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Evaluation of Combining Ability of Hibrid Maize in Diallel Crosses Under Low Nitrogen and High Plant Density Conditions

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Abstrak

Pembentukan hibrida persilangan dialel merupakan salah satu pendekatan pemuliaan tanaman untuk memperoleh kombinasi genetik unggul. Penelitian ini bertujuan mengevaluasi daya gabung umum (DGU) dan daya gabung khusus (DGK) galur jagung hasil persilangan dialel pada kondisi nitrogen rendah dan populasi tanam tinggi. Penelitian dilaksanakan pada tahun 2023 di Kebun Percobaan Fakultas Pertanian Universitas Hasanuddin di Moncongloe dan Bajeng serta Ponorogo. Pembentukan hibrida dilakukan menggunakan metode *partial half-diallel* dari 13 galur inbrida generasi lanjut yang memiliki karakter daun tegak dan semi-tegak sehingga dihasilkan 78 kombinasi hibrida. Seluruh hibrida dievaluasi pada empat kondisi lingkungan, yaitu kondisi normal di Ponorogo, kondisi normal di Bajeng, nitrogen rendah di Bajeng, dan populasi tanam tinggi di Bajeng. Analisis ragam menunjukkan bahwa lokasi, kombinasi persilangan, DGU, DGK, serta interaksi genotipe × lingkungan berpengaruh sangat nyata terhadap hasil biji jagung. Galur HDMT52 memiliki nilai DGU positif tertinggi dan stabil pada seluruh lingkungan pengujian, diikuti oleh AVLN118 dan Mpop27. Kombinasi persilangan 6 × 3, 14 × 4, dan 12 × 5 menunjukkan nilai DGK positif disertai hasil biji yang tinggi sehingga berpotensi dikembangkan sebagai hibrida unggul, sedangkan kombinasi 5 × 3 dan 13 × 8 menunjukkan nilai DGK negatif dengan hasil yang rendah. Hasil penelitian menunjukkan bahwa kombinasi genotipe dengan nilai DGK positif memiliki adaptabilitas yang lebih baik pada kondisi nitrogen rendah dan populasi tanam tinggi sehingga berpotensi dimanfaatkan dalam program pemuliaan jagung untuk menghasilkan varietas hibrida yang adaptif dan berdaya hasil tinggi.

Kata kunci: Jagung, Daya Gabung, Persilangan Dialel, Nitrogen Rendah, Populasi Tanam Tinggi

Abstract

The development of diallel hybrids is a widely used plant breeding approach for generating superior genetic combinations. This study aimed to evaluate the general combining ability (GCA) and specific combining ability (SCA) of maize inbred lines through diallel crosses under low-nitrogen conditions and high plant population density. The research was conducted in 2023 at the Experimental Farm of the Faculty of Agriculture, Hasanuddin University, Moncongloe, as well as in Bajeng and Ponorogo, Indonesia. Hybrids were developed using a partial half-diallel mating design involving 13 advanced inbred lines with upright and semi-upright leaf architecture, resulting in 78 hybrid combinations. The hybrids were evaluated under four environmental conditions, including normal conditions in Ponorogo, normal conditions in Bajeng, low nitrogen conditions in Bajeng, and high plant density conditions in Bajeng. Analysis of variance revealed that location, genotype, GCA, SCA, and genotype × environment interactions significantly affected grain yield. HDMT52 exhibited consistently high positive GCA effects across all testing environments, followed by AVLN118 and Mpop27. Hybrid combinations 6 × 3, 14 × 4, and 12 × 5 showed positive SCA effects accompanied by high grain yield, indicating their potential as promising hybrid candidates, whereas combinations 5 × 3 and 13 × 8 produced negative SCA effects and lower grain yield. The results demonstrate that hybrids with positive SCA effects possess superior adaptability under low nitrogen and high plant density conditions, making them valuable genetic resources for developing high-yielding and stress-adaptive maize hybrids.

Keywords: Maize, Combining Ability, Diallel Cross, Low Nitrogen, High Plant Density

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INTRODUCTION

Nitrogen is an essential nutrient for the growth and development of corn, influencing various important physiological processes, including photosynthesis, protein synthesis, and chlorophyll formation. However, excessive or unbalanced use of nitrogen fertilizers can cause various environmental problems, such as soil and water pollution, and impose economic burdens on farmers. Therefore, there is increasing global emphasis on sustainable agricultural practices, with a particular focus on improving nitrogen use efficiency (NUE) in corn production (Yuan et al, 2016).

Research conducted explored the base population for nitrogen use efficiency (NUE) in maize, revealing key quantitative trait loci (QTL) associated with NUE. While this research provides insight into the molecular mechanisms behind nitrogen utilization in maize, it did not explicitly address the development of maize populations with low N tolerance and suitable for high-density planting conditions. Existing research tends to focus more on identifying genes and molecular processes without delving into the process of breeding maize that can grow and perform well under resource-limited conditions, such as low nitrogen availability (Xue et al, 2016).

In contrast, planting with high plant populations in corn has the potential to increase yields but presents challenges related to competition for nutrient availability, particularly in the context of nitrogen availability and tolerance to low light. Planting populations affect various aspects of corn crops, including agronomic components and yield components. This research emphasizes the importance of optimizing plant populations to increase productivity, especially under nitrogen-limited conditions (Liu et al, 2011)

However, a significant knowledge gap in the literature lies at the intersection of these two factors: low N tolerance and adaptation to high populations. While individual studies have made significant contributions to nitrogen utilization and the effects of plant populations on maize, there is a gap in the development of maize populations that are broadly adaptable to low nitrogen conditions and high plant populations (Heinz et al, 2019).

Breeding for hybrid corn adaptive to dense populations and low N fertilization can be done through repeated selection. The basic technique in repeated selection is to recombine superior genotypes that have desired characteristics in a population from generation to generation until a population is obtained that has stable characteristics according to the selection target (Jafari et al, 2024).

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To achieve the goal of assembling varieties through repeated selection, selection is required using a selection agent or selection environment according to the targeted character, for example, for dense populations, selection is carried out in conditions of above-normal populations (> 71,000 plant/ha), salinity stress uses the NaCl selection agent, waterlogging stress is carried out in conditions of waterlogged plants and low fertilizer dose selection is carried out with various levels of fertilization. This selection is used for cross-pollinated plants, with the hope of not changing their genetic diversity much (Sciarresi, 2015).

A stable base population can be continued with the assembly of lines according to the intended environmental targets. Lines are carried out over several generations to obtain stable advanced lines with good combining ability according to the assembly objectives, such as varieties adaptive to dense populations and low nitrogen fertilization. Hybrids resulting from recombination between lines generally still have extensive genetic diversity so that selection is necessary according to the appropriate heterotic pair and then further evaluation to select candidate varieties that are adaptive to both abiotic stresses (Mukesh et al, 2024).

In addition to technical and biotic challenges, global climate change, including rising temperatures and shifting rainfall patterns, increases the risk of drought and crop failure. Limited subsidized fertilizers also force farmers to adopt input-efficient cultivation strategies, particularly nitrogen (N). Nitrogen is an essential nutrient that influences photosynthesis, protein synthesis, and chlorophyll formation (Meng et al, 2024).

However, excessive use of N fertilizer not only increases production costs but also causes environmental pollution and greenhouse gas emissions (Yuan et al, 2016). Therefore, developing corn varieties that are tolerant to low N fertilization is one solution to increase fertilizer efficiency while supporting sustainable agriculture.

Increasing corn productivity can also be achieved through planting with a dense population (>85,000 plants/ha), which allows for more optimal use of growing space and light (Liu et al, 2011). However, this system requires varieties with upright leaf ideotypes and adaptability to nutrient competition. A combination of tolerance to low N fertilization and adaptation to dense populations is an important strategy for reducing the yield gap at the farm level.

In this regard, the diallel crossing approach becomes highly relevant. This method allows the evaluation of general combining ability (GCA) and specific combining ability

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(SCA) in maize lines (Abd El Aty et al,2017) which helps identify superior crossing parents to produce high-yielding hybrids under abiotic stress conditions such as high population density and nitrogen limitation. In addition, diallel crossing provides important information about genetic variation that can be utilized for further selection (Vacaro et al, 2017).

By integrating low N fertilization strategies, dense populations, and breeding through diallel crossing, this research is expected to produce corn hybrids that are high in productivity, efficient in nitrogen utilization, adaptive to high planting densities, and support national food security while reducing dependence on corn imports.

METHODOLOGY

The research was conducted in 2023 at the Experimental Farm of the Faculty of Agriculture, Hasanuddin University, Moncongloe, as well as in Bajeng and Ponorogo, Indonesia. The formation of diallel hybrids used the partial half-diallel method from two sets of crosses consisting of 13 selected advanced generation lines, each with an upright leaf type with a small angle and a line with a slightly sloping leaf type with a medium leaf angle, resulting in 78 hybrids and 13 parental lines. Each line was planted in 8 rows with a spacing of 70 x 20 cm. For synchronization, the first planting was 4 rows, and the second planting was 4 rows with a 5-day interval. During the flowering process, the female and male flowers were isolated with paper covers and crossed with their respective heterotic pairs. At harvest time, each hybrid formed will be labeled for the purpose of evaluating special combining ability. The advanced-generation lines used in diallel crosses were previously tested lines with genetic advantages for high-density populations and low N fertilization (AVLN118, P9, Mpop27, BCY, HDMT52, HDMT 30, HR6, SNCGF, ELTWR03, pop23, HDMT16, CLYN-231, Mpop24). The results of the diallel crosses were then evaluated for their combining ability under four stress conditions.

RESULT AND DISCUSSION

The analysis results showed that the location effect (SITE) had a very significant effect on corn grain yield, with an F value of 578.2849 ($p < 0.01$). This indicates that environmental differences, such as soil conditions, rainfall, and temperature, contribute substantially to grain yield variation (Gama et al, 2021). The cross effect was also significant ($F = 1.81103$; $p < 0.001$), reflecting the presence of genetic variability between genotypes in influencing grain yield. Further analysis showed that the general combining ability (GCA) effect had a significant effect ($F = 11.72843$; $p < 0.01$), indicating that

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additive genetic effects play an important role in determining corn grain yield. In addition, the specific combining ability (SCA) effect was also significant (F = 4.3543; p < 0.01), indicating the contribution of non-additive genetic interactions, including dominance and epistasis.

Table 1. Analysis of Hybrid Variance Resulting from Diallel Crosses

	Df	Sum of Squares	Mean Square	F Value	Pr(>F)	
Location (SITE)	3	2037.808	679.2692	578.2849	3.7E-126	**
Cross	77	498.4839	6.473817	1.81103	0.000388	**
GCA	12	165.3184	13.77653	11.72843	0	**
SCA	65	332.4518	5.114644	4.354269	0	**
SITE : Cross	231	825.7464	3.57466	3.043229	7.22E-20	**
SITE : GCA	36	121.6551	3.379308	2.876919	4.41E-07	**
SITE : SCA	195	705.4907	3.617901	3.080042	0	**
Residual	308	361.7852	1.174627			

The interaction between location and cross (SITE: Cross) where the effect is significant (F = 3.043; p < 0.01), indicates that the grain yield performance of a particular cross is influenced by the environment (Akinwale et al, 2014). The interaction effect between location and GCA (SITE: GCA) is also significant (F = 2.876919; p < 0.01), indicating that the general combining ability of a particular genotype can differ between locations (Kenga et al, 2004). In addition, the interaction between location and SCA (SITE: SCA) is significant (F = 3.080042; p < 0.01), where the specific combining ability between genotypes is influenced by the environment (Singh et al, 2015). The relatively small residual value (361.7852) indicates that the model used is able to explain most of the variation in the data.

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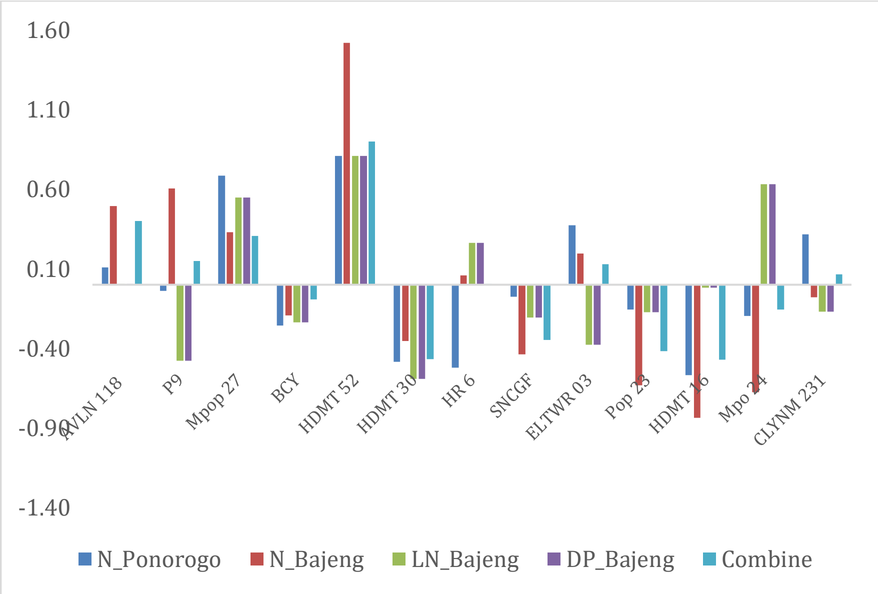

Figure 1**.** General combining ability of a number of hybrids resulting from diallel crosses

Overall, these results indicate that environmental effects contribute predominantly to corn grain yield, followed by significant influences from additive (GCA) and non-additive (SCA) genetic effects. Furthermore, genetic interactions with the environment (SITE: GCA and SITE: SCA) indicate the importance of considering environmental factors in genotype selection (Hallauer et al, 2010). In breeding programs, genotypes with high GCA values can be targeted for selection to improve yield stability across locations, while genotypes with high SCA values can be utilized for specific locations (Kenga et al, 2004).

Table 2. Analysis of The General Combining Ability of a Number of Hybrids Resulting

Parent No	Name Parent	NNP		NNB		LNB		DPB		Combined	
1	AVLN118	0.11		0.49	*	0.00		0.99	**	0.40	**
3	P9	-0.04		0.61	*	-0.48	**	0.49	**	0.15	
4	Mpop27	0.69	**	0.33		0.55	**	-0.34	*	0.31	**
5	BCY	-0.26		-0.19		-0.24	*	0.30		-0.09	
6	HDMT52	0.81	**	1.52	**	0.81	**	0.46	*	0.90	**
7	HDMT30	-0.48	**	-0.35		-0.59	**	-0.45	*	-0.47	**

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Parent No	Name Parent	NNP		NNB		LNB		DPB		Combined	
8	HR6	-0.52	**	0.06		0.26	*	0.20		0.00	
10	SNCGF	-0.08		-0.44		-0.21		-0.68	**	-0.35	**
11	ELTWR03	0.37	*	0.20		-0.38	**	0.31		0.13	
12	Pop23	-0.16		-0.63	*	-0.17		-0.72	**	-0.42	**
13	HDMT16	-0.57	**	-0.83	**	-0.02		-0.47	*	-0.47	**
14	Mpop24	-0.20		-0.68	**	0.63	**	-0.45	*	-0.16	
15	CLYNM231	0.32		-0.08		-0.17		0.34	*	0.06	

The results of the general combining ability analysis showed variability between genotypes in their contribution to corn grain yield in different environments. The environments tested included the normal location of Ponorogo (NNP), the normal location of Bajeng (NNB), the low nitrogen location of Bajeng (LNB), and the dense population of Bajeng (DPB). The effect of GCA is very important to consider in evaluating corn grain yield in different environments (Hallauer et al, 2010). The HDMT 52 genotype showed high positive GCA values across all environments. At the Ponorogo site, this genotype had a GCA value of 0.81 with a high significance level ($p < 0.01$), indicating consistent additive genetic contributions under optimal conditions in Ponorogo. Very high GCA values were also observed at the Bajeng (1.52; $p < 0.01$) and low-N Bajeng (0.81; $p < 0.01$) locations, indicating genetic stability for adaptation to the Bajeng site, both under normal and low-nitrogen stress conditions. Under the dense population treatment, the HDMT 52 genotype recorded a GCA value of 0.46, which was also significant ($p < 0.01$), indicating tolerance to dense populations. Overall, the echo GCA value of 0.90 ($p < 0.01$) confirmed the genetic superiority of this genotype for high and stable grain yields across a range of environments. This is in line with previous reports on the stability of corn yields at various test locations (Duvick et al, 2005). Other genotypes that showed good performance were AVLN 118 and Mpop 27. The AVLN 118 genotype produced significant GCA values in NNB (0.49; $p < 0.05$) and DPB (0.99; $p < 0.01$), as well as a positive overall contribution with a combined

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value of 0.40 ($p < 0.01$). This indicates the adaptation potential of AVLN 118 in the Bajeng location, especially in dense populations. The Mpop 27 genotype also showed significant GCA values in the NNP (0.69; $p < 0.01$) and LNB (0.55; $p < 0.05$) environments, with a combined value of 0.31 ($p < 0.01$), indicating good tolerance to the optimal conditions of Ponorogo and low nitrogen stress in Bajeng. In contrast, the HDMT 16 and HR 6 genotypes showed negative GCA values in almost all environments, including NNP (-0.57; $p < 0.01$) and NNB (-0.63; $p < 0.01$) for HDMT 16, and NNP (-0.52; $p < 0.01$) and NNB (-0.06; not significant) for HR 6. This indicates that these genotypes have a negative contribution to seed yield, making them less suitable for breeding programs aimed at increasing seed yield in various environmental conditions. These results indicate that genotypes with high positive GCA values such as HDMT 52, AVLN 118, and Mpop 27, have great potential to be used as parents in breeding programs. The HDMT 52 genotype, in particular, showed stable performance in various environments, both normal and stressful (low nitrogen and dense population). This line has great potential as a superior line for the development of environmentally adaptive hybrid varieties. This is in accordance with the parental selection approach reported by (Hallauer et al, 2010) for better seed yield stability. Furthermore, genotypes with negative GCA values, such as HDMT 16 and HR 6, are more suitable for use as gene sources for specific character improvement, rather than for directly increasing grain yield. Genetic interactions with the environment also need to be considered, especially in the selection of parents for specific locations. For example, the AVLN 118 line is more suitable for the Bajeng location, while Mpop 27 shows superiority under optimal conditions in Ponorogo and low nitrogen stress in Bajeng [26]. In breeding programs, a targeted environmental selection approach is crucial to ensure the selection of parents suited to site-specific conditions. Genotypes that consistently exhibit positive GCA values across environments, such as HDMT 52, can support the development of varieties with high and stable grain yields across a wide range of agroecological conditions (Duvick et al, 2005).

The SCA effect describes the non-additive interaction between a combination of male and female parents in a particular environment, reflecting the specific impact of that genetic combination on yield. A positive effect indicates that the male-female combination produces good yields, while a negative effect indicates lower yields. The significance value of the SCA effect is divided into two categories: first, effects with a p-value < 0.05 indicating the significance of the combination effect compared to the average, and second,

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effects with a p-value < 0.01 indicating very high significance. Combinations with consistent positive SCA effects across multiple locations are potential candidates for further development, as they indicate better adaptability to various environmental conditions (Singh et al, 2015). The combined effect (Combine) shows the average value of all SCA effects for each male-female combination across locations. Combinations with positive combined effects show good overall results, while those with negative combined effects tend to show poor performance. In addition, the relationship between SCA and grain yield shows that combinations with positive SCA effects at locations with environments often produce high yields, such as a 6 x 3 cross combination that yields 11.83 t/ha. Conversely, combinations with negative SCA effects, such as a 5 x 3 line cross, yield lower yields (8.05 t/ha), indicating that genetic-environmental interaction factors play an important role in achieving optimal yields (Kandel, 2020). Based on the combined analysis, the 6 x 3, 14 x 4, and 12 x 5 line cross combinations show positive combined effects and high yields, so they can be a priority for further development. On the other hand, combinations such as crossing 5 x 3 and 13 x 8 lines with combined effects and low yields have lower effectiveness.

CONCLUSION

The interaction between location and general combining ability (GCA) and specific combining ability (SCA) indicates that genotype performance is influenced by the environment, so genotype selection must consider specific environmental conditions. Genotype HDMT 52 exhibits high positive GCA values across a range of environments, both normal and stressed, indicating consistent yield stability. Genotypes AVLN 118 and Mpop 27 also demonstrate superior performance under certain conditions, making them potential candidates for breeding programs. Line cross combinations such as 6 x 3, 14 x 4, and 12 x 5 exhibit positive combining effects and high yields, and therefore can be prioritized for further development. While combinations such as 5 x 3 and 13 x 8, with low yields, are more effective and should be avoided. Genotype combinations with positive SCA effects produce high yields and demonstrate good adaptation to various locations. Conversely, combinations with negative SCA effects exhibit lower yields, making them less suitable for developing superior varieties.

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