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GENETICS OF PHOSPHORUS, POTASSIUM, MAGNESIUM AND CALCIUM CONTENT FOR BARLEY GRAIN IMPROVEMENT

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ABSTRACT

Studying the inheritance of phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) in barley grains is important because it enables the improvement of nutritional quality and crop yields through crop breeding. The aim of this study was to determine, through the application of diallel analysis, the mode of inheritance and components of genetic variance of P, K, and Mg in barley grains, as well as the combining abilities of the experimental material for these macroelements. The study was conducted at the experimental field of the Center for Small Grains in Kragujevac, Serbia. The plant material consisted of twelve F_1 hybrids resulting from a complete diallel cross between four winter barley varieties (Partizan, KG-6, HVW-247, NS-293). Hybridizations were performed through manual pollination, and the resulting F_1 hybrids, along with their parents, were sown in a randomized block design with three replications. The content of macroelements (P-phosphorus, K-potassium, Mg-magnesium and Ca-calcium) was quantified in the grain of the parents and the F_1 generation. Narrow sense heritability was highest for the potassium content (72.21%) and lowest for the magnesium (14.45%). The general combining ability (GCA) and the specific combining ability (SCA) were determined for the parents and the hybrids, respectively. The inheritance of the P and K content was mainly influenced by the additive genetic component, while the inheritance of Mg was predominantly influenced by the dominant genetic component. For Ca content, the additive-dominant inheritance model was not satisfactory due to inter-allelic interaction. VrWr regression analysis followed the previously established models of inheritance. The variety KG-6 was identified as the best general combiner for P, Mg, and Ca content, while NS-293 was found to be the best general combiner for K and Mg content in barley grains. These varieties can be effectively utilized as gene donors for enhancing the mineral composition in future F_1 hybrids.

Keywords: *Hordeum vulgare* L., diallel analysis, macroelements, regression analysis.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is cultivated worldwide and has a very large geographical range from mountain tops to the seashore, larger than that of other crop species (Newton *et al.,* 2011). Due to its great adaptability and tolerance to biotic and abiotic stress, it is the only cereal that can be grown in arid regions (Visioni *et al.,* 2023). Cultivated barley is mainly used as animal feed, for malt production and, to a lesser extent, for food production (Guerra *et al.,* 2022). Barley is a great source of nutrients, vitamins, minerals, and total phenolic compounds (Rohman *et al.,* 2024).

The world production of barley in 2023 was 145.08 million metric tons from an area of 46.3 million ha with an average productivity of 3.15 t/ha (FAOSTAT, 2023). The leading barley production states are the Russian Federation (21.02%), Spain (11.51%), Germany (10.81%), Canada (10.78%), France (10.31%), Australia (10.17%), Ukraine (8.94%), Turkey (8.33%) and UK (8.14%) (Verma *et al.,* 2022).

Barley has a significant role in crop production in Europe and more than 60% of the world's barley production is provided by Europe (Bindereif *et al.,* 2022). Regarding the production of barley hybrids, the largest areas for barley hybrids are located in France, Germany and the UK (Oberforster and Flamm, 2018), but the market share of hybrids was lower compared to other varieties until Syngenta's patented hybrid varieties (Longin *et al.,* 2012). Thus, the official EU database of registered plant varieties (last update: 30.05.2024.) reveals 13 barley hybrids. Hybrid barley is attractive for growing because of its good yield potential and especially yield stability under fluctuating environmental conditions compared to conventional varieties (Fernández-Calleja *et al.,* 2020). Besides barley hybrids offering possibilities of improving yield and stability under current and future climate conditions through heterosis, pyramiding strategic combinations of dominant major genes is easily achievable for them (Fernández-Calleja, 2022).

Mineral nutrients are essential elements for the growth and development of plants. Regarding essential nutrients concentrations in plant tissues, they can be divided into macro- and microelements. Macroelements encompass potassium (K), nitrogen (N), magnesium (Mg), phosphorus (P), sulfur (S) and calcium (Ca). At the same time microelements include copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), boron (B), chlorine (Cl) and molybdenum (Mo) (Houmani *et al.,* 2024). Macroelements are necessary for many metabolic processes such as protein synthesis, photosynthesis and cell homeostasis, while microelements have catalytic roles in different metabolic pathways and are required for the functions of proteins. Besides being necessary for optimal plant life cycle and productivity, mineral elements also serve as signaling molecules and mitigators of biotic or/and abiotic stress responses. That function of minerals could be of paramount importance in the context of present and future global climate change with adequate targeted breeding programs focused on climate adaptation (Khan *et al.,* 2023).

Most traits of economic importance in barley are quantitative in nature. Although, the macro and micronutrient content of grains is an important quality indicator, regardless of whether the grains are used for human or animal consumption, their concentrations appears to be genetically complex traits (Zeng *et al.,* 2016) and are quantitatively inherited. The content of macro and micronutrients in cereal grains is specific for the cultivar, but environmental factors and agricultural practices also have an influence (Omirou *et al.,* 2023) Extensive knowledge of the concentration of macro and micronutrients is important in order to understand their interrelationships and to create the elemental profile of the crop. Currently, the barley genome sequence, many genetic maps and genomic resources are available (Mascher *et al.,* 2017; Sato, 2020; Jayakodi *et al.,* 2024) and protein-coding genes and their regulatory network responsible for the processes of absorption, assimilation, storage, and mobilization of macro/micro-elements have been deciphered to some degree (Kumar *et al.,* 2021).

In the breeding and genetics of barley, information on the combining abilities of macroelement content in the grain has not been sufficiently investigated. This study was conducted to estimate the modes of inheritance of macroelements in the F_1 generation, the components of genetic variance and heritability, and the general and specific combining abilities of different barley genotypes and their F_1 combinations to evaluate their breeding potential for macroelment content as criteria for barley cultivar improvement.

MATERIALS AND METHODS

Plant Materials and Field Trials: The trials were carried out in the experimental field of the Center for Small Grains in Kragujevac, Serbia. The experimental plots are located at an altitude of 186 meters. The climate is temperate continental. The average annual precipitation is 530 mm and the average temperature is 11.5 °C. Precipitation is generally evenly distributed over the months during the barley vegetation period. The land is plain, i.e. its composition belongs to the smonica-type of soil in the process of decomposition. For the trials, the usual agronomic practices were followed on the uniform and well-prepared soil. Peas were grown as the previous crop in the crop rotation. The basal cultivation and the preliminary preparation of the soil were carried out in the traditional way.

The material selected for the full diallel analysis (including both direct and reciprocal crosses) consisted of four winter barley varieties (Partizan, KG-6, HVW-247, NS-293) and the corresponding 12 F_1 hybrids along with four parents. The parents were sown in a crossing block, and hybridizations were performed using emasculation followed by pollination. The F_1 hybrids resulting from the crossing program, along with their parents, were sown in a randomized block design with three replications. The genotypes were arranged in 2 m long rows, with a row spacing of 20 cm and a plant spacing of 5 cm within each row.

The agronomic practices included basic fertilization with 150 kg of NPK (15:15:15), two tillage operations, and manual weed control without the use of chemical protection. Harvesting was done manually when the plants reached full maturity. The grains were collected and subsequently prepared for laboratory analysis.

Laboratory analyses: The content of macroelements (P, K, Ca, and Mg) in barley grains was analyzed partly in the agrochemical laboratory of the Center for Small Grains in Kragujevac and partly in the Laboratory of physiology and agrochemistry of the Faculty of Agriculture in Zemun-Belgrade.

To determine the content of the elements, the samples were measured as a mixture of ground grains in cuvettes for burning in three replicates. The plant material was annealed at 550°C for 6 hours. The ash was dissolved in 2 ml 5N HN03, evaporated to a dry residue and reheated for 2 hours. The dry residue was dissolved in 5 ml 5N HCl and evaporated to dryness, then redissolved in 20 ml

0.5N HCl and filtered through a qualitative filter paper. From this common solution, phosphorus was determined colorimetrically by the molybdate method after 25-fold dilution with distilled H_20. Potassium was determined directly on the flame photometer from the diluted sample for phosphorus. Calcium and magnesium were measured directly by atomic absorption spectroscopy.

Statistical analysis: The variance of each trait was partitioned into the corresponding components of variation, including replicates, genotypes and years, for the parental varieties and for the progeny by factorial analysis of variance. Evaluation of the significance of differences within replicates, treatments and interactions was determined based on the F-test for two levels at 0.05 and 0.01. The ANOVA model of Johnson *et al.* (1955) was used to assess the heritability of traits as a ratio:

$$h^2 = \frac{Vg}{Vf}$$

The basic biometric parameters were calculated according to Kraljevic-Balalic *et al.* (1991). The method of Mather and Jinks (1971) was applied to evaluate the modes of gene action based on the values of the parents. The general and specific combining abilities of the studied parents in the diallel cross were determined according to Griffing (1956), method 1, according to the mathematical model 1:

$$X_{ij} = \mu + g_i + g_j + S_{ij} + r_{ij} + \frac{1}{bc} = \sum\sum e_{ijkl}$$

where μ is population mean, gi(gj) is effect of general combining ability (GCA) for the i-parents and for the j-parents, Sij is effect of specific combining ability (SCA) for the hybridization ixj, rij is reciprocal effect that includes reciprocal hybridizations between i-parent and j-parent and rij= -rji, eijkl is environmental effect related with ijkl-observation, b are blocks, c are individuals in each of the replicates.

The heritability h^2 was calculated according to Mather (1949).

a) in a broad sense, it shows the ratio of genetic and total, phenotypic variance:

$$H^2 = \frac{V_G}{V_F} = \frac{\frac{1}{2}D + \frac{1}{2}H1 - \frac{1}{4}H2 - \frac{1}{2}F}{\frac{1}{2}D + \frac{1}{2}H1 - \frac{1}{4}H2 - \frac{1}{2}F + E}$$

a) in a narrow sense, it shows the ratio of additive genetic variance to the total phenotypic variance:

$$h^2 = \frac{V_A}{V_F} = \frac{\frac{1}{2}D + \frac{1}{2}H1 - \frac{1}{4}H2 - \frac{1}{2}F}{\frac{1}{2}D + \frac{1}{2}H1 - \frac{1}{4}H2 - \frac{1}{2}F + E}$$

The components of genetic variance were calculated according to the methods of Jinks (1954), Hayman (1954) and Falconer (1981) using the following system of equations:

$$V_P = D + E$$

$$\overline{W}_r = \frac{1}{2}D - \frac{1}{4}E + \frac{1}{nE}$$

$$\overline{V}_r = D + H1 - F + (n+1)/2nE$$

$$V_m = \frac{1}{4D} + \frac{1}{4H1} - \frac{1}{4H2} - \frac{1}{4F} + 1/2nE$$

The ratio of the total number of dominant and recessive alleles:

$$\frac{K_D}{K_R} = \frac{\sqrt{4DH1} + F}{\sqrt{4DH1} - F}$$

Regression analysis was applied to determine the mode of inheritance of the quantitative traits studied. The expected regression line was calculated using the following formula:

$$W_{re} = (\overline{W_r} - b\overline{V}_r) + bV_r$$

The calculation of the limiting parabola was carried out according to the formula:

$$W_{re} = \sqrt{(VrVp)}$$

The regression coefficient was determined using the following formula:

$$b = \frac{\sum(x - \bar{x})\,(y - \bar{y})}{\sum(x - \bar{x})^2}$$

The following formulas were used to calculate the standard error of linear regression coefficient:

$$S_b = \sqrt{[Se^2/(x - \bar{x})^2]}$$

$$S_b = \sqrt{[\sum(y - \bar{y})]^2/(n - 2)}$$

The regression coefficients (β=1 and β=1/2) were tested according to Steel *et al.*, (1996): t = (b-1)/Sb for n^{-2} degrees of freedom.

RESULTS

ANOVA and mean values of macroelements content (parents and F_1): The results from the analysis of variance for the parents and F_1 crosses are presented in Table 1. The effects of the replications were not significant, whereas the influence of the genotypes on the variability of the macroelements was statistically highly significant.

The mean values of four macro elements content (P, K, Mg and Ca) were determined for the barley grains (4 parents and 12 F_1 hybrids) and statistical differences were evaluated using Tukey's multiple comparison test (Table 2).

The KG-6 variety had the highest phosphorus content (3300 mg/kg) in the mature grain, while the lowest content (2500 mg/kg) was found in the NS-293 variety. The hybrids of the F_1 generation had phosphorus content in the interval of the respective parents, and all values of the mean phosphorus content were in the interval between the highest and the lowest content achieved by the varieties.

The highest potassium content (3500 mg/kg) was found in the NS-293 variety and the lowest (2400 mg/kg) in the KG-6 variety. The average values of the F_1 hybrids also varied between the best and the worst parent in this case.

Table 1. One-way ANOVA of macroelements content in barley genotypes.

Macroelements	Replications		Genotypes	
	F	P-value	F	P-value
P	0.77	0.4	5.33	< 0.001
K	1.04	0.35	3.83	< 0.001
Mg	0.65	0.5	5.27	< 0.001
Ca	3.33	0.05	33.1	< 0.001

P: phosphorus, K: potassium, Mg: magnesium, Ca: calcium

Table 2. Mean values of macro elements content (mg/kg) in barley grain (parents and F_1).

Genotype	P	K	Mg	Ca
P1(Partizan)	3100^{a}	2600^{b}	900^{b}	700^{c}
S1 (1x2)	3000^{a}	2800^{b}	800^{c}	1000^{a}
S2 (1x3)	2900^{a}	3400^{a}	1000^{b}	1100^{a}
S3 (1x4)	3000^{a}	3100^{a}	900^{b}	900^{b}
R1 (2x1)	3200^{a}	2700^{b}	1000^{b}	500^{d}
P2 (KG 6)	3300^{a}	2400^{c}	1300^{a}	900^{b}
S4 (2x3)	3100^{a}	3300^{a}	900^{b}	900^{b}
S5 (2x4)	3200^{a}	3200^{a}	900^{b}	1200^{a}
R2 (3x1)	2900^{a}	2900^{b}	1100^{ab}	500^{d}
R4 (3x2)	3200^{a}	3200^{a}	1000^{b}	800^{b}
P3 (NS 293)	2500^{b}	3500^{a}	1000^{b}	500^{d}
S6 (3x4)	2800^{a}	2800^{b}	1200^{a}	600^{c}
R3 (4x1)	3200^{a}	3200^{a}	1000^{b}	500^{d}
R5 (4x2)	3000^{a}	3000^{ab}	900^{b}	500^{d}
R6 (4x3)	2900^{a}	3300^{a}	900^{b}	300^{e}
P4 (HVW-247)	2600^{b}	3000^{ab}	700^{c}	800^{b}

P: phosphorus, K: potassium, Mg: magnesium, Ca: calcium. Genotypes with the same letter are not significantly different in content for that specific element. Different letters indicate statistically significant differences according to Tukey's test at the 0.05 level.

The enrichment of magnesium in the grain of the varieties varied between 700 mg/kg for HVW-247 and 1300 mg/kg for KG-6. The F_1 hybrids did not show lower or higher values of the percentage content than the parent varieties.

The KG-6 variety had the highest calcium content (900 mg/kg), the lowest was found in the NS-293 variety (500 mg/kg). Characteristic for this element is that some F_1 hybrids showed higher or lower values of Ca content than the parent varieties. The hybrid KG-6 x HVW-247 had an average calcium content of 1200 mg/kg, and HVW-247 x NS-293 had only 300 mg/kg Ca. The mean value (300 mg/kg) of the hybrid HVW-247 x NS-293 was significantly lower in relation to its parents (Table 2).

The differences in the mean values of the content of the studied elements as well as their variation allowed further analysis of the nature of these traits. The inheritance types for all studied macroelements (Table 3) were simplified by adding their numerical representation. In the F_1 all modes of inheritance were observed, with the preponderance of superdominance (Table 3). Although all four types of inheritance were present in the studied plant material, the frequency of each inheritance mode varied. Superdominance emerged as the most frequently observed mode of inheritance across all four elements. It was represented in four cases for phosphorus, six for potassium, five for magnesium, and eight for calcium. Dominance was present for all four elements, occurring in two cases each for P and K, three cases for Mg, and two for Ca. Partial dominance was less common but still observed, with two cases for P and K, and one case each for Mg and Ca. Intermediate inheritance also appeared for all elements, with four cases for P, two for K, three for Mg, and one for Ca. Superdominance was the most prevalent overall, particularly in the case of Ca. In the cross HVW-247 x NS-293 for Ca content negative heterosis occurred.

Table 3. Representation of each inheritance type (superdominance, dominance, partial dominance, and intermediate) for P, K, Mg and Ca in twelve F_1 hybrids.

Mode of inheritance	P	K	Mg	Ca
Superdominance	4	6	5	8
Dominance	2	2	3	2
Partial dominance	2	2	1	1
Intermediate	4	2	3	1

P: phosphorus, K: potassium, Mg: magnesium, Ca: calcium

Components of genetic variance for macro elements content in barley grain: The analysis of the inheritance of the content of the studied macro elements made it possible to determine the mode of action of the genes that determine the expression of the macro elements. By dividing the genetic variance into additive and dominant (epistatic) component, the main effects of the genes controlling these traits were determined (Table 4).

The genes with additive effects had the greatest influence on the P content. The value D = 14.47 was greater than the dominant variance components H1 = 9.21 and H2 = 7.87, and F = 8.43 was significantly greater than zero, indicating that dominant genes had a major influence on the expression of P content. This assumption was confirmed by the frequency values of dominant (u = 0.69) and recessive alleles (v = 0.31). The H2/4H1 component confirmed the unequal distribution of dominant and recessive genes. At the same time, the value √1H1/D = 0.79 showed that the predominant mode of inheritance was partial dominance. According to the value of Kd/Kr= 2.13, it can be expected that the offspring would have a higher mean value than the mean parent when inheriting phosphorus.

According to the value of broad sense heritability (97.5%), phosphorus content is a highly heritable trait, and the rather high value of narrow sense heritability (64.3%) indicated good possibilities for breeding for this trait. During selection, it is possible to produce genotypes with higher values of phosphorus content than the average parent.

In the case of K, the value of the additive variance (D = 23.22) was greater than the values of the dominant variance (H1 = 18.52 and H2 = 17.28). Since the F value was positive (0.14), this trait was controlled by more dominant than recessive genes (u = 0.63 and v = 0.37). This fact was also confirmed by the ratio Kd/Kr = 1.01. High values of the broad sense (97.9%) and of the narrow sense heritability (72.2%) indicated the possibility of selection for increased potassium content in the grain of new barley lines.

Table 4. Components of genetic variance for the macro elements content in barley grain.

Variance component	P	K	Mg	Ca
D	14.77^{**}	23.22^{**}	6.19^{*}	2.72^{*}
H1	9.21^{*}	18.52^{**}	10.27^{**}	15.25^{**}
H2	7.87^{*}	17.28^{**}	6.07^{**}	9.12^{**}
F	8.43^{**}	0.14^{*}	9.86^{*}	1.05^{*}
E	0.15	0.36	0.06^{*}	0.20^{*}
u=p	0.69	0.63	0.82	0.81
v=q	0.31	0.37	0.18	0.19
H2/4H1	0.21	0.23	0.15	0.14
√H1/D	0.79	0.89	1.29	2.37
Kd/Kr	2.13	1.01	4.24	1.18
H^2	97.48^{**}	97.86^{**}	96.74^{**}	96.86^{**}
h^2	64.33^{**}	72.21^{**}	14.45	61.16^{**}

D: additive effect, H1 and H2: dominance effect, F: frequencies of dominant to recessive alleles in the parents, E: environment effect, u: the values of the dominant alleles, v: the value of the recessive alleles, H2/4H1: proportion of genes with positive and negative effects in the parents, √H1/D: average degree of dominance, Kd/Kr: ratio of the total number of dominant against recessive alleles, H^2: broad sense heritability, h^2: narrow sense heritability.

When analyzing the inheritance of Mg content, it can be seen that the dominant genes H1 = 10.27 and H2 = 6.07 had a somewhat greater influence on the average expression of this quantitative trait. The values of F = 9.86 and Kd/Kr = 4.24 confirmed the fact that significantly more dominant genes (u = 0.82%) than recessive genes (v = 0.18%) were involved in the determination of this trait. The ratio H2/4H1 = 0.15 deviated significantly from the theoretical value (0.25), which indicated that the distribution of dominant and recessive genes in the parents were asymmetrical. The extremely high proportion of dominant variance in the total variance was also reflected in the low value of the narrow sense heritability (14.45%), so that the conclusion can be drawn that the selection of plants for increased Mg content is a difficult breeding task.

The inheritance of Ca content in the F$_1$ barley hybrids was controlled mainly by dominant genes (H1 = 15.25 and H2 = 9.12) in relation to genes with additive effect (D = 2.72). A positive value of F = 1.05 means that the dominant genes had a greater influence on the interaction between additive and dominant genes. Since the value (Kd / Kr) was greater than zero, it was expected that the offspring on average had a higher value than the middle parent. High values were determined both for broad sense heritability (96.86%) and for narrow sense heritability (61.1%). This relatively high value of h^2 could be explained by a very low interaction between additive and dominant gene effects (F = 1.05). However, it should be noted that the heritability value could be much lower in a different population given the predominantly over-dominant inheritance of this trait in both directions (better and worse parents) (Table 3).

Combining ability analysis of the macroelements content: When breeding barley for an increased content of macro elements in the grain, in addition to determining the predominant mode of inheritance, the form of gene action and the heritability value, the selection of parent pairs for crossing also plays an important role. One of the methodological procedures for selecting suitable parents for the selection of superior offspring is the analysis of combining ability (Table 5). The values of the GCA express the average contribution of the parental genotypes created by diallel crossing.

For P content, the parents Partizan and KG-6 had highly significant positive GCA values, and the better general combiner was the variety KG-6. The variety NS-293 had a highly significant negative GCA value, as did the variety HVW-247. This is consistent with the high average P content of Partizan and KG-6 and the low average P content of NS-293 and HVW-247. The high significance of GCA in all four parents indicates a weaker influence of dominant and epistatic genes in the expression of P content.

The negative SCA value for P content in hybrid S1, which was created by crossing parents with positive GCA, illustrated the complexity of the gene system that codes for phosphorus accumulation. In this case, genes with a dominant effect, directly or through interaction, were carriers of a lower phenotypic value of the corresponding hybrid.

For K content, only the variety NS-293 was a good general combiner with a highly significant positive GCA value (2.2), while the varieties Partizan and KG-6 showed highly significant negative GCA values. The hybrid NS-293 x HVW-247, which showed negative heterosis in relation to the weaker parent, had the most pronounced negative GCA value (-2.38).

Table 5. Combining ability and ranking for macronutrient content in barley grain.

Combining ability	Genotype	P	Rank	K	Rank	Mg	Rank	Ca	Rank
GCA	P1	0.56**	2	-1.13**	3	-0.19	3	0.06	2
	P2	1.69**	1	1.50**	4	0.44	1	1.06**	1
	P3	-1.44**	4	2.2**	1	0.44	2	-0.81**	4
	P4	-0.81**	3	0.5	2	-0.69	4	-0.31	3
LSD 0.05		0.39	Se	0.61	Se	0.25	Se	0.45	Se
LSD 0.01		0.52	0.19	0.80	0.30	0.33	0.12	0.60	0.22
SCA	S1	-1.19**	6	-0.13	5	-0.94	5	-0.94**	5
	S2	-0.06	5	0.25	4	0.56	3	1.44**	1
	S3	1.31**	1	1.88**	1	0.69	2	-0.06	4
	S4	1.31**	2	1.63**	3	-1.06	6	0.94**	2
	S5	0.19	4	1.75**	2	-0.44	4	0.44	3
	S6	0.81**	3	-2.38**	6	1.06	1	-1.69**	6
LSD 0.05		0.55	Se	0.86	Se	0.35	Se	0.65	Se
LSD 0.01		0.74	0.27	1.15	0.42	0.47	0.17	0.88	0.32

**: significant at 0.01, *: significant at 0.05, GCA: general combining ability, SCA: specific combining ability.

In terms of Mg content, the varieties KG-6 and NS-293 stood out as good general combiners, while the variety HVW-247 (-0.69) was a significantly negative general combiner. The SCA values for most of the hybrids except S1 and S2 were statistically significant, but with a positive sign for S2, S3 and S6 and a negative sign for S1, S4 and S5.

According to the Ca content, the variety KG-6, which had the absolute highest content of this element in the grain, could be described as a good general combiner and the variety NS-293, with the lowest Ca content in the grain, as a statistically significant negative general combiner. The highest positive SCA value (1.44) was found in the hybrid Partizan x NS-293, i.e. a hybrid between one medium and one excellent combiner.

According to the ANOVA data for combining ability (Table 6), the GCA of the parents for the P content in the grain had a statistically highly significant influence on the total variation in combining ability, while SCA of hybrids was only significant. As the ratio of GCA/SCA was higher than unity it may be concluded that the additive gene action played an important role in the inheritance of P content. This is consistent with previous analyses showing that the additive component of genetic variance was more important for P content than the dominant component of variance, and also with a high value of narrow sense heritability.

Table 6. Analysis of variance (ANOVA) for combining ability of P, K, Mg and Ca content in barley grain.

	Df	P	K	Mg	Ca
GCA	3	15.71^{**}	22.17^{**}	2.37	5.08^{**}
SCA	6	3.47^{*}	7.71^{**}	2.72^{*}	2.71^{*}
REC	6	1.17	4.75^{**}	1.33	11.33^{**}
Error	30	0.15	0.36	0.06	0.20
GCA/(SCA+REC)	-	3.39	1.78	0.58	0.36

**: significant at 0.01, *: significant at 0.05, GCA: general combining ability, SCA: specific combining ability, REC: reciprocal effect, Df: degrees of freedom.

In the case of K, all three components of the variation in combining ability had a highly significant influence. In the ranking, the GCA comes first, then the SCA and finally the reciprocal. The ratio of the mean squares GCA/SCA is greater than one so the conclusion can also be drawn in this case that additive genes had a predominant influence on the inheritance of this trait. This is also consistent with the previously calculated components of additive and dominant variance as well as with narrow sense heritability, as they indicated that the genetic variance was predominantly additive.

The analysis of variance for the Mg content showed that only the specific combining ability had a significant influence on the variation (Table 6). SCA are the result of the effect of dominant and epistatic genes, so that the previously determined low heritability was consistent with the results of this analysis. For Mg content, the GCA/SCA ratio was less than one, which was consistent with the value of the average degree of dominance (1.29) and confirmed that genes with dominant and epistatic effects had a predominant influence on combining ability.

For Ca content, GCA and REC had highly significant mean square values. In this case, the reciprocal hybrid combinations had the highest influence on the combining ability. It was the interaction of cytoplasmic factors with nuclear genes that largely determined the expression of this trait. An example of this was the hybrid KG-6 x HVW-247, which had a Ca content more than twice as high (1200 mg/kg) as the reciprocal combination HVW-247 x KG-6 (500 mg/kg). Similar values were obtained for Partizan x KG-6 (1000 mg/kg and 500 mg/kg), NS-293 x HVW-247 (600 mg/kg and 300 mg/kg). Since the SCA of the reciprocal hybrids had a significantly higher value than the GCA, their ratio was less than one. This was consistent with the components of genetic variance in which dominant effects predominated over additive effects, thus confirming the superdominance in the inheritance of this trait.

Regression analysis (VrWr): The additive-dominant model of inheritance of quantitative traits makes it possible to divide genetic factors into their determining components. Regression analysis allows the identification of some types of epistasis in addition to the general identification of additive, dominant and epistatic effects of genes. The identification of the different types of epistasis is based on their characteristic deviation from the ideal VrWr graph. It should be emphasized that this model has limitations due to the existence of a large number of interactions that cannot be quantified. In addition, the regression analysis allows the evaluation of the genotypes of the parents used for the diallel in terms of the ratio of dominant and recessive genes.

The expected regression line for the P content $y = 1.57 + 0.94x$ intersected the y-axis above the coordinate origin (Figure 1a). That means partial dominance in the inheritance of P content. The b-value does not deviate significantly from zero (0.94), the additive-dominant model provides satisfactory results, and epistasis is not present. On the basis of the small area of the intercept between the expected regression line and the limiting parabola, it can be concluded that the expression of P content in barley grains was predominantly controlled by genes with additive effects. This agreed with all previous analyses. The varieties Partizan and KG-6 had the largest number of dominant genes, while the variety NS-293 had the largest number of recessive genes. Partizan and KG-6 also had the best GCA values, while NS-293 had the highest negative GCA value.

The regression line $y = 1.14 + 0.99x$ showed that inheritance of K was controlled by the additive genes action (Figure 1b). This was also consistent with all previous analyses. The variety HVW-247 had the most dominant genes for K content, while the variety KG-6 contained the largest number of recessive genes. The NS-293 variety had the highest positive value for GCA, while the KG-6 variety had the highest negative value. For the potassium content of barley, it can be concluded that due to the high heritability ($h^2 = 0.64$) it is possible to select progeny that on average have a higher content of the specified element in the grain than the parent varieties.

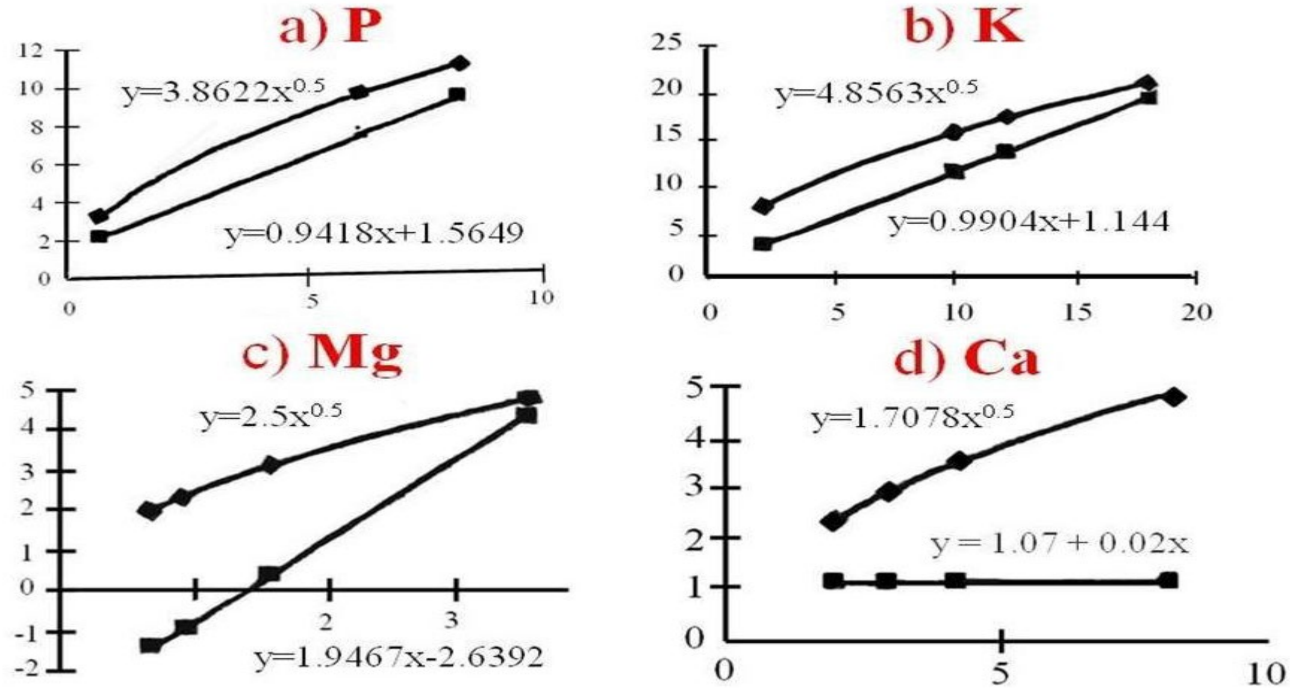

Figure 1. VrWr regression for the (a) P: phosphorus (b) K: potassium (c) Mg: magnesium (d) Ca: calcium.

The regression equation y = -2.64 + 1.95x showed that the value of the regression coefficient was greater than zero. The intersection of the regression line with the y-axis lied below the origin of the coordinates (Figure 1c), which corresponded to the average degree of dominance (1.29). The limiting parabola was quite far from the expected regression line, so the dominant gene effects were more important than the additive ones in the inheritance of this trait. The same conclusion was drawn based on the components of genetic variation, where H1>D. The parent with the most dominant genes was Partizan, and the one with the most recessive genes was HVW-247. As a result of this genetic system, the overall heritability was low, suggesting that selection for increased Mg content in the offspring was quite difficult.

The regression equation y = 1.07 + 0.02x showed that the slope of the regression line was close to zero and that there was a significant deviation from the additive-dominant model (Figure 1d). Epistatic gene effects had the strongest influence on the expression of Ca content. Interallelic interactions led to the formation of a large area between the expected regression line and the limiting parabola. To determine which of the parents initiates epistasis, one would have to remove them individually, but this would lead to unreliable data as the entire regression analysis would be based on only three parents and three hybrids. In this sense, there would be no improvement in research efficiency, which is consistent with previous analyzes showing that inter-allelic gene interactions had a pronounced effect on the inheritance of Ca content in barley grain.

DISCUSSION

The quality and the chemical composition of barley grains are influenced by many factors, such as genetic characteristics, environmental conditions, specific treatments, and agricultural practices (Panizo-Casado *et al.,* 2020). The content of macroelements in barley grain largely determines its quality and utility value. The obtained mineral elements content of the barley grain in this research was similar to those described by other authors (Rasmusson *et al.,* 1971; Platel *et al.,* 2010; Cieslik *et al.,* 2017). Due to the large number of polygenes and their interactions, the influence of environmental factors on the chemical properties of cereal grains can be considerable (Bao, 2014). Xue *et al.* (2016) found that micro-nutrients had higher genotype × environments interactions than macro-nutrients. When analyzing macro and micronutrient variation in wild barley, Abendroth *et al.* (2022) found that most micro- and macroelements had a modest degree of heritability, while heritability in our study was mostly high and moderate, except for Mg.

For breeders, the genetic nature of phenotypic variation is paramount, as is the evaluation of gene effects to determine the most beneficial breeding procedures to improve genetic material (Deb and Chowdhury, 2021). The mechanisms of genetic control of quantitative traits can be determined by diallel analyses, which are also commonly used in plant breeding to estimate GCA and SCA effects, heterosis, heritability and components of genetic variance. Additive gene actions are assessed based on the GCA variances and their effects, while non-additive gene actions (dominance, overdominance or epistasis) are indicated by the SCA effects (Gurmu *et al.,* 2018). GCA and SCA are important for the prediction of heterosis and serve as a theoretical basis for the selection of remarkable parents when creating hybrid varieties (Zhang *et al.,* 2015).

As a common feature of all macroelements investigated, it can be concluded from the components of genetic variance that genotypes of the progeny with a higher content of macroelements than the average parent are to be expected. In addition, an uneven distribution of genes with dominant and recessive effects was found in the parental varieties for all macroelements. Due to the somewhat lower values of narrow sense heritability for Mg and conditionally for Ca, it is more difficult to select superior genotypes for these elements than for the content of P and K. In general, the ANOVA of combining ability for macroelements gave a more detailed insight into the genotypes used and confirmed the previous results on the gene effects on macroelements content.

Regression analysis allows the identification of some types of epistasis in addition to the general identification of additive, dominant and epistatic effects of genes. Furthermore, the regression analysis allows the evaluation of the genotypes of the parents used in diallel with respect to the ratio between dominant and recessive genes. The VrWr regression analysis in this study showed that the additive-dominance model, as a theoretical assumption for conducting a diallel experiment enabled the alignment and interpretation of the gene action of three macroelements, but in the case of Ca inheritance, the given model was not suitable due to the presence of interallelic interaction. Additive gene action predominated in the inheritance of phosphorus and potassium, while dominance prevailed for magnesium. Partizan contained most dominant genes for Mg, the variety KG-6 for P, and HVW-247 for K and Cu. In contrast, most recessive alleles were present in cultivar KG-6 for K content, NS-293 for P and HVW-247 for Mg content. The simultaneous investigation of several generations in different environments can overcome the observed limitations in the analysis of gene effects controlling quantitative traits. Such experiments require considerable material effort, which may result in not being able to quantify all possible interactions in the end. In addition to high and stable productivity, the quality and nutrient enhancement of hybrids could also be valuable in the face of evolving agricultural challenges and market demands. The optimized and higher mineral concentration of barley grain in breeding new hybrids could be a significant characteristic for enriching human and animal consumption. Enhancing the mineral content of barley grain and the creation of better hybrids that would have fixed dominance and overdominance gene effects regarding macroelements content is a challenge and important goal for future barley hybrid breeding programs. By combining molecular markers with different quantitative traits, the location of the genes that have the strongest influence on the expression of a particular trait could be determined. In this way, the localized genes enable easier planning of the experiment and its monitoring in the selection process, both in classical and marker-assisted breeding.

Conclusion: Significant differences in the content of four elements (P, K, Mg and Ca) were found in the parents and F_1 hybrids, which affect the quality of barley grains. The GCA was determined for the parent varieties used and the SCA, both direct and reciprocal, for the hybrids. Phosphorus and potassium were primarily influenced by additive gene action, making them highly heritable and suitable targets for genetic improvement through selection. In contrast, magnesium and calcium were more affected by dominance and gene interactions, making their inheritance less predictable. All four minerals showed strong genetic control, though magnesium's low narrow-sense heritability suggests limited gains from selection alone. It was found that the best general combiner for P content in the grain of barley was the variety KG-6, for K content the variety NS-293, for Mg content the varieties KG-6 and NS-293 and for Ca content the variety KG-6. These varieties could be successfully used as gene donors for further improvement of the mineral composition characteristics of F_1 hybrids. The results of the VrWr regression analysis complemented the previously established inheritance models. The study demonstrates the potential of conventional breeding to enhance the nutritional quality of barley for food, feed, and malt production. By further integrating classical breeding methods with modern molecular tools, breeders can even more effectively select parental lines and create hybrids with increased macroelement content.

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