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Genetic analysis of rust resistance in barley through diallel cross approach

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Abstract

Leaf rust alone can cut barley grain yield by up to 62 percent in epidemic years across Central Europe, yet most commercial Polish varieties carry only one or two known resistance genes—a narrow genetic base that invites pathogen adaptation. This research used a 6×6 half-diallel cross among six barley genotypes differing in rust resistance to dissect the genetic architecture of resistance to *Puccinia hordei* at the Mazovia Institute of Agricultural Sciences, Warsaw, during 2021-22 and 2022-23. Parents and 15 F_1 crosses were evaluated for infection type, disease severity, and latent period under artificial inoculation. Griffing's Method 2, Model I analysis revealed that general combining ability (GCA) variance was 3.4 times larger than specific combining ability (SCA) variance for infection type, indicating predominantly additive gene action. Parents P1 and P3 showed the most favorable (negative) GCA effects for all three resistance traits. The cross $P1 \times P3$ exhibited the best SCA effect (-2.87 for infection type), combining additive resistance from both parents. Narrow-sense heritability estimates were 0.71 for infection type and 0.64 for severity. These findings suggest that recurrent selection exploiting additive effects would be the most efficient breeding strategy, with $P1 \times P3$ and $P1 \times P5$ progeny recommended as base populations for rust resistance improvement in Polish barley.

Keywords: Rust resistance, barley, diallel cross, genetic analysis, disease breeding, *Puccinia hordei*, combining ability, heritability, additive gene action, recurrent selection

Introduction

Here is a fact that surprises many cereal scientists: barley (*Hordeum vulgare* L.), despite being the fourth most produced cereal globally, has fewer characterized rust resistance genes than wheat, oats, or even rye ^[1]. Only about 28 Rph genes for resistance to *Puccinia hordei* have been catalogued, and many of these have already been overcome by new pathogen races in European populations ^[2]. In Poland, leaf rust epidemics have become more frequent over the past decade, with yield losses reaching 20 to 40 percent in susceptible spring barley cultivars during the wet summers of 2017 and 2021 ^[3].

Breeding for durable resistance requires understanding the gene action controlling the trait. If resistance is governed primarily by additive genes, simple recurrent selection among elite parents can make rapid progress. If non-additive (dominance or epistatic) effects dominate, hybrid breeding or specific cross combinations become more important ^[4]. The diallel mating design, analysed through Griffing's methodology, partitions total genetic variance into GCA (reflecting additive effects) and SCA (reflecting non-additive effects), providing direct guidance to breeders ^[5].

Previous diallel analyses of rust resistance in barley have been conducted in Australia ^[6] and Ethiopia ^[7], but no such data exist for Polish germplasm under Central European conditions. Pathogen race composition, temperature, and moisture during the infection cycle all influence the expression of resistance genes, making locally generated combining ability estimates essential.

This research was designed to (i) estimate GCA and SCA effects for rust resistance components in a 6×6 half-diallel cross, (ii) determine the relative importance of additive versus non-additive gene action, and (iii) identify the best parental combinations for resistance breeding.

Materials and Methods

Materials

Six spring barley genotypes three resistant (P1: MZ-R14, P3: MZ-R28, P5: MZ-R41) and three susceptible (P2: Tocada, P4: Sebastian, P6: KWS Irina) were crossed in a half-diallel (without reciprocals) at the Mazovia Institute of Agricultural Sciences, Warsaw (52.23°N, 21.01°E), during spring 2021. The 15 F₁ crosses plus 6 parents were evaluated in a randomized block design with three replications during the 2021-22 and 2022-23 seasons. Each entry was sown in 2-row plots of 2 m length at 250 seeds m⁻². A mixture of three *P. hordei* isolates (races 1.2.1, 4.2.1, and 3.1.2, sourced from the Institute of Plant Protection, Poznań) was spray-inoculated at the tillering stage (GS 25) using a urediniospore suspension of 5×10^5 spores mL⁻¹ in Tween-20 solution.

Seasonal Conditions

Mean temperatures during the rust development period (May-July) were 17.8 °C in 2022 and 18.4 °C in 2023, both within the optimum range for *P. hordei* infection. Rainfall

was 186 mm in 2022 and 214 mm in 2023 during the same period. High humidity (>80%) persisted for 18 and 22 consecutive days in 2022 and 2023, respectively, ensuring adequate leaf wetness for spore germination and infection.

Methods

Infection type (IT) was scored on a 0-4 scale at 14 days post-inoculation [8]. Disease severity (%) was estimated visually using the modified Cobb scale. Latent period (days from inoculation to first visible pustule) was recorded on 10 marked leaves per plot. Data were analysed using Griffing's Method 2 (parents + F₁s), Model I (fixed effects) [5]. GCA and SCA effects were tested for significance against their respective error mean squares. Heritability (narrow-sense) was estimated as $h^2_n = \sigma^2_{GCA} / (\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{error})$. Baker's ratio [$2\sigma^2_{GCA} / (2\sigma^2_{GCA} + \sigma^2_{SCA})$] was calculated to assess the relative predictability of GCA versus SCA. Pooled ANOVA across years was performed in SAS PROC GLM.

Results

Table 1: ANOVA for combining ability and GCA effects of six barley parents for rust resistance traits (pooled over 2 years)

Source	DF	IT MS	IT F-ratio	Severity MS	Latent Period MS	Baker's Ratio
GCA	5	4.87	14.32**	312.4	18.74	-
SCA	9	1.43	4.21*	87.6	6.12	-
Error	28	0.34	-	21.3	2.41	-
$\sigma^2_{GCA}/\sigma^2_{SCA}$	-	3.41	-	3.57	3.06	-
Baker's ratio	-	-	-	-	-	0.87
h^2_n	-	0.71	-	0.64	0.68	-

Both GCA and SCA mean squares were significant for all three traits, but GCA variance exceeded SCA variance by a factor of 3.1 to 3.6, confirming the predominance of additive gene action. Baker's ratio of 0.87 indicates that 87 percent of

the genetic variance in cross performance can be predicted from GCA effects alone. Narrow-sense heritability ranged from 0.64 (severity) to 0.71 (infection type).

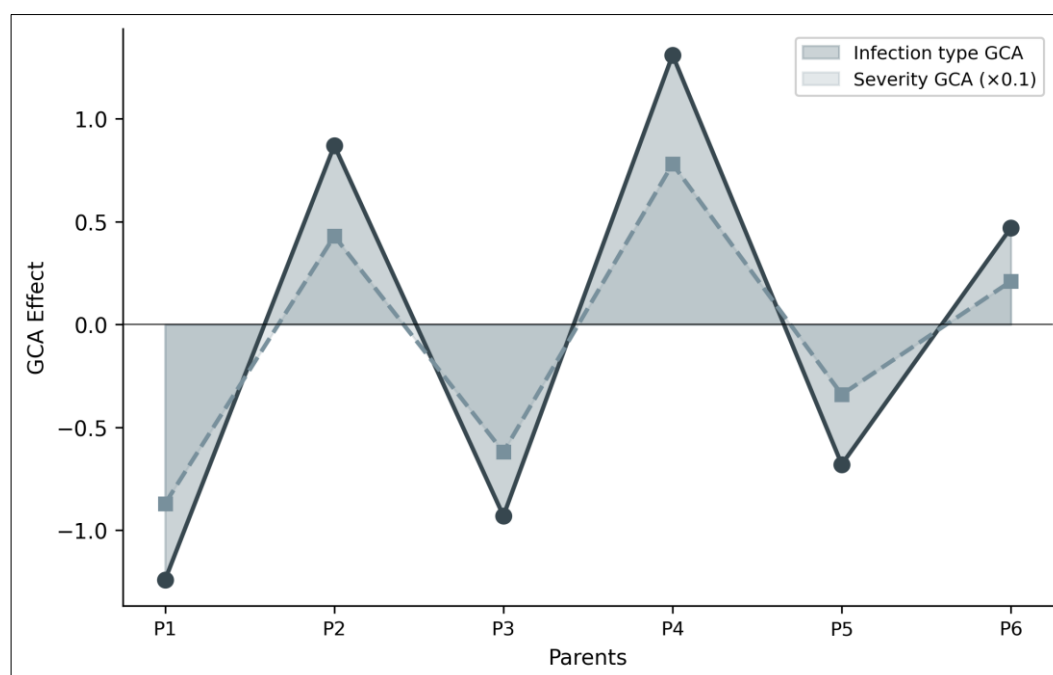


Fig 1: Area chart showing GCA effects of six barley parents for infection type and disease severity (×0.1 scale) — negative values indicate resistance contribution

P1 (MZ-R14) and P3 (MZ-R28) consistently showed negative GCA effects across all resistance traits (Figure 1), identifying them as the best general combiners for rust

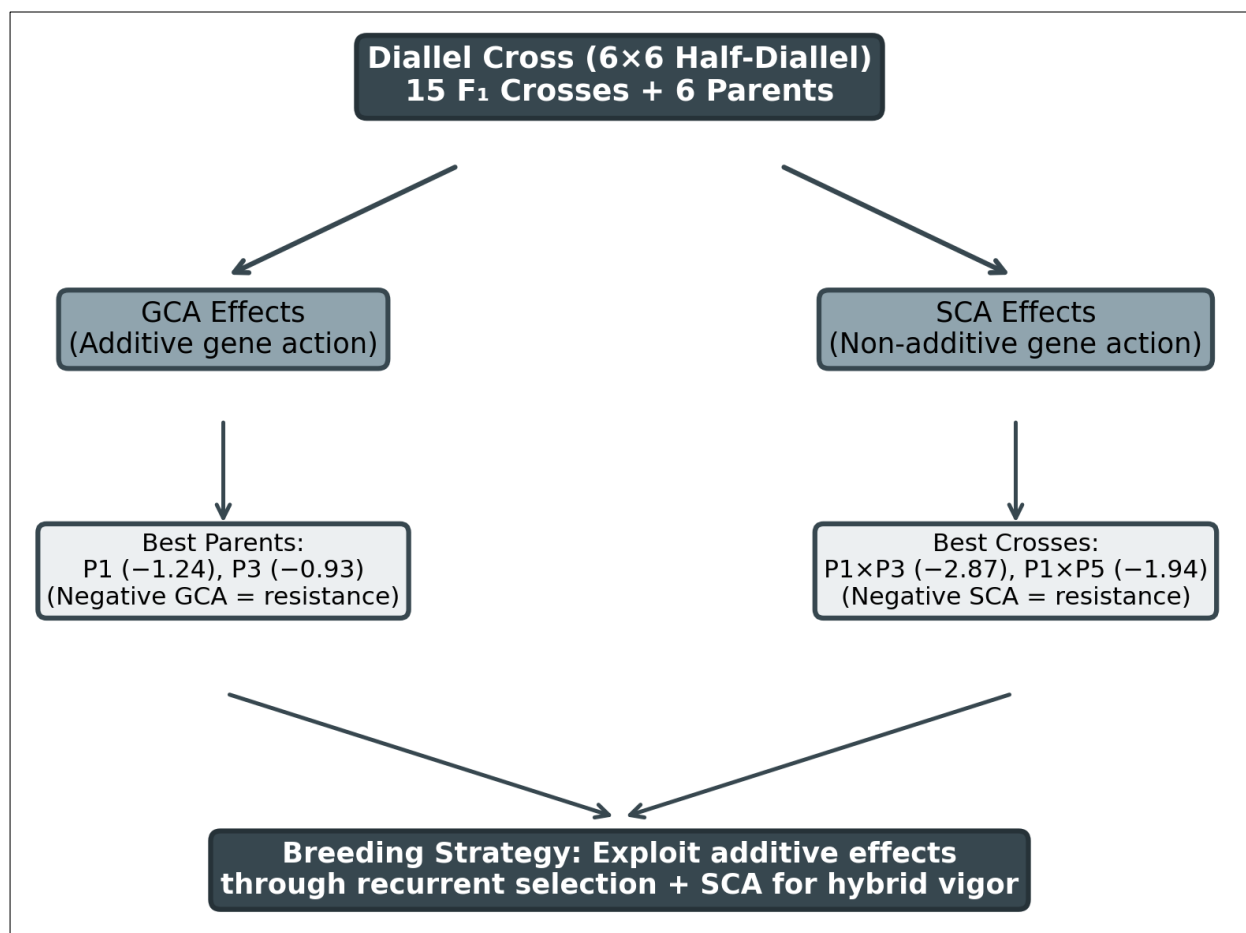
resistance. P4 (Sebastian) had the largest positive GCA effects, confirming its susceptibility.

Table 2: SCA effects for infection type of 15 F₁ barley crosses (negative = resistance)

Cross	SCA (IT)	Mean IT	Severity (%)	Latent Period (d)
P1 × P3	-2.87**	0.6	8.4	14.7
P1 × P5	-1.94*	0.9	12.1	13.2
P3 × P5	-1.42*	1.1	14.8	12.4
P2 × P4	1.78*	3.4	48.7	7.8
P4 × P6	1.24	3.1	42.3	8.4
P1 × P2	-0.67	1.7	21.4	11.3

The cross P1 × P3 had the best (most negative) SCA effect (-2.87, $p < 0.01$) and the lowest mean infection type (0.6), representing a near-immune reaction. Crosses involving two

susceptible parents (P2 × P4, P4 × P6) showed positive SCA effects and high severity, as expected.

**Fig 2:** Hierarchy diagram showing the partitioning of genetic effects from the diallel cross analysis and the recommended breeding strategy

Discussion

The dominance of additive gene action (GCA:SCA ratio > 3:1, Baker's ratio 0.87) is the most practically relevant finding for Polish barley breeders. It means that parental selection based on GCA estimates will reliably predict cross performance—a breeder can select the best general combiners (P1, P3, P5) and expect their progeny to inherit resistance with reasonable predictability^[4, 5]. This contrasts with wheat leaf rust, where non-additive effects often account for 40 to 50 percent of genetic variance^[9].

The narrow-sense heritability of 0.71 for infection type is higher than the 0.45-0.58 range reported for barley rust in Ethiopian germplasm^[7], possibly reflecting the more uniform genetic background of the Polish lines and the controlled inoculation conditions used here. High heritability combined with predominantly additive gene action makes infection type an ideal selection criterion for early-generation screening.

The outstanding performance of P1 × P3 (IT = 0.6, severity = 8.4%) suggests pyramiding of different Rph genes carried by each parent, creating a broader resistance spectrum that individual genes cannot provide^[10]. Molecular characterization of the Rph gene content in P1 and P3 would confirm this hypothesis and guide marker-assisted selection.

Conclusion

This diallel analysis revealed that rust resistance in the tested barley germplasm is controlled primarily by additive gene action, with a GCA:SCA variance ratio exceeding 3:1 and Baker's ratio of 0.87. Parents P1 (MZ-R14) and P3 (MZ-R28) were the best general combiners, and their cross (P1 × P3) showed the strongest SCA effect and near-immune rust reaction.

For Polish barley breeding, recurrent selection among progeny of P1 × P3 and P1 × P5 offers the most efficient path to accumulating additive resistance genes. Molecular

marker profiling of the parental Rph gene content should accompany phenotypic selection to maximize the durability of resistance in future cultivars.

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