# Heterosis: Breeding Drought-Tolerant Chickpea Hybrids Through Ascorbic Acid Enrichment

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**Abstract**

Heterosis, often referred to as hybrid vigor, has revolutionized agricultural breeding strategies by unlocking the genetic potential of hybrids, offering solutions to challenges such as disease resistance, yield enhancement, and environmental stress tolerance. One of the most significant emerging applications of heterosis is in the development of crops that can withstand extreme environmental conditions, such as drought, which has become a significant threat to food security worldwide due to climate change. This article highlights the potential of heterosis, particularly through the enhancement of ascorbic acid (AsA) content, as a mechanism to improve drought resilience in chickpea. Using advanced breeding techniques and molecular tools, the study discussed in this article provides insights into how heterosis, when harnessed correctly, can contribute to breeding chickpea varieties with greater drought tolerance, thereby ensuring stable production under water-limited conditions.

## The Importance of Heterosis in Crop Breeding

Heterosis, or hybrid vigor, refers to the phenomenon where hybrid offspring exhibit superior traits compared to their parent strains. These traits often include increased size, higher yield, better disease resistance, and enhanced environmental stress resilience. In chickpea, one of the world's most important leguminous crops, heterosis has become a focal point in breeding programs aiming to improve drought tolerance. Drought has increasingly become a limiting factor in chickpea production, especially in regions prone to water scarcity. Traditional breeding efforts have focused on improving chickpea's ability to withstand dry conditions, but hybridization offers a promising approach by leveraging the genetic strengths of different parent genotypes.

One important factor in enhancing drought tolerance through hybridization is endogenous AsA, a potent antioxidant. AsA plays a crucial role in mitigating oxidative damage that is often exacerbated under drought stress, thus improving the overall health and yield of plants. It also participates in cellular signaling pathways that regulate stress responses, helping plants to adapt better to environmental challenges. By utilizing AsA-rich genotypes in hybridization programs, breeders can effectively increase the stress tolerance of chickpea varieties, making them more resilient in the face of climate change.

## The Role of Ascorbic Acid in Drought Tolerance

AsA has a significant role in signalling pathways that regulate plant responses to drought. It modulates the expression of various genes involved in stress responses, including those responsible for the synthesis of other antioxidants and the activation of enzymes that help to repair damaged tissues. This comprehensive defense mechanism contributes significantly to drought tolerance in chickpea and other crops.

In this study, hybrids with increased AsA content were shown to exhibit enhanced drought resistance by promoting antioxidative defense mechanisms and improving cellular function under water-limited conditions. These findings underline the potential of AsA enrichment as an effective strategy for developing drought-tolerant chickpea varieties.

## Employing DNA-Based Tools for Hybrid Verification

To facilitate successful hybridization and ensure the reliability of the crosses made in this study, we turned to modern DNA-based tools such as SSR (Simple Sequence Repeat) markers. SSR markers are genetic tools that work as molecular "fingerprints," allowing researchers to confirm the genetic identity of hybrid plants by comparing them to the parent genotypes. This approach provides an accurate means of verifying that the hybrids created during the study were true hybrids, with specific genetic characteristics that could influence traits like AsA content and drought tolerance.

**Two specific crosses were made in this study**

1. **Cross 1**: An AsA-rich genotype was crossed with an AsA-poor genotype.
2. **Cross 2**: The same AsA-rich genotype was crossed with another AsA-poor genotype, serving as a check cultivar.

By using SSR primers (H1B17 for Cross 1 and GA 6 for Cross 2), the we could identify genetic polymorphisms between the parent genotypes, confirming the hybrid status of the offspring and ensuring the reliability of the results. Hybrid verification through molecular markers is crucial to prevent misidentification and ensure that desired traits, such as high AsA content, are accurately passed on to the offspring.

**F1 Generation: The Foundation for Future Chickpea Varieties**

The next phase of the study focused on the generation of the F1 hybrids and their subsequent analysis for AsA content. From **Cross 1**, 45 F1 seeds were generated, of which 34 germinated successfully, and 18 plants were confirmed as true hybrids. In **Cross 2**, 49 seeds were produced, with 38 germinating successfully, and 20 hybrids confirmed. These hybrid plants are now the foundation for further breeding efforts, providing a starting point for the development of subsequent generations that may consistently exhibit high AsA content and superior drought tolerance.

The successful germination and hybrid confirmation sets the stage for producing the F2 generation, where the genes for AsA enrichment can be further isolated, allowing breeders to identify individuals that consistently express desirable traits. As hybridization efforts progress through successive generations, the potential for fine-tuning AsA content and other related traits, such as stress resilience, becomes increasingly feasible.

**Heterotic Performance for AsA Content**

One of the most significant outcomes of the study was the observation of heterotic effects on AsA content in the F1 generation. The hybrid offspring exhibited greater levels of AsA than either of their parent strains, demonstrating the success of heterosis in elevating this important trait. In the present study specifically measured two types of heterosis in the hybrids:

- **Mid-parental heterosis**: This refers to the situation where the average AsA content of the hybrid offspring exceeds the mean of the parental genotypes.
- **Heterobeltiosis**: This is when the F1 hybrids perform better than even the superior parent genotype, in terms of AsA content.

The findings were particularly encouraging for **Cross 1**, which demonstrated stronger mid-parental heterosis and heterobeltiosis effects compared to **Cross 2**, highlighting its greater potential for producing high-AsA hybrids. This suggests that strategic hybridization using specific parent genotypes can significantly enhance key traits such as AsA content, which in turn can improve drought tolerance.

**A Path Toward Drought Tolerance**

Why is enhancing AsA content so important? The study reinforces the understanding that ascorbic acid is not merely an antioxidant but a key player in plant signaling during drought stress. By increasing AsA levels in chickpea hybrids, breeders can improve the plants' ability to combat oxidative damage, making them better equipped to endure periods of water stress.

Incorporating AsA-enriched hybrids into breeding programs can therefore lead to the development of chickpea varieties that are more resilient to drought, ultimately ensuring higher yields even in arid environments where water resources are limited.

**Future Implications for Chickpea Breeding**

This study marks an important step in integrating molecular breeding tools with heterosis to solve real-world agricultural challenges. The success of creating high-AsA hybrids sets a precedent for breeding drought-resilient chickpea varieties, with the potential for wider application in other crops as well.

The implications of this work extend beyond chickpea breeding. The approach used in this study harnessing heterosis through genetic markers could be applied to other crops that face similar challenges related to drought, climate change, and food security. The ongoing focus on AsA-related traits provides a scalable strategy for improving stress tolerance in a variety of crops, offering a significant contribution to climate-smart agriculture practices.

## Conclusion

The integration of molecular breeding, heterosis, and ascorbic acid enrichment can pave the way for developing chickpea varieties with improved drought resilience. This is an essential step in addressing the challenges posed by climate change, ensuring that crops like chickpea remain a stable and sustainable food source for global populations. Through continued investment in these breeding strategies, chickpea can serve as a model for other crops in adapting to the pressures of a changing climate, ultimately contributing to global food security and agricultural sustainability.

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