Review Article

Lettuce Genomics: Leveraging BSA/BSR Data Analysis Tools for Trait Mapping

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Abstract | Lettuce (*Lactuca sativa*) is a globally significant leafy vegetable valued for its nutritional benefits and economic impact. Advances in lettuce genomics, particularly through Bulk Segregant Analysis (BSA) and Bulk Segregant RNA-sequencing (BSR), have revolutionized trait mapping. These tools facilitate the identification of genetic markers and differentially expressed genes, advancing our understanding of traits like disease resistance, quality, and agronomic performance. This review synthesizes the principles, applications, and challenges of BSA/BSR in lettuce research. It highlights the role of these tools in improving lettuce breeding strategies and explores future directions, including multi-omics integration for more precise trait mapping and crop improvement. This study underscores the transformative potential of BSA/BSR in addressing agricultural challenges and enhancing lettuce production.

Keywords | Genomics, Genetic markers, Gene expression, Crop improvement, Disease resistance, Trait mapping

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INTRODUCTION

Lettuce (*Lactuca sativa*) is a major leafy vegetable crop cultivated worldwide for its nutritional value and economic significance. It is a staple in many diets due to its high content of vitamins, minerals, and dietary fiber, making it an essential component of a healthy diet (Sandoya *et al.*, 2024). The increasing demand for lettuce is driven by growing health-conscious consumer trends, as people seek to incorporate more vegetables into their meals to improve overall health and well-being. Economically, lettuce is a significant contributor to the agricultural industry (Jaeger *et al.*, 2023). It is widely grown in various climates and is a major cash crop in many regions. The global lettuce market is substantial, with production and consumption figures reflecting its importance in both local and international markets. The cultivation of lettuce also supports a range of agricultural activities, from seed production to post-harvest handling and marketing, providing employment and economic opportunities across the supply chain (Shatilov *et al.*, 2019; Islam *et al.*, 2021).

The field of lettuce genomics has made significant strides in recent years, building on a foundation of previous genetic

studies. Early efforts focused on understanding the basic genetic makeup of lettuce, including the identification of key genes and pathways involved in growth, development, and stress response (Oh *et al*., 2025). The sequencing of the lettuce genome has provided a wealth of information, enabling researchers to delve deeper into the genetic basis of various traits (Guo *et al*., 2023; Wang *et al*., 2024b). Trait mapping in lettuce is a critical area of research as it helps identify the genomic regions associated with specific characteristics that are important for crop improvement. These traits can range from disease resistance and nutritional content to agronomic performance (Wei *et al*., 2021). By mapping these traits, researchers can develop strategies to breed lettuce varieties that are more resilient, nutritious, and productive, thereby addressing the challenges faced by the agricultural sector and meeting the demands of consumers.

Bulk Segregant Analysis (BSA) and Bulk Segregant RNA-sequencing (BSR) are powerful tools that have revolutionized the process of trait mapping in plants, including lettuce. BSA is a technique that involves creating two pools (bulks) of individuals from a segregating population, one representing the extreme phenotype of interest and the other representing the opposite phenotype (Li and Xu, 2022; Wang *et al*., 2024a). By comparing the genetic makeup of these two bulks (pools of individuals with contrasting traits), researchers can identify genetic markers (SNPs) that are linked to the trait of interest. BSR takes this approach a step further by integrating RNA-sequencing technology with BSA (Wang *et al*., 2023, 2024c). This not only allows for the identification of genetic markers but also provides insights into the gene expression patterns associated with the trait. The experimental design of BSR involves creating the same two bulks as in BSA, but instead of genotyping, RNA is extracted and sequenced (Zhang and Panthee, 2020). The data analysis pipeline for BSR includes steps such as read alignment, variant calling, and differential expression analysis, which help in identifying genes that are differentially expressed between the two bulks (Li *et al*., 2022). The advantages of BSR over traditional BSA include the ability to detect genes that are differentially expressed, providing a more comprehensive understanding of the genetic and transcriptional basis of the trait. This makes BSR a valuable tool for lettuce genomics, as it can help identify the functional genes underlying important traits, thereby facilitating more targeted breeding efforts (Li *et al*., 2019).

Despite the significant advancements in lettuce genomics and the growing use of BSA/BSR techniques, there is a notable lack of comprehensive reviews that synthesize the current state of these tools, specifically in the context of lettuce trait mapping. To address this, the specific objectives of this review article are to provide a detailed overview of BSA/BSR data analysis tools, evaluate their applications in lettuce genomics, and discuss the challenges and future directions in this field.

Principles of BSA/BSR data analysis tools

Bulk Segregant Analysis (BSA) and Bulk Segregant RNA-sequencing (BSR) are advanced tools that have significantly enhanced the process of trait mapping in plants, particularly in lettuce (Varshney *et al*., 2016). BSA is a technique that involves creating two extreme phenotypic bulks from a segregating population derived from a cross between two parental lines that differ in the trait of interest (Liu *et al*., 2012). These bulks are created by pooling DNA from individuals that exhibit extreme phenotypes of the trait, such as high resistance and high susceptibility to a disease. The genetic basis of BSA relies on comparing the frequency of genetic markers in these two bulks to identify markers linked to the trait of interest (Nguyen *et al*., 2019; Goettelmann *et al*., 2024). Various types of genetic markers can be used in BSA, including single nucleotide polymorphisms (SNPs), simple sequence repeats (SSRs), insertions/deletions, and restriction fragment length polymorphisms (RFLPs). SNPs are particularly popular due to their high density and ease of detection (Zou *et al*., 2016; Majeed *et al*., 2022).

Bulk Segregant RNA-sequencing (BSR) builds upon BSA by integrating RNA-sequencing technology. This integration allows for the identification of not only genetic markers but also differentially expressed genes (DEGs) between the two bulks (Du *et al*., 2017). The experimental design for BSR involves collecting tissue samples from the extreme phenotypic bulks at the appropriate developmental stage, extracting RNA, and performing high-throughput RNA-Seq to generate transcriptome data (Sun *et al*., 2019; Guan *et al*., 2022). Bioinformatics tools (Minimap2, STAR, NanoCount, and edgeR) are then used to align the RNA-Seq reads to a reference genome, quantify gene expression levels, and identify DEGs (Andersson, 2017). The advantage of BSR over traditional BSA is its ability to provide insights into gene expression patterns, leading to higher mapping resolution and more accurate identification of causal genes. This makes BSR a powerful tool for understanding the molecular mechanisms underlying complex traits in lettuce, thereby accelerating the development of improved varieties (Rao *et al*., 2019; Deshpande *et al*., 2023).

Applications of BSA/BSR in lettuce trait mapping

Disease resistance traits

Lettuce is susceptible to various diseases, and BSA/BSR has been instrumental in mapping genes related to disease resistance (Table 1). For example, Simko *et al*. (2013) used

BSA to identify QTLs conferring resistance to downy mildew in legacy cultivars of lettuce. This study helped pinpoint genomic regions associated with resistance, which can be used in breeding programs to develop disease-resistant varieties. Similarly, Xiong *et al.* (2023) through genome assembly and analysis, we have provided insights into the non-host resistance to downy mildew in a wild relative of lettuce, *Lactuca saligna*. These findings have significant implications for lettuce breeding programs, enabling the development of disease-resistant lettuce varieties.

Quality-related traits

The quality of lettuce is a key factor influencing consumer preferences. BSA/BSR has been instrumental in mapping genes associated with various quality-related traits (Su *et al.*, 2020). Regarding leaf color, BSA/BSR has enabled the identification of genes that determine the pigmentation of lettuce leaves (Table 1). For example, by comparing bulks of plants with different leaf colors, genetic markers linked to the synthesis of chlorophyll and other pigments have been detected (Simko, 2023). In terms of texture, genes influencing the crispness and tenderness of lettuce leaves have been mapped. This is achieved by analyzing the differential expression of genes related to cell wall composition and structure in bulks of plants with varying textural qualities (Shi *et al.*, 2024). For nutritional content, BSA/BSR has helped in locating genes responsible for the accumulation of vitamins and antioxidants. By examining the gene expression patterns in bulks of lettuce with high and low levels of vitamin C or antioxidants, candidate genes involved in the biosynthesis and transport of these nutrients have been identified (Zhang *et al.*, 2017, 2021; van Treuren *et al.*, 2018). As for shelf-life, BSA/BSR has been used to map genes that affect the post-harvest senescence of lettuce. Genes related to the regulation of ethylene production and the maintenance of cellular integrity during storage have been pinpointed. The potential of using these mapped genes to improve the overall quality of lettuce products is immense (Zhou *et al.*, 2024). Breeders can select for plants with desirable combinations of these quality traits, resulting in lettuce varieties that not only meet consumer expectations in terms of appearance and taste but also offer enhanced nutritional value and longer shelf-life, reducing post-harvest losses and increasing the marketability of lettuce products (Chadwick *et al.*, 2024; Zhang *et al.*, 2024).

Agronomic traits

Agronomic traits such as plant growth rate, yield, and tolerance to abiotic stresses are crucial for the economic viability of lettuce cultivation (Zhang *et al.*, 2023b; Khaleeq *et al.*, 2023). BSA/BSR has been applied to map genes related to these traits. In the case of plant growth rate, by creating bulks of fast-growing and slow-growing lettuce plants from a segregating population, BSA/BSR has facilitated the identification of genetic markers associated with genes that regulate cell division, elongation, and overall plant development (Kozik *et al.*, 2017). For yield, genes influencing factors such as leaf size, number of leaves, and plant architecture have been mapped. BSR has provided insights into the gene expression patterns that

Table 1: Applications of BSA/BSR in lettuce trait mapping.

Trait category	Specific trait	BSA/BSR application	Key findings	References
Disease resistance	Downy mildew resistance	Identified QTLs associated with resistance in legacy cultivars	Pinpointed genomic regions for breeding disease-resistant varieties	(Simko *et al.*, 2013b)
	Non-host resistance	Genome assembly and analysis of *Lactuca saligna*	Insights into resistance mechanisms in Lactuca saligna	(Xiong *et al.*, 2023b)
Quality-related	Leaf color	Mapped genes controlling chlorophyll and pigment synthesis	Identified genes responsible for leaf pigmentation	(Simko, 2023b)
	Texture	Analyzed genes related to cell wall composition	Mapped genes influencing crispness and tenderness of lettuce leaves	(Shi *et al.*, 2024a)
	Nutritional content	Identified genes for vitamin C and antioxidant accumulation	Discovered genes involved in nutrient biosynthesis and transport	(Zhang *et al.*, 2021b)
	Shelf-life	Mapped genes regulating ethylene production and cellular integrity	Identified genes that delay post-harvest senescence	(Zhou *et al.*, 2024)
Agronomic traits	Growth rate	Identified markers linked to cell division and elongation	Mapped genes regulating plant development and growth rate	(Reyes-Chin-Wo *et al.*, 2017)
	Yield	Analyzed gene expression patterns related to leaf size and plant architecture	Identified genes contributing to higher yield potential	(Wei *et al.*, 2021a)
	Abiotic stress tolerance	Mapped genes for drought and heat tolerance	Discovered genes involved in stomatal regulation and heat shock protein production	(Oyebamiji *et al.*, 2024a)

contribute to higher yield potential. Regarding tolerance to abiotic stresses like drought and heat, BSA/BSR has helped in locating genes that enable lettuce plants to withstand these adverse conditions (Wei *et al*., 2021; Oyebamiji *et al*., 2024). For example, genes involved in the regulation of stomatal opening and closing, osmotic adjustment, and heat shock protein production have been identified. These findings contribute to the development of more productive and stress-tolerant lettuce varieties (Damerum *et al*., 2021; Zhang *et al*., 2023a). Breeders can use the identified genes and markers to select for plants with improved agronomic traits, resulting in lettuce varieties that can thrive under a wider range of environmental conditions, ensuring stable yields and reducing the vulnerability of lettuce cultivation to climate change and other abiotic stress factors (Khaleeq *et al*., 2024b).

Factors affecting the Accuracy of BSA/BSR data analysis

Quality of the reference genome

The accuracy of BSA/BSR data analysis in lettuce is highly dependent on the quality of the reference genome. A well-assembled and annotated reference genome serves as the cornerstone for reliable genetic mapping. In the context of lettuce, which has a complex genome with repetitive sequences and a relatively large size, a high-quality reference genome is crucial for precise alignment of sequencing reads and accurate identification of genetic variants (Rhie *et al*., 2021). A poorly assembled reference genome may lead to misalignment of reads, resulting in incorrect identification of genetic markers and misinterpretation of data. For instance, ambiguous regions in the genome can cause false-positive associations between genetic markers and traits (Wang *et al*., 2024b). On the other hand, a complete and accurate reference genome can significantly enhance the mapping resolution, allowing for the fine mapping of genes and the identification of true causal genes with greater precision. It enables researchers to distinguish between closely linked genetic markers and to pinpoint the exact genomic regions associated with the traits of interest, thereby facilitating more effective breeding strategies (Shi *et al*., 2024; Workum *et al*., 2024).

Experimental design

Proper experimental design plays a pivotal role in BSA/BSR studies. The selection of appropriate parental lines is fundamental, as they should exhibit clear and contrasting phenotypes for the trait under investigation. The size and composition of the segregating population are also critical factors (Zhang *et al*., 2023b). A larger population size increases the statistical power to detect genetic markers associated with the trait, reducing the likelihood of false negatives. The number of individuals in each bulk should be carefully determined to ensure that the bulks are representative of the extreme phenotypes. Environmental variations can introduce noise into the phenotypic measurements, making it difficult to accurately segregate the population into the two bulks (Zhang *et al*., 2017). Phenotypic measurement errors can also lead to incorrect assignment of individuals to the bulks, affecting the reliability of the genetic analysis. Genetic background noise, which includes the presence of other linked or unlinked genes that may influence the trait, can complicate the identification of the target gene (Lee *et al*., 2023). To mitigate these issues, careful experimental design is essential. This includes controlling environmental conditions as much as possible, using robust phenotyping methods to minimize measurement errors, and employing statistical methods to account for genetic background noise (Zhang *et al*., 2021, 2023b; Workum *et al*., 2024; Khaleeq *et al*., 2024a). By addressing these factors through thoughtful experimental design, the accuracy and reliability of BSA/BSR data analysis can be substantially improved, leading to more meaningful and actionable results in lettuce trait mapping.

Comparison with traditional trait mapping methods

Traditional trait mapping methods in lettuce, like linkage mapping and quantitative trait locus (QTL) analysis using individual-based genotyping, have long been the cornerstone of genetic research. However, they come with significant drawbacks (El-Esawi, 2015). Linkage mapping often requires large populations and extensive genotyping efforts, which are time-consuming and costly. The process can span multiple generations, delaying the identification of relevant genes (Fukuda *et al*., 2017). Additionally, the mapping resolution is relatively low, making it difficult to precisely pinpoint the genes responsible for complex traits. With QTL analysis, the large number of individual genotyping data points can be overwhelming to manage and analyze, and the results may not always translate well into practical breeding applications due to the broad genomic regions associated with QTLs (Meng *et al*., 2021).

In contrast, BSA/BSR techniques offer several distinct advantages. Firstly, they are highly efficient, drastically reducing the time required to identify trait-associated genomic regions. By pooling samples, the need for extensive individual genotyping is minimized (Gurdon *et al*., 2019). Secondly, the cost is significantly lower, as fewer sequencing runs and genotyping assays are needed. This makes it more accessible for research teams with limited budgets (Dziechciarkova *et al*., 2004). Thirdly, BSA/BSR can handle complex traits more effectively. The ability to capture genetic and gene expression differences simultaneously provides a more comprehensive view of the trait's genetic basis (Fukuda *et al*., 2017). For

example, in mapping a trait influenced by multiple genes and environmental factors, BSA/BSR can tease out the key players involved, whereas traditional methods might struggle to do so. Overall, BSA/BSR has revolutionized lettuce trait mapping by overcoming many of the limitations of traditional approaches (Franco *et al*., 2015; Wang *et al*., 2024c).

Challenges and solutions in BSA/BSR-based lettuce trait mapping

BSA/BSR techniques, while powerful, face several technical challenges in lettuce trait mapping. One significant challenge is low sequencing coverage, which can lead to incomplete data and reduce the power to detect genetic markers linked to traits of interest (Hintum, 2003). High levels of genetic heterogeneity within lettuce populations can also complicate the identification of true associations, as it may mask the effects of specific genes or introduce false positives. Additionally, the complexity of trait inheritance, particularly for polygenic traits, can make it difficult to pinpoint specific genomic regions responsible for the trait (Zhang *et al*., 2021). For example, in a study by Hartman *et al*. (2014), the authors noted the challenges of mapping abiotic stress QTL in lettuce crop-wild hybrids due to the complex genetic background and environmental interactions.

To overcome these technical challenges, several solutions and strategies can be employed. Increasing sequencing depth is a straightforward approach to improve data quality and reduce the likelihood of false negatives (Ichikawa *et al*., 2010). Advanced bioinformatics algorithms can help in more accurately identifying genetic markers and filtering out noise from the data. For instance, the use of machine learning algorithms can enhance the detection of subtle genetic variations associated with complex traits (Li *et al*., 2022; Samim *et al*., 2023). Furthermore, integrating multiple data sources, such as genomics, transcriptomics, and proteomics, can provide a more comprehensive view of the genetic architecture underlying the traits. This multi-omics approach has been shown to improve the accuracy of trait mapping in other crops and can be similarly applied to lettuce. For example, a study by Huo *et al*. (2016) demonstrated the effectiveness of combining BSA with whole-genome sequencing to identify lettuce seed germination mutants rapidly. By leveraging these strategies, researchers can enhance the reliability and resolution of BSA/BSR-based trait mapping in lettuce, leading to more robust and actionable genetic insights for breeding programs.

Application in lettuce breeding

BSA/BSR-based trait mapping has significant potential in accelerating lettuce breeding programs. The identified genes and markers can be used in marker-assisted selection (MAS) and genomic selection (GS) to develop new lettuce varieties with improved traits. For instance, a study by Li *et al*. (2024) identified QTL associated with abiotic stress tolerance in lettuce, which can be used to develop more stress-tolerant varieties. Additionally, the use of BSA/BSR in mapping taste and flavor traits in lettuce has provided breeders with tools to improve the palatability and nutritional value of lettuce (Chadwick *et al*., 2024). As lettuce genomics research continues to advance, BSA/BSR will play a crucial role in crop improvement by facilitating the rapid identification and integration of desirable traits into breeding programs. The future of lettuce genomics and breeding is thus intertwined with the continued evolution and application of BSA/BSR and related omics technologies.

CONCLUSION AND RECOMMENDATIONS

The application of BSA/BSR data analysis tools in lettuce genomics has significantly advanced our understanding of the genetic basis of various traits in lettuce. These tools have not only identified genetic markers linked to important agronomic, quality, and disease resistance traits but have also provided insights into the underlying gene expression patterns. The integration of BSA/BSR with other omics technologies has further enhanced the resolution and accuracy of trait mapping, leading to the discovery of novel genes and regulatory networks. For instance, a comprehensive study characterized a vast number of structural variations (SVs) in the lettuce genome, providing a valuable genomic resource for future breeding efforts. Additionally, the development of a super-pangenome for lettuce has expanded our view of the gene repertoire, revealing loci that are not present in the reference genome and offering a strong basis for research into gene presence/absence variation (PAV), copy-number variation (CNV), and other variations underlying important biological traits.

The future of lettuce genomics is promising, with several exciting directions on the horizon. The continued integration of BSA/BSR with metabolomics and proteomics will provide a more comprehensive understanding of trait inheritance, leading to the discovery of novel genes and regulatory networks. This multi-omics approach will be crucial in unraveling the complex genetic architecture of traits such as water-use efficiency (WUE) and yield under different environmental conditions. Furthermore, the application of BSA/BSR in marker-assisted selection (MAS) and genomic selection (GS) will accelerate the development of new lettuce varieties with improved traits. The identified genes and markers can be used to target specific breeding goals, such as enhancing

disease resistance, improving nutritional content, and increasing stress tolerance. Overall, while we have made significant strides, the journey in lettuce genomics and breeding is far from over. Continued research, innovation, and collaboration will be essential to fully harness the potential of BSA/BSR and related technologies, ensuring a prosperous future for lettuce production and consumption.

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NOVELTY STATEMENT

This review synthesizes the current state of BSA/BSR tools in lettuce genomics. It underscores their role in advancing trait mapping and breeding strategies. The integration of BSA/BSR with multi-omics approaches is highlighted as a key innovation for precise trait mapping and crop improvement.

AUTHOR'S CONTRIBUTION

SN, AA, and MAA proposed a general idea and were responsible for writing the review.
NK, SR, and SM contributed to the review's conception and design.
BK, MA, TR, and MNM reviewed all manuscript drafts and provided extensive editing.
All authors have read and approved the final version of the manuscript.

Availability of data and material

Not applicable.

Conflict of interest

The authors have declared no conflict of interest.

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