Research article

A 7×7 diallel cross for developing high-yielding and saline-tolerant barley (*Hordeum vulgare* L.)

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ABSTRACT

In this experiment, F_1s produced from a 7×7 half-diallel cross along with their parents were evaluated to develop high yielding and saline-tolerant barley lines. The investigation focused on the general combining ability (GCA) of parents, specific combining ability (SCA) of offspring, genetic action, and heterosis of eight quantitative variables. Genetic analysis and potence ratio suggested that different degrees of dominance controlling the inheritance of the studied traits. Significant GCA and SCA variances suggested the presence of both additive and non-additive gene actions controlling the traits. However, a GCA:SCA ratio lower than 1 indicated the preponderance of the non-additive gene action involved in the expression of the traits. The parents P_5 and P_6 possess the genetic potential favorable for early and short stature in their F_1s. Conversely, P_2 and P_4 were more likely to produce short F_1s with high yield potential. Based on the mean performance, SCA, and heterobeltiosis, crosses $P_2 \times P_3$, $P_2 \times P_7$, $P_3 \times P_4$, $P_4 \times P_5$, $P_5 \times P_6$, and $P_6 \times P_7$ were selected as promising F_1s for earliness, short stature, and high yield potential. These crosses are recommended for further breeding to obtain early-maturing and high-yielding segregants. To identify saline-tolerant F_1s, screening was conducted in saline media prepared in half-strength Hoagland solution. The salinity stress involved exposing F_1s to 100 mM NaCl for first 10 days, and followed by an increase to 150 mM until maturity. Among the F_1s, five crosses ($P_1 \times P_2$, $P_2 \times P_3$, $P_3 \times P_5$, $P_4 \times P_6$, and $P_4 \times P_7$) exhibited promising signs of saline tolerance based on a comprehensive evaluation of healthy seed set, K^+/Na^+ ratio, root volume, generation of reactive oxygen species ($O_2^{\bullet-}$ and H_2O_2), and activities of key antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR). These crosses will undergo further evaluation in the next filial generation to confirm heritable saline tolerance.

1. Introduction

Barley (*Hordeum vulgare* L.) belonging to the Gramineae family, having chromosome number $2n = 2x = 14$, is an annual plant [1]. Globally barley occupied the fourth largest position among the cereals in terms of production after rice, wheat, and maize [2]. This crop is renowned for its multipurpose uses, such as human food, animal feed, malting, and fermenting materials [3]. It is a great source of nutrients, vitamins, minerals, and total phenolic compound. It can be cultivated in a wide range of environments including salinity

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and drought [4] with low input agriculture [5]. In Bangladesh, barley was traditionally cultivated since 1970. However, after 1980's, its cultivation gradually decreased due to lack of high-yielding hull-less varieties. At present, 172 metric ton barley was produced from 183.4 ha of land [6]. However, this inherently saline tolerant crop can play significant role in food and nutrition security as more than one million ha of salt affected coastal land remains fallow in barley growing season. At the same time, barley can be grown in drought prone area with minimum water. Cultivation of both high-yielding and salinity tolerant varieties can help in increasing food and nutritional security. Moreover, one of the aims of Sustainable Development Goal (SDG, objective-2) "End hunger, achieve food security and improved nutrition and promote sustainable agriculture'' is intrinsically related with food, nutrition, society, economy, and environment [7]. To achieve the target of SDG, barley can play an important role as it is highly nutritious, gluten free, low Glycaemic Index (GI) value, less disease and insect infestation, and own salt and drought tolerant trend. Considering such importance as well as upcoming climate change, Bangladesh Agricultural Research Institute (BARI) emphasized on research to develop high-yielding and saline-tolerant varieties of barley.

Heritability of different agricultural crops is considered as an important factor for the successful breeding program. Additionally, to realize the mode of gene effects, inheritance, magnitude, and interaction are crucial to articulate an efficient breeding program for generating superior genotypes [8]. For improving barley genotypes, manipulation of its genetic variability is needed in order to ensure the dimensions of adaptation, the possibility of presenting new gene introduction, and ultimately, to increase of the genetic gain in the subsequent generations [9]. The objective of genetic improvement of barley is to maximize the desirable genes in the same genotype or the same variety.

During research, emphasis should be given on the selection of biotic and abiotic stress tolerance of varieties with yield stability [10]. For genetic improvement, the selection of hybrids is more practical than their parents, though time consuming [2]. Understanding the mode of gene effects such as inheritance, magnitude and interaction is crucial to design an effective breeding program for development of superior genotypes [8]. Diallel analysis is one of the mating designs from which combining ability can be predicted [11]. For determining the ability of the genotypes to be involved or not in a breeding program, combining ability analysis is an effective method [12]. The general combining ability (GCA) of parents and the specific combining ability (SCA) of offspring could be analyzed in the F_1 generation. The GCA value of parents is considered as a useful selection criterion for the identification of the parents with favorable alleles for desirable characters, and the SCA value of hybrids is considered for selection of hybrids with expected traits [2]. In the F_1 generation, the assessment of agronomic characters and yield performance is very crucial for investigating the genetic determination of hybrids [13]. This assessment provides valuable information about the mode of gene action and the nature of transgression of traits [14]. The mode of gene action in F_1 generation is classified as additive and non-additive expression [15]. For an effective breeding program and genetic improvement of crops aimed at achieving high yield, such findings could prove valuable.

On the other hand, salinity stress is the most detrimental and complex stress, leading to alterations in ion homeostasis, particularly potassium (K^+). This metabolic imbalance affects the growth, development, and yield of crop species worldwide [16]. Beside ionic imbalance, it induces osmotic stress, cross-talk with reactive oxygen species (ROS), and hormonal imbalances, thus, impacts integrated effect in signals transaction pathways [17,18]. Among the ROS, superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), the most studied ROS, whose excessive production can oxidize DNA, proteins, enzymes, carbohydrates, and lipids leading to cell death [16,19]. On the other hand, plants possess integral protective systems to control the ROS generation through enzymatic and non-enzymatic antioxidants. Among the enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) play important roles. These enzymes are induced during different abiotic stresses, including salinity, to enhance the tolerance to oxidative stress by encountering the burst of ROS [20,21]. It is important that plants showing better tolerance exhibit lower ROS production, along with an improved antioxidant system [22–24]. Therefore, these parameters can serve as biomarkers during the selection of F_1 and subsequent generations in a breeding program to develop saline-tolerant varieties.

Considering these factors in account, we have designed the study to develop high-yielding barley from diallel progenies through better understanding of gene action governing trait inheritance. Additionally, we have evaluated GCA of parents and SCA of offspring under non-saline field conditions. In our selection process, we utilized biomarkers such as the accumulation of Na^+ and K^+, ROS generation, activities of key antioxidant enzymes, and root phenotype to identify the best salt-tolerant F_1s for further segregation.

Table 1
Salient features of the parents used in diallel crosses.

Parents	Major characters	Source
INBYT/18−E−19 (P_1)	Bold seeded, long spike, tall, and high yielder	ICARDA
INBON/18-L-53 (P_2)	Bold seeded, medium saline tolerant, medium dwarf, with profuse tillering, and large spike	ICARDA
INBYT/18-E−9 (P_3)	Medium height, late, medium saline tolerant, and large spike	ICARDA
INBYT/18-E−6 (P_4)	Medium early, short, medium saline tolerant, long spike, and high yielder	ICARDA
BARI barley-9 (P_5)	Drought tolerant, medium height, bold seeded, and high yielder	BARI
INBYT/18-E−25 (P_6)	Waxy and erect leaf, long spike and medium height, and bold grained	ICARDA
BARI barley-7 (P_7)	Saline tolerant, dwarf, medium early, and bold seeded	BARI

2. Materials and methods

2.1. Plant materials and experimental sites

Seven barley (*Hordeum vulgare* L.) genotypes INBYT/18-E−19 (P_1), INBON/18-L-53 (P_2), INBYT/18-E−9 (P_3), INBYT/18-E−6 (P_4), BARI barley-9 (P_5), INBYT/18-E−25 (P_6), and BARI barley-7 (P_7) were crossed in 7 × 7 diallel fashion in a hybridization program. The F_1s, along with their parents, were evaluated at a non-soil Research field of Plant Breeding Division of Bangladesh Agricultural Research Institute (BARI, 23.987281°N and 90.407802°E). To identify barley genotypes tolerant to salinity, the F_1s were evaluated in saline solution within a hydroponic system under greenhouse conditions of the same division. The salient features of the genotypes are presented in Table 1. Parents P_2, P_3, P_4, and P_7 were used as the sources of both saline tolerance and high yield. Parent P_7 was chosen for its dwarfing characteristics. On the other hand, parent P_6 was used as a source of drought tolerance, while P_1 was selected as a high-yielding parent.

2.2. Land preparation and field layout for high yield

In the following year, the F_1s and parents were evaluated in the field conditions. Mechanical ploughing and laddering were used to prepare the soil for the plant's cultivation. The experiment was carried out in a Randomized Complete Block Design (RCBD) with three replications. In each replication, the seeds of parents and F_1s were sown in two 5-m rows spaced 25 cm apart. Seeds were sown continuously in line. After thinning, a plant-to-plant distance of 10 cm was maintained.

2.3. Saline treatment for screening salt-tolerant F_1s

Seven-day-old F_1 seedlings were transferred to a hydroponic system under greenhouse conditions following a completely randomized design with three replications. After a three-day hardening period, seedlings were exposed to 100 mM NaCl solution prepared with half-strength Hoagland solution for 10 days. The concentration of NaCl was then increased to 150 mM and continued until maturity. The saline media were changed twice a week.

2.4. Crop husbandry for field evaluation

Fertilizers were applied at the rate of 100, 60, and 40 kg ha^{-1} of N, P, and K, respectively, for field experiments. Half the dose of nitrogen was applied as a basal dose along with the full doses of phosphorus and potash. The remaining nitrogen was top-dressed just after first irrigation, coinciding with the crown root initiating (CRI) stage (25 days after sowing). Second irrigation was applied at flower initiating stage. Weeding was performed at both the CRI and vegetative growth stages. Thinning and plant protection measure were implemented as needed throughout the cropping duration. To control foot rot, Provax 200WP was applied at the rate of 5 g L^{-1} at 30 days after sowing.

2.5. Data collection for field experiment

Data were collected on various agronomic traits, such as days to flowering (DF), days to maturity (DM), number of tillers per plant (TP), plant height (PH), panicle length (PL), number of grains per plant (NGPP), 1000-grain weight (1000-GW), and yield per plant (YPP). For each of these parameters, observations were obtained from 10 randomly selected plants of each genotype (parents and F_1s) per replication.

2.6. Data collection for saline-tolerant barley

To assess salinity stress tolerance, data were collected from the top most expanded leaves after 50 days of salinity exposure. Specifically, measurements were taken for Na$^+$ (ppm), K$^+$ (ppm), reactive oxygen species (ROS), and key antioxidant enzymes' activity. Additionally, root volume (cc) was measured at maturity.

2.6.1. Protein extraction and Quantification

For salinity experiment, protein extraction was carried out from fresh leaves following the method described by Rohman et al. [25]. Briefly, leaf tissue (0.5 g) was homogenized in 1 ml 50 mM ice-cold K–P buffer (pH 7.0) containing 100 mM KCl, 1 mM ascorbate, 5 mM β-mercaptoethanol, and 10 % (*w/v*) glycerol. The centrifugation of homogenates was performed at 11,500×*g* for 10 min at 4 °C, and the resulting supernatants were used for enzyme activity assay. Protein content was measured spectrophotometrically following the method described by Bradford et al. [26], using BSA as standard.

2.6.2. Measurement of Na$^+$ and K$^+$

Sodium (Na$^+$) and potassium (K$^+$) contents were measured by extracting sap from freshly harvested shoots using a tissue sap extractor (Horiba, Japan). The Na$^+$ (ppm) and K$^+$ (ppm) contents were determined using compact Na$^+$ ion (Horiba-731, Japan) and K$^+$ ion (Horiba-722, Japan) meters, respectively, following the method described by Rohman et al. [27].

2.6.3. Measurement of root volume

The root volume of different genotypes was measured using a 1000 cc measuring cylinder by calculating the difference in water volumes.

2.6.4. SDS-PAGE and gel staining of enzymatic activity

Non-denaturing polyacrylamide gel electrophoresis (PAGE) was used to separate equal amounts of protein (50 μg), following the
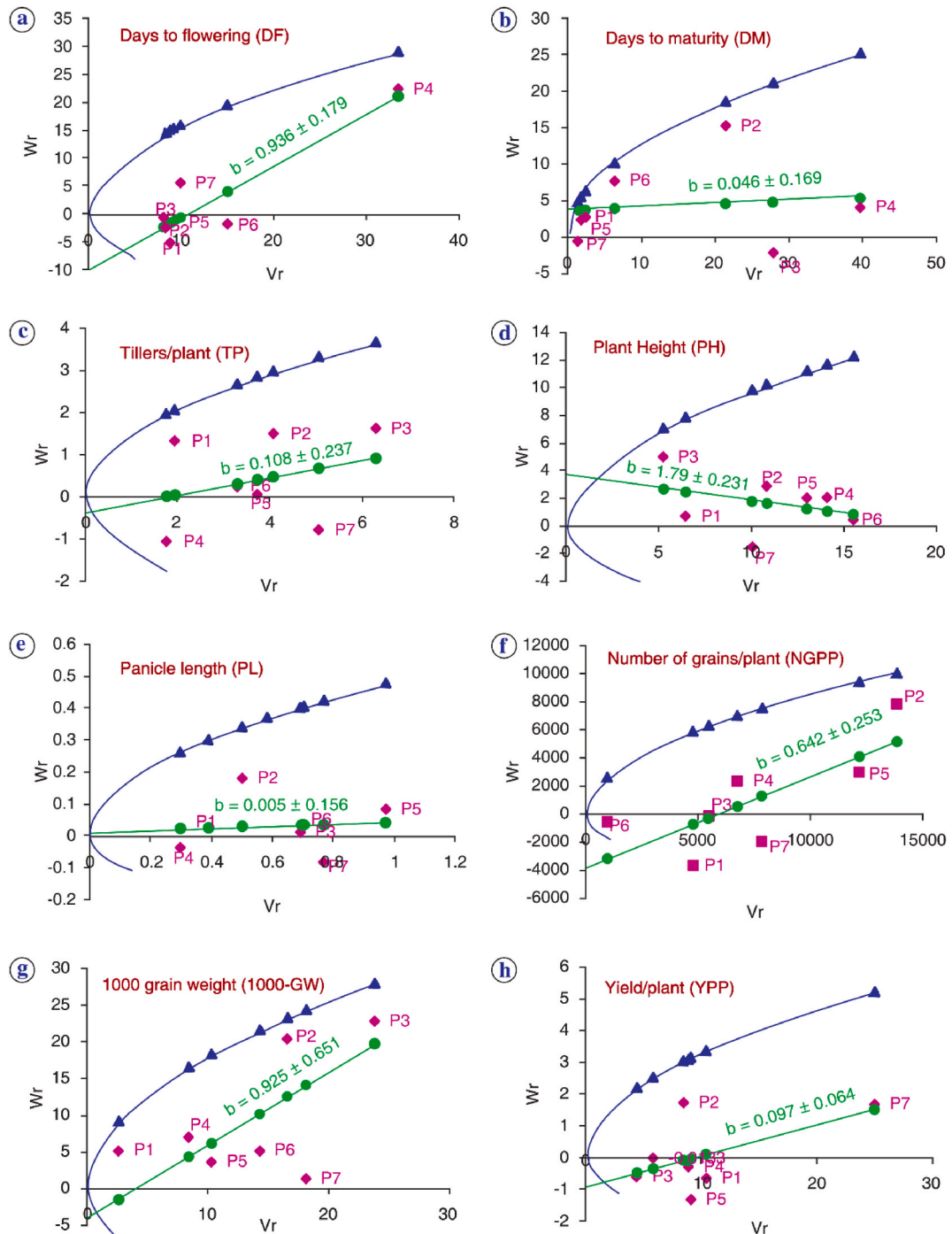

Fig. 1. Vr-Wr and relative distribution of gene of parents for important traits (a) days to flowering (DF), (b) days to maturity (DM), (c) tillers/plant (TP), (d) plant height (PH, cm), (e) panicle length (PL, cm), (f) Number of grains/plant (NGPP), (g) 1000 grain weight (1000-GW, g), and (h) yield/plant (YPP, g) in barley.

method developed by Laemmli [28]. Isoenzymes of SOD, CAT, POD, APX, and GR were subsequently determined using the procedures described in Islam et al. [29].

2.6.5. Assessment of the contents of superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2)

Superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) contents were determined in fully expanded barley leaves following the procedures outlined by Elstner and Heupel [30] and Yang and Wang [31], respectively. The $O_2^{\bullet-}$ content was expressed as nmol g^{-1} fresh weight min^{-1}, while H_2O_2 content µmol g^{-1} fresh weight.

2.7. Statistical analysis

Diallel data from field experiment were analyzed using the TNAUSTAT statistical package [32]. We followed the diallel Model II and Method 2 of Hayman [33] and Griffing [34] approaches as described by Nadarajan et al. [35]. Randomized Complete Block Design (RCBD) was followed during the analysis of data. For mean comparison, a significance level of $P \leq 0.05$ was considered using least significant different (LSD) test. Preparation of Vr-Wr graphs and calculation of the potence ratios were performed in MS Excel. Additionally, numerical data of Na$^+$, K$^+$, $O_2^{\bullet-}$, H_2O_2, and root volume from the salinity experiments were analyzed using the statistical software Statistix 10, following completely randomized design (CRD). $P \leq 0.05$ was considered as significant level using LSD test.

3. Results

3.1. ANOVA for Hayman's approach for different traits of barley genotypes (parents and crosses)

The analysis of variance (ANOVA) using Hayman's approach revealed significant differences ($P \leq 0.01$) among the barley genotypes for all the traits studied (Supplementary Table 1). These results indicated substantial genetic variation prevail among the genotypes. Consequently, the data were processed for further genetic and combining ability analysis.

3.2. Direction and order of dominance by the graphical approach

The direction and order of dominance were analyzed using Hayman [33]'s graphical approach. The genetic diversity of parental lines for a trait can be described by examining the distribution of parental array points along the regression line in the Vr-Wr graph [36]. Therefore, the distribution of parental array points in the Vr-Wr graph and the relative distribution of genes among parents for different important traits were presented in Fig. 1. Genetic diversity was evident among the parents, as the parental array points were scattered along the regression line for all traits. In the present investigation, the regression line intersected the vertical axis (Wr) above the origin for days to maturity [DM] (Fig. 1b), plant height [PH] (Fig. 1d), and panicle length [PL] (Fig. 1e), suggesting these three traits were controlled by partial dominance of the genes. Conversely, the regression line intersected Wr below the origin for days to flowering [DF] (Fig. 1a), tillers per plant [TP] (Fig. 1c), number of grains per plant [NGPP] (Figs. 1f), 1000-grain weight [1000-GW], and yield per plant [YPP] (Fig. 1g) implying that the overdominance controlled these traits. Again, the position of parents in Vr-Wr graph reflects the order of dominance [36]. The parents P$_1$, P$_2$, P$_3$, P$_5$, and P$_7$ cluster near the point of origin (Fig. 1a), demonstrating the existence of dominant genes for the trait DF in these mentioned parents. Similarly, parents P$_1$, P$_5$, and P$_7$ possess dominant genes for DM; P$_1$ and P$_4$ exhibit dominance for PH; P$_4$ controls PL; P$_1$ and P$_6$ contribute to the NGPP; P$_1$, P$_4$, and P$_5$ play a role in 1000-GW; and P$_3$, P$_4$, and P$_5$ influence YPP (Fig. 1a–h). Conversely, for the trait DF and DM, parent P$_4$; for TP, parents P$_3$ and P$_7$; for PH, parents P$_4$, P$_5$, and P$_6$; for PL, parent P$_5$; for NGPP, parents P$_5$ and P$_2$; for 1000-GW, parents P$_2$ and P$_3$; and for YPP, parent P$_7$ were the furthest from the origin (Fig. 1a–h), implying that these parents like carry mostly recessive genes for the respective traits.

Table 2
Adequacy of additivity of Hayman's method for various traits in 7 × 7 F$_1$ half-diallel crosses of barley.

Traits	t^2	Regression analysis		Conclusion
		b_0	b_1	
DF	0.115^{NS}	5.22^S	0.353^{NS}	Adequate
DM	6.45^S	0.270^{NS}	5.66^S	Inadequate
TP	2.23^{NS}	0.456^{NS}	3.76^S	Partial adequate
PH	2.30^{NS}	−0.772^{NS}	5.09^S	Partial adequate
PL	7.92^S	0.032^{NS}	6.38^S	Inadequate
NGPP	0.276^{NS}	0.253^{NS}	1.41^{NS}	Adequate
1000-GW	2.30^{NS}	1.42^{NS}	0.116^{NS}	Partial adequate
YPP	56.96^S	1.51^{NS}	14.05^S	Inadequate

S and NS indicate significant and non-significant, respectively. The model is considered fully adequate if all the scaling tests are found in favor of assumptions, and partially adequate if one of the tests fulfils the assumptions. Failure of all tests completely invalidates the genetic model. DF = days to flowering, DM = days to maturity, TP = number of tillers per plant, PH = plant height, PL = panicle length, NGPP = Number of grains per plant, 1000-GW = thousand grains weight and YPP = yield per plant.

3.3. Assumptions of diallel analysis and tests of adequacy

The validity of information obtained from a group of genotypes using the diallel method relies on the following assumptions: (i) diploid segregation of chromosomes, (ii) homozygosity of parents, (iii) absence of reciprocal effects, (iv) absence of epistasis, (v) no multiple allelism, and (vi) independent distribution of genes among parental genotypes. Homozygous diploid inbred lines of barley were used in this diallel crossing program (Table 1). To assess the adequacy of the additive–dominance model and validate the diallel assumptions underlying the genetic model, datasets of various traits were subjected to two scaling tests: t^2 test (F test at 5,5 degrees of freedom) and regression analysis. These tests specifically address the last three assumptions. According to Mather and Jinks [37], the regression coefficient (b) is expected to be significantly different from zero ($b \neq 0$), but not necessarily equal to unity ($b = 1$). Failure of this test indicates the presence of epistasis and the data may be unfit for further genetic analysis. A non-significant value in the t^2 test also confirms the absence of non-allelic interaction, implying that the genes act independently for random association. If both tests favor the assumptions, the genetic model is declared fully adequate. If one test fulfils the assumptions, it is considered partially adequate. However, if both tests fail, the additive–dominance model is completely invalidated. In our analyzed dataset, traits like DF and NGPP fully satisfied the assumptions (Table 2). Conversely, traits such as TP, PH, and 1000-GW partially met the assumptions. However, DM, PL, and YPP appeared inadequate in fulfilling the assumptions.

3.4. Nature of genetic variance

The analysis of genetic variance components (Table 3) indicated that the additive variance (D) is significant for days to flowering (DF), number of grains per plant (NGPP), and thousand grain weight (1000-GW). The dominance variance (H_1) is significant for all the traits studied except days to maturity (DM), whereas overall dominance effect (h^2) is significant for DM, NGPP, and 1000-GW. Therefore, the traits DF, NGPP, and 1000-GW are highly controlled by both additive and dominance gene action. The dominant effect (H_1) is more pronounced than the additive component (D). Additionally, the dominance in NGPP and 1000-GW is reflected by a high degree of dominance effect, which considers the sum total of overall loci in the heterozygous state (h^2). Notably, the dominance falls within the overdominance range because $(H_1/D)^{0.5} > 1.0$ for all traits.

The environmental variance (E) is non-significant for all the traits, except for PH. Although E is significant for PH, it is lower than D and H_1. The proportion of dominant and recessive alleles explains whether the distribution of these alleles in the overall parents is symmetrical ($p = q = 0.5$) or asymmetrical ($p \neq q$). All the traits under study exhibited an asymmetrical distribution of dominant and recessive alleles. The values of the proportion of dominant and recessive alleles for all traits are greater than 1, indicating an excess of dominant alleles ($p < q$) in the overall parents [38]. The distribution of dominant and recessive alleles in parents is further recognized by the relative size of dominance variance (H_1) and the proportion of $\pm$ genes (H_2). In the present study, $H_1 \neq H_2$ for all traits, indicating an asymmetry in the distribution of alleles among parents. Furthermore, $H_1 > H_2$ for all traits suggesting an excess of dominant alleles over recessive alleles. The distribution of dominant and recessive alleles in parents is also confirmed by the direction of mean covariance of additive and dominant effects (F) [9]. Since $F > 0$ for all studied traits, it indicated that dominant alleles are more frequent than recessive alleles ($p > q$) in parents. The proportion of dominant genes with positive or negative effects in parents can be determined by the ratio of $H_2/4H_1$ with the maximum theoretical value 0.25. In the present study, the ratio of $H_2/4H_1$ is not equal to 0.25, and it is positive for all studied traits. This indicated that dominant genes have both decreasing and increasing effects on all traits. The estimates of narrow-sense heritability (h^2_{NS}) were low (<30 %) for all traits except DF (33 %) and 1000-GW (44 %).

3.5. Potence ratio or dominance effect

The potence ratio of different traits of a single cross specified the existence of various degrees of dominance effects. According to Ghosh et al. [39], a potence ratio of +1 indicated complete dominance; when the potence ratio lies between −1 and +1, a partial

Table 3
Genetic variance components for 8 traits in a 7 × 7 half-diallel cross of barley.

Variance components	DF	DM	TP	PH	PL	NGPP	1000-GW	YPP
D	26.27**	12.23ns	1.89ns	5.58ns	0.12ns	6660*	31.45**	0.49ns
H_1	68.30**	46.11ns	14.13**	34.58**	2.31**	31088**	48.38*	40.06*
h^2	4.48ns	40.81*	1.33ns	2.75ns	−0.05ns	36810**	30.87*	5.01ns
E	1.02ns	4.12ns	0.29ns	4.19*	0.10ns	664.84ns	1.28ns	0.61ns
$(H_1/D)^{0.5}$	1.61	1.94	2.73	2.49	4.40	2.16	1.24	9.03
Prop. Dom/rec gene	3.02	1.53	1.53	1.65	1.43	2.02	2.02	1.26
H_2	40.96**	40.06ns	12.96**	28.73*	1.95**	26405**	36.68*	37.07*
F	42.62**	9.90ns	2.18ns	6.84ns	0.18ns	9733ns	26.42ns	1.01ns
$H_2/4H_1$	0.15	0.22	0.23	0.21	0.21	0.21	0.19	0.23
h^2_{NS} (%)	33	23	11	17	20	10	44	11

*and ** indicate significant at $P \leq 0.05$ and ≤ 0.01, respectively. D = Additive variance, H_1 = Dominance variance, $h.^2$.
=Overall dominance effect, E = Environmental variance, $(H_1/D)^{0.5}$ = Mean degree of dominance, Prop. Dom/rec gene = Proportion of dominant to recessive genes, H_2= Proportion of $\pm$ genes, F = Mean covariance of additive and dominant effects, $H_2/4H_1$= Proportion of genes with $\pm$ effects, and h^2_{NS}= Heritability in narrow sense. Additional details are given in the footnotes of Table 2.

dominance is indicated. If the potence ratio exceeds ± 1, it indicates overdominance and a potence ratio equals to 0 suggest the absence of dominance. The potence ratio of 21 F_1 crosses for 8 agronomic traits were presented in Table 4. For DF, the potence ratio ranged from -95 in $P_1 \times P_7$ to 17 in $P_2 \times P_3$. Among the crosses, seven exhibited partial dominance (with values between -1 and $+1$), while the remaining 14 crosses showed overdominance (with values exceeding ± 1). For DM, the potence ratio ranged from -8.67 ($P_1 \times P_6$) to 18 ($P_1 \times P_5$). Among these, five crosses displayed partial dominance, and the remaining 16 crosses showed overdominance. In case of TP, the potence ratio ranged from -80.33 ($P_5 \times P_7$) to 53 ($P_1 \times P_3$). Among the crosses, only $P_3 \times P_7$ indicated complete dominance ($+1$), six exhibited partial dominance, and 14 showed overdominance. In terms of PH, the potence ratio spanned from -21.84 ($P_1 \times P_7$) to 3.99 ($P_1 \times P_2$). Interestingly, no cross displayed complete dominance ($+1$) or absence of dominance (0). Five crosses exhibited partial dominance, while remaining 16 showed overdominance. For PL, the potence ratio ranged from -11.12 ($P_3 \times P_7$) to 37.22 ($P_4 \times P_5$). Among the crosses, eight showed partial dominance and 13 showed overdominance. Regarding NGPP, the potence ratio varied from -37 ($P_3 \times P_5$) to 465.67 ($P_4 \times P_5$) where four crosses displayed partial dominance, and 17 showed overdominance. The potence ratio of 1000-GW ranged from -3.09 ($P_3 \times P_7$) to 7.39 ($P_3 \times P_4$). Among the crosses, 14 showed overdominance, and seven exhibited partial dominance. Finally, the potence ratio of YPP ranged from -101.44 ($P_2 \times P_7$) to 84.12 ($P_4 \times P_6$). Two crosses exhibited partial dominance, while the remaining 19 showed overdominance.

3.6. Analysis of variance of GCA and SCA

Analysis of variance for combining ability revealed highly significant ($P \leq 0.01$) estimates of mean sum of squares for both general combining ability (GCA) and specific combining ability (SCA) of all the studied traits (Table 5). The ratio of GCA to SCA was less than 1 (Table 5), indicating that GCA played a more dominant role in the inheritance of all the studied traits.

3.7. General combining ability (GCA) effects

The GCA effects and the per se performance of parents for different traits were shown in Table 6. None of the parents were found to be a good general combiner for all the traits studied. A wide range of variability in GCA effects was observed among the parents. In the present study, the parents P_5 and P_6 emerged as good general combiners for days to flowering due to their significantly negative GCA effects and low per se values. This indicated these parents can be valuable breeding materials for developing early flowering varieties. In contrast, parents P_2, P_4, and P_7 exhibited higher per se values along with significant GCA effects. The parent P_3 showed a significant negative GCA value for DM along with the lowest per se value suggesting it is good combiner for early maturity. Conversely, P_2 had the highest per se values and significant GCA for late maturity. For TP, parents P_4 and P_6 showed significant positive GCA effects with high per se performance (11.5 and 12.15, respectively), making them valuable combiners for high tillering in barley. Parents P_2 and P_5 were identified as good combiner for short stature due to significant and negative GCA value for PH. For PL, parents P_5 and P_6 displayed promise for breeding long panicles based on their high per se performance and significant GCA values. Parent P_1 stood out as a good combiner for high grain number due to its significant GCA and high per se performance. On the other hand, parents P_2 and P_4 exhibited significantly higher GCA effects, suggesting their potential as good combiners for developing high-yielding barley varieties.

Table 4
Potence ratios of different traits in 7×7 diallel crosses in barley.

Crosses	DF	DM	TP	PH	PL	NGPP	1000-GW	YPP
$P_1 \times P_2$	-1.25	-1.27	3.15	-1.19	0.10	-2.25	-0.33	6.89
$P_1 \times P_3$	-0.29	0.44	53.00	-1.02	-9.68	-1.36	-1.32	-2.78
$P_1 \times P_4$	-1.00	-3.60	0.73	-1.31	-0.15	-1.34	-1.85	-1.79
$P_1 \times P_5$	-1.67	18.00	-0.21	-3.46	0.60	-3.61	-2.75	-4.57
$P_1 \times P_6$	2.87	-8.67	0.08	-1.30	4.32	2.68	-0.27	-0.54
$P_1 \times P_7$	-95.00	-1.10	-1.27	-21.84	35.18	136.00	1.67	10.71
$P_2 \times P_3$	17.00	-1.03	10.33	0.22	-0.30	-7.50	-1.59	1.35
$P_2 \times P_4$	-1.10	2.95	0.77	0.57	4.71	5.43	1.18	2.40
$P_2 \times P_5$	1.32	-1.19	-2.24	-0.67	5.00	-3.43	-0.88	0.24
$P_2 \times P_6$	1.60	-0.62	1.02	-6.66	-1.79	-0.68	-0.25	1.97
$P_2 \times P_7$	-1.65	1.14	3.03	1.30	0.86	2.89	0.92	-101.44
$P_3 \times P_4$	-3.70	-7.07	3.30	1.23	-0.004	43.00	7.39	-20.17
$P_3 \times P_5$	-0.90	1.63	0.30	-0.52	-2.79	-37.00	-2.87	3.07
$P_3 \times P_6$	-0.71	4.83	-3.00	0.51	-0.19	-0.52	-1.30	-5.77
$P_3 \times P_7$	-0.21	-2.00	1.00	1.24	-11.12	-0.12	-3.09	5.15
$P_4 \times P_5$	-1.36	-0.83	-4.47	-15.99	37.22	465.67	-2.47	14.18
$P_4 \times P_6$	-1.21	-1.88	0.59	2.53	4.07	-1.43	-0.19	84.12
$P_4 \times P_7$	-0.29	-0.004	5.93	-5.33	-1.95	-2.29	0.86	-6.47
$P_5 \times P_6$	0.56	-4.50	-3.00	2.40	-6.33	-1.17	1.69	-19.05
$P_5 \times P_7$	2.43	-0.005	-80.33	-1.47	-0.81	-0.20	3.92	-2.55
$P_6 \times P_7$	1.38	1.43	-4.78	3.99	17.61	2.14	3.18	2.51

Additional details are given in the footnotes of Table 2.

Table 5

Mean sum of squares for GCA and SCA of 8 traits in a 7 × 7 diallel cross in barley.

Sources of variation	df	Mean sum of square							
		DF	DM	TP	PH	PL	NGPP	1000-GW	YPP
GCA	6	22.48**	21.35**	2.24**	11.60**	0.51**	4806**	41.39**	3.85**
SCA	21	14.05**	14.63**	3.59**	10.43**	0.59**	8860**	12.36**	10.15**
Error	54	1.04	4.15	0.30	4.25	0.11	682.30	1.32	0.628
GCA:SCA		0.183	0.182	0.066	0.132	0.094	0.056	0.403	0.038

** Significant at $P \leq 0.01$ level. The full name of the traits is given in the footnotes of Table 2.

Table 6

Estimates of general combining ability (GCA) effects and the per se performance of seven parents for different traits in 7 × 7 diallel cross of barley. Values for per se performance are included in parentheses.

Parents	Traits							
	DF	DM	TP	PH	PL	NGPP	1000-GW	YPP
P_1	−0.47ns	0.32 ns	−0.89**	0.41ns	0.08ns	38.47**	−2.22**	−0.48ns
	(63)	(102.5)	(9.03)	(92.4)	(9.17)	(624)	(30.15)	(19.06)
P_2	2.57**	2.58**	−0.13ns	−1.30*	−0.21*	−24.46**	4.41**	1.01**
	(68.67)	(111.17)	(9.47)	(85.43)	(8.13)	(440)	(44.93)	(20.3)
P_3	−0.21ns	−2.53**	−0.23ns	1.05ns	−0.19ns	−28.20**	−0.60ns	−0.96**
	(69)	(99.5)	(9.07)	(94.93)	(9.47)	(480)	(38.05)	(18.53)
P_4	0.87**	−0.50ns	0.48**	−0.67ns	−0.04ns	3.28ns	0.82*	0.55 *
	(75.33)	(104.17)	(11.5)	(90.88)	(8.53)	(490)	(36.23)	(18.25)
P_5	−1.76**	0.76 ns	0.10ns	−1.34*	−0.24*	−9.35ns	−0.40ns	−0.11ns
	(62)	(102.17)	(12)	(89.98)	(8.5)	(490)	(33.6)	(17.54)
P_6	−1.87**	−0.13ns	0.62**	0.29ns	0.39**	6.43ns	−1.09**	0.18ns
	(60.67)	(101.5)	(12.15)	(87.97)	(9.02)	(646)	(27.92)	(18.17)
P_7	0.87**	−0.50ns	0.06ns	1.56*	0.21*	13.84ns	−0.91*	−0.19ns
	(63.33)	(99.17)	(12)	(92.2)	(9.14)	(623)	(31.5)	(20.2)
SE (gi)	0.32	0.63	0.17	0.64	0.10	8.06	0.35	0.24

* and ** indicates significant at $P \leq 0.05$ and 0.01 level, respectively. ns− non-significant. The full names of the traits are given in the footnotes of Table 2.

3.8. Specific combining ability (SCA) effects

The SCA effects along with per se performance of F$_1$ crosses for eight traits are presented in Table 7. No single F$_1$ emerged as superior across all studied traits. For DF, crosses P$_1$ × P$_2$, P$_1$ × P$_4$, P$_2$ × P$_3$, P$_3$ × P$_6$, and P$_4$ × P$_6$ exhibited significant negative SCA effects and lower per se flowering time (desirable). Interestingly, these F$_1$s often exhibited contrasting SCA effects for days to maturity, further suggesting their earliness. Moreover, crosses P$_1$ × P$_6$, P$_2$ × P$_4$, P$_2$ × P$_5$, and P$_2$ × P$_7$ showed significant SCA effects and lower per se values. These crosses mostly involved parents with contrasting GCA effects (average × high, high × low, and low × average). Increased tillering potential was observed in several crosses. For the trait TP, P$_1$ × P$_7$, P$_2$ × P$_5$, P$_2$ × P$_7$, P$_3$ × P$_4$, P$_3$ × P$_6$, P$_4$ × P$_5$, and P$_6$ × P$_7$ showed significant and positive SCA effects, suggesting higher tillering compared to their parents. These crosses involved various combinations (low × average, low × high, and high × average) of GCA effects. Out of 21 F$_1$s, five crosses (P$_1$ × P$_6$, P$_1$ × P$_7$, P$_2$ × P$_3$, P$_4$ × P$_5$, and P$_5$ × P$_7$) exhibited significant negative SCA effects for PH, along with lower per se performance. This indicated these F$_1$s might be shorter than their parents, potentially desirable for some breeding programs. These crosses involved parents with different combinations (average × average, average × high, low × high, low × low, and low × high) of GCA. Ten crosses (P$_1$ × P$_2$, P$_1$ × P$_5$, P$_2$ × P$_3$, P$_2$ × P$_7$, P$_3$ × P$_4$, P$_3$ × P$_5$, P$_4$ × P$_5$, P$_4$ × P$_6$, P$_4$ × P$_7$, and P$_5$ × P$_6$) exhibited significant and positive SCA effects for both NGPP and YPP. For NGPP, these crosses involved various combinations of parental GCA effects, including low × low, low × high, and high × low for NGPP, and mostly low × low, low × high, and average × low for GPP. In contrast, the cross P$_6$ × P$_7$ exhibited significant positive SCA for PL, 1000-GW, and YPP, with a high × high GCA effects for the traits. This suggested that both parents (P$_6$ and P$_7$) likely possess favorable alleles for these traits, contributing to the superior performance of the F$_1$. Based on a combined analysis of per se performance and SCA effects, five crosses emerged as promising candidates for further breeding selection during segregation: P$_2$ × P$_3$, P$_2$ × P$_7$, P$_4$ × P$_5$, P$_5$ × P$_6$, and P$_6$ × P$_7$. These crosses exhibited desirable traits, including earliness, short stature, and high yield potential, F$_1$s and recommended for further selection during segregation.

3.9. Determination of heterobeltiosis

Heterosis, or hybrid vigor, is defined as the progeny having higher average values than the mean of its two parents. Heterobeltiosis, a specific form of heterosis, occurs when hybrid surpasses its best parent. Given that barley is a self-pollinated crop, large-scale hybrid seed production is very cumbersome for the plant breeder, leading to a discouragement of hybrid programs. Therefore, in this hybridization program, heterobeltiosis was considered to compare the F$_1$s for developing high-yielding inbred barley variety.

Table 7

Specific combining ability (SCA) effects and the per se performance of 21 crosses for different traits in 7 × 7 diallel cross in barley. Values for per se performance are included in parentheses.

Cross	Traits							
	DF	DM	TP	PH	PL	NGPP	1000-GW	YPP
$P_1 \times P_2$	−4.80**	−1.58ns	−0.32ns	2.46**	−0.13ns	98.69**	0.70ns	3.56**
	(62.33)	(101.33)	(9.93)	(93.07)	(8.6)	(739)	(35.07)	(23.97)
$P_1 \times P_3$	0.65ns	2.53**	−0.22ns	−0.59ns	−0.85**	13.43ns	−0.45ns	1.10**
	(65)	(100.33)	(9.930	(92.37)	(7.9)	(650)	(28.9)	(19.53)
$P_1 \times P_4$	−2.43**	0.50ns	0.31ns	1.40**	−0.03ns	−21.39*	−3.21**	−0.57ns
	(63)	(100.33)	(11.17)	(92.63)	(8.9)	(646)	(27.57)	(19.38)
$P_1 \times P_5$	0.54ns	−1.76*	−0.28ns	4.80**	−0.07ns	143.57**	−2.42**	2.49**
	(63.33)	(99.33)	(10.2)	(95.37)	(8.63)	(799)	(27.13)	(21.77)
$P_1 \times P_6$	2.65**	−2.54**	−0.54*	−4.90**	0.10ns	−66.20**	−0.13ns	−1.19**
	(65.33)	(97.67)	(10.47)	(87.3)	(9.43)	(605)	(28.73)	(18.37)
$P_1 \times P_7$	5.57**	−0.84ns	1.96**	−3.34**	0.65**	−9.61ns	0.66ns	−5.68**
	(71)	(99)	(12.40)	(90.13)	(9.8)	(669)	(29.7)	(13.52)
$P_2 \times P_3$	−1.72**	−0.73ns	0.42ns	−2.12*	0.54**	36.35**	0.05ns	0.69*
	(65.67)	(99.33)	(11.33)	(89.13)	(9)	(610)	(36.03)	(20.61)
$P_2 \times P_4$	−0.46ns	−4.76**	−0.36ns	0.17ns	0.66**	−4.46ns	−1.97**	−4.62**
	(68)	(97.33)	(11.27)	(89.7)	(9.27)	(600)	(35.43)	(16.81)
$P_2 \times P_5$	3.50**	−2.02*	2.33**	0.37ns	−1.01**	−41.83**	−1.88**	−1.53**
	(69.33)	(101.33)	(13.57)	(89.23)	(7.4)	(550)	(34.3)	(19.24)
$P_2 \times P_6$	4.94**	0.87ns	−2.33**	4.64**	0.33*	5.06ns	−1.17*	0.27ns
	(70.67)	(103.33)	(9.43)	(95.13)	(9.37)	(613)	(34.32)	(21.33)
$P_2 \times P_7$	1.54**	−3.76**	3.36**	1.43ns	0.21ns	180.98**	−3.63**	4.83**
	(70)	(98.33)	(14.57)	(93.2)	(9.07)	(797)	(32.03)	(25.52)
$P_3 \times P_4$	−5.35**	−11.65**	2.78**	−1.48ns	0.37**	98.61**	−1.96**	1.72**
	(60.33)	(85.33)	(14.3)	(90.4)	(9.0)	(700)	(30.43)	(21.19)
$P_3 \times P_5$	−0.72ns	0.42ns	−1.04**	−0.02ns	−0.79**	81.24**	−1.73**	0.76*
	(62.33)	(98.67)	(10.1)	(91.18)	(7.63)	(670)	(29.43)	(19.56)
$P_3 \times P_6$	−1.28**	−1.69*	3.57**	0.39ns	0.15ns	2.13ns	−4.08**	−1.77**
	(61.67)	(95.67)	(15.23)	(93.23)	(9.2)	(607)	(26.4)	(17.32)
$P_3 \times P_7$	−0.35ns	2.01*	−2.04**	1.15ns	−1.41**	−51.94**	−5.99**	−3.65**
	(65.33)	(99)	(9.07)	(95.27)	(7.47)	(560)	(24.67)	(15.06)
$P_4 \times P_5$	−4.46**	2.05*	1.02**	−6.19**	0.50**	103.09**	−0.92ns	2.65**
	(59.67)	(102.33)	(12.87)	(83.3)	(9.07)	(723)	(31.67)	(22.96)
$P_4 \times P_6$	−5.02**	−0.94ns	−0.74**	1.98*	−1.42**	43.98**	−0.62ns	0.97**
	(59)	(100.33)	(11.63)	(93.1)	(7.78)	(680)	(31.28)	(21.58)
$P_4 \times P_7$	0.57ns	2.64**	−1.55**	2.68**	0.40**	65.91**	3.82**	5.29**
	(67.33)	(101.67)	(10.27)	(95.07)	(9.42)	(709)	(35.9)	(25.51)
$P_5 \times P_6$	0.28ns	−0.32ns	0.31ns	0.95ns	1.39**	36.61**	4.89**	3.96**
	(61.67)	(100.33)	(12.3)	(91.4)	(10.39)	(660)	(35.57)	(23.9)
$P_5 \times P_7$	−0.13ns	0.38ns	−3.40**	−2.25**	−0.26*	−87.46**	−2.42**	−4.09**
	(64)	(100.67)	(8.03)	(89.47)	(8.56)	(543)	(28.43)	(15.48)
$P_6 \times P_7$	−0.35ns	−0.73ns	0.47*	5.18**	0.68**	−36.57**	5.24**	1.87**
	(63.67)	(98.67)	(12.43)	(98.53)	(10.13)	(610)	(35.4)	(21.72)
SE(Sij)	0.42	0.83	0.22	0.84	0.13	8.06	0.47	0.32

* and ** indicates significant at $P \leq 0.05$ and 0.01 level, respectively. ns− non-significant. The full names of the traits are given in the footnotes of Table 2.

Heterobeltiosis can occur in either positive or negative sense, which one will be accepted depends on desired traits [40]. In the present study, heterobeltiosis was determined (Table 8), and it was found that out of 21 crosses, 12 crosses ($P_1 \times P_2$, $P_1 \times P_3$, $P_1 \times P_4$, $P_2 \times P_3$, $P_2 \times P_4$, $P_3 \times P_4$, $P_3 \times P_5$, $P_3 \times P_6$, $P_3 \times P_7$, $P_4 \times P_5$, $P_4 \times P_6$, and $P_4 \times P_7$) for DF, eight crosses ($P_1 \times P_2$, $P_2 \times P_3$, $P_2 \times P_4$, $P_2 \times P_5$, $P_2 \times P_6$, $P_2 \times P_7$, $P_3 \times P_4$, and $P_3 \times P_6$) for DM, and two crosses ($P_2 \times P_3$ and $P_4 \times P_5$) for PH exhibited significant negative heterobeltiosis. Among them, five crosses ($P_1 \times P_2$, $P_2 \times P_3$, $P_2 \times P_4$, $P_3 \times P_4$, and $P_3 \times P_6$) exhibited significant negative heterobeltiosis for both DF and DM. Additionally, the cross $P_2 \times P_3$ showed significant negative heterobeltiosis for DF, DM and PH. Therefore, this cross is recommended as early and short stature F$_1$. In contrast, five crosses ($P_2 \times P_3$, $P_2 \times P_5$, $P_2 \times P_7$, $P_3 \times P_4$, and $P_3 \times P_6$) for TP, two crosses ($P_5 \times P_6$ and $P_6 \times P_7$) for PL, nine crosses ($P_1 \times P_2$, $P_1 \times P_5$, $P_2 \times P_3$, $P_2 \times P_4$, $P_2 \times P_7$, $P_3 \times P_4$, $P_3 \times P_5$, $P_4 \times P_5$, and $P_4 \times P_7$) for NGPP, one cross ($P_6 \times P_7$) for 1000-GW, and eight crosses ($P_1 \times P_2$, $P_1 \times P_5$, $P_2 \times P_7$, $P_3 \times P_4$, $P_4 \times P_5$, $P_4 \times P_6$, $P_4 \times P_7$, and $P_5 \times P_6$) for YPP showed significant positive heterobeltiosis. Considering the overall feature of heterobeltiosis, the crosses $P_2 \times P_3$, $P_2 \times P_7$, $P_3 \times P_4$, and $P_4 \times P_5$ emerged as the most promising F$_1$s for further evaluation obtaining desirable segregants.

3.10. Determination of salinity tolerance in F$_1$s

Salinity tolerance is a complex qualitative trait involving significant physiological, biochemical and molecular interventions. To identify suitable saline-tolerant barley genotypes, the F$_1$s obtained from the 7 × 7 diallel crossing scheme were subjected to a two-stage

Table 8
Determination of heterobeltiosis of F_1s for different traits in 7×7 diallel cross in barley.

Cross	DF	DM	TP	PH	PL	NGPP	1000-GW	YPP
$P_1 \times P_2$	−9.22 **	−8.85 **	4.93 ns	0.72 ns	−6.25 ns	18.43 **	−21.96 **	18.06 **
$P_1 \times P_3$	−5.80 **	−2.11 ns	9.56 ns	−2.70 ns	−16.55 **	4.17 ns	−24.05 **	2.47 ns
$P_1 \times P_4$	−16.37 **	−3.68 ns	−2.90 ns	0.25 ns	−2.98 ns	3.63 ns	−23.92 **	1.68 ns
$P_1 \times P_5$	0.53 ns	−3.09 ns	−15.00 *	3.21 ns	−5.89 ns	28.04 **	−19.25 **	14.24 *
$P_1 \times P_6$	3.70 ns	−4.72 ns	−13.85 *	−5.52 ns	2.83 ns	−6.44 ns	−4.70 ns	−3.57 ns
$P_1 \times P_7$	12.11 **	−3.41 ns	3.33 ns	−2.45 ns	6.83 ns	7.21 ns	−5.71 ns	−33.06 **
$P_2 \times P_3$	−4.83 *	−10.64 **	19.72 *	−6.11 *	−4.93 ns	27.08 **	−19.81 **	1.55 ns
$P_2 \times P_4$	−9.73 **	−12.44 **	−2.03 ns	−1.29 ns	8.64 ns	22.59 **	−21.14 **	−17.18 **
$P_2 \times P_5$	0.97 ns	−8.85 **	13.06 *	−0.83 ns	−12.94 *	12.38 ns	−23.66 **	−5.21 ns
$P_2 \times P_6$	2.91 ns	−7.05 **	−22.36 **	8.15 *	3.88 ns	−5.15 ns	−23.62 **	5.09 ns
$P_2 \times P_7$	1.94 ns	−11.54 **	21.39 **	1.08 ns	−0.77 ns	27.81 **	−28.71 **	25.73 **
$P_3 \times P_4$	−19.91 **	−18.08 **	24.35 **	−4.78 ns	−4.93 ns	42.86 **	−20.02 **	14.34 *
$P_3 \times P_5$	−9.66 **	−3.43 ns	−15.83 *	−3.95 ns	−19.37 **	36.73 **	−22.65 **	5.55 ns
$P_3 \times P_6$	−10.63 **	−5.75 *	25.38 **	−1.79 ns	−2.82 ns	−6.19 ns	−30.62 **	−6.53 ns
$P_3 \times P_7$	−5.31 *	−0.50 ns	−24.44 **	0.35 ns	−21.13 **	−10.16 ns	−35.17 **	−25.42 **
$P_4 \times P_5$	−20.80 **	−1.76 ns	7.22 ns	−8.34 *	6.37 ns	47.62 **	−12.60 **	25.82 **
$P_4 \times P_6$	−21.68 **	−3.68 ns	−4.25 ns	2.45 ns	−13.68 **	5.15 ns	−13.67 **	18.22 **
$P_4 \times P_7$	−10.62 **	−2.40 ns	−14.44 *	3.11 ns	3.14 ns	13.80 *	−0.92 ns	26.33 **
$P_5 \times P_6$	−0.54 ns	−1.79 ns	1.23 ns	1.57 ns	15.27 **	2.06 ns	5.85 ns	31.56 **
$P_5 \times P_7$	1.05 ns	−1.47 ns	−33.06 **	−2.96 ns	−6.31 ns	−12.83 *	−15.38 **	−23.36 **
$P_6 \times P_7$	0.53 ns	−2.79 ns	2.33 ns	6.87 *	10.91 *	−5.67 ns	12.38 *	7.56 ns
SE	1.44	2.88	0.77	2.92	0.46	36.94	1.63	1.12

* and ** indicates significant at $P \leq 0.05$ and 0.01 level, respectively. ns− non-significant. The full names of the traits are given in the footnotes of Table 2.

salinity stress regime. The first ten days involved exposure to 100 mM NaCl, followed by 40 days at 150 mM NaCl, both in half-strength Hoagland solution. Following 50 days of salinity stress imposition, various indicators were measured in leaves to assess salinity tolerance in the F_1s. These indicators included Na^+ and K^+ content, root volume, $O_2^{\bullet-}$ and H_2O_2 accumulation, and the activity of antioxidant enzymes involved in their metabolism. Data were then analyzed and presented in Table 9. Since seed set under salinity stress was the primary selection criterion, only five F_1s produced healthy and viable seeds. Consequently, these five F_1s were excluded from diallel analysis.

The accumulation of Na^+, K^+, and their ratio, as well as the generation of $O_2^{\bullet-}$ and H_2O_2, and root volume, significantly differed among the F_1s (Table 9). F_1s such as $P_1 \times P_2$, $P_2 \times P_3$, $P_3 \times P_5$, $P_4 \times P_6$, and $P_4 \times P_7$ exhibited significantly lower Na^+, $O_2^{\bullet-}$, and H_2O_2 contents. Additionally, these crosses showed higher K^+ contents, favorable K^+/Na^+, and increased root volume. Interestingly, these crosses also produced healthy seeds (data not shown). On the contrary, crosses like $P_1 \times P_3$, $P_1 \times P_6$, $P_1 \times P_7$, $P_2 \times P_4$, $P_2 \times P_5$, $P_2 \times P_6$, $P_2 \times P_7$, $P_3 \times P_4$, $P_3 \times P_7$, $P_5 \times P_6$, $P_5 \times P_7$, and $P_6 \times P_7$ displayed higher Na^+, $O_2^{\bullet-}$, and H_2O_2 concentrations along with lower K^+ levels

Table 9
Concentration of Na^+, K^+, K^+/Na^+, $O_2^{\bullet-}$, H_2O_2, and root volume in salinity treated F_1s. All measurements, except for root volume, were taken from uppermost expanded leaves at 50 days of stress imposition. Root volume was measured at maturity. Data represent the mean of three observation $\pm$ standard error (SE). Significant differences ($P \leq 0.05$) among crosses within a column are indicated by different letters.

Crosses	Na^+ (ppm)	K^+ (ppm)	K^+/Na^+	$O_2^{\bullet-}$	H_2O_2	Root volume (cc)
$P_1 \times P_2$	$1800^g \pm 58$	$1140^a \pm 38$	$0.63^a \pm 0.036$	$8.40^i \pm 0.53$	$9.09^{ef} \pm 0.42$	$90.17^a \pm 6.5$
$P_2 \times P_3$	$2033^f \pm 72$	$983^{bc} \pm 47$	$0.48^{cd} \pm 0.040$	$10.90^h \pm 0.19$	$9.99^{de} \pm 0.40$	$83.83^{a\text{-}c} \pm 6.4$
$P_3 \times P_5$	$1700^g \pm 58$	$1067^{ab} \pm 33$	$0.63^a \pm 0.039$	$7.71^i \pm 0.25$	$9.48^{ef} \pm 0.57$	$88.33^a \pm 3.2$
$P_4 \times P_6$	$1830^g \pm 123$	$950^c \pm 29$	$0.53^{bc} \pm 0.053$	$10.64^h \pm 0.26$	$7.05^g \pm 0.35$	$91.50^a \pm 2.1$
$P_4 \times P_7$	$1800^g \pm 58$	$1050^{ab} \pm 29$	$0.59^{ab} \pm 0.035$	$8.80^i \pm 0.28$	$8.09^{fg} \pm 0.27$	$86.47^{ab} \pm 3.2$
$P_1 \times P_4$	$2400^{de} \pm 44$	$850^{de} \pm 26$	$0.36^{ef} \pm 0.018$	$12.88^{e\text{-}g} \pm 0.14$	$9.53^{ef} \pm 0.29$	$77.17^{b\text{-}d} \pm 2.1$
$P_1 \times P_5$	$2200^{ef} \pm 58$	$933^{cd} \pm 29$	$0.43^{de} \pm 0.029$	$10.99^h \pm 0.23$	$11.69^{cd} \pm 0.79$	$74.83^{cd} \pm 3.2$
$P_3 \times P_6$	$2367^{de} \pm 33$	$803^{ef} \pm 29$	$0.34^f \pm 0.014$	$11.84^{gh} \pm 0.35$	$9.75^{ef} \pm 0.65$	$77.33^{bc} \pm 3.3$
$P_4 \times P_5$	$2067^f \pm 88$	$940^{cd} \pm 42$	$0.46^{cd} \pm 0.034$	$11.97^{f\text{-}h} \pm 0.23$	$10.11^{de} \pm 0.28$	$77.17^{b\text{-}d} \pm 3.4$
$P_1 \times P_3$	$2900^{ab} \pm 58$	$660^{gh} \pm 35$	$0.23^{hi} \pm 0.016$	$15.61^b \pm 0.22$	$14.31^{ab} \pm 0.98$	$54.83^{f\text{-}h} \pm 3.2$
$P_1 \times P_6$	$2800^{ab} \pm 58$	$507^{ij} \pm 26$	$0.18^i \pm 0.007$	$14.68^{bc} \pm 0.37$	$12.87^{bc} \pm 0.51$	$50.50^{f\text{-}i} \pm 3.3$
$P_1 \times P_7$	$2717^{bc} \pm 73$	$550^{ij} \pm 35$	$0.20^{hi} \pm 0.015$	$17.59^a \pm 0.13$	$14.31^{ab} \pm 0.34$	$44.83^{hi} \pm 3.4$
$P_2 \times P_4$	$2800^{ab} \pm 58$	$500^j \pm 29$	$0.18^i \pm 0.014$	$13.06^{e\text{-}g} \pm 0.65$	$15.68^a \pm 0.71$	$60.83^{ef} \pm 3.2$
$P_2 \times P_5$	$2767^b \pm 33$	$567^{h\text{-}j} \pm 39$	$0.21^{hi} \pm 0.016$	$13.92^{c\text{-}e} \pm 0.20$	$14.39^{ab} \pm 0.58$	$56.83^{e\text{-}g} \pm 3.8$
$P_2 \times P_6$	$2833^{ab} \pm 88$	$603^{hi} \pm 29$	$0.22^{hi} \pm 0.017$	$13.20^{d\text{-}f} \pm 0.23$	$13.98^{ab} \pm 0.19$	$52.83^{f\text{-}i} \pm 3.4$
$P_2 \times P_7$	$3000^a \pm 58$	$553^{ij} \pm 37$	$0.19^i \pm 0.016$	$15.47^{b\text{-}d} \pm 0.62$	$13.84^{ab} \pm 0.58$	$46.50^{g\text{-}i} \pm 3.3$
$P_3 \times P_4$	$2533^{cd} \pm 88$	$657^{gh} \pm 29$	$0.26^{gh} \pm 0.016$	$15.56^b \pm 0.79$	$13.05^{bc} \pm 0.36$	$56.67^{e\text{-}g} \pm 3.2$
$P_3 \times P_7$	$2900^{ab} \pm 100$	$733^{fg} \pm 61$	$0.25^{g\text{-}i} \pm 0.021$	$18.5^a \pm 0.78$	$15.75^a \pm 1.23$	$44.00^i \pm 3.5$
$P_5 \times P_6$	$2700^{bc} \pm 58$	$567^{h\text{-}j} \pm 30$	$0.21^{hi} \pm 0.015$	$14.70^{bc} \pm 0.32$	$12.89^{bc} \pm 0.46$	$57.10^{ef} \pm 3.2$
$P_5 \times P_7$	$2800^{ab} \pm 58$	$573^{h\text{-}j} \pm 39$	$0.20^{hi} \pm 0.010$	$14.45^{b\text{-}d} \pm 0.40$	$14.40^{ab} \pm 0.59$	$44.17^i \pm 3.3$
$P_6 \times P_7$	$2367^{de} \pm 120$	$713^c \pm 26$	$0.31^{bc} \pm 0.023$	$12.56^{fg} \pm 0.37$	$11.59^{cd} \pm 0.51$	$66.83^{de} \pm 3.2$

and root volume, without any seed settings. Therefore, based on healthy seed setting, higher K^+/Na^+ ratio, higher root volume, and lower ROS generation, five crosses ($P_1 \times P_2$, $P_2 \times P_3$, $P_3 \times P_5$, $P_4 \times P_6$, and $P_4 \times P_7$) were grouped as saline tolerant (Table 10). Four crosses ($P_1 \times P_4$, $P_1 \times P_5$, $P_3 \times P_6$, and $P_4 \times P_5$) exhibited few deformed seeds, comparatively lower K^+/Na^+ ratio and root volume and relatively higher ROS generation, categorized as medium tolerant. The remaining crosses, which did not produce any seeds, showed higher Na^+ levels and ROS, grouped as salinity sensitive (Table 10).

3.11. Analysis of enzymatic antioxidants using native in-gel electrophoresis

To investigate the ROS regulatory mechanism, the activities of several enzymatic antioxidants, including SOD, CAT, POD, APX, and GR were analyzed in leaves of all five tolerant F_1s, two representative medium tolerant F_1s ($P_1 \times P_5$ and $P_3 \times P_6$), and two salt-sensitive F_1s ($P_2 \times P_5$ and $P_2 \times P_6$) through native in-gel electrophoresis. The phenotypes of the F_1s are shown in Supplementary Fig. 1). Native in-gel electrophoresis revealed only one SOD isozyme in all the crosses, although band intensity varied across crosses (Fig. 2 and Supplementary Fig. 2A). The susceptible crosses displayed the weakest SOD activity, visualized as faint bands, compared to all the tolerant and one medium tolerant cross ($P_3 \times P_6$) that exhibited stronger bands. Similar to SOD, only one CAT isozyme was detected with variable activity among the F_1s (Fig. 2 and Supplementary Fig. 2B). The susceptible crosses again showed the lowest CAT activity, while the tolerant crosses (except $P_4 \times P_7$) and the medium tolerant crosses exhibited significantly higher activity visualized as more intense bands. Activity of POD also varied among the crosses of barley (Fig. 2 and Supplementary Fig. 2C). Two POD isozymes were appeared in all the genotypes. Interestingly, tolerant crosses displayed a stronger induction of these isozymes compared to susceptible and medium tolerant crosses. On the contrary, three APX isozymes were detected in in all F_1s (Fig. 2 and Supplementary Fig. 2D). Notably, tolerant crosses exhibited significantly denser APX activity as compared to the others groups, with substantially higher activity observed in $P_4 \times P_7$. Unlike other enzymes, glutathione reductase (GR) activity showed a different pattern (Fig. 2 and Supplementary Fig. 2E). While two tolerant crosses ($P_4 \times P_6$ and $P_4 \times P_7$) showed the activity level similar to the susceptible crosses, the remaining three tolerant ($P_1 \times P_2$, $P_2 \times P_3$, and $P_3 \times P_5$) and medium tolerant crosses exhibited intensified GR activity. This suggested that GR may play a more complex role in salt tolerance, potentially varying among genotypes.

4. Discussion

In genetics and plant breeding, the mean performance of the F_1 generation conveys a fundamental idea about crossbreeding. In the case of barley, the F_1 generation, also known as the first filial generation, represents the initial hybridization of two genetically distinct parents. The genetic diversity resulting from parents used in a diallel cross is particularly valuable in breeding programs, as it can lead to introduce desirable traits that may not exist in either of the parental lines. In the present study, we used seven genetically diverse patents (Table 1) to produce F_1s. The mean performance of the genotypes exhibited significant variation at $P \leq 0.01$ (Supplementary Table 1). Therefore, a diallel analysis was performed to investigate genetic action and combining ability to identify high-yielding barley F_1s.

In the Vr-Wr graph, the parental array points scattered along the regression line indicated existing genetic diversity among the parents, as expected for the function of parental order of dominance. On the contrary, the array points remain in a cluster form, reflect minimum genetic diversity [38]. From the graphical model proposed by Hayman (Fig. 1), it was observed that genetic diversity was present among the parents for all the traits. The average degree of dominance of genes controlling these traits can be detected based on the intercept of the regression line on the Wr axis in relation to the origin (0) [41]. Specifically, if the straight line intercepts the vertical axis (Wr) above the origin, it indicates partial dominance. If the regression line passes below the origin, it signifies overdominance, and if it passes through the origin, it represents complete dominance of the genes controlling the respective trait [36,42,43]. In the current study, the traits DM, PH, and PL exhibited partial dominance of the genes. Conversely, DF, TP, NGPP, 1000-GW, and YPP exhibited overdominance of the genes. This conclusion is based on the regression line intersecting the vertical axis (Wr) below the origin. These findings align with previous researches by Chaudhari et al. [44] and Nagar et al. [45], who also reported overdominance behavior for days to 50 % heading, number of spikelets per spike, and thousand grain weight in bread wheat. Rohman et al. [46] observed overdominance behavior in plant height, days to 50 % tasseling, days to 50 % silking, and 100-GW in maize.

It is necessary to test the assumption of the additive-dominance model to confirm the inheritance of a particular trait. In this study, to assess the additive-dominance model and the validity of underlying assumptions of the diallel analysis, data sets for various traits were subjected to two scaling tests: t^2 and regression analysis (Table 2). Our analyses revealed that traits such as DF and NGPP fully satisfied the assumptions of the additive dominance model, while TP and 1000-GW only partially met these assumptions. Conversely,

Table 10
Grouping of 21 F_1 generations of barley on the basis of salinity tolerance.

| Group	Number of F_1s	List of genotypes	Criteria
Tolerant	05	$P_1 \times P_2$, $P_2 \times P_3$, $P_3 \times P_5$, $P_4 \times P_6$, and $P_4 \times P_7$	Healthy seed production, higher K^+/Na^+, higher root volume and lower ROS generation
Medium tolerant	04	$P_1 \times P_4$, $P_1 \times P_5$, $P_3 \times P_6$, and $P_4 \times P_5$	Deformed and few seed settings, comparatively lower K^+/Na^+ and root volume and comparatively higher ROS generation
Sensitive	12	$P_1 \times P_3$, $P_1 \times P_6$, $P_1 \times P_7$, $P_2 \times P_4$, $P_2 \times P_5$, $P_2 \times P_6$, $P_2 \times P_7$, $P_3 \times P_4$, $P_3 \times P_7$, $P_5 \times P_6$, $P_5 \times P_7$, and $P_6 \times P_7$	No seed setting or died before seed setting or flowering, lower K^+/Na^+ and root volume and higher ROS generation
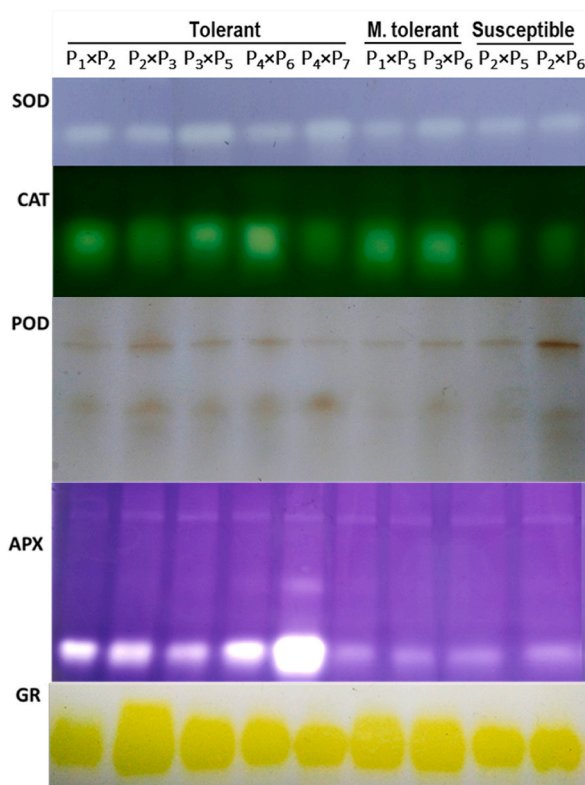
Fig. 2. In-gel native activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) in selected barley F_1s. 50 μg protein loaded per lane. For SOD, 10 % SDS-PAGE was used, and the remaining enzymes (CAT, POD, APX, and GR) were separated in 8 % SDS-PAGE. This figure was prepared by cropping the portions containing bands from the original gel images. The complete, non-adjusted gel images were provided as supplementary materials in Supplementary Fig. 2.

DM, PL, and YPP appeared inadequate to fulfil the assumptions, suggesting that epistasis is present for these three traits, limiting the additive-dominance model. Consequently, a significant inter-allelic interaction can influence the phenotypic expression of these traits. In the Vr-Wr graphs, parents positioned in the middle likely possess equal frequencies of both dominant and recessive alleles, while those located at edges likely harbor recessive alleles [42,45]. Thus, parents positioned closer to the origin likely contain higher frequency of dominant alleles, while those farther away likely harbor more recessive alleles.

To further investigate the contributions of additive and dominance effects to the studied traits, we analyzed the genetic components of variance along with other relevant genetic parameters (Table 3). The observed significant values for both additive variance (D) and dominance variance (H_1 and h^2) across all environments suggest the presence of both additive and dominance gene action the studied traits [44]. However, if $D > H_1$ for a particular trait, it indicates that the additive component plays a more predominant role in controlling that trait compared to dominant component. Conversely, a significant value of h^2 reflects a high degree of dominance effects associated with heterozygote gene loci [47]. While environmental effects significantly influence the yield potential of genotypes, the additive or dominance effects can sometimes be strong enough to mask these environmental effects. The proportion of dominant genes to positive and negative gene effects ($H_2/4H_1$) provides insight into the distribution of positive and negative alleles among the parents [38]. In the present study, most of the traits appeared to be governed by overdominance, as $(H_1/D)^{0.5}$ is greater than 1 (Table 3). However, in graphical presentation of the Vr-Wr graph, partial dominance was suggested for DM, PH, and PL (Fig. 1). Nonetheless, in additivity test suggested that the inheritance of both DM and PL affected by inter-allelic interaction, limiting the additive dominance (Table 2). On the contrary, the genetic parameters from Table 3 revealed a significant influence of the environmental component (E) in expressing the phenotype of PH. This information is also crucial to consider when illustrating partial dominance in a graphical approach. Additionally, other genetic parameters such as the proportion of dominant to recessive genes, H_2, F, and $H_2/4H_1$ further suggested that inheritance of DM, PH, and PL is governed by overdominance effects (Table 3). Dominance gene action had a greater influence than additive gene action for all studied traits. For most traits, dominance effects (H_1) appeared to be greater than additive effects (D), suggesting a potentially stronger influence of dominant genes. However, some traits like DF, NGPP, and 1000-GW exhibited significant values for both D and H_1, indicating involvement of both additive and dominance gene effects for these traits. The observed average degree of dominance being greater than 1 could indicated the existence of overdominance for all traits. This result is in agreement with earlier researches [44]. However, it is important to consider that this value can be influenced by epistasis (gene interactions) and not solely dominance. The positive F values indicated that several dominant genes likely contribute to the expression of all the traits. The $H_2/4H_1$ ratio, which reflects the distribution of positive and negative alleles among the parents, was

less than the expected value (0.25) for all traits. This suggests an uneven distribution of alleles, potentially influencing gene actions. Low narrow-sense heritability (<30 %) observed for most of the studied traits, coupled with dominance variance (H_1) exceeding additive variance (D), suggesting that these traits are governed by dominance type genetic effects. This aligns with previous findings [9] and supports the potential value of heterosis breeding in barley for these traits, where exploiting dominance effects can be beneficial [48]. Traits like days to flowering (DF) and 1000-grain weight (1000-GW) exhibited moderate heritability (33 % and 44 %, respectively). This indicates these traits were influenced by both additive and dominant genes [45]. Similar observations were reported by Chaudhari et al. [44] in bread wheat for the same traits.

The potence ratio, a measure of dominance, was calculated for the studied traits in barley (Table 4). The results revealed varying degrees of dominance among the traits, ranging from partial to overdominance in the heritance of the traits. These findings are in agreement with observations by Ghosh et al. [9], supporting the role of dominance gene action in these traits.

Significant differences observed among genotypes for the studied traits (Supplementary Table 1) indicate a wide range of genetic variability present among the genotypes. This genetic diversity serves as the raw material for generating novel recombination of the traits in the offspring (segregating generation) [49]. In the present study, the observed variation increases the chance of isolating new recombinants with potentially desirable traits. General and specific combining ability (GCA and SCA) play a substantial role in the expression of the traits tested in the F_1 generation [50]. Zhan et al. [51] demonstrated that the value of GCA is a good indicator for understanding the nature of parental lines. Combining ability analysis revealed that significant mean squares for both GCA and SCA for all studied traits (Table 5). This indicate the involvement of both additive and non-additive gene effects in controlling these traits [52, 53]. The GCA/SCA ratio is being less than one suggests a predominance of non-additive gene action for controlling these traits in barley. This aligns with previous findings in barley [52,54–56]. Similar observations of non-additive gene action have been reported in maize for kernel number per row [57] and grain yield [58,59].

Estimation of GCA of parents is crucial for identifying those parents with the potential to produce high-yielding hybrids through heterosis [2]. In the case of barley, pure lines with significant and negative GCA effects are considered as good general combiners for DF, DM, and PH. These parents contribute genes that promote earliness and shorter stature in F_1s, leading to improved crop management and potentially higher yields. However, for yield and its components, parents with significant and positive GCA effects are considered as good general combiners. In the present investigation, the parents P_5 and P_6 could serve as good sources for developing early and short stature hybrids. Parent P_5 exhibited significant negative GCA effects for DF and PH, indicating its potential to contribute earliness and shorter stature to offspring (Table 6). The non-significant positive GCA value for DM suggests minimal influence on this trait. Similar to P_5, parent P_6 exhibited significant negative GCA for DF, making it a valuable parent for breeding early maturing hybrids. The non-significant negative GCA for DM and non-significant positive GCA for PH indicate a neutral effect on these traits (Table 6). Parents P_2 and P_4 displayed negative GCA for PH and positive GCA for yield and yield components. These suggests they could contribute favorable genes for both short stature and yield potential of the F_1s. Significant positive GCA for PH lead to producing taller hybrid which have the tendency of lodging [2]. Our findings align with previous studies by Amer et al. [52] and Moustafa et al. [49], who highlighted the importance of selecting parents with significant positive GCA for 1000-GW and grain yield for improving barley yield in breeding programs.

Including at least one parent with strong GCA effects, especially the female parent, during the crossing can enhance heterosis in the F_1s [60]. Similar conclusion also illustrated by Xingming et al. [61]. Even if the parents exhibit average GCA effects, offspring with high SCA effects can be valuable for breeding program [62]. These promising crosses can be used for further breeding cycles to potentially develop superior lines. A significant proportion of the SCA effects expressed by low × low crosses can be ascribed to dominance × dominance type of non-allelic gene action, resulting in overdominance, and these effects are non-fixable [9]. In high × high cross combinations, additive × additive interactions are desirable for early segregants in the early advanced generation. Conversely, in high × low, or low × high cross combinations, predominantly additive effects are present in the good combiner, while a complementary epistatic effect may be observed in the poor combiner. These two gene actions work together to maximize gene expression [63]. Importantly, the term 'high' stands for significant GCA effects in the desired direction, while 'low' indicates a non-significant GCA effect, either negative or positive. The SCA effects of the crosses did not exhibit specific trends in cross combinations between parents with high, medium, and low GCA effects in this study. However, for desirable traits like NGPP and YPP, most F_1s were produced from low × low or low × high, and occasionally from high × low GCA combinations. Here, any combination of parents may result in hybrid vigor over the parents, which could be due to dominance, overdominance, or epistatic gene action. Therefore, the crosses showing desirable SCA effects can be used in the future breeding programs. In the present study, considering the per se performance and SCA value (Table 7), the crosses $P_2 × P_3$, $P_2 × P_7$, $P_4 × P_5$, $P_5 × P_6$, and $P_6 × P_7$ were selected as early, short-statured, and high-yielding F_1s, and recommended for further evaluation to obtain desirable segregants.

The degree of heterosis or hybrid vigor is typically assessed in F_1 hybrids using mid-parent heterosis (MPH) and best-parent heterosis (BPH), or heterobeltiosis in self-pollinated crops. BPH refers to the superiority of the F_1 hybrid over its better parent. Since barley is a self-pollinated crop with limited yield potential due to low heterosis, its breeding techniques also critical [1]. In the current study, we estimated the range of heterobeltiosis in F_1 hybrids (Table 8) to select better F_1s. Preethi et al. [64] noted that for days to flowering and for days to first harvest, negative heterosis is ideal. This aligns with the primary objective of any breeding program: to help growers to capture the early market maximize economic benefits. Considering the overall features of heterobeltiosis, the crosses $P_2 × P_3$, $P_2 × P_7$, $P_3 × P_4$, $P_4 × P_5$ could be selected as promising F_1s and should undergo further evaluation in the filial generation.

Salinity tolerance is a complex trait, both genetically and physiologically. Plant breeders have had very limited success in increasing the tolerance of crops to salt stress. Genotypically, salt tolerance is achieved by managing cytoplasmic ionic balance, which involves ion homeostasis and compartmentalization, osmotic adjustment, and improved antioxidant metabolism, including a higher

capacity of ROS scavenging [65,66]. High absorptions of Na^+ and Cl^- can lead to disproportionation, impairing the uptake of other essential ions such as K^+ and Ca^{2+}. This inhibition negatively affects plant growth and productivity. Tolerant plants employ a number of strategies to restrict Na^+ movement, favoring the retention of K^+ and reducing the K^+/Na^+ ratio in the tissues [67–69]. In this study, the tolerant barley crosses maintained higher K^+ level as well as favorable K^+/Na^+ ratio, and greater root volume compared to susceptible ones (Table 9). Similar findings of tolerant genotypes with lower Na^+/K^+ ratio have also been reported in mustard [70] and cucumber [16]. Additionally, tolerant barley genotypes exhibit inherently higher root growth under saline conditions [71].

Salinity is one of the most significant abiotic stresses threatening the global food security. Growth inhibition and biomass reduction are common consequences of salinity-induced stress in plants [72]. Salinity-mediated osmotic stress alters various physiological and molecular processes [73]. To ensure cellular survival, plants deploy an antioxidant-mediated protective system though signalling networks to adapt to salt stress. Key enzymes such as SOD, CAT, POD, APX, GPX, and GR play essential role to protect cellular organelle from oxidative damage [20]. The toxic effects resulting from excessive $O_2^{\bullet-}$ production can be mitigated by SOD activity, which rapidly converts $O_2^{\bullet-}$ to H_2O_2 [74]. The H_2O_2 produced in this way, as well as through non-enzymatic pathways, is detoxified by the activities of CAT, POD, APX, and GPX. In our study, we observed genotypic variation in ROS production, where higher levels of $O_2^{\bullet-}$ and H_2O_2 were noticed in sensitive F_1s (Table 9), suggesting a higher possibility of cellular death. F_1s with higher SOD activity in tolerant crosses, particularly in $P_3 \times P_5$ and $P_4 \times P_7$ (Fig. 2 and Supplementary Fig. 2A), highlight the importance of SOD in reducing $O_2^{\bullet-}$ levels. Previous studies have also reported genotypic variation in SOD activity, with lower $O_2^{\bullet-}$ levels in maize [23,75]. However, CAT is a potent antioxidant enzyme with a high turnover rate and a low affinity for H_2O_2, while APX exhibits a high affinity for H_2O_2 compared to CAT and other antioxidant enzymes. These enzymes are frequently employed by plants to fine-tune H_2O_2 levels [76]. In our study, the activities of CAT and APX were very prominent in tolerant barley F_1s (Fig. 2 and Supplementary Fig. 2). Thus, the activities of these two enzymes played significant role in reducing H_2O_2 levels in tolerant barley hybrids (Tables 9 and 10). Cultivar differences in cucumber under salinity have also been reported regarding the accumulation of H_2O_2 [77]. In our previous study, we observed that salt-sensitive maize seedlings exhibited higher ROS production [23]. Notably, in the F_1 hybrid $P_4 \times P_7$, we observed the densest APX activity with comparatively lower CAT activity, resulting in a slightly higher H_2O_2 concentration. Conversely, the F_1 hybrid $P_4 \times P_6$, which had higher activities of both CAT and APX, exhibited lower H_2O_2 levels (Fig. 2, Supplementary Fig. 2 and Table 9). Similarly, in the moderate tolerant group, F_1 hybrids $P_1 \times P_5$ and $P_3 \times P_6$ exhibited higher CAT activity and least APX activity, resulting in comparatively higher H_2O_2 levels than $P_4 \times P_7$. These results suggest that a combination multiple enzymes contributes to better H_2O_2 reduction capacity. However, POD activity varied among barley F_1 hybrids and showed better activity in both tolerant and susceptible F_1s. Additionally, GR plays a crucial role in recycling cellular glutathione homeostasis, as reduced glutathione is critical for maintaining antioxidative activities in plant [20]. In this study, the tolerant and medium salt tolerant F_1s exhibited better GR activity, particularly in the crosses like $P_2 \times P_3$, $P_3 \times P_5$, $P_1 \times P_2$, $P_1 \times P_5$, and $P_3 \times P_6$ (Fig. 2 and Supplementary Fig. 2E). However, the F_1 hybrid $P_2 \times P_3$, which had a lower K^+/Na^+ ratio, showed comparatively lower CAT activity than the tolerant F_1s (Table 9). Previous reports have also highlighted higher GR activity in salt-tolerant genotypes of wheat and maize [23,78]. Therefore, in addition to antioxidants and ROS, considering K^+/Na^+ ratios and root phenotypes are crucial for selecting salt-tolerant F_1 hybrids in barley.

5. Conclusion

This study investigated the genetic basis of various traits and salinity tolerance in F1 barley hybrids derived from seven parental lines. Diallel analysis revealed the involvement of both additive and dominance gene effects for most studied traits. However, dominance effects played a more prominent role, suggesting the potential value of heterosis breeding in barley. The GCA analysis identified parents suitable for developing early maturing, short-statured, and high-yielding hybrids. Considering genetic action, per se performance, SCA effects, and heterobeltiosis, the crosses $P_2 \times P_3$, $P_2 \times P_7$, $P_3 \times P_4$, $P_4 \times P_5$, $P_5 \times P_6$, and $P_6 \times P_7$ were identifies as promising F_1s. These crosses exhibited earliness, short-statured, and high yield potential, making them suitable candidates for advancing to subsequent generations to obtain desirable segregants. Salinity tolerance was associated with maintaining a favorable K^+/Na^+ ratio, higher root volume, and enhanced antioxidant enzyme activity, particularly SOD, CAT, APX, and GR. Based on healthy seed setting, favorable K^+/Na^+ ratios, and the generation of $O_2^{\bullet-}$ and H_2O_2 levels, five F_1 hybrids $P_1 \times P_2$, $P_2 \times P_3$, $P_3 \times P_5$, $P_4 \times P_6$, and $P_4 \times P_7$ were selected as saline tolerant. Overall, the study provides valuable insights for breeding barley varieties with improved yield potential and salinity tolerance.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Md Motiar Rohman: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shahnewaz Begum:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation. **Mohammed Mohi-Ud-Din:** Writing – review & editing, Visualization, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34278.

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