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Review Article

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The New Phenotyping Algorithms for Seven Genetic-Physiological Systems That Control the Characteristics of the Maximum Yields of Future Plant Varieties

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Abstract: Global warming and the growing rise in the price of bakery and feed grains make the problem of hereditary increase in the drought resistance of grain crops one of the most important tasks of ensuring the food security of mankind. Based on the dismemberment of the complex structure of the properties of drought resistance of cereals (phenotyping), the limitations of the canonical genecentric approach and approaches of molecular genetics to solving the problem of a radical hereditary increase in drought resistance are shown. A priority epigenetic approach to hereditary increase in drought resistance is proposed, based on the Theory of Ecological-Genetic Organization of Quantitative Traits (TEGOQT), which operates not with components of productivity, but with seven genetic-physiological systems (GPS), whose positive contributions increase yields: 1) attractions; 2) microdistributions of the attracted plastic substances between grains and chaff in the ear; 3) adaptability (drought, cold, frost, heat, salt resistance, etc.); 4) horizontal immunity; 5) "payment" by dry biomass for the limiting factor of soil nutrition (N, P, K…); 6) tolerance to thickening of agrophytocenosis; 7) hereditary variability in the duration of the phases of ontogenesis. This article examines one of the subcomponents of a complex GPS - adaptability, in particular - drought resistance, in the formation of which at least 22 component traits are involved.

Keywords: Cereals, drought tolerance, phenotyping, eco-genetic (epigenetic) nature of productivity traits, management of the selection of parental pairs for hybridization.

INTRODUCTION

Currently, there are two directions in the study of pathways - "genes - traits" - a direct path from gene to trait - genotyping - GT, [Фурта, Е.Ю. *et al*., 2018] and the opposite way - phenotyping - PhT), [Tuberosa R, 2012]. GT is a movement along the path from DNA - to mRNA - proteins - metabolic pathways - components of complex traits - to the very resulting complex traits - productivity and yield. Classical Mendelism, Quantitative Trait Genetics (QTG) and Molecular Genetics are mainly concerned with GT.

However, N.I. Vavilov (the first in the world) feel doubt about ability of classical Mendelism to describe the inheritance and development in the ontogeny of quantitative characters (QC). He emphasized: ***"We will not be surprised if a thorough study of the heredity of quantitative traits leads to a radical revision of simplified Mendelian concepts"*** [Вавилов, Н.И, 1965].

Today it is known [Попов, Е.Б . *et al*., 2020] that classical Mendelism (together with QTG and molecular genetics) describes no more than 10% of the phenomena of hereditary transmission (HT), hereditary realization (HR) and hereditary variability (HV). 40% of such phenomena are described by epigenetics, in particular its branch - ecological genetics. 60% of such phenomena describe biosymmetric inheritance and econics. Thus, the Genocentric paradigm ("everything from genes") and the Central dogma of molecular genetics ("everything from DNA and RNA") today have lost 90% of the HT, HR and HV phenomena from their field of vision, and yet at the beginning of the 21st century they claimed to be 100% description of these phenomena.

New Theory of fractal organization of four scientific clusters - biosymmetrics, genetics, epigenetics, econics [Попов, Е.Б. *et al*., 2020] strictly describes all the phenomena of HT, HR and HV, while giving strict quantitative predictions.

The absence in the GT algorithms in 90% of cases of an synonymous ("track") "genes-character" led to the need to create the opposite GT process - phenotyping - PhT. PhT is the process of dismembering the complex characters of a phenotype into their elements, then the dismemberment of the elements into smaller components, and so on until this dissociation reaches a specific protein (group of proteins) studied in the course of GT. PhT today is a very important tool for studying the mechanisms of determining the characteristics of the productivity of agricultural plants, which has already confirmed its good possibilities for plant breeding to increase productivity and yield.

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Traditionally, breeders select according to the phenotypic value of a trait, and each productivity trait has a phenotypic variability visible to the eye, including those invisible to the eye - ecological (modification), genotypic, genetic (additive) and epigenetic variability. With very unreliable visual phenotypic selection in segregated populations, it is practically very difficult to see with the eye and "catch" the unique individuals that will give rise to the future crop breakthrough variety.

(Note: we use the term "genotypic" in the broad sense of the word, ie we include chromosomal (genome), plastid (plastom) and cytoplasmic (plasmon) hereditary factors).

In addition, in breeding, traditionally, the division of complex characteristics of productivity (phenotyping) is carried out according to the elements of the structure of the yield - the total mass of a plant is divided by the mass of vegetative organs and the mass of ears. The mass of the ears is divided by the number of ears, the number of spikelets in an ear, the number of grains in a spikelet, the mass of grains per plant, the mass of chaff per plant, etc. We propose a different approach.

Grounding of New Phenotyping Algorithms for Seven Genetic-Physiological Systems

The priority PhT developed by us has fundamental features.

Seven genetic-physiological systems (GPS) have been discovered in plants [Драгавцев, В.А. *et al*., 1998], which can be controlled by PhT algorithms to radically increase the productivity and yields of new varieties.

1) GPS of attraction ("absorption") of photosynthetic products from stems and leaves into an ear (cereals), a basket (sunflower), an ear (corn), fruits and berries, etc.
2) GPS of microdistributions of plastic substances between grain and chaff in an ear, or kernel and husk in sunflower seed, etc.
3) GPS of adaptability (general adaptability to a specific field, zone and year of testing, or adaptability to a specific lim-factor, in the case of a provocative background - drought, cold, heat, salinity, soil pH, etc.),
4) GPS of horizontal (polygenic) immunity,
5) GPS of "payment" with dry biomass of the plant for a low dose of the lim-factor of soil nutrition (nitrogen, phosphorus, potassium, etc.),
6) GPS of tolerance to the thickening of the phytocenosis.
7) GPS of genetic variability of lengths phases ontogenesis

Our PhT algorithms and our breeding technology do not work with traits - components of productivity, which is generally accepted today. The indicators of productivity in our PhT technology serve as abscissas and ordinates of two-dimensional coordinates, in which the contributions of different GPS are multidirectional ("orthogonal"), which makes it possible to eliminate all noises (reducing the efficiency of selection in the field in hundreds times) and accurately recognize and select a positive hereditary shift existing in an individual according to any of the seven GPS [Кочерина, Н.В. *et al*., 2008].

Let's consider the most important GPS for Russia: adaptability systems. Let's start with the systems of drought resistance, due to the weakness of which in modern varieties of grain crops, Russia annually suffers a loss from droughts in the amount of 8-17 billion rubles. So in 2015, droughts caused damage to the Russian Federation in the amount of RUB 16 billion. In Australia, in 2003, due to the drought, wheat production fell from 24 to 9 million tons, or by 62.5%.

Is it possible to significantly increase the hereditary drought tolerance of cultivated cereals? To answer this question, it is necessary, as required by the open N.I. Vavilov The law of homologous series in hereditary variability [Вавилов, Н.И, 1965], to find the facts of the presence of increased drought resistance in wild relatives of traditional grain crops. It is known that some cereals (Graminea) are superior in drought tolerance to cultivated varieties of cereals. Namely - steppe timothy (Phleum phleoides, Wib.), Common bent (Agrostis vulgaris, With.), Feather grass (Stipa capillata, L.), chiy (Lasiagrostis splendens, Kunth.), Bulbous bluegrass (Poa bulbosa, L.) , fescue (Festuca ovina, L., F. sulcata, L.), awnless bonfire (Bromus inermis, Leyss.), sterile bonfire (Bromus sterilis, L.), comb wheatgrass (Agropyrum cristatum, L.), wild barley (Hordeum sponmaneum, C. Koch.) - grow on dry soils, where no cultivar of cereals can exist. Wild species have been increasing drought tolerance through natural selection for hundreds of thousands of years. Modern geneticists, physiologists and breeders are obliged to create new drought-resistant varieties in the next decade.

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The drought tolerance of cereals (even for one type of drought) has a complex nature. But there are many types of droughts themselves. The resulting property of "drought tolerance" to a particular type of drought is determining by many different components:

1) Orthotropicity and penetration depth of the root system [Декалб, 2013- Sears, E.R, 1953].
2) Labile "center of gravity" of the root system [Декалб, 2013].
3) The depth of the tillering node, determined by the balance of hormones [Ауземус, Э.Р. *et al*., 1970].
4) Osmotic pressure in root hairs [Russel, M.B, 1959- Кузнецов, В.В. *et al*., 2011].
5) Energy of transport of soil solutions [Levitt, J; Курсанов, А.Л, 1976].
6) Energy of enzymatic reactions [Финчем, Дж, 1968].
7) Temperature corridors of catalytic activity of nodal enzymes [Финчем, Дж, 1968].
8) General plant energy, ATP synthesis [Bonner, J, 1965].
9) The efficiency of the membranes [Скулачёв, В.П, 1989].
10) The total surface of the leaves in relation to their volume and mass [Медведев, С.С, 2004].
11) The thickness and density of the cuticle [Ауземус, Э.Р. *et al*., 1970].
12) The number of stomata per unit of leaf area and their sizes [Ort, D.R. *et al*., 2014].
13) Osmotic mode of their opening and closing [Ort, D.R. *et al*., 2014].
14) Leaf pubescence (hair density, hair length, light reflection, hair stiffness), [Ауземус, Э.Р. *et al*., 1970].
15) The ability of a leaf to curl in drought [Кузнецов, В.В. *et al*., 2011].
16) Vertical orientation of leaves [Кузнецов, В.В. *et al*., 2011].
17) Short and narrow leaves [Ауземус, Э.Р. *et al*., 1970].
18) A shift in the critical phase of ontogenesis from the “impact” of a typical stressor at a typical time [Ауземус, Э.Р. *et al*., 1970].
19) The intensity of the formation of metabolic water [Кузнецов, В.В. *et al*., 2011].
20) Optimal structure of chloroplasts for drought conditions [Кузнецов, В.В. *et al*., 2011].
21) Assimilation of a unit volume of $CO2$ per unit of moisture loss.
22) Energy costs for the attraction process [Dragavtsev, V.A, 2002].

In the literature, works on the PhT of the “drought tolerance” trait for elementary components begin to appear [Chen, D. *et al*., 2014].

Each of the listed components of the complex trait “drought tolerance” cannot be determined in any way by one or two Mendelian oligogens. For example, consider the genetic determination of the component - "osmotic pressure in root hairs". It is known [Alberts, B. *et al*., 1994] that the total number of genes expressed in human cells is about 24000, of which about 11000 are present in cells of any type. If this principle is also valid for plants, then it can be assumed that in each cell of the root hair there is a pool of products of more than 10,000 genes, and each product makes its own (positive or negative) contribution to the resulting osmotic pressure of hair cells, which makes it possible to extract the soil solution from a semi-dry substrate. ...

It is impossible to collect 10 000 genes in one variety, the products of which have the maximum osmotic activity, using traditional breeding methods (paired crosses and selection for phenotypes). The remaining components of drought tolerance are most likely determined by a smaller number of genes; however, for none of the 22 listed components, modern genetics have not yet found distinct discrete histograms of Mendelian segregations. N.L. Udolskaya [Удольская Н.Л, 1936] showed as early as 1936 that the degree of drought tolerance of a variety changes in ontogenesis and depends on a combination of factors causing drought. Drought-resistant varieties can become non-drought-resistant in other conditions with a different distribution of precipitation.

It is firmly established that productivity and yield are determined not by genes of quantitative traits (QTL), but by the effects of genotype-environment interaction (GEI), which are emergent (re-emerging) properties of high levels of organization (ontogenetic, phytocenotic) and are absent at the molecular level [Драгавцев, В.А, 2013]. Until now, special (specific) genes for productivity, yield, yield homeostasis (variety’s plasticity), horizontal immunity, species immunity, drought, winter, heat, cold resistance and etc. [Драгавцев, В.А, 2013]. Our theory states that they are unlikely to ever be found, since specific QTLs for each trait of productivity do not exist in nature.

It makes no sense to look for specific genes of consciousness, since the processes responsible for

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consciousness, long before the transition to the molecular level, decay to ordinary reactions that occur, for example, in the liver [Стент, Г, 1974].

Currently, against the backdrop of a fading belief in the omnipotence of molecular manipulations to increase productivity and crop yields, there are many GMO lobbyists who propose to radically increase drought resistance through transgenosis (production of GMOs). But transgenic methods can work with only one oligogen, and so far no oligogens have been found that can significantly increase the drought resistance of plants. To date, very few Mendelian genes have been described in plants - only 1-3% of the total number of genes in the genome of a species. The products of the remaining 97% of genes, firstly, are still almost unknown, and secondly, they are in complex interactions with each other, but most importantly, with constantly changing (even during the day) limiting environmental factors, as a result of which, when the lim- factor, these products alternately determine both the components and the resulting property - "drought resistance", which has non stable ("passport") genetics,
but "wandering" genetics, depending on the change in the lim-factor of the environment.

Unfortunately, some genetics, including molecular and genetic engineering

consider the most complex property - "drought tolerance", which forms over time against the background of differential gene activity in the phases of ontogenesis and is determined by "wandering" sets of gene products when changing lim-environmental factors - as a trait that is genetically controlled in the same way as elementary Mendelian traits. This does not take into account the diversity of drought types and phases of ontogeny, in which different elements of productivity are laid. The founder of the Russian scientific school of plant physiology, D.A. Sabinin [Сабинин, Д.А, 1963] called to study laying of different organs and components of productivity according to phases of ontogenesis.

Breeders and agronomists traditionally assess the drought tolerance of a variety by the grain yield, although there are varieties with the same dry biomass per unit area (i.e., in fact, with the same drought tolerance), but with different GPS attractions, due to which different grain yields will be obtained. Consequently, grain yield cannot be an objective and strict measure of drought tolerance. Thus, firstly, it is necessary in each specific case to take into account the type of drought and the phase of ontogenesis, in which it “hit” the plants, and to describe it in terms of its strength and duration. Secondly, “resistance”, in our opinion, is a biologically very uncertain term (this is a physical concept), therefore it is more appropriate to use the terms “drought tolerance”, or “drought productivity” (we think the latter concept is the most adequate). A strict quantitative measure of drought productivity should be the value of the decrease in dry biomass of a plant against a background of drought in comparison with the dry biomass of a plant of the same cultivar under comfortable conditions, or the ratio of these values - the index of drought productivity.

Note that the yield of biomass per unit area of an agrophytocenosis ("drought yield") is determined not only by the components of drought resistance, but also by the polygenes of competitiveness and tolerance to thickening of the phytocenosis, i.e. this criterion is much less stringent than the drought productivity of dry plant biomass.

Unfortunately, the drought-induced expression of the so-called “dormant” genes, similar to the inducible genes of heat and cold shock proteins, has not yet been sufficiently studied [Жученко, A.A, 2010]. The reaction of plants to water deficit by the synthesis of abscisic acid (ABA) is known, which covers the stomata, stops the growth of the aboveground part of the plant and stimulates the penetration of the root system into the depths of the soil to its moist horizons [Maximov, N.A, 1929]. With water deficiency, the synthesis of osmotically active substances (mono- and oligosaccharides and amino acids, mainly proline, betaines, polyhydric alcohols and various stress proteins (osmotine, dehydrins) is enhanced.

Of particular importance is proline, the synthesis of which sharply increases against the background of drought [Кузнецов, В.В. *et al*., 1999; Yamada, M. *et al*., 2005]. It was found that the RD29 (responsive to degidration) genes increase expression during osmotic shock, cooling, water deficiency, i.e. they respond to the effects of various stressors [Кузнецов, В.В. *et al*., 1999].

Of the 12 phases of wheat ontogeny, for a simplified consideration, we will consider the tillering phase. In the indicated phase, the action of drought sharply reduces the number of meristematic tubercles, from which the trait “number of grains in an ear” develops later. In most of the grain zones of Siberia, spring-summer

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droughts are common (no rain throughout June). At the same time, instead of the desired 80-90 grains, 15-20 are laid in the ear. The second most important phase is grain filling. The first night frosts in Siberia are from August 8-10. Weak cold resistance of varieties in this phase leads to grain feeble and a sharp decrease in yields.

FINDINGS AND DISCUSSIONS

We found that there are two serious obstacles to hereditary increase in drought productivity: the difficulty of creating combinations of the best polygenes in one variety and the very low efficiency of accurate identification of genotypes by their phenotypes during visual selection in segregated generations. The second problem is practically eliminated by our innovative selection technology, built on the principle of background traits and "orthogonal" (multidirectional) identification of individual genotypes [Dragavtsev, V.A. *et al*., 1977]. In our technology, productivity traits [Dragavtsev, V.A, 2002], as such, are not used for the visual detection of plants possessing the GPS required for the breeder. The traits serve as two-dimensional coordinates in which the contributions of GPS become multidirectional, which makes it possible to eliminate all noises that sharply reduce the efficiency of individual identification of genotypes in the field, and to accurately find plus deviations for any of the seven GPS, including one subsystem of GPS adaptability - drought productivity.

In this paper, we present our priority approach for solving the problem of fast (without change of generations) identification of positive shifts in drought productivity. We believe that this process should be controlled not at the molecular level (DNA and Mendelian oligogens), but at the level of initiation and formation of each of the 22 components of the resulting drought productivity and assessment for each a component of its additive proportion of variance in a particular set of varieties. After finding cultivars carrying additive polygenes with the maximum contribution to each component of drought productivity, one should try to combine these polygenes in one future cultivar by means of diallelic crosses of 22 cultivars with additive genes with maximum positive contributions to drought productivity. To solve this problem, we propose to use the developed by V.A. Dragavtsev and A.B. Dyakov a new model of quantitative trait, which replaced the traditional, but poorly working, model of Ronald Almer Fisher, [Кочерина, Н.В. *et al*., 2008]. The new model is formalized in the corresponding algorithms and programs [Михайленко, И.М. *et al*., 2013].

Our Theory of Ecological-Genetic Organization of Quantitative Characters (TEGOQC) and 24 new prognostic consequences from it have shown that geneticists, physiologists and breeders may well ignore the complexity of combinations of many gene products that affect the work of GPS and work only with the seven GPS , instead of the need to work with each of the 120 000 common wheat genes (as genomic editing enthusiasts try to do). Seven GPS are sufficiently additive and capable of combining together in one future breakthrough variety. Of course, they are not 100% additive, but the interactions between them do not greatly interfere with the historical trend of breeding increase in yields [Драгавцев, В.А, 2003]. Our point of view is confirmed by the facts of the creation of winter wheat varieties in the Kuban and Moscow region with yields of more than 10 t / ha based on traditional breeding technologies (without the use of genetic engineering, genomic editing, common genomics, proteomics and metabolomics).

We used the PhT algorithms described above in the field. Created 4

breakthrough varieties of spring bread wheat (Grenada, Ikar 2, Atlanta 2), [Новохатин, В.В. *et al*., 2019; Новохатин, В.В. *et al*., 2020] and breakthrough variety of naked grains - Gremme 2U. For the first time in the world, methods have been created for accurate forecasting of signs and values of genotypic correlation coefficients between productivity traits against the background of different dynamics of limiting environmental factors [Dragavtsev, V.A. *et al*., 2021; Драгавцев, В.А, 2012].

In a compact presentation of the Theory of the Ecological-Genetic Organization of Quantitative Characters – TEGOQC [41], it is shown that for a sharp rise in the efficiency of plant breeding in the Russian Federation, it is necessary to build the world's first engineering structure in the Russian Federation - the Breeding Phytotron, which will be able to create dynamics of limiting environmental factors in its climatic chambers for any geographic point of the Russian Federation and the entire Earth.

Phytotronic breeding technologies, which are conveyed in the Breeding Phytotron, will be able to conduct breeding to improve the adaptability of

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each phase of ontogenesis (fundamentally new "phase breeding"); reduce the time required for the design of breakthrough innovative varieties by 2-3 times; will increase the accuracy of identification ("recognition") of the best genotypes during selection - up to 1000 times; accelerate selection for thickening tolerance by 2-3 times; will provide an increase in the yield of the new variety over the standard one not by 3-4 centners / ha, as is now happening at field breeding centers of the Russian Federation, but by 10-12 or more centners / ha; will reduce the volume of crosses hundreds of times (practiced today at field selection centers of the Russian Federation); will reduce the cost of environmental testing by 3-4 times. But the main thing is that they will dramatically speed up the breeding process (in the field under the conditions of the Russian Federation, only one generation of cereals can be grown per year, and in the phytotron - 3-4, and even 5 with continuous illumination, this has been confirmed by many experiments).

Currently, in the USA, Europe, China and Japan the number of publications in the field of PhT systems of drought tolerance has sharply increased [Araus, J.L, 2012; Berger, B. *et al*., 2010; Dhondt, S. *et al*., 2013; Fiorani, F. *et al*., 2013; Klukas, C. *et al*., 2014; Sellammal, R. *et al*., 2014; Sozzani, R. *et al*., 2011; Xiong, L. *et al*., 2006; Furbank, R.T. *et al*., 2011]. To take a leading position in the world in the export of crop-breakthrough varieties (export of varieties from the Russian Federation can give the country an economic effect comparable to the export of certain energy carriers), it is urgently necessary to build the world's first Breeding phytotron in the Russian Federation.

In the staff of the Breeding Phytotron, only with the help of which it is possible to quickly and radically raise the drought productivity of our grain crops, there should be a department of PhT drought productivity of cereals, consisting of at least 6 research groups (three to four specialists in each), which are in artificial climate chambers will study genotypic variability between varieties (for example, wheat) for each of the components of drought tolerance in each phase of ontogenesis, assess the additive variability of each component, select parents with the maximum additive plus value of each component, and subsequent crosses, combine the best additive plus deviations for each component - together in one future variety.

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