

MECHANIZED KERNEL HARVESTING POTENTIAL IN ISOGENIC CORN DOUBLED HAPLOID (DH) LINES

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Keywords: Corn, DH lines, Mechanical kernel harvesting, Combining ability, Heritability

Abstract

An incomplete diallel cross design was taken to evaluate the combining ability and application potential of the DH lines. The results revealed significant differences in General Combining Ability (GCA) among the DH lines, with JM104 and JM108 exhibiting high dehydration rates and a strong tendency to form high-yield hybrids. Comprehensive analysis of GCA across agronomic traits indicated that JM104 possesses high combining ability and application value, followed by JM108 and JM105. Superior combinations with high Total Combining Ability (TCA) effects and rapid dehydration rates included JM104×JF004, JM104×PHB1M, and JM108×JF004, which require further multi-year and multi-location trials to fully explore their potential. Genetic parameter analysis suggested that traits such as plant height, ear height, ear length, and kernel rows per ear should be selected in early generations. When configuring hybrids, DH lines with superior traits such as plant height, ear height, ear length, and ear diameter should be selected as parents, with comprehensive consideration of GCA for ear diameter, kernel moisture content, and dehydration rate, as well as Specific Combining Ability (SCA) of the hybrids. Specifically, the enhancement of husk leaf quantity should focus on SCA selection.

Introduction

Maize (*Zea mays* L.), also known as corn in North American (Niu 2023). Its widespread cultivation has driven the development of various industries, including the food industry, animal husbandry, and the pharmaceutical sector (Zhang *et al.* 2023). With the trend towards agricultural mechanization, breeding efforts have gradually shifted to developing varieties suitable for mechanical harvesting, promoting the advancement of early-maturing, fast-dehydrating, and densely-planted varieties (Hao *et al.* 2021, Jiang *et al.* 2023). Traditional methods for inbred line selection are time consuming and inefficient, typically requiring 4-6 years to develop a pure line. In contrast, doubled haploid (DH) technology allows for the production of homozygous inbred lines within 2-3 generations, significantly shortening the breeding cycle and becoming a crucial tool in modern maize breeding (Lv *et al.* 2023). Combining ability, a key indicator for evaluating the utility of inbred lines, plays a vital role in parental selection and the utilization of heterosis (Jin *et al.* 2021, Wang *et al.* 2022, Jiao *et al.* 2023).

Mechanical kernel harvesting refers to the machinery to harvest grains, such as corn, instead of unusual labour. This method is common in development combination is becoming increasingly important in others to improve efficiency and reduce labour (Cui *et al.* 2019). The combination of inbred lines with comprehensive properties suitable for mechanical harvesting and high GCA and SCA effects is more likely to achieve high yields through combining force analysis. Practical experience has demonstrated that inbred lines with high coordination ability exhibit better heterosis. Therefore, selecting inbred lines with high combining ability is a prerequisite for breeding high-quality maize varieties, and rational combinations can fully exploit the potential of superior inbred lines (Lu *et al.* 2018, Peng *et al.* 2021, Lian *et al.* 2024).

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This study focuses on maize DH lines from the same genetic background, analyzing their combining ability and genetic parameters to screen for DH lines with high combining ability and superior agronomic traits. The results will provide theoretical support and breeding references for developing new maize varieties suitable for mechanical grain harvesting, thereby promoting the selection of mechanized grain harvesting varieties and advancing the modernization of the maize industry.

Materials and Methods

Eight superior Doubled Haploid (DH) lines were used as female parents, while five inbred lines from the NSS group- PH4CV, PHB1M, PH5AD, PH1CRW, and the self-selected line JF004- were used as male parents. A total of 40 hybrid combinations were generated using the NCII genetic mating design.

The experiment was conducted in 2023 at the maize breeding experimental field of Jilin Agricultural Science and Technology University. A randomized block design was employed with three replications. Each new hybrid combination was planted in a four-row plot with a row length of 5 meters and a plant spacing of 0.65 meters, achieving a planting density of 75,000 plants per hectare. Field management practices, including fertilization and irrigation, were consistent with those of conventional experimental fields.

In each plot, 10 individual plants were randomly selected for field investigation. Traits measured included plant height and ear height. Indoor seed analysis was conducted to determine ear length, ear diameter, kernel rows per ear, husk leaf number, kernel moisture content, and dehydration rate. The determination of the test items was based on the method of Wu *et al.* (2022).

Experimental data were organized using Excel 2022. Variance analysis was performed using SPSS 19.0 software, and combining ability analysis for traits showing significant differences among combinations was conducted using an incomplete diallel cross model.

Results and Discussion

Variance analysis was conducted on eight agronomic traits across 40 hybrid combinations, as shown in Table 1. The results demonstrated that the differences in these traits among the combinations were highly significant ($P < 0.01$), confirming substantial genetic variation in agronomic performance among the tested hybrids. Additionally, the combining ability variance analysis revealed that the general combining ability (GCA) variances for all traits of both P1 and P2 reached highly significant ($P < 0.01$) or significant at $P < 0.05$ levels. Similarly, the specific combining ability (SCA) variances for the traits of $P1 \times P2$ also exhibited significant or highly significant differences, underscoring the critical roles of both GCA and SCA in determining trait performance.

Subsequently, a GCA analysis was conducted for plant height, ear height, ear diameter, kernel rows per ear, husk leaf number, kernel moisture content, and dehydration rate across the eight DH lines. The results revealed inconsistent performance of the same traits among different DH lines (Table 2).

For plant height and ear height, the GCA effects were negative for JM102 (-3.845, -3.794), JM104 (-0.911, -3.604), JM105 (-2.453, -3.320), and JM107 (-1.740, -3.425). These four DH lines exhibited a certain inhibitory effect on plant height and ear height, potentially enhancing lodging resistance. In terms of ear length, DH lines JM105 (7.615) and JM108 (1.839) demonstrated high GCA effects, indicating their potential to significantly increase ear length when used as parents. For ear diameter, DH lines JM104 (5.825) and JM108 (3.975) showed high GCA effects, suggesting their utility in breeding varieties with thicker ears.

Table 1. Variance analysis of each trait.

Source of variation	Plant height	Ear height	Ear length	Ear diameter	Kernel rows per ear	Husk leaf number	Kernel moisture content	Dehydration rate
Block	0.809	2.821	0.561	0.310	0.746	0.524	0.377	1.290
Combination	10.185**	7.868**	9.002**	4.228**	25.359**	8.612**	3.532**	11.962**
P1	6.063**	8.258**	10.425**	3.696*	7.377**	2.786*	2.987*	5.221**
P2	5.850**	6.799**	5.243**	7.967**	3.464**	2.995*	3.703*	4.815**
P1 × P2	4.262**	2.825**	3.299**	1.673*	12.098**	5.588**	2.091**	5.649**

*, ** indicate significant at 0.05% and 0.01% level, respectively.

Table 2. General combining ability (GCA) effect values of the eight DH lines.

Inbred line	Plant height	Ear height	Ear length	Ear diameter	Number of rows per ear	Number of husk leaves	Grain moisture content	Dehydration rate
JM101	2.558	3.600	-2.852	0.139	3.688	1.112	0.076	-4.081
JM102	-3.845	-3.794	-1.674	-3.130	2.414	3.540	-0.678	-3.871
JM103	4.686	5.846	-2.034	0.568	-8.087	1.264	-6.482	-6.650
JM104	-0.911	-3.604	-1.373	5.825	3.424	4.382	10.898	19.083
JM105	-2.453	-3.320	7.615	0.026	10.520	1.534	-2.132	0.399
JM106	1.233	0.228	-0.183	-1.446	-8.438	-3.708	0.921	-6.041
JM107	-1.740	-3.425	-1.338	-5.957	-0.794	-9.759	-1.363	-4.620
JM108	0.472	4.470	1.839	3.975	-2.727	1.635	-1.239	5.781

Regarding husk leaf number and kernel rows per ear, DH lines JM103 (1.264, -8.087) and JM108 (1.635, -2.727) exhibited positive SCA values for husk leaf number but negative SCA values for kernel rows per ear. Among the three DH lines analyzed for dehydration rate, JM104 (19.083) displayed the highest GCA effect, making it a promising candidate for breeding varieties suitable for mechanical grain harvesting.

The specific combining ability (SCA) effect values for each trait are shown in Table 3. The combination with the highest SCA effect values for plant height and ear height is JM103 × PH5AD (4.781, 6.998). The combination with the highest SCA effect value for ear length is JM108 × PH1CRW (5.521). The combination with the highest SCA effect value for ear diameter is JM106 × PH4CV (5.420). The combination with the highest SCA effect value for the number of rows per ear is JM105 × PH1CRW (14.686). The combination with the highest SCA effect value for the number of husks is JM108 × JF004 (8.968). The combination with the highest SCA effect value for dehydration rate is JM104 × JF004 (29.906). The SCA effect values indicate that there are differences in agronomic traits among different hybrid combinations, and there are also differences among traits within the same combination. Therefore, when assembling hybrid combinations, attention should be paid to the magnitude of the specific combining ability coefficients.

Through the analysis of general combining ability (GCA) and specific combining ability (SCA) in Tables 2 and 3, it can be seen that selecting parents with positive GCA values can result in hybrid combinations with large negative SCA values, while selecting parents with negative GCA values can also result in hybrid combinations with large positive SCA values. Therefore, when selecting and breeding superior varieties, it is advisable to first choose DH lines with high GCA as parents, and then moderately use combinations where both parents have moderate combining ability but high specific combining ability.

Table 3. Specific combining ability effect value of hybrid combinations.

Combination	Plant height	Ear height	Ear length	Ear diameter	Number of kernel rows	Number of husk leaves	Grain moisture content	Dehydration rate
JM101×PH4CV	1.325	2.677	0.723	1.359	-5.816	-8.402	-4.787	4.407
JM101×PHB1M	2.194	-2.024	-0.506	-3.244	6.005	4.671	2.583	-3.078
JM101×PH5AD	-3.397	5.914	1.748	0.577	0.829	-5.337	-7.617	11.630
JM101×PH1CRW	-1.623	-2.564	-3.364	3.100	-10.676	6.757	6.227	-0.529
JM101×JF004	1.501	-4.003	1.398	-1.792	9.658	2.311	3.594	-12.430
JM102×PH4CV	-1.928	-2.498	4.494	-2.731	7.760	5.014	-3.936	-1.784
JM102×PHB1M	-1.139	3.433	2.758	-0.827	-5.901	3.424	2.950	2.683
JM102×PH5AD	2.559	-0.493	-2.649	0.515	2.762	-3.550	-4.638	-2.899
JM102×PH1CRW	0.608	3.407	-4.140	2.495	-12.038	0.032	3.129	2.166
JM102×JF004	-0.100	-3.849	-0.463	0.548	7.417	-4.920	2.496	-0.167
JM103×PH4CV	-5.471	-4.435	4.155	0.698	4.641	8.807	-1.230	-1.339
JM103×PHB1M	-1.703	-2.730	-4.158	3.505	4.160	-7.785	-2.278	4.746
JM103×PH5AD	4.781	6.998	2.469	-0.161	1.181	2.855	-0.838	-2.806
JM103×PH1CRW	-0.637	-3.936	-3.045	-1.822	-2.417	0.622	7.045	5.385
JM103×JF004	3.119	4.003	0.580	-2.220	-7.565	-4.498	-2.700	-5.986
JM104×PH4CV	-0.468	4.448	-1.264	-1.151	-3.795	-2.065	-5.788	-18.367
JM104×PHB1M	2.082	-0.312	0.533	-0.177	7.587	2.750	0.818	6.037
JM104×PH5AD	0.451	-3.893	1.930	-0.461	-2.422	0.917	-4.447	-8.933
JM104×PH1CRW	-2.668	1.081	0.579	-2.354	8.919	-1.485	7.200	-8.543
JM104×JF004	0.603	-1.323	-1.778	4.142	-10.289	-0.116	2.217	29.906
JM105×PH4CV	2.659	0.831	-2.170	-0.232	-1.225	2.806	3.517	2.329
JM105×PHB1M	-0.870	1.153	2.863	-1.736	-8.076	4.418	0.039	0.182
JM105×PH5AD	-2.462	-3.189	-5.255	-4.964	1.137	3.091	6.434	-1.879
JM105×PH1CRW	1.145	-0.586	3.486	1.664	14.686	-3.356	-5.939	1.204
JM105×JF004	-0.472	1.792	1.077	5.268	-5.522	-6.959	-4.051	-1.836
JM106×PH4CV	-1.312	-1.737	-0.390	5.420	0.928	5.351	4.722	7.386
JM106×PHB1M	-0.539	1.705	-0.254	-1.426	0.118	-14.443	-5.248	-0.691
JM106×PH5AD	-1.130	-3.416	2.175	1.620	1.972	2.518	8.356	1.989
JM106×PH1CRW	3.531	1.077	0.439	-2.364	-2.395	3.403	-4.973	-0.289
JM106×JF004	-0.552	2.371	-1.970	-3.150	-0.623	3.171	-2.857	-8.396
JM107×PH4CV	3.628	2.293	-4.308	-3.157	-2.653	-4.778	1.393	5.100
JM107×PHB1M	-2.202	2.248	4.084	-0.246	-2.254	3.575	-3.584	-5.917
JM107×PH5AD	0.875	-2.552	1.528	4.117	-1.280	-0.112	8.085	6.294
JM107×PH1CRW	-1.574	-0.105	0.544	-0.371	-6.634	-0.828	-9.383	1.127
JM107×JF004	-0.727	-1.885	-1.848	-0.343	12.821	2.143	3.489	-6.604
JM108×PH4CV	1.557	-1.679	-1.240	-0.107	0.159	-6.734	6.108	2.268
JM108×PHB1M	2.175	-3.473	-5.320	4.152	-1.639	3.390	4.721	-3.963
JM108×PH5AD	-1.578	0.631	-1.946	-1.245	-4.179	-0.381	-5.335	-3.396
JM108×PH1CRW	1.218	1.625	5.521	-0.349	11.555	-5.143	-3.306	-0.523
JM108×JF004	-3.372	2.896	3.004	-2.452	-5.896	8.968	-2.188	5.612

The combination JM104 $\times$ JF004 has the highest dehydration rate (389.613) and total combining ability (TCA) effect value (20.164), followed by JM104 $\times$ PHB1M (308.555, 9.035) and JM108 $\times$ JF004 (298.589, 7.666) (Table 4). Further experimental evaluations can be conducted to assess the yield potential, stability, and adaptability of these three high-yielding combinations, thereby exploring their application potential. The ranking of the TCA effect values for dehydration rate across the 40 hybrid combinations is largely consistent with the ranking of the dehydration rates in the F1 generation, indicating a high utilization rate of TCA and good production performance, which can better reflect the actual production level of the varieties. Among the top 10 combinations ranked by TCA effect values for dehydration rate, JM104 is involved in 4 combinations, JM108 in 3, JM105 in 2, and JM102 in 1. The results demonstrate that TCA is a comprehensive reflection of GCA and SCA. Therefore, when assembling high-quality hybrid combinations, particular attention should be paid to the magnitude of the total combining ability.

The GCA variances for plant height (67.680%), ear height (76.191%), ear length (74.410%), and ear diameter (81.130%) are larger than their respective SCA variances (32.320, 23.810, 25.590, and 18.870%) (Table 5). The variation ratios for these four traits are all above 2, indicating that additive genes play a dominant role in these traits. Therefore, when selecting hybrid combinations, it is essential to use superior DH lines as parents. The GCA variances for the number of rows per ear and dehydration rate are 1.406 to 1.568 times greater than their SCA variances, suggesting that these traits are primarily controlled by additive genes but are also influenced by non-additive effects. The GCA variance for the number of husks is smaller than its SCA variance, indicating that non-additive effects dominate this trait. When improving the number of rows per ear, attention should be paid to the selection of specific combining ability.

There are significant differences in general combining ability (GCA) among various traits of doubled haploid (DH) lines from the same lineage, suggesting that GCA can serve as a marker for trait performance in the breeding of new varieties (Liu *et al.* 2022). The findings of this study indicate that JM104 exhibits the highest dehydration rate GCA among the eight DH lines, followed closely by JM108. JM104, as a parent, can produce maize hybrids with high yield, medium plant height, low ear position, thicker ear, increased number of ear rows and bract leaves; Meanwhile, JM108 and JM105 have great potential for application, and as parents, they may also produce combinations with better traits. Previous research has shown that selecting germplasm with high GCA for specific traits does not necessarily result in high SCA values in hybrids. However, high GCA in parents makes it easier to develop superior combinations that meet breeding objectives (Feng *et al.* 2021). In this study, the combinations JM104 $\times$ JF004 and JM101 $\times$ PH5AD exhibited excellent SCA for dehydration rate. The combinations JM104 $\times$ JF004, JM104 $\times$ PHB1M, and JM108 $\times$ JF004 demonstrated high dehydration rates and total combining ability effect values, making them promising candidates. These three combinations should undergo multi-year, multi-location trials to determine their application value and adaptability.

This study aligns with previous research analyzing the combining ability of ear-related traits in maize DH lines (Xiong *et al.* 2022). The experimental results suggest that for traits such as plant height, ear height, ear length, and ear diameter, priority should be given to parents with outstanding GCA. For kernel rows and dehydration rate, both parental GCA and hybrid SCA should be considered. Traits like plant height, ear height, ear length, and kernel rows exhibited broad-sense and narrow-sense heritability above 50%, making them suitable for early-generation selection. In contrast, ear diameter, husk leaf number, and dehydration rate showed low narrow-sense heritability, making them unsuitable for early-generation selection.

Table 4. Ranking of dehydration rate and general combining ability effect value of hybrid combinations.

Combination	Dehydration rate	Ranking	General combining ability
JM104×JF004	389.613	1	20.164
JM104×PHB1M	308.555	2	9.035
JM108×JF004	298.589	3	7.666
JM105×JF004	267.440	4	3.389
JM102×JF004	261.125	5	2.522
JM104×PH1CRW	256.089	6	1.831
JM108×PH4CV	255.860	7	1.799
JM104×PH5AD	253.106	8	1.421
JM108×PHB1M	251.990	9	1.268
JM105×PHB1M	248.988	10	0.855
JM101×PH5AD	246.792	11	0.554
JM102×PHB1M	244.695	12	0.266
JM107×JF004	243.681	13	0.127
JM108×PH1CRW	243.266	14	0.070
JM103×PHB1M	242.956	15	0.027
JM105×PH4CV	242.936	16	0.025
JM103×JF004	240.251	17	-0.344
JM106×PH4CV	239.581	18	-0.436
JM104×PH4CV	238.053	19	-0.646
JM107×PH4CV	237.486	20	-0.724
JM101×PH4CV	237.105	21	-0.776
JM106×JF004	235.881	22	-0.944
JM105×PH1CRW	234.393	23	-1.149
JM108×PH5AD	234.260	24	-1.167
JM107×PH5AD	232.529	25	-1.404
JM106×PHB1M	231.234	26	-1.583
JM101×JF004	230.850	27	-1.636
JM101×PHB1M	230.195	28	-1.725
JM103×PH1CRW	227.430	29	-2.105
JM102×PH1CRW	226.364	30	-2.251
JM105×PH5AD	224.871	31	-2.456
JM102×PH4CV	222.592	32	-2.769
JM107×PH1CRW	222.024	33	-2.847
JM107×PHB1M	221.997	34	-2.851
JM101×PH1CRW	219.313	35	-3.220
JM106×PH5AD	218.629	36	-3.313
JM103×PH4CV	216.924	37	-3.547
JM106×PH1CRW	215.139	38	-3.793
JM102×PH5AD	212.032	39	-4.219
JM103×PH5AD	205.510	40	-5.115

Table 5. Estimation of genetic parameters for each trait.

Genetic parameters	Plant height	Ear height	Ear length	Ear diameter	Number of kernel rows	Number of husk leaves	Grain moisture content	Dehydration rate
General combining ability Variance V_g (%)	67.680	76.191	74.410	81.130	58.440	43.110	60.190	61.060
Specific combining ability Variance V_s (%)	32.320	23.810	25.590	18.870	41.560	56.890	39.810	38.940
Broad-Sense heritability H_B^2 (%)	77.090	71.870	74.970	54.330	89.900	72.890	47.740	79.920
Narrow-Sense heritability H_N^2 (%)	52.170	54.760	55.790	44.080	52.530	31.420	28.740	48.800

Developing high-quality maize DH lines is only a foundational step in hybrid breeding; the study of combining ability is the key. In this experiment, stable pure lines were obtained within just two growth cycles through induction and doubling, significantly shortening the time required for inbred line development and improving breeding efficiency. Since different ecological environments and planting densities can affect the combining ability and heterosis of inbred lines, future efforts should focus on strengthening the selection and evaluation of DH lines across multiple dimensions.

Acknowledgements

This work was supported by Jilin City Department of Science and Technology Project (#20230501010).

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(Manuscript received on 03 June 2025; revised on 30 July 2025)