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Enhancing and comparison of yield components through diallel analysis in F_1, F_2, F_3 and F_4 barley (*Hordeum vulgare* L.) populations

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Developing high-yielding cultivars requires understanding genetic variation in yield and its components. The study aimed to investigate the genetic structure and inheritance of key traits to identify suitable parents and promising hybrid combinations across F_1–F_4 generations, using a randomized complete block design with three replications (2019–2023). Data analysis included Griffing Method I, Model 1, general combining ability (GCA) and specific combining ability (SCA) analysis, full diallel variance analysis, Jinks-Hayman diallel hybrid analysis, and heterosis-heterobeltiosis ratios. The Arcanda cultivar showed strong general combining ability, while the Arcanda/Asparuh and Alena/Asparuh hybrid combinations exhibited significant specific combining ability. In the F_1–F_4 evaluations, Arcanda × Asparuh achieved the highest yields (30.43 g plant^{-1}; 340.40 g 1 m^{-1}; 8500.0 and 9151.0 kg ha^{-1}), with mean heterosis and heterobeltiosis values of 37.32% and 23.07%, respectively. Alena × Asparuh also performed strongly, particularly in F_3 (8459.0 kg ha^{-1}), and exhibited high heterosis in F_1. High heterosis and heterobeltiosis values, especially for grain yield and thousand kernel weight indicate substantial potential for genetic improvement. To assess the kinship of the parental lines, iPBS-retrotransposon primers were used. The Alena and Arcanda cultivars showed 78% similarity, while the Asparuh cultivar showed 71% similarity to the other parents. In conclusion, Arcanda × Asparuh and Alena × Asparuh consistently combined high yield with genetic stability, making them strong candidates for breeding high-performing barley cultivars. Delaying selection to the later F_3–F_4 generations increases the accuracy of identifying and stabilizing superior hybrids, thereby maximizing genetic potential and enhancing agricultural productivity.

Keywords *Hordeum vulgare* L., Diallel analysis, Combining ability, Genetic diversity, Heterosis

Barley (*Hordeum vulgare* L.) stands as one of the earliest domesticated and fundamental crops of the ancient world[1]. Barley's significance in crop production, especially in non-irrigated regions, lies in its versatile role as both a crucial livestock feed and a primary raw material for malt production. Additionally, it is being investigated for its potential applications in human food products, broadening its agricultural and economic importance[2]. In 2023, barley was cultivated on 3.3 million ha in Türkiye, representing one-third of the total cereal production area (11.5 million ha). With a production of 9.2 million tons, barley is the second most produced cereal after wheat (22 million tons). Historical yields ranged from 2000–2250 kg ha^{-1} in the early 1990s to 2520 kg ha^{-1} on average over the past five years (2019–2023)[3].

The successful utilization of hybrid cultivars relies on the presence of economically significant heterosis, adequate cross-pollination to ensure cost-effective hybrid seed production, and an efficient, reliable system for developing the female parent[4]. Furthermore, understanding gene action, inheritance patterns, magnitude of

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effects, and interactions is essential for designing effective breeding strategies to develop superior genotypes[4]. Heterosis is an important agricultural phenomenon in which hybrid genotypes exhibit enhanced growth, productivity, earliness, quality, and other traits compared to their parents and its application has expanded to an increasing number of crop species[5]. In contrast, heterobeltiosis refers to the superiority of hybrids specifically over the parent with the highest trait value.

Identifying genetically superior parents is a crucial prerequisite for developing promising lines[6]. To design an effective breeding program for developing superior genotypes, it is crucial to understand the mode of inheritance, the magnitude of gene effects, and their interactions[7]. This knowledge not only aids in selecting the most suitable breeding approach but also guides the development of appropriate varieties for specific conditions. The genetic mechanisms involved in the expression of yield and its components are consistent and reliable in the F_1 generation[8]. Diallel analysis, a potent genetic tool, evaluates combining ability of parental lines' combining ability and sheds light on complex trait interactions. Through a broad mating scheme with diverse parental lines, it estimates both general and specific combining abilities. This analysis unveils modes of inheritance, genetic architecture, and the significance of yield component traits. Consequently, it informs the selection of parental components for hybridization with scientific precision[9,10]. To analyze gene effects related to yield and its components, various genetic models have been proposed, particularly second-degree statistical models[11]. Diallel analysis may be performed out using Jinsk-Hayman and Griffing methods. The Jinks-Hayman method is used to determine heritability, genetic components, and gene effects, while the Griffing method estimates specific combining ability (SCA), general combining ability (GCA), and reciprocal effects. In addition, the methods can be utilized to estimate additive and non-additive effects. The Jinks-Hayman[12] and Griffing[13] methods are usually performed together for assessment of supporting data[14]. In combining ability tests, high SCA values indicate gene dominance, while elevated GCA levels suggest additive gene effects. If both GCA and SCA are insignificant, gene epistasis plays a significant role in trait control[15]. By evaluating their general combining ability (GCA) and specific combining ability (SCA) values across multiple traits, this approach helps identify the most promising candidates[16,17].

For the effective identification of genes in barley breeding, crosses are performed between genetically distinct parents that exhibit contrasting phenotypic responses to the trait of interest[18]. DNA fingerprinting of parental lines and their hybrids, particularly when combined with molecular markers, has proven to be an efficient and powerful technique in breeding programs[19]. Considering the results of molecular analysis, high-performance hybrids can be obtained by increasing the genetic distance between parental lines[20]. Inter-primer binding site (iPBS) primers are retrotransposon-based molecular markers that amplify the region between two reverse-aligned retrotransposons, which are bound by the opposite transcriptase marker regions[21]. This marker system is suitable for any plant species due to the common presence of a tRNA addition as the binding region for the opposite transcriptase in long terminal repeat (LTR) retrotransposons, and it does not require prior sequence information[22].

To improve barley genotypes, manipulating genetic variability is essential to enhance adaptation, facilitate the introduction of new genes, and ultimately increase genetic gain in subsequent generations[23]. The primary goal of barley genetic improvement is to maximize the accumulation of desirable genes within a single genotype or variety[24]. Heritability in various agricultural crops is a key factor in the success of breeding programs. The investigation of heterosis significantly influences the breeding methodologies for varietal improvement and sheds light on the usefulness of parents in breeding programs[25]. The performance of hybrids is assessed by their improvement over the mid-parent (heterosis) and the better parent (heterobeltiosis). Taking these factors into account, this study was designed to develop high-yielding barley varieties from diallel progenies, aiming to enhance the understanding of the genetic mechanisms governing trait inheritance. Therefore, this present study was aimed to: 1) evaluate yield and its components across successive generations (F_1 to F_4), 2) discover genetic structures within progeny (F_1, F_2 and F_3), 3) identification of superior parental combinations based on GCA and SCA for yield traits, 4) assessment of the heterosis, and heterobeltiosis of yield and yield components, and 5) determining genetic diversity between parental varieties with iPBS markers.

Materials and methods

Field trails and investigated traits

A field trial was carried out at the experimental site in the Gumusova District, Duzce Province, Türkiye (40°50′23.2" N, 30°58′24.3" E; elevation 160 m). The study was carried out from October 2019 to July 2023. In the study, three two-rowed barley varieties (*Hordeum vulgare* L.), namely Arcanda, Alena, and Asparuh, were used as parents and reciprocally hybridized based on a diallel cross using Griffing's Method I, Model 1. The three parent varieties and their resulting six hybrids (Arcanda/Alena, Arcanda/Asparuh, Alena/Arcanda, Alena/Asparuh, Asparuh/Arcanda, Asparuh/Alena) were grown in a randomized complete block design with three replications. The seeds of F_1 progenies and their parents were sown in 2 rows, each 1 m long, spaced 30 cm apart, with an intra-row spacing of 10 cm, in early November 2019. The seeds of F_2 progenies and their parents were sown in 2 rows, 1 m long, spaced 20 cm apart early November 2020. The seeds of F_3 progenies and their parents were sown in 3 rows, each 5 m long, spaced 20 cm apart in early November 2021, and F_4 progenies and their parents were sown in 5 rows, each 5 m long, spaced 20 cm apart in early November 2022 (Fig. 1).

Agronomic practices from sowing to harvest including fertilization and weed control were applied for the experiment for each year (2019–2023). Ten main plants were selected randomly from each replication for parents and F_1s to F_4s progenies to take measure of the investigated traits: spike length (SL), number of grains per spike (NGS), and grain weight per spike (GWS). Thousand kernel weight (TKW) was calculated based on the average weight of 4 randomly selected samples of 100 kernels from each plot. Grain yield (GY) was calculated as grams (g) per plant in the F_1 generation, grams (g) per 1 m row in the F_2 generation, and as kilogram (kg) per hectare (ha) in the F_3 and F_4 generations.

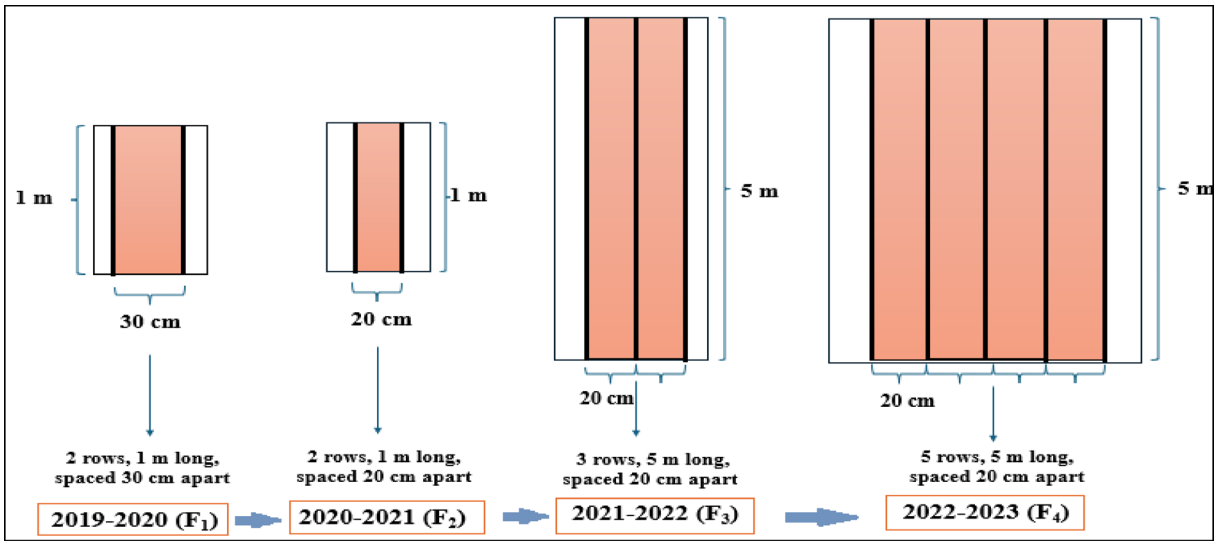

Fig. 1. Experimental plot design across generations F_1–F_4 (2019–2023).

No	Primer	Primer Sequence	Tm (°C)
1	2074	GCTCTGATACCA	50
2	2384	GTAATGGGTCCA	50
3	2245	GAGGTGGCTCTTATACCA	50
4	2402	TCTAAGCTCTTGATACCA	50
5	2382	TGTTGGCTTCCA	50
6	2381	GTCCATCTTCCA	50
7	2246	ACTAGGCTCTGTATACCA	50
8	2257	CTCTCAATGAAAGCACCA	50
9	2278	GCTCATGATACCA	50
10	2386	CTGATCAACCCA	50

Table 1. Name, sequences and melting temperature of the iPBS-retrotransposon primers.

Molecular analysis

In this study, genetic variation among the three barley varieties was assessed using iPBS markers. iPBS markers were chosen because they provide genome-wide coverage by targeting retrotransposon insertion sites, exhibit high levels of polymorphism in barley and other cereals, and do not require prior sequence information. These properties make them a reliable and representative tool for evaluating genetic diversity. Ten iPBS-retrotransposon primers were used to assess the genetic similarity among the parents. DNA isolation was carried out using the CTAB protocol[26]. The DNA concentration of the genotypes was measured using a NanoDrop spectrophotometer and diluted to 5 ng/µL. The samples were stored at − 20 °C until polymerase chain reaction (PCR) analysis. The details of the iPBS-retrotransposon primers are provided in Table 1. Genomic DNA extracted from the barley genotypes and the iPBS-retrotransposon primers were used for PCR analysis. The PCR reaction mixture consisted of 10 × PCR buffer (1 µL), $MgCl_2$ (1 µL), dNTP (1 µL), primer (1.5 µL), Taq polymerase (0.15 µL), DNA (2.5 µL), and ddH2O (2.85 µL). The amplification protocol was as follows: 3 min at 95 °C, 15 s at 95 °C, 1 min at 50 °C, 1 min at 68 °C, followed by a final extension for 5 min at 72 °C with 30 cycles, and the reaction was then stored at 4 °C[27]. PCR products were analyzed by 1.5% (w/v) agarose gel electrophoresis using 0.5 × Tris–Borate-EDTA (TBE) buffer. The gels were stained with ethidium bromide and visualized under UV light. To ensure the reliability and reproducibility of the molecular data, the entire iPBS-retrotransposon marker analysis was conducted using two independent biological replicates for each parental genotype (Arcanda, Alena, and Asparuh). The resulting amplification profiles were highly consistent across these replicates. Furthermore, to minimize subjective bias in data interpretation, the DNA banding patterns on all agarose gels were scored independently by two of the authors (H.G. and M.F.C.). Any discrepancies in scoring between the two evaluators were resolved by a joint re-examination of the gel image until a consensus was reached. The representative gel images presented in this manuscript were selected from this verified dataset. The original, full-length, and uncropped gel images for all primers and replicates are provided in the Supplementary Information (Supplementary Figure S1) to ensure full data transparency.

Statistical analysis

The mean data were compared using the least significant difference (LSD) test at significance level of $P \leq 0.05$. The heterosis (Ht) and heterobeltiosis (Hb) were calculated according to the method suggested by Chang and Smith[28] and Fonseca and Patterson[29]. Meanwhile, the estimates of combining ability variances and effects were calculated using Griffing's Method I, Model I[13]. Estimating the components of variation and determining the nature of gene effects in the studied traits were performed using the diallel biometric approach as outlined by Jinks and Hayman[12]. The data obtained from the trials were analyzed using the TARPOPGEN statistical package[30].

The resulting DNA banding patterns were analyzed using TotalLab TL120 software (TotalLab Ltd., Gosforth, Newcastle upon Tyne, UK). For the iPBS amplification products, each locus was scored in a binary format, where "1" indicated the presence and "0" the absence of a band. After that using this matrix, a dendrogram was generated to illustrate the similarities among the barley genotypes by applying the Unweighted Pair Group Method with Arithmetic Average (UPGMA) in NTSYSpc 2.21q software[31]. The polymorphism information content (PIC) for each iPBS-retrotransposon primer used in the study was calculated according to the formula $PIC = 1 - \Sigma Pi^2$, where Pi represents the frequency of the ith allele in the three barley genotypes investigated[32].

Results

The variance analysis for all the investigated traits is given in Table 2, with agronomic traits presented in Fig. 2 and the high-performing barley crosses per generation for grain yield (GY) and yield components summarized in Table 3. Genetic parameters are given in Table 4, and the GCA effects of parents, SCA of hybrids, and Ht and Hb values are presented in Figs. 3, 4 and 5, respectively.

Agronomic traits

Significant differences among genotypes were found (Table 2). A comprehensive evaluation was conducted to assess various traits across four generations (F_1–F_4) of barley crosses involving the parental genotypes Arcanda, Alena, and Asparuh.

Arcanda demonstrated consistently superior performance across traits, reinforcing its value as a breeding parent, while Alena excelled in GWS and Asparuh contributed genetic diversity with progressive improvement over generations. Among parents, Arcanda generally outperformed Alena and Asparuh. In SL, Arcanda/Asparuh and Alena/Asparuh ranked highest in early generations and maintained competitive values in later ones. NGS improved in the early generations, with Asparuh/Alena and Alena emerging as top performers by F_4. For GWS, Arcanda/Asparuh and Alena/Asparuh consistently ranked among the best, while Arcanda/Alena achieved notable gains in the final generation. TKW was led by Arcanda/Asparuh and Asparuh/Arcanda in F_1, with Alena/Arcanda and Asparuh/Arcanda dominating in F_4. In terms of grain yield, Arcanda/Asparuh achieved 30.43 g plant^{-1} in F_1, 340.40 g 1 m^{-1} in F_2, 8500.0 kg ha^{-1} in F_3, and 9151.0 kg ha^{-1} in F_4, consistently ranking first. Alena/Asparuh also maintained strong performance, reaching 8459.0 kg ha^{-1} in F_3 (Fig. 2; Table 3).

SL: Spike length, NGS: Number of grains per spike, GWS: Grain weight per spike, TKW: Thousand kernel weight, GY: Grain yield.

F	Source of Variance	DF	SL	NGS	GWS	TKW	GY
F_1	Replication	2	0.640370	3.06704	0.0324825	0.07704	0.4399
	Genotype	8	1.961759*	45.96954**	0.1130372*	44.18204**	176.6148**
	Error	16	0.62412	1.3600	0.039495	0.3058	1.940
	C.V. (%)		6.59	3.87	10.06	1.27	8.40
F_2	Replication	2	0.360370	5.79704	0.0066280	4.12890	149.086
	Genotype	8	1.741481**	18.84787**	0.0695681**	10.49188**	5930.517**
	Error	16	0.17954	1.4083	0.008813	0.61213	133.36
	C.V. (%)		4.57	4.25	7.25	1.94	5.04
F_3	Replication	2	0.1403704	0.597037	0.0008874	0.26037	894.17
	Genotype	8	0.5273148**	6.265648**	0.0374468**	31.36704**	27508.90**
	Error	16	0.054537	0.64995	0.003658	1.0554	1476.3
	C.V. (%)		2.56	3.16	5.28	2.68	5.12
F_4	Replication	2	0.137778	1.0882	0.0165963	0.91000	1344.95
	Genotype	8	1.401667**	4.1055*	0.3518701**	62.84000**	45058.10**
	Error	16	0.12486	2.48454	0.016572	1.0775	2335.7
	C.V. (%)		3.40	5.06	7.51	2.46	6.39

Table 2. Analysis of variance for grain yield and yield components in 3 × 3 full-diallel crosses of F_1–F_4 barley generations. ** Significant at the $P < 0.01$ probability level, * Significant at the $P < 0.05$ probability levels. SL: Spike length, NGS: Number of grains per spike, GWS: Grain weight per spike, TKW: Thousand kernel weight, GY: Grain yield.

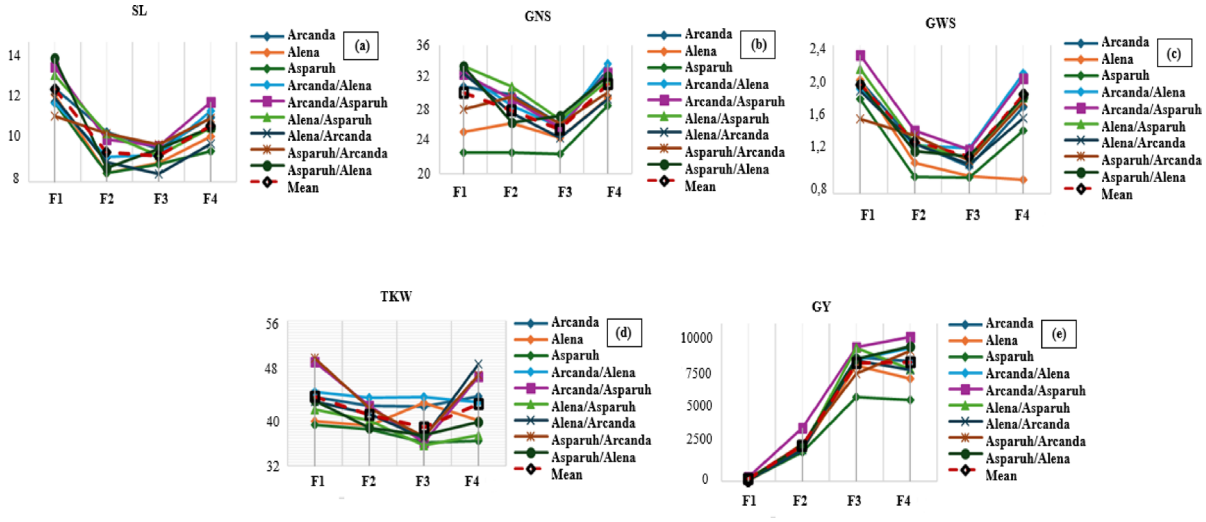

Fig. 2. Means of spike length (**a**), number of grains per spike (**b**), grain weight per spike (**c**), thousand kernel weight (**d**) and grain yield (**e**) components of parents and their F_1–F_4 progeny in 3 × 3 full diallel cross of barley.

F	SL	NGS	GWS	TKW	GY
F_1	Asparuh × Alena (13.30 cm)	Alena × Asparuh / Asparuh × Arcanda (30.87)	Arcanda × Asparuh (2.300 g)	Asparuh × Arcanda (49.73 g)	Arcanda × Asparuh (30.43 g plant^{-1})
F_2	Alena × Asparuh / Asparuh × Arcanda (10.06 cm)	Alena × Asparuh (30.87)	Arcanda × Asparuh (1.484 g)	Arcanda × Alena (43.22 g)	Arcanda × Asparuh (340.40 g 1 m^{-1})
F_3	Asparuh × Arcanda (9.60 cm)	Asparuh × Alena (27.20)	Arcanda × Alena (1.291 g)	Arcanda × Alena (43.40 g)	Arcanda × Asparuh (8500.0 kg ha^{-1})
F_4	Arcanda × Asparuh (11.43 cm)	Arcanda × Alena (33.67)	Arcanda × Alena (2.10 g)	Alena × Arcanda (48.77 g)	Arcanda × Asparuh (9151.0 kg ha^{-1})

Table 3. High-performing barley crosses per generation for grain yield and yield components.

Genetic parameters

In this study, the genetic parameters of SL, NGS, GWS, TKW, and GY were examined across F_1, F_2, and F_3 generations in barley. The analysis provided comprehensive insights into the genetic control and variation of these traits across different generations (Table 4).

Environmental effects (E) on SL decreased markedly from F_1 to F_3, suggesting a diminishing environmental influence over successive generations. For NGS, environmental influence peaked in F_2 before declining in F_3. GWS showed minimal environmental influence across all generations. TKW experienced a temporary increase in environmental influence in F_2, followed by a slight reduction in F_3. In contrast, GY showed a substantial rise in environmental influence by F_3, indicating greater sensitivity to environmental conditions compared with other traits.

The additive genetic variance (D) reflects the proportion of total genetic variance attributable to the additive effects of alleles. For SL, additive variance increased from F_1 to F_2 before declining in F_3, indicating a reduced contribution of additive effects in later generations. NGS displayed high additive variance in F_1, which declined steadily by F_3. GWS exhibited consistently low additive variance across all generations. In TKW, additive variance fluctuated, with a marked increase in F_3. GY showed the most substantial rise in additive variance by F_3, suggesting that selection in later generations could effectively capture and exploit these additive effects for yield improvement.

Frequencies of dominant to recessive alleles in the parental population decreased for SL from F_1 to F_3, indicating a reduction in dominance influence across generations. NGS also showed a marked decline, while GWS remained relatively stable with minor changes. TKW displayed fluctuating values, alternating between negative and positive, suggesting shifts in dominant direction. In contrast, GY showed a substantial increase by F_3, reflecting the persistence of dominance effects in yield traits despite a general decline in heterosis for other characteristics. Dominance effects (H_1 and H_2) quantify the contribution of dominant genetic factors to trait expression. Both parameters indicated declined across generations for SL, NGS, GWS, and TKW, indicating a reduction in dominance influence as selection progressed. In early generations, dominance effects were more pronounced, particularly for yield-related traits, but diminished in later generations as additive effects became more influential. For GY, dominance effects remained relatively high compared with other traits, suggesting that heterotic potential persisted for yield even when it declined for spike-related traits.

The difference between additive and dominance variance ($D - H_1$) reflects the balance between these two genetic components. Across most traits, values indicated a decline in dominance influence over successive generations. For SL and NGS, early generations showed a stronger dominance component, which progressively

Genetic parameters	SL			NGS			GWS			TKW			GY		
	F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2	F_3
E	0.217	0.068	0.021	0.517	0.632	0.215	0.013	0.003	0.001	0.093	0.334	0.322	0.591	45.036	470.534
D	0.683	1.080	0.151	17.842	12.674	3.848	0.036	0.047	0.005	6.322	4.310	13.724	8.834	463.990	18.797.813
F	0.842	0.802	0.267	29.079	15.674	5.660	0.076	0.031	0.016	−2.027	1.549	−8.351	30.141	1.515.658	33.981.709
H_1	5.143	2.422	0.725	79.123	29.474	13.600	0.241	0.092	0.068	50.057	4.637	21.754	310.489	8.324.056	44.422.010
H_2	4.814	2.470	0.482	63.333	23.722	11.420	0.170	0.097	0.052	53.119	4.115	39.979	281.390	6.346.165	23.113.777
$(D-H_1)$	−4.459	−1.342	−0.574	−61.280	−16.800	−9.752	−0.205	−0.045	−0.063	−43.735	−0.326	−8.029	−301.655	−7.860.065	−25.624.196
$(H_1/D)^{1/2}$	2.743	1.497	2.192	2.106	1.525	1.880	2.584	1.400	3.553	2.814	1.037	1.259	5.929	4.236	1.537
$(H_2/4H_1)$	0.234	0.255	0.166	0.200	0.201	0.210	0.176	0.263	0.190	0.265	0.222	0.459	0.227	0.191	0.130
KD/KR	1.579	1.660	2.356	2.262	2.350	2.285	2.361	1.623	2.496	0.892	1.419	0.611	1.808	2.255	3.854
h^2	0.061	0.336	0.056	58.888	10.304	4.715	−0.011	0.074	0.060	35.034	2.787	11.073	140.298	1.985.388	16.556.942
$K(h^2/H_2)$	0.013	0.136	0.116	0.930	0.434	0.413	−0.063	0.764	1.157	0.660	0.677	0.277	0.499	0.313	0.716
h^2_n	0.282	0.629	0.747	0.815	0.692	0.461	0.554	0.660	0.708	0.966	0.831	0.857	0.868	0.911	0.867
H^2_b	0.117	0.364	0.217	0.255	0.436	0.304	0.143	0.394	0.088	0.108	0.493	0.304	0.030	0.062	0.604
GCA	0.675 ns	14.708**	8.784**	20.678**	16.108**	2.709 ns	0.391 ns	11.285**	3.164 ns	215.764**	47.006**	64.428**	46.281**	33.226**	19.694**
SCA	3.895*	9.152**	12.868**	69.236**	17.555**	22.032**	1.030 ns	12.974**	19.088**	233.296**	7.619**	12.532**	146.736**	54.665**	25.468**
Resip. Effect	3.819*	5.304*	7.059**	7.118**	7.396**	1.869 ns	6.341**	0.552 ns	6.105**	8.157**	6.750**	23.773**	65.242**	41.769**	11.093**
GCA/SCA	0.17	1.61	0.68	0.30	0.92	0.12	0.38	0.87	0.17	0.92	6.17	5.14	0.31	0.61	0.77

Table 4. Genetic parameter values calculated for the traits examined in F_1, F_2 and F_3 generations. ** Significant at the $P < 0.01$ probability level, * Significant at the $P < 0.05$ probability levels.

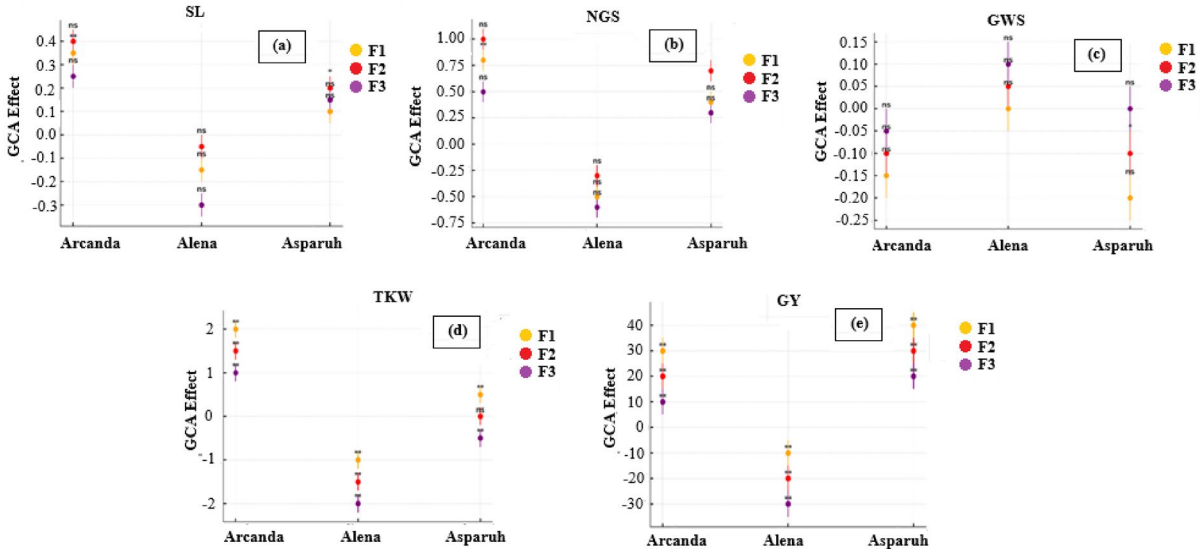

Fig. 3. Estimates of general combining ability (GCA) effects for spike length (**a**), number of grains per spike (**b**), grain weight per spike (**c**), thousand kernel weight (**d**) and grain yield (**e**) in barley hybrids.

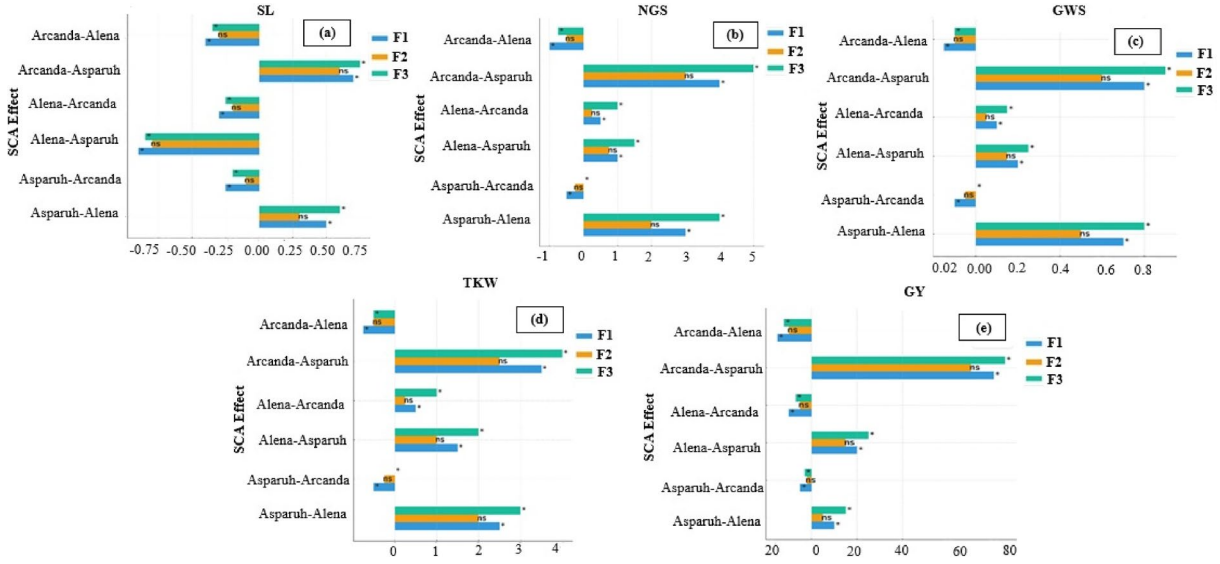

Fig. 4. Estimates of specific combining ability (SCA) effects for spike length (**a**), number of grains per spike (**b**), grain weight per spike (**c**), thousand kernel weight (**d**) and grain yield (**e**) in barley hybrids.

diminished in later generations. GWS maintained a small but consistent dominance effect throughout. TKW exhibited marked dominance influence initially, which was substantially reduced by the final generation. Similarly, GY displayed strong dominance effects in the early generation, but these declined sharply over time, suggesting a shift toward additive genetic control in later cycles of selection. The average degree of dominance $(H_1/D)^{1/2}$ describes the relative contribution of dominance compared with additive effects, where values greater than 1 indicate overdominance and values less than 1 indicate partial dominance. Across traits, SL and NGS exhibited strong overdominance in early generations, which declined by F_3. GWS fluctuated but maintained dominance influence across generations. TKW showed reduced dominance effects in later generations. GY displayed pronounced overdominance in F_1, which diminished substantially by F_3, indicating a shift toward additive genetic control as selection advanced.

Proportion of genes with positive and negative effects $(H_2/4H_1)$ reflects the balance between dominant and recessive alleles in the parental population. Values for most traits remained relatively stable across generations, although SL and GY showed a gradual decline by F_3, indicating a shift in allele distribution. TKW displayed an increase over time, suggesting a growing contribution of dominant alleles. These patterns provide insights into population genetic structure, supporting informed selection to maintain favorable allele combinations.

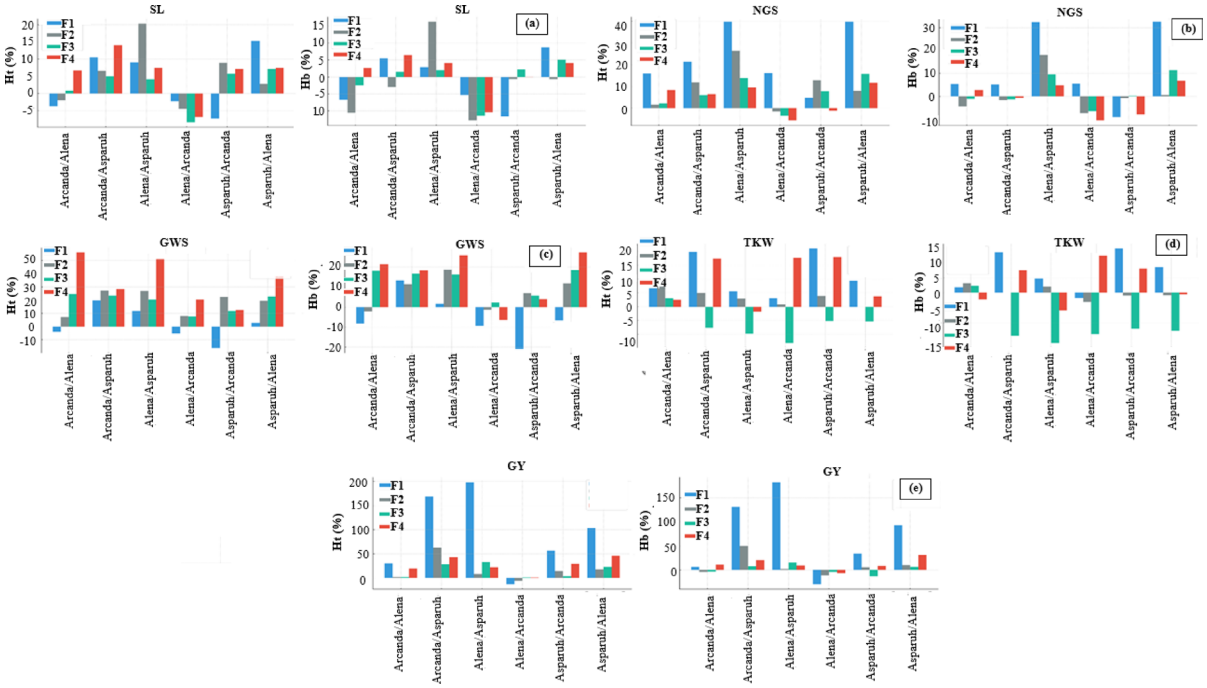

Fig. 5. Estimates of heterosis and heterobeltiosis for spike length (**a**), number of grains per spike (**b**), grain weight per spike (**c**), thousand kernel weight (**d**) and grain yield (**e**) in barley hybrids.

Dominant alleles (KD) represent the frequency or proportion of dominant alleles in the population, while recessive alleles (KR) represent the frequency or proportion of recessive alleles. A KD/KR ratio greater than one indicates a predominance of dominant alleles, favoring the expression and selection of dominant traits. Across traits, SL and GY showed an increasing KD/KR ratio over generations, suggesting a shift toward greater dominance. NGS maintained consistently high ratios, whereas GWS exhibited moderate fluctuations. TKW displayed lower ratios, indicating a stronger influence of recessive alleles. These patterns highlight that while dominance effects remain important for certain traits, additive gene effects become increasingly relevant for sustainable genetic improvement.

The dominance effect (h^2) represents the proportion of total phenotypic variance attributable to dominance variance. Across traits, dominance effects were generally strong in the early generation (F_1) but declined progressively in later generations, indicating a shift toward additive genetic control. SL showed moderate dominance influence, peaking in the intermediate generation before decreasing. NGS and GY exhibited particularly high dominance in F_1, followed by sharp reductions in subsequent generations. GWS maintained low dominance effects throughout, whereas TKW displayed high initial dominance that diminished over time. This overall trend highlights the decreasing role of dominance effects and the increasing relevance of additive genetic variance for sustained breeding progress.

Narrow-sense heritability (h^2n) reflects the proportion of phenotypic variance attributable to additive gene effects, which are critical for effective selection in breeding programs. Across traits, h^2n generally increased over successive generations, indicating a growing contribution of additive variance to trait expression. SL and NGS maintained moderate to high h^2n values, while GWS, TKW, and GY showed strong additive genetic control, particularly in later generations. Broad-sense heritability (H^2b), which accounts for additive, dominance, and epistatic effects, also increased for most traits over generations, suggesting an overall strengthening of genetic influence. While some traits, such as GWS and TKW, displayed fluctuations in H^2b, GY exhibited a marked rise in later generations, highlighting the increasing stability of genetic control. These patterns suggest that, as breeding cycles progressed, additive effects became more prominent, supporting the potential for sustained genetic gains through selection.

General combining and specific combining

GCA reflects the average performance of a parent based on its additive genetic contribution to its offspring. High GCA values indicate that a parent consistently transmits favorable alleles, making it ideal for breeding programs focused on additive genetic improvement. For SL, GCA values were non-significant in F_1 but significant in F_2 and F_3, indicating strong additive genetic control. NGS showed significant GCA values in F_1 and F_2, but non-significant in F_3, suggesting reduced additive effects. For GWS, GCA values were non-significant in F_1 and F_3 but significant in F_2, showing variability in genetic control. TKW had consistently high GCA values across generations, highlighting strong and consistent additive effects. GY had strong GCA values across all generations, indicating robust additive genetic control.

GCA values reflect the additive genetic contribution of parents to their offspring. Arcanda consistently showed positive GCA values, indicating strong additive contributions across multiple traits and generations. Significant GCA values were found for Arcanda in F_2 and F_3 for SL (Fig. 3a), in F_1 and F_2 for NGS (Fig. 3b), in F_2 and F_3 for GWS (Fig. 3c), in F_1 and F_2 for TKW (Fig. 3d), and in F_2 and F_3 for GY (Fig. 3e). Alena exhibited high positive GCA values, especially in early generations for NGS and SL. Significant GCA values for Alena were in F_1 and F_2 for SL (Fig. 3a), in F_1 for NGS (Fig. 3b), in F_2 for GWS (Fig. 3c), in F_1 for TKW (Fig. 3d), and in F_3 for GY (Fig. 3e). In contrast, Asparuh often had negative GCA values, indicating a less favorable additive genetic contribution. Negative GCA values for Asparuh were found in F_1 and F_2 for SL (Fig. 3a), in F_1 and F_2 for NGS (Fig. 3b), in F_1 and F_2 for GWS (Fig. 3c), in F_2 for TKW (Fig. 3d), and in F_2 and F_3 for GY (Fig. 3e).

SCA reflects the performance of specific cross combinations based on non-additive gene effects, such as dominance and epistasis. High SCA values indicate that combinations of parents produce superior offspring due to these non-additive interactions. For SL, SCA values were significant across generations, indicating that specific parent combinations result in superior SL due to non-additive gene effects. NGS had high SCA values, suggesting the importance of non-additive effects. For GWS, SCA values were non-significant in the F_1 but significant in the F_2 and F_3, showing the importance of non-additive effects in these generations. TKW exhibited high SCA values, particularly in the F_1 generation, indicating the crucial role of non-additive effects. GY had significant SCA values across generations, highlighting the importance of non-additive effects in improving yield.

The specific combining ability (SCA) values for various traits highlight the significance of non-additive gene effects across different crosses. For SL, the Arcanda/Asparuh cross consistently showed positive SCA values in F_2 and F_3, indicating beneficial non-additive effects, while the Arcanda/Alena cross had negative SCA values (Fig. 4a). For NGS, the Arcanda/Asparuh cross had significant positive SCA values in F_1, F_2, and F_3, reflecting the importance of non-additive effects (Fig. 4b). The Alena/Asparuh cross also showed strong positive SCA values in F_1 and F_2 (Fig. 4b). For GWS, the Arcanda/Asparuh cross had positive SCA values in F_2 and F_3, while the Alena/Asparuh cross showed a positive SCA value in F_2 (Fig. 4c). For TKW, the Arcanda/Asparuh cross demonstrated strong positive SCA values in F_1 and F_2, and the Arcanda/Alena cross had a positive SCA value in F_2 (Fig. 4d). Regarding GY, the Arcanda/Alena cross had high positive SCA values in F_1, F_2, and F_3, emphasizing the critical role of non-additive effects in enhancing yield (Fig. 4e).

The analysis of GCA and SCA values provides insights into genetic control and breeding potential. High GCA values indicate strong additive effects, making certain parents, like Arcanda, ideal for breeding programs. High SCA values highlight the significance of non-additive effects, suggesting specific parent combinations, such as Arcanda/Alena and Arcanda/Asparuh, can produce superior offspring.

Reciprocal effects

Reciprocal effects significantly influenced various traits across generations. For SL, the effects increased over time, indicating a growing influence. NGS showed significant effects initially but diminished in later generations. GWS had significant effects in early and late generations, but not in the middle generation. TKW showed strong effects across all generations, while GY had highly significant effects throughout. These results suggest that the direction of crossing significantly impacts trait expression, highlighting the importance of maternal and paternal contributions.

Heterosis (Ht) and heterobeltiosis (Hb)

The data analysis across all generations (F_1–F_4) highlights significant trends in Ht and Hb for various barley crosses and traits. For SL, the Alena/Asparuh cross showed strong performance throughout, particularly F_2 with Ht of 20.30% and Hb of 16.22%, and in F_4 with Ht of 7.49% and Hb of %4.09, while Arcanda/Asparuh exhibited notable results in F_4 with Ht of 14.02% and Hb of 6.41% (Fig. 5a). In terms of NGS, Alena/Asparuh excelled across generations, with peak performance in F_3 (Ht: 39.60%, Hb: 32.43%) and consistent results in F_4 (Ht: 9.53%, Hb: 4.83%), while Asparuh/Alena showed robust performance in F_4 with Ht of 11.64% and Hb of 6.72% (Fig. 5b). For GWS, Arcanda/Asparuh demonstrated high Ht in F_4 (28.16%) and Hb (18.34%), and Asparuh/Alena also showed significant Ht (52.24%) and Hb (27.32%) in F_4, indicating strong hybrid vigor (Fig. 5c). TKW showed Arcanda/Asparuh achieved high values in F_1 with Ht of 19.80% and Hb of 13.45%, although TKW results are more variable across generations (Fig. 5d). For GY, Arcanda/Asparuh stands out in F_1 with exceptional Ht (168.96%) and Hb (131.30%), while Asparuh/Alena consistently performs well, particularly in F_4 with Ht of 46.10% and Hb of 31.44%, and Alena/Asparuh shows significant results in F_3 (Ht: 33.20%, Hb: 15.30%) (Fig. 5e).

Molecular analysis

iPBS-retrotransposon markers were used to determine genetic diversity of the barley genotypes used as parents in current study. Ten iPBS-retrotransposon were produced 70 polymorphic alleles with 7 alleles average, and the most polymorphic one was primer 2257 with 0.543 PIC value and 14 allele numbers. The average PIC value was 0.39, while the polymorphism rate of the primers was 59.93% (Table 5). The gel pictures of the primers iPBS 2246 and iPBS 2257 are shown in Fig. 6.

A dendrogram was also produced using alleles obtained from iPBS-retrotransposon primers (Fig. 7). According to the dendrogram, Arcanda and Alena were found more like each other (78%) than Asparuh (71%).

Discussion

This research investigated agronomic traits, genetic parameters, heterosis and heterobeltiosis across F_1–F_4 generations using 3×3 full-diallel analysis. This approach evaluated genetic variation and inheritance patterns, providing insights into genetic architecture and improvement potential in barley cultivars. Differences among

No	iPBS Primer	Number of Polymorphic Allele	Polymorphism Rate	PIC Value
1	2074	7	63.64	0.444
2	2384	3	75.00	0.500
3	2245	7	63.64	0.475
4	2402	9	64.29	0.476
5	2382	4	40.00	0.289
6	2381	7	46.67	0.304
7	2246	6	46.15	0.333
8	2257	14	77.78	0.543
9	2278	10	58.82	0.405
10	2386	3	33.33	0.185

Table 5. Performance of the iPBS-retrotransposon primers on barley parents.

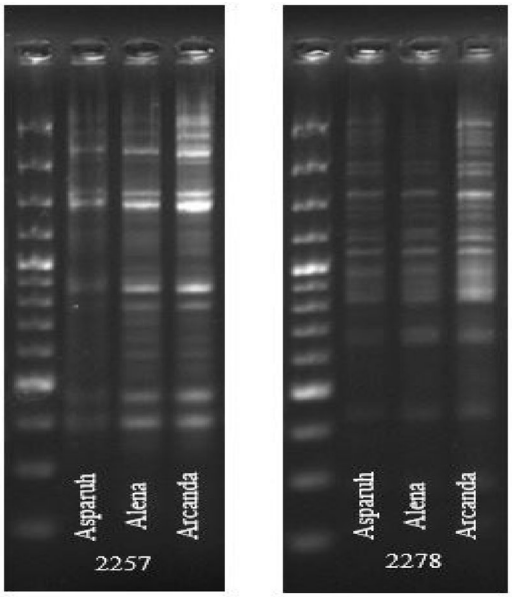

Fig. 6. Representative Gel pictures of the primers iPBS 2246 and iPBS 2257. Lane 1: DNA ladder (100 bp). Lanes 2–4: PCR products of Asparuh, Alena, and Arcanda genotypes, respectively. The genotype names are indicated above each lane. These images are representative examples selected from two independent biological replicates, which showed consistent banding patterns. The full, uncropped gel images are available in Supplementary Figure S1.

the genotypes (F_1–F_4) for the traits examined were statistically significant, consistent with the findings of Eshghi and Akhundova[33], Madić et al.[34], Metwali[35], and Bouchetat and Aissat[23]. The mean values for all traits measured in the hybrids (F_1–F_4 generations) were greater than the average values recorded in their parents, except for the TKW in the F_3 generation. This aligns with findings by Eshghi and Akhundova[33] for GY, and Bouchetat and Aissat[23] for PH and GY. The variation between hybrid and parental performance can be attributed to the combined effects of genetic factors and environmental influences, which together influence phenotypic performance, leading to either the enhancement or reduction of the trait.

The average trait values across generations (F_1–F_4) were analyzed to understand the progression and stability of these traits in the hybrids. For SL, the averages were 12.11 cm in F_1, 9.41 cm in F_2, 9.19 cm in F_3, and 10.58 cm in F_4, indicating an initial decline followed by an increase. NGS showed an initial decrease from 32.02 in F_1 to 26.06 in F_3, followed by an increase to 31.59 in F_4. GWS averaged 1.981 g in F_1, 1.364 g in F_2, 1.206 g in F_3, and 1.874 g in F_4, demonstrating improvement after F_3. TKW values were 44.94 g in F_1, 40.84 g in F_2, 37.49 g in F_3, and 43.52 g in F_4, highlighting a decrease in F_2 and F_3 before increasing in F_4. GY showed substantial improvement, increasing from 19.18 g plant^{-1} in F_1 to 7833.10 kg ha^{-1} in F_3, and stabilizing at 8112.00 kg ha^{-1} in F_4. These genetic differences align with findings from related agronomic studies[36,37].

The analysis of gene effects using the Griffing method[13] revealed significant contributions from both GCA and SCA effects across the majority of evaluated traits. The GCA effects were significant for all traits, except for SL in the F_1 generation, NGS in the F_3 generation, and GWS in the F_1 and F_3 generations. Similarly, the SCA effects were significant for all traits and generations, except for GWS in the F_1 generation. In agreement with

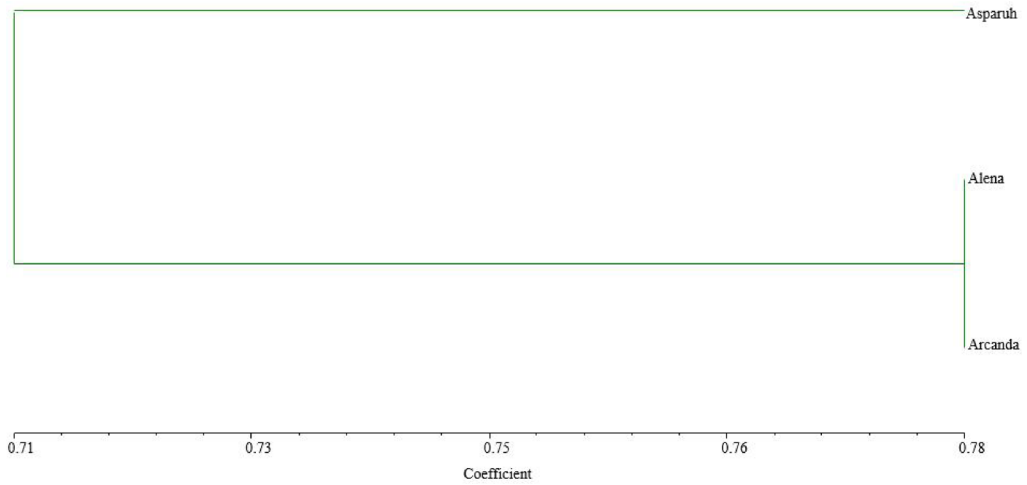

Fig. 7. A dendrogram used to visualize kinship of the barley genotypes.

these findings, Madić et al.[34] and Zhang et al.[38] reported that the analysis of variance of combining abilities in barley revealed significant GCA and SCA variances for all traits. Furthermore, Panwar and Sharma (2019) found that GCA was significant for all traits in barley, while SCA effects were significant for all traits except for days to 75% maturity. These findings indicate that both additive and non-additive gene effects should be considered in breeding programs, and that exploiting high SCA effects to develop superior hybrids can be particularly effective when non-additive variance predominates.

In the F_1, F_2, and F_3 generations, the GCA/SCA variance ratio was found to be less than one for all the characters studied, except SL in the F_2 generation and TKW in the F_2 and F_3 generations. The GCA/SCA ratio provides insight into the relative importance of additive versus non-additive effects. Similar results were obtained by Amer et al.[39], Madić et al.[34], Rohman et al.[24]. In previous barley gene action studies, trait transmission has been demonstrated that non-additive effects are more essential than additive effects, notably for one trait, showing predominance of dominance-type gene action[17,40]. These results imply that breeding programs should focus on exploiting heterosis and selecting superior hybrid combinations in early generations for traits under strong non-additive control, while applying additive-based selection for traits such as TKW where GCA effects are more prominent.

Arcanda demonstrated positive and significant GCA effects for SL, GWS, and TKW in F_2 and F_3, as well as for NGS and GY in F_1 and F_2, underscoring its value as an effective general combiner for these traits. Alena exhibited negative GCA effects in F_2 for SL, TKW, and GY, but displayed positive effects in F_3 for TKW and GY. Asparuh consistently demonstrated negative GCA effects for NGS, GY, and TKW, as well as for SL and GWS except in F_1. Arcanda is the best genotype for breeding these traits. Alena/Asparuh had positive SCA for SL and NGS in all generations, while Arcanda/Asparuh was positive for these traits in F_2 and F_3. Both crosses showed positive SCA for GWS in F_2 and F_3. For TKW, Arcanda/Asparuh and Asparuh/Alena were positive in F_1, and Arcanda/Alena, Arcanda/Asparuh, and Asparuh/Alena in F_3. For GY, Arcanda/Asparuh and Alena/Asparuh had positive SCA in all generations. Negative SCA values were noted for Asparuh/Arcanda in multiple traits. Arcanda/Asparuh and Alena/Asparuh hybrids consistently showed positive SCA across traits and generations. Considering all traits and generations, Arcanda/Asparuh and Alena/Asparuh consistently expressed positive SCA effects, reinforcing their potential as elite parental combinations in hybrid-oriented breeding programs.

These results are validated by the Jinks-Hayman model[12], which applies to all evaluated parameters. The analysis of genetic parameters across the F_1, F_2, and F_3 generations revealed that the additive effect (D) was not significant for any of the traits studied. In contrast, the dominance components (H_1 and H_2) were positive and non-significant for all traits. Notably, the value of H_1 was consistently higher than D, indicating a greater influence of over-dominance effects compared to additive effects. This observation was further confirmed by the net dominance component (h^2), which was positive and non-significant for all traits except for GWS in the F_1 generation. Additionally, the average degree of dominance $(H_1/D)^{1/2}$ was higher than unity for all traits, suggesting the presence of over-dominance gene effects. These findings highlight the significance of non-additive gene effects, particularly over-dominance, in the inheritance of the traits studied.

Heritability analysis across F_1, F_2, and F_3 generations showed higher H^2b than h^2n for all traits. For SL, H^2b was higher compared to h^2n. The NGS also had higher H^2b heritability. GWS showed moderate heritability with H^2b values higher than h^2n. TKW had the highest heritability among the traits, with H^2b values significantly higher than h^2n. GY demonstrated high H^2b heritability but lower h^2n values. The results indicate a strong genetic influence but suggest limited effectiveness for selective breeding due to the relatively low additive genetic variance.

Heterosis analysis revealed consistently positive mid-parent heterosis (Ht) for most traits across generations, with variable heterobeltiosis (Hb) responses depending on the trait and cross. Grain yield (GY) exhibited the most pronounced heterotic advantage, particularly in Arcanda/Asparuh (Ht = 168.96%, Hb = 131.30% in F_1) and Alena/Asparuh (Ht = 198.39%, Hb = 181.97% in F_1), sustaining high heterosis through later generations.

GWS also showed strong positive heterotic expression, notably in Arcanda/Asparuh and Alena/Asparuh, with Ht values exceeding 20% in multiple generations. In contrast, TKW displayed lower and sometimes negative Hb in F_3, indicating a reduced potential for hybrid superiority in certain cycles. For SL and NGS, heterosis was moderate, with Alena/Asparuh and Arcanda/Asparuh frequently achieving above-average performance. These results highlight Arcanda/Asparuh and Alena/Asparuh as elite combinations capable of exploiting non-additive gene action to achieve superior hybrid performance, particularly for GY and GWS. The sustained heterotic performance across generations underscores their potential for integration into long-term breeding programs aimed at maximizing yield gains. Previous studies by Pesaraklu et al.[17] and Moustafa et al.[41] reported high heterotic responses for grain yield in barley. The genetic diversity of the barley genotypes used in the current study was revealed by iPBS-retrotransposon primers. Ten primers generated 70 polymorphic alleles and similarity of the barley genotypes were determined which is crucial to cross diverse genotypes to expand genetic base. Arcanda and Alena were more similar to each other with a 78% similarity and 22% dissimilarity which is still well enough to obtain variation via crossing each other. Asparuh was relatively more distinct to Arcanda and Alena with 29% dissimilarity percentage. Our results provide direct evidence for the principle that parental genetic distance can drive heterotic performance. The iPBS marker analysis revealed that the Asparuh cultivar was the most genetically distinct parent, sharing only 71% similarity with Arcanda and Alena (Fig. 7). Correspondingly, the two most superior hybrid combinations for grain yield heterosis were Arcanda × Asparuh and Alena × Asparuh (Fig. 5e). This strong correlation between the molecular diversity data and the agronomic performance data underscores the utility of marker-assisted parental selection for maximizing heterosis in barley breeding programs. Previous studies by Ahmed et al.[42], Güngör et al.[43], and Yeken et al.[44] reported the recognition of substantial genetic variability among the genotypes underscores the tactical benefit of crossing genetically diverse individuals to improve segregation and selection methods. Moreover, underscore the potential of iPBS and SCoT markers as a cost-effective and time-efficient instrument for accelerating the development of novel hybrid combinations in plant breeding.

While this study provides valuable insights into the genetic control of yield components in barley, its limitations must be acknowledged to contextualize the findings. First, as suggested by the reviewer, the research was conducted at a single experimental site in Duzce, Türkiye. This approach, while allowing for detailed generational analysis, restricts the assessment of genotype × environment (G × E) interactions, which are known to significantly influence quantitative traits like grain yield. Second, the parental pool was limited to three two-rowed barley cultivars. Although these parents were selected to provide genetic contrast, a broader set of germplasms would be necessary to generalize our conclusions about combining ability and heterosis across a wider range of barley genetic backgrounds. Consequently, the performance of the identified superior crosses, such as Arcanda/Asparuh, should be validated in multi-environment and multi-year trials before their widespread recommendation. Future research should therefore focus on evaluating these promising hybrid combinations across diverse agro-ecological zones and expanding the diallel analysis to include a larger, more diverse panel of parental lines. Such studies, potentially integrated with genomic selection tools, would further refine the identification of loci underlying key agronomic traits and accelerate the development of broadly adapted, high-yielding barley cultivars. Despite the limitations noted, this study advances understanding of the genetic architecture underlying key agronomic traits in barley, clarifying the contrasting contributions of additive and non-additive gene action across generations. The identification of high-potential parental combinations, particularly Arcanda/Asparuh and Alena/Asparuh, provides a strong foundation for developing hybrid-focused breeding strategies aimed at improving grain yield and associated traits. These outcomes offer practical guidance for future breeding programs by promoting the targeted exploitation of heterosis for traits governed by non-additive effects, while applying additive-based selection for traits where additive variance predominates.

Conclusions

Crossbreeding in barley led to significant variation in agronomic traits, highlighting the importance of genetic studies. Among the crosses, Arcanda/Asparuh proved to be the most promising, exhibiting superior grain yield along with consistently high values for spike length, grain weight per spike, and thousand-kernel weight across generations. Arcanda proved to be an excellent general combiner, while both Arcanda/Asparuh and Alena/Asparuh exhibited superior specific combining ability for multiple traits. Selection is recommended in later generations (F_3–F_4) to stabilize desirable characteristics. Significant heterosis and heterobeltiosis for grain yield and thousand kernel weight confirm the potential for genetic improvement through hybrid breeding. Genetic diversity revealed by iPBS-retrotransposon markers highlights the importance of crossing genetically distinct parents to broaden the genetic base and accelerate the development of high-yielding, well-adapted cultivars. The findings of this study enhance the understanding of the genetic determinants of key agronomic traits in barley and provide a practical framework for breeding programs that integrate heterosis exploitation with targeted selection.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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