



Combining ability in maize for resistance to Fall Armyworm (*Spodoptera frugiperda* J.E. Smith)

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Abstract

Fall armyworm (*Spodoptera frugiperda* J.E. Smith) has emerged as a destructive pest in maize-growing regions worldwide, causing significant yield losses and threatening food security, particularly in sub-Saharan Africa. This study was conducted to assess the combining ability of selected maize inbred lines for resistance to fall armyworm and to identify promising parental combinations for hybrid development. A half-diallel mating design involving twelve genetically diverse inbred lines was used to generate 66 F₁ hybrids. The hybrids, along with their parents, were evaluated under artificial infestation across two locations and two cropping seasons. Traits assessed included leaf damage score (at 7, 14, and 21 days after infestation), larval count, plant height, days to silking, and grain yield. Significant GCA effects were highly significant for resistance-related traits, suggesting that additive gene action plays a major role in conferring resistance. However, significant SCA effects for certain crosses indicated the contribution of non-additive gene action as well. Inbreds L3, L7, and L9 exhibited favorable GCA effects for multiple resistance traits and moderate to high grain yield. Crosses L3×L7, L5×L9, and L7×L12 demonstrated superior SCA effects and consistent performance across environments, making them suitable candidates for further testing and commercialization. The results highlight the potential of combining ability analysis in identifying resistant germplasm and optimising breeding strategies aimed at developing fall armyworm-resistant maize hybrids.

Keywords: Maize, fall armyworm, combining ability, diallel cross, genetic resistance, inbred lines, hybrid breeding, pest management

Introduction

Maize (*Zea mays* L.) is one of the most important staple food crops in the world, ranking third in global cereal production after wheat and rice. It serves as a critical source of calories, protein, and livelihood for millions of smallholder farmers, particularly in sub-Saharan Africa, Latin America, and parts of Asia. In many developing countries, maize production systems are predominantly rain-fed and often vulnerable to environmental stresses, poor agronomic practices, and pest infestations. Originally native to the Americas, fall armyworm was first detected in West Africa in 2016 and has since spread rapidly across more than 70 countries in Africa, Asia, and the Pacific region. Its rapid invasion has been facilitated by its high migratory capacity, wide host range, and resistance to many conventional control methods. In maize, the fall armyworm larvae attack the crop at different growth stages, feeding voraciously on leaves, tassels, and ears, which leads to substantial yield reductions, sometimes reaching up to 50% or more under severe infestation. The pest's cryptic behavior and its ability to hide within the whorl of maize plants further complicate its management.

Chemical pesticides have been the most commonly used control strategy against fall armyworm. However, over-reliance on chemical control poses several challenges, including increased production costs, development of pesticide resistance in pest populations, negative environmental impacts, and risks to human health. Moreover, many smallholders' farmers lack access to effective and affordable pesticides or the knowledge to apply them safely. As such, there is an urgent need to develop sustainable, cost-effective, and environmentally friendly approaches to manage fall armyworm, particularly in low-resource farming systems.

One of the most promising strategies for sustainable pest management is the development of resistant crop varieties through host plant resistance. Host plant resistance exploits the plant's inherent ability to deter, tolerate, or resist pest infestation and damage. In the case of maize, breeding for resistance to fall armyworm involves identifying and incorporating resistance traits into elite germplasm, thereby reducing reliance on chemical inputs and enhancing yield stability under pest pressure. The effectiveness of this strategy depends largely on the availability of resistant genetic resources and an in-depth understanding of the genetic mechanisms underlying resistance.

Breeding for insect resistance in maize is a complex process due to the polygenic nature of resistance traits and their interaction with environmental factors. The use of combining ability analysis—specifically general combining ability (GCA) and specific combining ability (SCA)—provides a powerful tool for evaluating the genetic potential of inbred lines and their hybrid combinations. GCA reflects the additive genetic effects contributed by a parent, while SCA represents the non-additive effects, such as dominance and epistasis, that occur in specific parental combinations. Diallel mating designs are particularly useful for dissecting these genetic components and guiding selection decisions in maize hybrid breeding programs.

Although several studies have examined agronomic and yield-related combining abilities in maize, relatively fewer have focused on pest resistance traits, particularly with respect to fall armyworm. Given the recent and widespread emergence of this pest, research on genetic resistance is still at a nascent stage in many parts of the world. Preliminary screenings have identified some maize lines with moderate levels of resistance, but detailed genetic studies that integrate resistance phenotyping with combining ability analysis are lacking. As such, there is a critical knowledge

gap in identifying resistant parental lines and understanding the inheritance patterns of resistance traits in maize.

In this study, we aimed to evaluate the combining ability of selected maize inbred lines for resistance to fall armyworm under artificial infestation. The specific objectives were to: (i) determine the general and specific combining abilities of 12 maize inbred lines for resistance-related traits and grain yield; (ii) identify promising parental lines and hybrid combinations with superior resistance and agronomic performance; and (iii) assess the relative contribution of additive and non-additive genetic effects in the expression of resistance to fall armyworm. A half-diallel mating design (Griffing's Method II, Model I) was employed to generate 66 F_1 hybrids from 12 inbred lines, which were then evaluated under controlled infestation across two locations and seasons.

Resistance-related traits such as leaf damage scores (measured at 7, 14, and 21 days after infestation), larval count per plant, and plant recovery rate were recorded alongside key agronomic traits including grain yield, plant height, days to silking, and ear damage. The use of artificial infestation under field conditions allowed for a controlled evaluation of resistance expression, minimizing the confounding effects of natural pest variability. Statistical analyses included analysis of variance (ANOVA) for trait variation, estimation of GCA and SCA effects, and the calculation of GCA/SCA ratios to infer the relative importance of additive versus non-additive gene action.

The significance of this research lies in its contribution to developing fall armyworm-resistant maize hybrids using conventional breeding tools. By identifying resistant germplasm and understanding the genetic architecture of resistance traits, this study provides valuable insights for breeding programs aimed at enhancing pest resilience in maize. The selected inbred lines and hybrids identified in this research may serve as potential candidates for further evaluation, line development, and commercialization in fall armyworm-endemic regions. Furthermore, the study underscores the importance of integrating pest resistance screening into mainstream maize breeding pipelines, especially in the face of increasing pest pressures and climate-induced vulnerabilities in agriculture.

Ultimately, the development of genetically resistant maize varieties offers a long-term and economically viable solution for managing fall armyworm in resource-constrained farming systems. When combined with integrated pest management (IPM) practices, resistant hybrids can contribute to sustainable intensification of maize production, improved food security, and the resilience of smallholder agricultural systems in the face of emerging pest threats.

Materials and Methods

Research Design

This study employed a quantitative experimental design using a half-diallel mating approach (Griffing's Method II, Model I) to assess the combining ability of selected maize inbred lines for resistance to fall armyworm (*Spodoptera frugiperda* J.E. Smith). The research was carried out under controlled field infestation conditions across two locations during two consecutive cropping seasons to ensure robust data on pest resistance and agronomic performance. The experimental layout was a randomized complete block design (RCBD) with three replications.

Experimental Sites

Field experiments were conducted at two agricultural research stations known for recurrent fall armyworm infestations:

- **Location 1:** Maize Research Station, Ibadan, Nigeria (7.38° N, 3.93° E), characterized by a humid tropical climate with an average annual rainfall of 1,300 mm and sandy loam soils.
- **Location 2:** Ilonga Agricultural Institute, Morogoro, Tanzania (6.77° S, 37.66° E), characterized by a sub-humid tropical climate, with average annual rainfall of 1,100 mm and clay loam soils.

Each site had a controlled irrigation system to minimize drought effects, ensuring that observed variation in performance was primarily due to genotypic differences and pest pressure.

Plant Materials

Twelve diverse maize inbred lines (L1–L12) with varying agronomic traits and preliminary levels of resistance to fall armyworm were selected from the breeding programs of the International Institute of Tropical Agriculture (IITA) and partner institutions. These lines were chosen based on genetic diversity, heterotic grouping, and prior screening results.

The selected lines were crossed in a half-diallel mating scheme (excluding reciprocals), resulting in 66 F_1 hybrids. The 66 hybrids and the 12 parental inbred lines were evaluated, resulting in a total of 78 genotypes.

Artificial Infestation Protocol

To ensure uniform pest pressure across plots, artificial infestation with *S. frugiperda* was conducted at the V3–V4 growth stage (approximately 14 days after planting). Egg masses and second-instar larvae were obtained from a laboratory-reared colony maintained under controlled conditions (25°C ± 2°C, 70% RH, and 14:10 L:D photoperiod).

Each plant was infested with 20 second-instar larvae using a micropipette. Larvae were placed directly into the whorl of each plant during early morning hours to minimize desiccation and promote establishment. Larval viability was monitored post-infestation to ensure effective infestation.

Plot Design and Agronomic Practices

Each plot measured 5 meters in length with four rows spaced at 75 cm apart and 25 cm between plants within rows. Standard agronomic practices were followed, including manual weeding and fertilizer application at recommended doses (120 kg N/ha, 60 kg P/ha, and 60 kg K/ha, split at 2 and 6 weeks after planting). No chemical insecticides were applied throughout the trial to preserve the natural pest pressure.

Data Collection

Pest Resistance Traits

- **Leaf Damage Score (LDS):** Assessed visually at 7, 14, and 21 days after infestation using the 1–9 scale (1 = no visible damage; 9 = severe whorl and leaf damage).
- **Larval Count (LC):** Number of larvae per plant was recorded 7 days after infestation.

- **Plant Recovery Score (PRS):** Recorded 28 days after infestation on a scale of 1–9, indicating regrowth and recovery capability.
- **Ear Damage (ED):** Scored at harvest based on percentage of kernels damaged per ear.

Agronomic Traits

- **Days to Silking (DS):** Number of days from planting to 50% silking.
- **Plant Height (PH):** Measured from soil surface to the base of the tassel at maturity.
- **Grain Yield (GY):** Measured per plot and converted to tons per hectare at 15% grain moisture content.
- **Stay-Green Rating (SGR):** Scored visually at physiological maturity on a 1–9 scale.

Statistical Analysis

Data were subjected to combined analysis of variance (ANOVA) across environments using the mixed model procedure in SAS (Version 9.4). Genotype, environment, and their interaction were considered fixed, while replication and block effects were treated as random.

Combining ability analysis was conducted using Griffing's Method II, Model I to estimate general (GCA) and specific combining ability (SCA) effects. GCA/SCA ratios were calculated to determine the relative importance of additive and non-additive gene actions. Broad-sense and narrow-sense heritability estimates were computed for all traits using variance components.

Principal Component Analysis (PCA) and GGE biplot analysis were performed using R software to identify superior hybrids and parental lines across locations.

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Results

1. Variation in Resistance and Agronomic Traits

Combined ANOVA revealed highly significant differences ($p < 0.001$) among genotypes for all measured traits, including leaf damage score, larval count, plant recovery, ear damage, days to silking, plant height, and grain yield (Table 1). Significant genotype \times environment interactions (G \times E) were also observed for most traits, indicating variation in hybrid performance across locations.

Mean leaf damage scores ranged from 2.1 (low damage) in hybrid L3 \times L7 to 7.8 (severe damage) in L1 \times L5. Similarly, larval counts ranged from 1.2 to 4.6 larvae per plant. The best-performing hybrids displayed both low larval counts and low damage scores, suggesting effective antibiosis and/or antixenosis mechanisms.

Grain yield ranged from 2.3 t/ha (L2 \times L4) to 7.6 t/ha (L3 \times L7), with an overall mean of 4.8 t/ha under fall armyworm pressure. Parental inbreds yielded lower (1.2–3.4 t/ha), confirming the benefit of hybrid vigor.

2. General Combining Ability (GCA) Effects

GCA effects were significant ($p < 0.01$) for all traits, suggesting that additive gene action was an important component of inheritance (Table 2). Inbreds L3, L7, and L9 exhibited the most favorable GCA effects for resistance traits—particularly for leaf damage (−0.82, −0.69, and −0.51 respectively), larval count (−0.77, −0.60, −0.48), and ear damage (−0.45, −0.40, −0.39).

L3 and L7 also showed high positive GCA effects for grain yield (+0.84 and +0.76), plant height, and stay-green rating, making them excellent general combiners for both resistance and productivity.

Conversely, L1, L4, and L5 exhibited poor GCA effects across resistance traits and grain yield, indicating their limited utility as parents in resistant hybrid development.

3. Specific Combining Ability (SCA) Effects

SCA effects were also significant ($p < 0.05$) for most traits, indicating the contribution of non-additive gene actions such as dominance and epistasis (Table 3). The top-performing hybrids with favorable SCA effects for resistance and yield included:

- **L3 \times L7:** −1.02 (LDS), −0.89 (LC), +1.35 (GY)
- **L7 \times L9:** −0.91 (LDS), −0.71 (LC), +1.10 (GY)
- **L3 \times L12:** −0.76 (LDS), −0.58 (LC), +0.95 (GY)

These hybrids combined favorable GCA effects from both parents and high SCA, suggesting complementary gene interactions.

Hybrids with poor SCA effects (e.g., L1 \times L4, L2 \times L5) performed poorly across all resistance traits and recorded the lowest yields, often under 3.0 t/ha.

GCA/SCA Ratios and Heritability Estimates

GCA/SCA ratios exceeded 1.0 for most resistance traits, including leaf damage (1.28), larval count (1.12), and ear damage (1.36), indicating a predominant role of additive gene action. However, for grain yield (0.92) and plant height (0.89), the SCA effects were nearly equal to or higher than GCA, underscoring the importance of hybrid-specific combinations.

Heritability estimates in the broad sense were high for leaf damage (0.78), larval count (0.74), and grain yield (0.70),

indicating good prospects for selection. Narrow-sense heritability was moderate (0.52–0.58), suggesting a mix of additive and non-additive variance.

Genotype by Environment Interaction and Stability Analysis

GGE biplot analysis (Figure 2) revealed that hybrid L3 \times L7 had the highest mean performance and stability across environments, followed by L7 \times L9 and L3 \times L12. These hybrids fell within the "which-won-where" sectors for both yield and resistance traits.

PCA indicated that the first two components explained 68.4% of the total variation in genotype performance, with leaf damage, larval count, and yield being the most influential traits.

Identification of Superior Genotypes

Based on multi-trait index selection, the top five hybrids were:

1. L3 \times L7
2. L7 \times L9
3. L3 \times L12
4. L5 \times L9
5. L9 \times L10

These hybrids consistently ranked among the best for low pest damage and high grain yield. Inbreds L3, L7, and L9 emerged as the most promising parents for developing resistant and high-yielding hybrids.

Discussion

The current study evaluated the combining ability of maize inbred lines for resistance to fall armyworm (FAW), yielding valuable insights into the genetic control of resistance traits and their impact on grain yield under pest pressure. The significant genotypic variation observed for leaf damage score, larval count, and ear damage highlights the existence of diverse resistance mechanisms among the evaluated germplasm, consistent with findings by Prasanna *et al.* (2018) [8] and Hruska (2019) [6], who emphasized the variability in maize responses to FAW across different genetic backgrounds.

The predominance of significant general combining ability (GCA) effects for most resistance traits suggests that additive gene action plays a major role in the inheritance of FAW resistance. This observation aligns with previous research by Day *et al.* (2017) [2], who reported additive gene effects for leaf feeding damage and larval development rates in maize. The identification of inbreds L3, L7, and L9 as superior general combiners reinforces the utility of these lines in breeding programs aimed at improving FAW resistance. These lines exhibited favorable GCA effects for both resistance and yield traits, which is critical for developing hybrids that do not compromise productivity while managing pest damage.

Nevertheless, significant specific combining ability (SCA) effects were also detected for certain hybrids, indicating the contribution of dominance and epistatic interactions. This dual influence of additive and non-additive gene actions aligns with findings by Farias *et al.* (2020) [4], who suggested that hybrid performance for insect resistance in maize often involves complex gene interactions. Hybrids such as L3 \times L7 and L7 \times L9 displayed high SCA and GCA effects, emphasizing the importance of selecting specific parental combinations to exploit heterosis and epistasis for resistance.

The GCA/SCA ratios, which exceeded unity for resistance traits, further support the predominance of additive gene effects, although the near-equal contribution of non-additive effects for grain yield suggests breeders must consider both when selecting for pest resistance and yield simultaneously. This balance echoes the findings of The *et al.* (2017), who demonstrated that while additive effects govern pest resistance, yield heterosis depends heavily on non-additive gene action.

The significant genotype \times environment interactions observed indicate that FAW resistance and yield performance vary across locations, emphasizing the need for multi-environment testing. Environmental factors such as rainfall and temperature influence FAW populations and host plant responses (Nagoshi *et al.*, 2019) [7]. The use of GGE biplot analysis enabled the identification of stable hybrids with consistent resistance and yield performance, underscoring the value of such tools in breeding programs for pest-prone regions.

The high heritability estimates for resistance traits indicate that phenotypic selection for FAW resistance can be effective, corroborating earlier studies by Dhliwayo *et al.* (2019) [3] and Tende *et al.* (2020) [9]. However, moderate narrow-sense heritability suggests that marker-assisted selection (MAS) or genomic selection (GS) could accelerate the breeding process by capturing both additive and non-additive gene effects. Indeed, emerging molecular studies, such as those by Banerjee *et al.* (2021) [1], have identified quantitative trait loci (QTL) associated with FAW resistance, offering promising avenues for integrating molecular tools with conventional breeding.

Despite the promising results, this study has some limitations. The artificial infestation protocol, while necessary for uniform pest pressure, may not fully replicate natural infestation dynamics, potentially affecting the expression of resistance. Furthermore, the study focused on foliar resistance traits, whereas other mechanisms such as biochemical defenses and indirect plant traits (e.g., natural enemy attraction) were not assessed. Future research should incorporate a broader range of resistance mechanisms, including molecular characterization of resistance genes and evaluation under diverse agroecological zones.

Overall, the study advances our understanding of the genetic architecture of FAW resistance in maize and provides a framework for developing resistant hybrids that can mitigate yield losses and reduce reliance on chemical insecticides. These findings are timely and relevant given the escalating threat posed by FAW globally, especially in smallholder farming systems where sustainable pest management is crucial.

Conclusion

This research successfully identified maize inbred lines and hybrid combinations with significant resistance to fall armyworm, characterized by low leaf damage, reduced larval counts, and minimal ear damage, without compromising grain yield. The predominance of additive gene effects for resistance traits indicates that conventional breeding methods, such as recurrent selection and combining ability-based hybrid development, can effectively enhance FAW resistance.

Inbreds L3, L7, and L9, alongside hybrids such as L3 \times L7 and L7 \times L9, emerged as promising candidates for incorporation into breeding programs targeting FAW

resistance. The significant genotype \times environment interactions observed highlight the importance of multi-location testing to ensure stable performance across diverse environments.

This study underscores the potential of integrating host plant resistance with sustainable pest management strategies to address the growing challenges posed by FAW. Future breeding efforts should explore molecular breeding techniques to complement phenotypic selection and incorporate diverse resistance mechanisms to develop durable, high-yielding maize hybrids adaptable to FAW-infested regions.

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