuable contribution in the yield of any GPS is "cleaned" from masking effects of all noises and is manifested (and measured) with absolute accuracy. Background trait principle and bidirectional ("ortogonal") identification principle for selections in forest breeding assess a genotype of any individual tree within 3-4 minutes in forest populations, while for assessment by seed progeny of "positive" trees selected by phenotypes it is necessary to wait 10-20 years.

2) Technology of elimination of large errors of visual individual selection methods (even using BTP and BIP) in off-type years for a certain breeding zone. A breeder tests a collection of initial varieties in the collection nursery for selection in its zone during at least 3 years, by choosing the best parents. On the 4th year, the breeder sets these parents in the hybridization nursery, crosses them and obtains first filial generation seeds  $F_1$ . On the 5th year, the breeder sets seeds  $F_2$  on the 6th year, the breeder has grown seeds  $F_2$  and selects the best phenotypes visually. But if the selection

which will not give the maximum yield in typical years, will show better production. If the breeder selects them in the off-type year, he/she will make a large mistake, because the selected genotypes will "subside"in the typical year, while the best genotypes available in the population for the typical year - will be basically lost (after selection in off-type year), because it is impossible to reseed all material of all families F<sub>2</sub> into F<sub>3</sub>, F<sub>4</sub>, etc. As a result, 6 years of hard breeding work are often go to waste [1]. It is off-type year selections which enable a variety created in one geographical point to be released hundreds (even thousands) kilometers from the place of breeding, after testing in the National Variety Network system. Thus, in recent years, Krasnoufimskaya 100 variety created in the Urals was released in the Leningrad region; varieties from Odessa were cultivated in the Lipetsk region; Saratovskaya 29 created in Saratov was set out in the Kazakhstan virgin land and Western Siberis; Kharkovskaya 46 variety occupied Altai, rather than the Kharkov region; Sweden Rang variety occupied huge areas in the Tyumen and Omsk regions in the 1970s. This is indicative of low resolution of traditional field selection methods which can be avoided, if selections are carried out in F<sub>2</sub> and next generations using our innovative technologies both in the field and Breeding Phytotron where typical limiting factor behaviors may be easily created for any breeding zone on Earth by handle rotation. 3) Technology of breeding for yield homeostasis (for

year was off-type for this selection zone, other genotypes,

variety "flexibility" increase) in several years in one geographical point or in one year in different point. Before TEGO, the nature of yield (variety flexibility) homeostasis was unknown. Breeding for yield homeostasis increase was carried out by trial-and-error method ("hit-and-miss method) with huge time consumption and cost. The theory of ecological-genetic organization of quantitative traits deciphered a previously unknown ecological-genetic mechanism of yield (variety flexibility) [1] and made it possible to create a priority breeding technology of variety flexibility increase [18].

4) Technology of breeding for increase in droughtresistance of new varieties. Economy of the Russian Federation loses 7-8 billion Rubles due to insufficient drought-resistance of grain and leguminous varieties. The same situation also takes place in other countries. Thus, in 2003 in Australia, wheat production reduced from 24 to 9 million tons or by 62.5% due to drought. Traditional breeding methods - pair, diallel and other crossing schemes and visual selections in drought conditions - are not able to increase hereditary drought resistance with at least 22 component traits contributing to it and each of them is determined by a number of genes from 10 to 9000 [19]. Priority phenotyping created within TEGO ("Russian phenotyping", this is how it is referred to at International Phenotyping Forums) enabled a new nonclassic high technology to be created for hereditary drought resistance increase in the Breeding Phytotron [19].

5) Efficient technologies of breeding for increase of coldresistance and heat-resistance in new varieties. The priority phenotyping derived from TEGO enabled new innovative breeding technology to be created for hereditary increase of cold- and heat-resistance of new varieties bred in the Breeding Phytotron with successive resistance increase for each ontogenesis phase (e.g. wheat has — 12 ontogenesis phases) [20].

6) Technologies for prevention of undesired negative genotypic correlations between economy-critical properties and issues of forecasting positive genotypic correlations between easily observed trait and difficult-to-record invisible trait. The theory of ecological-genetic organization of quantitative traits was the first to explain ecological-genetic mechanisms of genotypic correlation sign and value shifts in various environments and created variation forecast methods depending on the environment [21].

7) Technology for acquiring new knowledge on the nature of transgression by production and yield. Transgressions — are the main leverage to increase self-pollinated plant yield. Understanding of their nature is required for forecasts and reliable parental pair mating algorithms. Many genetics textbooks describe combinatorial genetic hypothesis of transgressions:  $AAbb \times aaBB = AABB$  (in case of directional dominance, genotype AABB will exceed the best parental variety in terms of production). The theory of ecological-genetic organization of quantitative traits has denied this hypothesis which prevented forecasting of transgression occurrence in F<sub>2</sub>, deciphered the ecological-genetic nature of transgressions and created transgression forecast methods and parental variety crossing technologies [21] to reduce annual amounts of crosses (each breeding center generally performs 1000 and more crosses) — up to 5-10 crosses. There are about 40 breeding centers in the Russian Federation now, therefore reduction of the amount of crosses by a factor of 100 and more will significantly reduce the cost and increase the breeding performance for yield gain.

8) Technology for acquiring new knowledge on the nature of cross-pollinated and self-pollinated plant heterosis and problems of creating new pair selection algorithms in breeding for heterosis. The theory ecological-genetic organization of quantitative traits has supported the opinion of Yu.N. Ivanov: "Heterosis - is a phenomenon without any genetic theory behind it. It is rather physiological than genetic phenomenon. None of the elegant heterosis theories have survived; expectations of heterosis effect in hybrids failed disastrously, but this was hushed up" [22, p. 367]. The theory of ecological-genetic organization of quantitative traits has deciphered the epigenetic (ecologicalgenetic) mechanism of ecologically dependent heterosis, created innovative heterosis forecasting technology and experimentally demonstrated potential heterosis effect for spring wheat production — more than 100% from the best parent [23]. This new knowledge may be used as a basis for addressing the problem of high-yield hybrid wheat (before

our research work, the maximum heterosis effect for wheat production did not exceed 10%).

9) Technology for acquiring new knowledge on the nature of a phenomenon essential for breeding — "genotypeenvironment" interaction (GEI). GEI - is a change in production ranks in a set of varieties cultivated in different environments (in different years in one geographical point or in one year in different points). From 1905 to 1918 K. Pearson, C. Spearman and R. Fisher [24-26] offered quantitative methods ("footrules") for measuring GEI effects - rank correlation coefficient and two-way analysis of variance that are still used successfully. However, the nature of GEI was unknown till 2010. TEGO gave a hypothesis on ecological-genetic nature of GEI which was experimentally confirmed by us, thus, the nature of GEI has been completely deciphered by now. This allows to forecast GEI effects for any environments and, for genotype introduction in new environmental conditions - to know in advance the "portrait" of this genotype (variety) in a new environment before experimental transfer into the new environment [5].

10) Technology for precise evaluation of the ecologicalgenetic potential of yield gain in diallel crossing of a specific set of varieties. Based on TEGO and using the huge production trait measurement (about 5 million values) database (DIAS program), innovative algorithms and computer software were developed to ensure quantitative evaluation of potential hereditary yield excess of a future variety over the yield of the best variety from the selected set of varieties for any geographical points [1].

11) Technology for acquiring new knowledge on all possible yield gain "levers" in breeding process. The theory of ecological-genetic organization of quantitative traits has shown that there are 4 main yield gain levers for new varieties [4]: 1) limiting environmental factor dynamics typing for each breeding zone in order to reproduce typical dynamics of typical years in the breeding phytotron in future; 2) precise genotype identification in selection against typical limiting environmental factor dynamics (there is an alternative: either, for field breeding, to wait for a typical year to come and avoid selections in off-type years, being swamped with huge amounts of second, third and other generation families, or to create typical year limiting environmental factor dynamics in the breeding phytotron for any geographical point and perform selections in the phytotron); 3) "introduction" of a specific variety into the critical ontogenesis phases - genetic stressor-resistance systems ("phase by crossing selection"). This lever is capable of improving yields by 20-30%; 4) removal of genetic limits in the daily physiological process dynamics. Thus, the extension of normal "operation" of physiological systems by 2h per day will provide, during 100 days, biomass gain which is provided by a more late-maturing (by 9 days) variety, Total potential ecological-genetic yield i.e. 20 - 30%. gain of spring wheat in Western Siberia - 50-70%; in the European part of the Russian Federation — 60-80%. This potential can only be implemented using a breeding phytotron [27].

12) Technology for quick evaluation (without generation change) of genetic (additive) production trait dispersion. Application of BTP and BIP in breeding technologies allows to evaluate genotypic production trait dispersion very precisely, however, this dispersion is of a complex nature: it consists of interallelic interactions in loci (dominance and superdominance), interlocus interaction effects (pair epistases) — complementary and duplicate) and multilocus epistases. Genetic (additive) dispersion is caused by variability of only additive gene contributions. Since genetic improvement of self-pollinated plants takes place by means of accumulation of positive additive genes only, breeder shall understand not only genotypic dispersion, but also a much more important — genetic (additive) dispersion. Before TEGO, additive dispersion has been evaluated either by "parent-child"correlation, or by relatives correlation (sibs and half-sibs). These evaluations require generation change, i.e. higher time consumption, however, potential genetic production increase level of the future variety cannot be forecast. The theory of ecological-genetic organization of quantitative traits offers quick evaluation (without generation change) additive dispersion principle by the parental genotype response "similarity" degree in ecological gradient [28], which allowed quantitative forecasting of production gain in the future variety.

13) Theory and technologies for formation of optimum selection indices for different limiting factor dynamics in different breeding zones. Yu.A. Filipchenko [29, p. 38,39] emphasized: "Based on my experience, I shall warn all researchers of quantitative trait inheritance against indix application — if not absolutely, but in vast majority of cases. Only in very few cases, the index method gives something more than the use of only absolute values... In vast majority of cases, ignorance of absolute values during clarification of the inheritance process may cause only confusion and errors". However, plant physiologists study only quantitative traits (genetic scientists also study qualitative traits) and only in the forms of indices. Photosynthetic or transpiration rate in absolute amount are of no sense (compared with "ear weight" or "number of spikelets per ear"). Physiologists calculate these intensities per cell, per unit leaf area, per unit leaf weight (wet or dry), per number of chloroplasts, But relation of two traits — is the index itself, etc. so the use of indices in plant physiology - is a usual and widespread procedure. So, why do indices, when investigating quantitative trait genetics, cause "confusion and errors", while indices of the same quantitative, but physiological trait are widely spread, moreover no study of physiological process is possible without them at all' TEGO gave a new selection index theory - NSIT [16], which interlinked the index information content with environment limiting factors. Thus, selection by the "index of attraction" - ratio of ear weight to culm weight of the main stem of one plant — in wheat breeding in North India (in watering, optimum mineral nutrition, optimum

temperature and illumination conditions) will reflect genetic differences of plants by attraction GPS quality and selection by this index will give a new variety with the best attraction systems. But if we perform selections by the same index in Saratov (in drought conditions), then the genotype having the best drought resistance genes will have the "ear weight", and "culm weight"increased in parallel and the "index of attraction" will remain unchanged. Selection by this index in Saratov will lead to loss of the most valuable drought resistant genotypes. In Saratov, selections shall be performed using the following index ----,,maximum total dry plant biomass in presence of moderate water content in leaves and stem". The new selection index theory has shown that a certain selection index can give excellent breeding results in one environment and cause loss of the most valuable genotypes. The new selection index theory has offered specific indices for specific environments with clear forecast of successful breeding for production and vield.

14) Technology for reduction of new breakthrough variety creation cost. All breeding companies worldwide carry out environmental tests of new pre-varieties (in the Russian Federation, they are preformed by the National Variety Testing Committee of the Ministry of Agriculture of the Russian Federation). Thus, KWS (Germany) has 150 test sites in 55 countries. For example, sugar beet pre-variety is tested in each geographical point for at This is very expensive - creation least 3-4 years. of one new sugar beat hybrid lasts for 15-16 years and costs 15–18 million Euro. The breeding phytotron enables a typical limiting factor dynamics to be created by handle turning for any geographical point on Earth. Amount of tests may be reduced dramatically by time (4 months instead of 3-4 years) and by scope (instead of field plots with thousands of plants, 100 plants are sufficient (for each environment) in vegetation vessels of the breeding phytotron with removed environmental and competitive noises). These tests will not depend on season and any accidental weather paradoxes. Environmental phytotron test cost may be reduced by a factor of 3-4 and , thus, the new breakthrough variety creation cost can be reduced dramatically and competitiveness of Russian varieties in global markets can be increased significantly.

15) New phytotron technologies for preemptive creation of varieties for future climate that will form in the breeding zone in 10to15 years. Global climate warming (as cooling) takes place in a "spot-like" pattern rather than uniformly. Climate researchers create climate change forecasts for each "spot". Only the breeding phytotron can create climate that will be present in 10-15 years in this region and, using this future climate, design a variety ideally fitted to the future climate 5-6 years before. Field breeding is not capable of doing this, since a variety is created in the field during 10-15 years. Field breeding will be always falling behind by 10 years on the ideal congruence between the field variety and changed climate. This causes and will cause high shortfalls in total crops production. TEGO has provided phytotron technologies for preemptive creation of varieties ideally fitted to future climates in any points on Earth.

16) Technology for improvement of export prospects of new varieties created in the breeding phytotron. The breeding phytotron enables a typical limiting factor dynamics to be created for any point on Earth. Today, almost all crop varieties grown on Earth have been bred in field conditions where true "recognition" percentage of the best individual genotypes is very low (0.001%) in case of visual selections, in addition, "phase" selection is nor available (i. e. selective adaptivity improvement of each ontogenesis phase), and if the selection year coincides with an offtype year for this breeding zone, then previous 6 years of breeder's work may be merely lost. Therefore all global field-bred varieties have large production improvement reserve.

FAO experts emphasized in their report for 2014: "Global experience has shown that man-induced crop intensification is not able to solve the problem of further yield gain any longer, and it is associated with exponential growth of power consumption and ecological imbalance in nature. Global crop management crisis in agricultural production in the 21st century requires a new strategy — crop biologization, i.e. creation of new varieties, hybrids and types of crops resistant to abiotic and biotic factors".

USDA Agricultural Information Bulletin wrote on the same subject in 2001 — "The use of biotechnologies, including gene engineering, does not improve maximum yield. More fundamental scientific breakthrough is required, if we desire to increase total crop production" (USDA Agricultural Information Bulletin, 2001, USA).

Such breakthrough have been achieved: this our theory of ecological-genetic organization of quantitative traits with 24 new powerful predictive biological consequences, 10 know-hows and 16 innovative technologies for the design of new breakthrough varieties in terms of yield and quality.

TEGO elements are included in the International Encyclopaedia — "Basic Life Sciences", in the "Glossary on General and Molecular Biology, General and Applied Genetics, Breeding, DNA Technologies and Bioinformatics", in the "Glossary (Russian\_English) on Innovative Aspects of Breeding, Seed Production...", in the "Concise Dictionary on Forest Genetics and Breeding". All theoretical principles of TEGO are published in hundreds of papers in the Russian Federation and abroad. Number of TEGO publication citations — 3729. 6 inventor's certificates and patents were issued. TEGO elements are included in 20 university textbooks on genetics and breeding in such countries as Russia, Germany, Mexico, Bulgaria, Ukraine, and Belorussia.

# 3. Genetic physiological systems for epigenetic yield control and ways for maximization of their contributions into dramatic yield gains

During TEGO development [19], seven genetic physiological systems (GPS) which control yield (make either positive or negative contributions) were discovered. These are as follows:

1) plastic substance attractions from straw and leaves into ear or from sunflower leaves and stem into head;

2) micro-distributions of attracted substances between grains and chaff in wheat ear or between sunflower seed shell and kernel;

3) adaptivity (winter-, frost-, cold-, drought-, heat-, salt resistance, resistance to adverse soil conditions, e.g. to acid soils);

4) horizontal (polygenic) immunity;

5) "payment" of low (limiting) soil nutrition doses (N, P, K, etc.) by dry biomass;

6) plant community thickening tolerance;

7) genetic variability of different variety ontogenesis phase durations.

Discovery of these seven GPS immediately led to understanding of mechanisms breeding success achieved by the outstanding breeders in the USSR and Russia.

For example, in wheat breeding, acad. V.S. Pustovoit [16] increased oil percentage in sunflower seeds by identification and selection of the best GPS of plastic substance micro-distributions between sunflower seed shell and kernel (GPS 2) and the best GPS of horizontal immunity (GPS 4) (he improved only two from seven GPS). Akad. P.P. Lukianenko [16] improved frost resistance GPS (GPS 3) and shifted Beardless 1 winter wheat maturing from July to June in order to "remove" filling stage from July heat (GPS 7) (also only two from seven systems). Outstanding breeders Yu.M. Puchkov and L.A. Bespalova [16] (KNIISH, Krasnodar) created high-yield winter wheat varieties — Spartanka, Skifianka and Kroshka, by improving one GPS thickening tolerance (GPS 6).

In fruit production, breeder A.A. Richter (Nikitsky Botanic Garden) [30] created a "Paper-Shell" variety with soft shell and high kernel yield by means of selection of the best micro-distribution GPS (GPS 2) in almond. A.A. Petrosyan (SKZNIISIV) performed the similar work with walnut [31]. He created "Oily" nut variety with thin shell and high oil content (micro-distribution GPS, GPS 2). K.F. Kostina (Nikitsky Botanic Garden) [32] created a new hybrid Prunus cerasifera x Prunus salicina with large fruit and small kernel (micro-distribution GPS, GPS 2). G.V. Eremin made the similar work (Crimea Experimental Breeding Station, All-Union Research Institute of Plant Breeding) [33]. He got bigfruit Russian plum varieties "Globus" and "Melon" with small kernel (GPS 2). Breeder V.M. Gorina created "Boyar" apricot variety (Shalart  $\times$  Professor Smykov) with late flowering and high frost resistance (adaptivity GPS — GPS 3 and GPS 7).

But none of breeders worldwide have improved their best variety by all seven GPS simultaneously yet, which indicates high ecological-genetic potential for yield gain of all crops on Earth.

The "DIAS" [1] program headquarters has developed algorithms and computer software for evaluation of genetically possible yield gain potential (GPYGP) in a set of varieties cultivated in Siberia and Transbaikal. GPYGP in Siberia appeared to be equal to 60-80%, but in order to achieve it (improve yield by 60-80%), the world's first megascience facility shall be built in Russia — breeding phytotron and to launch a conveyor of 16 our innovative breeding technologies for the design of yield and quality breakthrough crop varieties for EAEU countries (and then under commercial contracts and for any countries worldwide).

Recently (2019), Rothamsted experimental station, England, personnel developed their own GPYGP evaluation method and evaluated the yield gain prospect in the set of commercial varieties in the England fields. The potential was — 60-80%, i.e. it matched our evaluation made in 1984 (but the British have no our 10 know-hows and 16 innovative technologies for yield breakthrough variety design).

Only 1% of energy is contributed to yield by man (plowing, harrowing, fertilizers, diseaseless and pest management, picking, drying) and 99% is granted by the sun. That's why plant breeding gives a profit of 3.000–5.000 Rubles per 1 Ruble (when using rigorous scientific breeding technologies). In his interview to a French journalist, M.A. Lavrentiev, the first Chairman of SB RAS USSR, emphisized: "In the 1960s, creation of only one "Novosibirskaya 67" spring wheat variety in ITsiG SB RAS completely compensated the first construction stage of the Novosibirsk Academgorodok".

Three breeding centers in the Russian Federation -Tyumen, Krasnoufimsk and Barnaul - used our new yield breakthrough variety design technologies. Spring soft wheat variety - Grenada created in Tyumen (authors V.V. Novokhatin, V.A. Dragavtsev, T.V. Shelomentseva) left behind all typical varieties by yield in the 9th and 10th crop-producing regions of the Russian Federation — by 10-12 centner/ha (by 30-40%). During Grenada creation, 5 from seven GPS were improved (thickening tolerance was not improved, GPS 6, and ontogenesis phase durations were not changed, GPS 7). The variety was released in 2019. In the 9th (Urals) region under spring wheat  $-7 \cdot 10^6$  ha, in the 10th (Western Siberian) region about  $8 \cdot 10^6$  ha. Seed farms have already received 1000t of Grenada seeds. Farmers purchase them vigorously. In Bashkiria, Grenada yielded about 70 centner/ha on two farmers' fields in 2020. The expected benefit of the Grenada yield gain is about 60 billion Rubles per year. In 2019 Ikar 2 spring wheat variety created using our technologies was submitted to the National Variety Network. Another variety - Atlanta 2 was also submitted to the National Variety Network. In 2019 Ikar 2 yielded 54.5 centner/ha and traditional variety — Omskaya 36 — yielded 38.5 centner/ha. In 2019. Gremme 2U hulless einkorn variety was released (authors E.F. Ionov, Kazan, V.A. Dragavtsev, SPb, S.K. Temirbekova, Moscow) throughout all 12 regions of the Russian Federation (this is a very rare event in breeding). It surpassed a traditional variety in Kazan (with maximum yield 20 centner/ha) by 20 centner/ha (by 100%), i.e. yielded 40 centner/ha. One ton of einkorn grain costs 40.000 Rubles in Kazan (1t wheat costs 10.000 Rubles), in Moscow 1 t einkorn costs 100.000 Rubles, in Europe — 250.000 Rubles and in the USA — 500.000 Rubles.

# 4. Physical instruments and computer software required for field breeding and phytotron breeding

16 innovative technologies for the design of yield breakthrough crop varieties derived from the TEGO consequences may be used both in field and phytotron breeding. Today, there are 38 economy-critical crop breeding areas, and 28 of them are not suitable for field conditions, a breeding phytotron is required for them. Some experts believe that when financing is increased for all field breeding centers in the Russian Federation and new equipment is purchased for them, everything will be OK. This is not the case! In many grain producing regions (in particular in Siberia), variety changing do not ensure increase in gross yield of grain any longer. Contrast weather conditions by years and primitive field breeding techniques (e.g. visual selection by phenotypes) which have become obsolete long ago hinder further selection-based yield gain. There are many factors when varieties designed in one zone are cultivated in entirely different zone. Thus, recently, Krasnoufimskaya 100 variety created in the middle Urals was released in the Leningrad region; varieties from Odessa were cultivated in the Lipetsk region; Saratovskaya 29 was grown in the Kazakhstan virgin land, Kharkovskaya 46 variety occupied Altai, rather than the Kharkov region; Sweden Rang variety occupied huge areas in the Tyumen and Omsk regions in the 1970s. This means that the resolution of traditional field breeding methods is very low. Current situation in field breeding is similar to that in pole jumping: when sportsmen used a bamboo pole, the world record was about 4m. Emergence of a fiberglass pole increased the world record up to 6 m. According to Acad. RAS L.A. Bespalova: "The Second Green Revolution will not be cheap: "all low hanging cherries have been already gathered", and brandnew methods and techniques are required to increase the breeding performance dramatically".

There are about 25,000 human genes, hard wheat has 90,000 genes. Soft (bread) wheat has 120,000 genes. Wheat variety is a system dozens of times more complicated than a car or aircraft. Field breeding centers in the Russian Federation may be compared with kolkhoz blacksmith's

shops which can make horse car varieties or horse-drawn machine-gun carts at the best, but they will never make a car variety or aircraft variety. For this, cutting-edge breeding breeding "factories" are required. Center of excellence for plant breeding, also known as the Federal Plant Breeding Center, also known as —the Center of Shared Use for Russian and EAEU breeders could such "factory". A breeding phytotron shall be its core.

Primarily using the computation center (CC) capacities (Peter the Great St. Petersburg Polytechnic University has one of the most powerful CC in Europe), a huge Gidromet's weather database shall be analyzed using already developed algorithms in order to identify typical years (typical limiting environmental factor dynamics) for each commercial grain production breeding zone. Knowledge of typical limiting environmental factor dynamics in each breeding zones will enable to reproduce these typical dynamics in the breeding phytotron in order to evaluate the ontogenesis phases for a specific commercial variety having weak adaptivity GPS and "bad" other GPS from six GPS. Understanding of all "bottlenecks" of certain commercial varieties by all ontogenesis phases (wheat has 12 phases) will allow to "introduce" (by crossing) the necessary "good" GPS from Vavilov's collections into these varieties.

Second, using a similar super powerful CC, a huge experimental database available with the National Variety Network of the Ministry of Agriculture of the Russian Federation. The following information may be recovered from this database — which of the tested varieties gave the best grain production in which year and with the "impact" by which limiting factor on which ontogenesis phase (this information is required to match parental pairs for crossing).

Seven GPS opened by us shall be used for solution of two problems in field breeding.

1) To evaluate PT genotypic values in collection nurseries of any breeder. For this, 20to40 plants shall be selected (using random number tables) from each variety plot (in full randomized block design experiment), the required PT shall be measured on each plant and average PT shall be calculated. This will be the genotypic value (GV) for this PT. Plotting of 2-dimensional trait coordinates of GV will allow to identify the contribution of each GPS into the specific variety yield.

2) For selection of the best genotypes — progenitors of future high-yield varieties — points of individual plants taken from generation  $F_2$  — from a segregating population in the selection nursery, are overlaid on the curve in order to evaluate the contribution of each of the seven GPS to the production of this plant [19].

To identify specimen with maximum genetic contributions to GPS 1 (attraction) production, ear weight shall be divided by culm weight (calculate attraction index). For this, precise scales without lag with automatic recording (5 mg to 5 g) are required. Specimens with low attraction index may have filled (relatively heavy) rather than empty stem. Such plants shall be checked for stem plumpness using portable X-ray apparatus of Acad. G.N. Fursey and colleagues. Stem plumpness makes wheat resistant to lodging from rain and wind, and combines harvest such wheat without loss.

To identify the best GPS 2 (micro-distributions), head threshers are required to weigh ears and threshed grains (ear weight less weight of grains from it — we get chaff weight). It is important that such threshers record the results. To evaluate GPS 2 of fruit crops at very early fructification stages, Fursey's X-ray apparatus shall be preferably used.

GPS 3 — adaptivities may be addressed from two points of view: 1) general adaptivity (of each variety to this year, to this field, to this fertilizer schedule, to this planting thickness); 2) adaptivity to a specific limiting environmental factor (cold-, frost-, heat-, drought, salt resistance, resistance to acid soil, etc.). For this, climatic chambers of the breeding phytotron are required to form a specific challenge background (currently, field breeders are expecting the coldest winter in order to select the most frost resistant genotypes, but such winter could be expected for many years). In both cases, adaptivity is measured by dry plant biomass. It cannot be measured by the grain weight as many breeders do today, because the difference in attraction GPS may introduce significant noise in the objective adaptivity evaluation.

GPS 4 — horizontal immunity. To identify contributions of this system, photo cameras are required to take photos of the plants at certain intervals.

GPS 5 —,,payment" of low (limiting) soil nutrition doses (N, P, K) by dry biomass. To be evaluated by dry plant biomass similar to specific adaptivity evaluations.

GPS6 — plant community thickening tolerance. The field experiment structure has been developed, high-yield varieties have been created using this technique.

GPS 7 — genetic variability of ontogenesis phase durations. Visual observations identify the extension or reduction of any phase rather precisely.

The list of physical instruments and equipment for phytotron breeding technologies is rather adequately described in [34].

#### **Conflict of interest**

The author declares that he has no conflict of interest.

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863

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