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ECOLOGICAL AND GENETIC FEATURES OF PROTEIN CONTENT CONTROL IN SPRING BARLEY VARIETIES IN THE CONDITIONS OF THE NORTHERN STEPPE OF UKRAINE

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Abstract

This work aims to establish the ecological and genetic features of protein content control in spring barley varieties under the conditions of the Steppe of Ukraine. Heiman's genetic analysis allows us to judge the level of additive genetic variability. Under the influence of soil and air drought in 2021, high protein content of grain was noted in parental varieties - 13.5%, in hybrids - 13.3%. Under favorable conditions of 2022 - 12.2% and 11.5%, respectively. In the cool and rainy 2023, the protein content of both is equal - 12.1%. It was found that protein accumulation in spring barley grain depends to a greater extent on the hydrothermal conditions of the year (93.31%) than on the genetic characteristics of varieties and hybrids. In contrasting growing conditions, some advantages of the additive effect of genes in protein inheritance are noted; in optimal conditions, allelic and non-allelic interactions of genes contribute to the heritability of grain protein. A strategy for selection based on protein content in spring barley varieties is proposed for breeding, making it possible to improve the source material.

Key words: *gene, hybrid, protein content, selection strategy, spring barley, variety.*

INTRODUCTION

In determining the food (forage) value of spring barley (*Hordeum vulgare* subsp. *distichon* L.), an important role is played by the protein content in the grain. The total protein content of spring barley grain varies widely from 7 to 25%, according to studies (Evans et al., 1999; Steiner et al., 2011) the average level of protein content in grain in spring barley varieties is between 9-13%.

Proteins are high-molecular nitrogen-rich compounds that are built into peptides based on L-amino acids. In spring barley grains, proteins are represented by a variety of forms with functional responsibility for the structural organization of cells and organelles, metabolic activity, and the supply of nitrogen to the embryo in the process of grain germination (Musselman, 2003; Ragaee et al., 2006; Siebenhandl-Ehn et al., 2011; Finnie et al., 2014; Rybalka et al., 2016; Lukinac et al., 2022). According to research results (Schelling, 2003; Grausgruber, 2008; Horash et al., 2008; Kumar et al., 2013; Haley, 2015), grain protein is one of the main indicators of the brewing qualities of spring barley. The creation of varieties capable of accumulating less protein, other things being equal, is important for the selection of malting spring barley. The complexity of selection to reduce the protein content in spring barley is determined by the polygenic nature of the trait and its significant variability under the influence of environmental conditions. The main limiting factors affecting the accumulation of protein in grain are: the first factor - soil drought, in which growth processes decrease, and protein biosynthesis increases; the second factor is the lack of nitrogen and mineral nutrients in the soil, which also leads to a decrease in protein content; the third is a lack of heat and light during plant growth, while the percentage of protein decreases or remains at the same level.

Numerous studies conducted in malting spring barley-producing countries have shown that the content of extract, protein and enzymes in spring barley grain strongly depends on the variety (Savin et al., 2004; Rooney et al., 2023), but environmental conditions have a decisive influence (Gong, 2014; Solonechna, 2015;

Kuzina, 2020; Kendal, 2021). The influence of external conditions on chemical and technological indicators is so significant that the same variety can be brewer's in one year, forage in another. The use of high levels of mineral nutrition (especially nitrogen) leads to a significant increase in protein content in grain, so the assessment and selection for brewing qualities must be carried out based on starch content, grain size and extractability.

Other factors affecting the accumulation of protein in grain include precursors, sowing dates, cultivation zones, fertilizers, technological measures of spring barley cultivation (Hadjichristodoulou, 1990; Passarella, 2002; Shrimali et al., 2017; Kozachenko et al., 2020; Bondareva et al., 2021). When studying the combining ability of spring barley varieties, the predominance of additive effects of genes in determining the protein content of spring barley grain is shown, although the allelic and non-allelic interaction of genes has a significant influence (Aksel, 1963; Gudzenko, 2012; Zviahintseva et al., 2012; Singh et al., 2013; Zhang et al., 2015; Kompanets et al., 2019; Medimagh, 2019; Patial, 2019; Vashchenko et al., 2021; Zymogliad, 2021).

The question of the nature of inheritance of grain proteins and the system of genetic control of this indicator has been poorly studied.

The purpose of this work is the genetic analysis of the control trait of protein content in spring barley varieties, to determine the limiting factors for the selection of adaptive spring barley varieties.

Based on certain systems of genetic control, a selection strategy for protein content in spring barley varieties is proposed. The research hypothesis was confirmed during the creation of the spring barley variety Stalyy through the implementation of systematic crossings and selection from the obtained source material.

MATERIALS AND METHODS

The research was carried out in the field crop rotation of the Donetsk State Agricultural Science Station of the National Academy of Agrarian Sciences of Ukraine (DSASS NAAS) during 2021-2023.

The years of research were characterized by contrast in terms of temperature and humidity. In 2021, the annual amount of precipitation was only 238 mm, that is, the year was dry (180-270 mm). The calculation of the hydrothermal coefficient (HTC) during the grain pouring period showed the lowest value over the years of research (0.56).

In 2022, compared to the average long-term indicators, the amount of precipitation and air humidity were increased, that is, the year is considered wet (450-550 mm) based on the amount of precipitation (536 mm). The hydrothermal coefficient during grain pouring was 1.63.

The year 2023 turned out to be unstable in terms of temperature and moisture supply. April and May was characterized by excessive moisture, but the temperature regime was lower than the average long-term indicators by -1.1°C, which could not but affect the vegetation of plants. Since the beginning of June, the average daily temperature has been increasing rapidly (+2.4°C to the average long-term indicators), which led to severe drought and a decrease in the hydrothermal coefficient index to 0.2. The hydrothermal coefficient during the grain pouring period was 1.02.

The object of research was 5 varieties and 20 intervarietal hybrids F1 of spring barley, obtained in diallel crossings:

- Partner variety. Originator is the DSASS NAAS. Early ripening, steppe ecotype, valuable. The direction of use is grain, fodder. Adapted to drought conditions, heat-resistant. Designed for intensive growing technologies;
- Donetskyy 14 - a medium-ripening variety of the steppe ecotype. Originator is the DSASS NAAS. It is distinguished by ecological plasticity, tolerance to soil and wind droughts. Weakly susceptible to powdery mildew, helminthosporiosis, root rot, volatile and hard soot. A variety of grain fodder and food use;
- Halaktyk is a variety of the steppe ecotype, brewery. Originator is the Institute of Breeding and Genetics - National Center for Seed Production and Variety Research UAAS. It is resistant to flying soot, dwarf rust, reticular helmintosporiosis. Mildly affected by powdery mildew (0-0.2%), striped helminths (0-5%);
- Adapt is a variety of the steppe ecotype, originator of the Institute of Breeding and Genetics - National Center for Seed Production and Variety Research UAAS. It is precocious,

valuable. Drought resistance is 8-9 points. Weakly susceptible to striped helmintosporiosis, volatile and hard soot, powdery mildew. Resistant to laying;

- Stalyy variety. Originator is the DSASS NAAS. Steppe ecotype, medium ripe. The potential yield is 6.0 t/ha. Resistance to lying down is 7.5 - 9 points. Drought resistance is 8-9 points. The variety is universal, plastic. Effectively responds to increased doses of fertilizers, resistant to diseases and lodging.

The genetic analysis was performed according to the program of the PPP "OSGE" "Elite Systems gr." (1992), compiled in the Laboratory of Genetic Basis of Breeding at the Institute of the Institute of Plant Breeding, the main parameters of Heiman were determined (Griffing, 1956; Aksel, 1963; Heiman, 2001). Genetic control was evaluated based on the analysis of Heiman graphs (dependence of Wr on Vr - covariance and variance, respectively) and parameters: P3 - correlation coefficients between the sum of Wr + Vr and the mean values of the trait in parental forms, which characterizes the direction of dominance; P6 - averages in the level of dominance of alleles of all loci in the population ($\sqrt{H1/D}$); P9 is the average frequency of alleles of all loci (1/4 H2/H1); P13 is the ratio of the total number of dominant and recessive genes in parental forms ($\sqrt{4D \cdot H1}+F/\sqrt{4D \cdot H1}-F$), F - characterizes the ratio of the total number of dominant genes to the total number of recessive genes in parental varieties, where D, H1 and H2 - components of variation caused by the action of genes with additive effects, dominant and recessive genes, respectively; h2/H2 - reflects the number of genes that control the trait and show dominance. The estimation of the effects of the general combinatorial ability (GCA) and the variance of the specific combinatorial ability (SCA) were calculated according to the Griffing method as described by Litun et al. (1992). Statistical processing of the obtained data was carried out by generally accepted methods of analysis using the Microsoft Excel (2007) package.

RESULTS AND DISCUSSIONS

A significant variation of the hydrothermal conditions of the year in our research affected the accumulation of protein in the grains of spring barley varieties (Table 1).

Table 1. Protein content in grains of spring barley varieties, %

Variety	Year			Average (2021-2023)
	2021	2022	2023	
Partner	13.1	12.2	14.0	13.1
Donetskyy 14	13.9	12.9	13.3	13.3
Halaktyk	14.0	11.2	10.8	12.0
Adapt	13.5	12.6	10.2	12.1
Stalyy	12.8	12.2	12.3	12.4
Average	13.5	12.2	12.1	12.6
$LSD_{0.5}$	0.39	0.82	1.0	

Under the influence of soil and air drought in 2021, the growth processes of plants decreased to a greater extent than the main processes of biosynthesis, as a result of which the highest grain protein content was noted, primarily in tall genotypes. The sign varies from 12.8 to 14.0%, that is, the range between the extreme values was 1.2%.

Under favorable conditions in 2022, the protein content in grain decreased by 1.3% on average and varied from 11.2 (Halaktyk) to 12.9% (Donetskyy 14). The difference between varieties was 1.7%.

A rather peculiar response of the cultivars from protein accumulation was found in the cool and rainy 2023. First of all, the differences between the cultivars were wider. Thus, high protein content was noted in the Partner variety (14.0%), and the lowest in Adapt (10.2%), that is, the range between the extreme genotypes was 3.8%. It is interesting to note that increased grain protein content was observed in varieties prone to lodging.

The analysis of group average varieties (Table 1) and F1 hybrids (Table 2) showed that in the first two years, the protein content of the latter was slightly lower.

In 2021, the trait in parental varieties was 13.5%, in hybrids - 13.3%; in 2022 - 12.2 and 11.5%, respectively. This indicates the partial dominance of the parental variety with reduced protein content. In 2023, the protein content of those and others is equal - 12.1%, which implies the predominance of the intermediate type in the inheritance of the trait. The noted features regarding the difference in the protein content in the grain of varieties by year are characteristic of their hybrids. Thus, in 2021, the difference was 1.3%, in 2022 - 1.8%, and in 2023 - 3.2%.

Table 2. Protein content in grains of F1 spring barley hybrids, %

F1 spring barley hybrids	Year			Average (2021-2023)
	2021	2022	2023	
Partner/ Donetskyy 14	13.3	11.6	12.5	12.5
Partner/ Halaktyk	12.6	12.1	12.7	12.5
Partner/ Adapt	12.9	12.2	11.7	12.3
Partner / Stalyy	12.9	12.3	11.7	12.3
Donetskyy 14/ Partner	13.5	11.7	12.3	12.5
Donetskyy 14/ Halaktyk	13.9	10.9	13.1	12.6
Donetskyy 14/ Adapt	13.5	10.5	11.7	11.9
Donetskyy 14/ Stalyy	13.6	11.1	11.8	12.2
Halaktyk/ Partner	13.4	10.8	12.0	12.1
Halaktyk/ Donetskyy 14	13.3	11.6	12.2	12.4
Halaktyk/Adapt	13.8	11.9	11.9	12.5
Halaktyk/Stalyy	13.8	12.1	10.9	12.3
Adapt/Partner	12.9	12.1	10.4	11.8
Adapt/ Donetskyy 14	13.1	11.7	11.4	12.1
Adapt/Halaktyk	13.2	11.4	13.6	12.7
Adapt/Staliy	13.0	11.8	11.7	12.2
Stalyy/Partner	12.9	11.0	12.6	12.2
Stalyy/ Donetskyy 14	13.0	11.5	13.0	12.5
Stalyy/Halaktyk	13.6	11.0	13.0	12.6
Stalyy/Adapt	13.8	11.4	12.8	12.7
Average	13.3	11.5	12.1	12.3
$LSD_{0.5}$	0.27	1.06	0.67	

The indicator of hybrids is slightly different depending on the direction of crossings. So, in 2021, the protein content in direct crosses was 13.3%, in reverse - 13.2%; in 2022 - 11.6% and 11.4% and in 2023 - 12.3% and 12.5%, respectively. At the same time, in the first year, 4 hybrids in direct crosses and 2 in backcrosses had better protein content, in the second year - 5 and 3, in the third year - 4 and 5, respectively. These data indicate that both the cytoplasmic effect and nuclear-plasma interactions are significant in the variability of the indicator.

It should be noted that, if the F1 varieties and hybrids do not meet the requirements for the quality of spring barley for brewing under conditions of drought of medium intensity, then the Halaktyk variety and almost all hybrids meet the requirements under optimal conditions. In cool and rainy conditions, relatively low grain protein content was shown by Galactic and Adapt varieties, as well as 50% of F1 hybrid combinations.

The results of variance analysis (Table 3) showed that protein accumulation in spring barley grain depends to a greater extent on the conditions of the year (93.31%) than on the genotypes of varieties and hybrids (2.80%). The interaction of factors also makes a significant contribution to the variability of the trait (3.49%).

Table 3. The share of influence of factors on the variability of grain protein content

Factor	mS	Ff	Ft	%
Conditions of the year (A)	52.86	237.84	3.07	93.31
Genotype (B)	1.59	7.14	1.60	2.80
Interaction (A x B)	1.98	8.90	1.49	3.49
Error	0.22	-	-	0.39

mS - middle square; Ff, Ft - Fisher criteria

When studying the combining ability of varieties according to their hybrids (Table 4), based on the proportional ratio, the variation of GCA and SCA, it follows that in rather contrasting conditions in 2021 and 2023.

Table 4. Combination ability of spring barley varieties in terms of grain protein content

A source of variability	2021		2022		2023	
	mS	%	mS	%	mS	%
GCA	0.431*	67.2	0.270*	18.2	1.314	43.2
SCA	0.089*	13.9	0.859*	57.9	1.027	33.8
reciprocal effect	0.121*	18.9	0.355*	23.9	0.696	23.0
*Reliable at P≤ 0.05						

In the inheritance of spring barley grain protein, some advantages have additive effects of genes. Variations of the GCA amounted to 67.2 and 43.2% of the total variability of the trait, respectively.

Under optimal conditions in 2022, a noticeable advantage in the inheritance of grain protein content comes from allelic and non-allelic gene interactions (the share of SCA variation was 57.9%).

The unique hydrothermal conditions during the years of research were also reflected in the assessments of the effects of GCA varieties (Table 5).

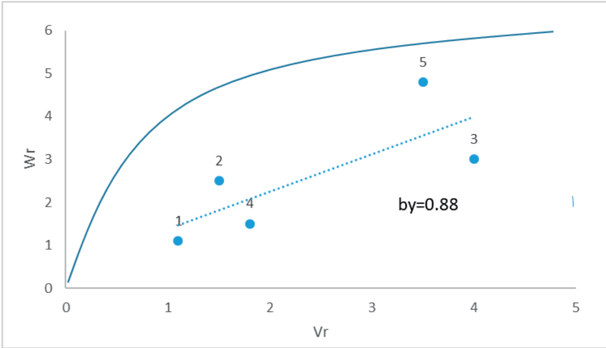
Table 5. Estimates of the effects of GCA of spring barley varieties based on protein content in grain

Variety	Year			Average (2021-2023)
	2021	2022	2023	
Partner	-0.28	0.16	0.24	0.04
Donetskyy 14	0.17	-0.05	0.33	0.15
Halaktyk	0.23	-0.26	-0.05	-0.03
Adapt	-0.02	0.17	-0.60	-0.15
Stalyy	0.10	-0.02	0.07	0.05
Error	-0.28	0.16	0.24	

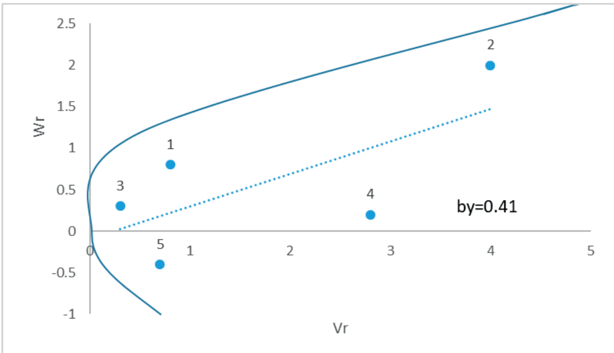
In drought conditions, the protein content of hybrids of the Donetskyy 14 and Halaktyk varieties increases significantly, while it decreases in the Partner variety; under optimal conditions, the Partner and Adapt varieties have positive evaluations, but the indicator of the Halaktyk variety hybrids decreases significantly; in rainy and cool conditions, hybrids of tall varieties Partner and Donetskyy 14 have increased protein, and hybrids of Adapt variety have reduced protein. Thus, in the latter case, positive evaluations have genotypes prone to laying down.

The analysis of Heiman's graphs and genetic parameters (Figure 1) allows us to note the significant lability of systems of genetic control of grain protein (the relationship between covariance Wr and variance Vr is expressed through the linear regression coefficient). Under optimal conditions, intralocus overdominance (P6 = 1.52), slight additivity between loci plus complementary (recessive) epistasis was noted. The movement of the Stalyy variety from the recessive to the dominant zone is observed.

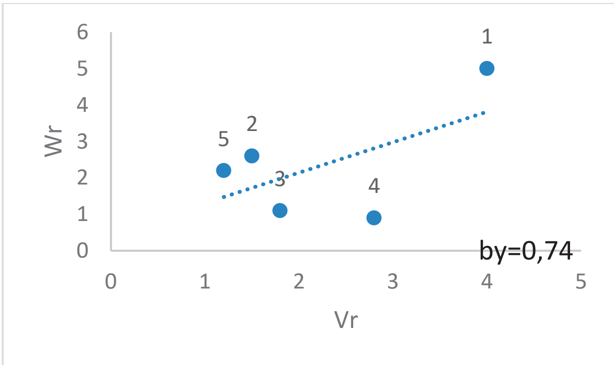
Heiman's genetic parameters based on the protein content of grain by years of research are presented in Table 6.



2021



2022



2023

Figure 1. Dependence between covariance (Wr) and variance (Vr) according to the grain protein content of spring barley varieties for 2021-2023 (1 - Partner, 2 - Donetskyy 14, 3 - Halaktyk, 4 - Adapt, 5 - Stalyy)

In cool and humid conditions, as well as during drought, incomplete dominance was observed in loci (P6 = 0.95), and additivity plus minor complementary epistasis was observed between loci. Under these conditions, protein content is reduced by dominant genes and increased by recessive genes. Compared to 2022, the Partner variety moved from the dominant zone to the recessive one, and Donetskyy 14, on the contrary, from the recessive to the dominant one.

It should be noted that in loci showing dominance, the frequency of plus and minus alleles is constantly asymmetrical (P9 = 0.17-0.22), and in parental varieties dominant genes predominate (P13 = 1.99-3.81).

Table 6. Heyman's genetic parameters based on protein content in spring barley grain

Year	Parameter			
	r(Wr+Vr)/Xp P3	$\sqrt{H_1/D}$ P6	¼ H_2/H_1 P9	$\sqrt{4D \cdot H_1}+F/\sqrt{4D \cdot H_1}-F$ P13
2021	-0.21±0.43	0.78	0.17	1.99
2022	0.75±0.19	1.52	0.19	2.53
2023	0.78±0.17	0.95	0.22	3.81

Thus, the accumulation of protein in spring barley grain largely depends on the hydro-thermal conditions of the year: in dry conditions its content increases, in optimal conditions it decreases, and with a lack of heat and excess precipitation, differentiation of the experimental material is noted both in terms of both increased and decreased levels.

In the declining signs, judging from the mutual correlation between the GCA and SCA variants, it is indicated that there is a predominance of the additive effects of genes in contrasting minds during the growing season; in the optimal ones, there is allelic and non-alelic interaction of genes.

Genetic control systems for protein accumulation in spring barley grains during the growing season reveal significant lability. It was indicated with internal locus interaction in 2021 and 2023 births of non-dominance, in 2022 births - over dominance. Interaction between 2021 and 2023 will have additivity + epistasis, and in 2022 epistasis + additivity. The sign is controlled by two genetic systems, and the meaning is ensured until reassignment. Not only a redefinition of the genetic system of the trait as a whole was noted, but also of the genetic formulas of specific varieties. The reason for this is the determination of signs by different genes: just as dryness genes produce dryness genes, then when there is a lack of positive temperatures, coldness genes are produced. However, both the first and the second increase the protein content.

Only in conditions close to optimal for the region, the percentage of protein decreases. But even under these conditions, the selection of low-protein forms is difficult, since over dominance and complementary epistasis were found in the genetic control of the trait.

These facts make it possible to make assumptions about selection in later generations of hybrids (F4-F6). Adapt grade can be used as a source to reduce the protein content in spring barley grain, and Donetskyy 14 grade to increase it.

As a result of the research, a new raw material was created, which is evaluated based on the protein content in the grain.

CONCLUSIONS

The performed analysis of the genetic control of protein content in spring barley varieties showed that it is due to the interaction between loci by the type of additivity and epistasis and incomplete or type of over dominance within the loci.

The research of varieties and hybrids determined the limiting factors for the selection of adaptive varieties of spring barley in changing environmental conditions. Under the action of the limiting factor, variety-specific properties are manifested, which increases the variances of the specific combining ability and the reciprocal effect. In such cases, an additional system of non-allelic interaction of genes or complementary epistasis is connected.

Based on certain systems of genetic control, a selection strategy for protein content in spring barley varieties is proposed, from which the following options are offered:

- non-directional dominance. Selection is made on the basis of both dominant and recessive alleles, so that these alleles increase or decrease the trait. At the same time, recessive alleles are better, since recessive homozygotes appear already in the F2 generation of hybrids;
- direction of dominance. Selection is based on only one type of allele, either dominant or recessive. Dominant alleles increase the trait, the intensity of selection in F2 should be low, since heterozygotes fall along with homozygotes. In this situation, it is necessary to reseed the breeding material and carry out the selection of the necessary homozygotes in later generations of hybrids (F4-F6);
- over dominance. Selection of elites is possible in F2 or F3, but its intensity will be determined

depending on which alleles control the trait - dominant or recessive. In the first case, the intensity of selection is low, in the second - high. In practice, the evaluation of the breeding material according to the qualitative characteristics of spring barley grain is performed in later generations, when the analysed samples are homozygous, have a certain yield value and there is a sufficient number of seeds for biochemical analysis.

The obtained information on the variability of traits, the combinatorial ability of varieties and genetic analysis according to Heiman allows us to judge the high level of additive genetic variability and provides the possibility of improving the studied indicator through selection.

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