



MOLECULAR IDENTIFICATION OF ABIOTIC STRESS-TOLERANT
RASPBERRY (*RUBUS IDAEUS* L.) GENOTYPES USING DNA MARKER
TECHNOLOGIES

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Abstract. Raspberry (*Rubus idaeus* L.) is an economically important fruit crop whose productivity is increasingly threatened by abiotic stress factors such as drought and salinity. This review summarizes current knowledge on morphological characteristics, genetic diversity, and molecular mechanisms underlying stress tolerance in raspberry. Particular attention is given to the role of DNA marker technologies, including SSR and SNP markers, in identifying stress-responsive genes and quantitative trait loci (QTLs). The application of marker-assisted selection (MAS) in breeding programs is also discussed as an effective approach for improving stress tolerance. Overall, integrating molecular tools with physiological traits provides new opportunities for developing resilient raspberry cultivars under changing environmental conditions.

Keywords: Raspberry, *Rubus idaeus*, abiotic stress, DNA markers, SSR, SNP, marker-assisted selection.

Аннотация. Малина (*Rubus idaeus* L.) является экономически важной плодовой культурой, продуктивность которой все чаще снижается под воздействием абиотических стрессовых факторов, таких как засуха и засоление. В данном обзоре обобщены современные данные о морфологических особенностях, генетическом разнообразии и молекулярных механизмах устойчивости малины к стрессам. Особое внимание уделено роли ДНК-маркерных технологий, включая SSR и SNP маркеры, в выявлении генов, ответственных за стрессоустойчивость, и количественных признаков (QTL). Также рассмотрено применение маркер-ассистированной селекции (MAS) как эффективного подхода для повышения устойчивости к стрессам. В целом, интеграция молекулярных методов с физиологическими характеристиками открывает новые возможности для создания устойчивых к неблагоприятным условиям сортов малины.

Ключевые слова: малина, *Rubus idaeus*, абиотический стресс, ДНК-маркеры, SSR, SNP, маркер-ассистированная селекция.

Annotatsiya. Malina (*Rubus idaeus* L.) iqtisodiy jihatdan muhim meva ekini bo‘lib, uning hosildorligi qurg‘oqchilik va sho‘rlanish kabi abiotik stress omillari ta‘sirida tobora kamayib bormoqda. Ushbu sharh maqolada malinada morfologik belgilar, genetik xilma-xillik va stressga chidamlilikning molekulyar mexanizmlari bo‘yicha zamonaviy ilmiy ma‘lumotlar umumlashtirilgan. Xususan, SSR va SNP kabi DNK marker texnologiyalarining stressga javob beruvchi genlar hamda miqdoriy belgilar (QTL) ni aniqlashdagi ahamiyati yoritilgan. Shuningdek, markerga asoslangan seleksiya (MAS) usulining stressga chidamli navlarni yaratishdagi samaradorligi ko‘rib chiqilgan. Umuman olganda, molekulyar yondashuvlarni fiziologik belgilar bilan integratsiya qilish iqlim o‘zgarishi sharoitida barqaror malina navlarini yaratishda muhim ahamiyat kasb etadi.

Kalit so‘zlar: malina, *Rubus idaeus*, abiotik stress, DNK markerlar, SSR, SNP, markerga asoslangan seleksiya.

Introduction. Raspberry (*Rubus idaeus* L.) is a perennial fruit crop belonging to the Rosaceae family, widely recognized for its nutritional and commercial value. The species is believed to have originated in Europe and parts of northern Asia, where wild forms still occur in natural ecosystems. Over time, raspberry cultivation has expanded globally, including North America, South America, and Asia, driven by increasing demand for fresh fruits and processed products[1].

The domestication and spread of raspberry have been influenced by natural selection and human-mediated breeding. The introduction of elite cultivars and hybridization among geographically distant germplasm have contributed to the development of diverse genetic resources. Today, raspberry is cultivated in a wide range of agroecological zones, which has further enhanced its adaptability to different environmental conditions[2].

Raspberry (*Rubus idaeus* L.), belonging to the genus *Rubus* within the Rosaceae family, is considered one of the valuable fruit crops due to its high nutritional and economic importance. This diploid species ($2n = 14$) contains a wide range of bioactive compounds, including vitamins, minerals, ellagic acid, and raspberry ketones, which contribute to its functional food value. Raspberry is widely cultivated across many countries, with well-established production systems integrating cultivation, processing, and commercialization.

Genetic diversity plays a crucial role in plant adaptation, survival, and breeding improvement. In raspberry, considerable genetic variability has been developed through long-term selection and hybridization among different geographical regions. However, traditional classification based on morphological traits is often unreliable due to environmental influences, which limits its effectiveness in accurately distinguishing genotypes. Therefore,

molecular approaches have become essential tools for assessing genetic diversity and understanding evolutionary relationships[3].

Molecular marker technologies provide stable, reproducible, and high-resolution methods for studying genetic variation. Compared to conventional phenotypic markers, molecular markers are not affected by environmental factors and allow precise identification of genotypes and trait-associated loci. Over time, molecular markers have evolved into different generations, including early restriction-based markers, PCR-based markers, and high-throughput sequencing markers. Among these, PCR-based markers remain widely used due to their efficiency and cost-effectiveness[4].

Simple sequence repeat (SSR) markers are particularly valuable due to their high level of polymorphism, codominant inheritance, and multi-allelic nature. These markers are widely applied in genetic diversity analysis, mapping studies, and breeding programs. However, their application may be limited by the availability of loci and relatively higher costs. On the other hand, sequence-related amplified polymorphism (SRAP) markers target coding regions of the genome and provide an efficient and economical alternative for detecting genetic variation. Although SRAP markers are relatively simple to use, they may present challenges related to reproducibility[5].

Despite the advantages of individual marker systems, the combined use of SSR and SRAP markers has proven to be more effective in genetic studies. SSR markers provide precise information on allelic variation and genetic relationships, while SRAP markers enable efficient screening of functional genomic regions. This complementary approach enhances the accuracy of genetic analysis and supports more effective breeding strategies[3].

In raspberry research, molecular marker applications are still developing compared to major crops. Many studies have primarily focused on morphological traits or single-marker systems, which limits the understanding of complex genetic mechanisms. Additionally, markers such as ISSR, although useful for detecting polymorphism, have certain limitations due to their dominant inheritance pattern, which restricts their ability to distinguish heterozygous genotypes[5].

The integration of different molecular marker systems provides a more comprehensive understanding of genetic diversity and improves the efficiency of breeding programs. These approaches allow early identification of desirable genotypes, facilitate gene mapping, and accelerate the development of new cultivars. As a result, molecular marker technologies are becoming increasingly important in advancing raspberry breeding towards more precise and efficient selection strategies.

ABIOTIC STRESS TOLERANCE: GENERAL OVERVIEW

Abiotic stresses, including drought, salinity, temperature extremes (both heat and cold), and nutrient imbalances, represent major environmental constraints limiting the growth, productivity, and fruit quality of raspberry (*Rubus idaeus* L.). These stress factors negatively

influence plant development by disrupting key physiological and biochemical processes such as photosynthesis, transpiration, water uptake, and ionic homeostasis. For instance, drought stress reduces leaf water potential and stomatal conductance, leading to decreased carbon assimilation, while salinity stress causes ionic toxicity and osmotic imbalance that impair nutrient absorption and metabolic activity[6].

In raspberry plants, exposure to abiotic stress often results in reduced vegetative growth, poor flowering, decreased fruit set, and lower yield. Additionally, stress conditions can alter fruit composition, affecting important quality parameters such as sugar content, acidity, and antioxidant compounds. The severity of these effects depends on stress intensity, duration, and the genetic background of the genotype.

The ability of raspberry plants to tolerate abiotic stress is governed by complex genetic and molecular mechanisms involving multiple genes, transcription factors, and signaling pathways. Stress perception triggers a cascade of molecular responses, including the activation of stress-responsive genes, synthesis of protective proteins, and accumulation of osmolytes such as proline and soluble sugars. Antioxidant defense systems, including enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), also play a critical role in mitigating oxidative damage caused by stress conditions[7].

Molecular marker technologies have become essential tools for dissecting the genetic basis of abiotic stress tolerance. Markers such as SSR (simple sequence repeats), SNP (single nucleotide polymorphisms), and ISSR (inter-simple sequence repeats) enable the identification of genomic regions and quantitative trait loci (QTLs) associated with stress-responsive traits. These markers facilitate marker-assisted selection (MAS), allowing breeders to identify and select tolerant genotypes at early developmental stages without relying solely on phenotypic evaluation under field conditions[5].

Furthermore, advances in genomics and high-throughput sequencing technologies have enhanced the identification of candidate genes involved in stress tolerance. Integrating molecular marker data with physiological and phenotypic traits provides a more comprehensive understanding of plant responses to environmental stress. This integrative approach is particularly important for perennial crops like raspberry, where breeding cycles are relatively long.

Overall, understanding the genetic and physiological mechanisms underlying abiotic stress tolerance is crucial for developing improved raspberry cultivars with enhanced resilience. The application of molecular tools not only accelerates breeding programs but also contributes to sustainable production systems under changing climatic conditions.

DROUGHT STRESS TOLERANCE

Drought stress is a major environmental constraint limiting raspberry (*Rubus idaeus* L.) production, especially in regions characterized by irregular precipitation and increasing temperature fluctuations. Water deficit conditions lead to reduced cell turgor, limited leaf

expansion, and decreased photosynthetic activity due to stomatal closure, ultimately resulting in lower biomass accumulation and fruit yield.

Raspberry plants exhibit a range of adaptive responses to drought stress at physiological and molecular levels. Key mechanisms include regulation of stomatal conductance to minimize water loss, enhancement of root system architecture to improve water uptake, and accumulation of osmoprotectants such as proline and soluble sugars, which help maintain cellular osmotic balance. In addition, drought stress induces the expression of stress-responsive genes, including those encoding dehydrins, aquaporins, and transcription factors involved in abscisic acid (ABA)-mediated signaling pathways[8].

Molecular marker technologies have been widely applied to identify genomic regions associated with drought tolerance. For instance, SSR markers have been effectively used in several fruit crops, including raspberry and related species, to assess genetic diversity and to detect loci linked with traits such as root development and water-use efficiency. Similarly, SNP markers, due to their high density and genome-wide distribution, have been employed in association mapping studies to identify candidate genes involved in drought response(1-rasm).

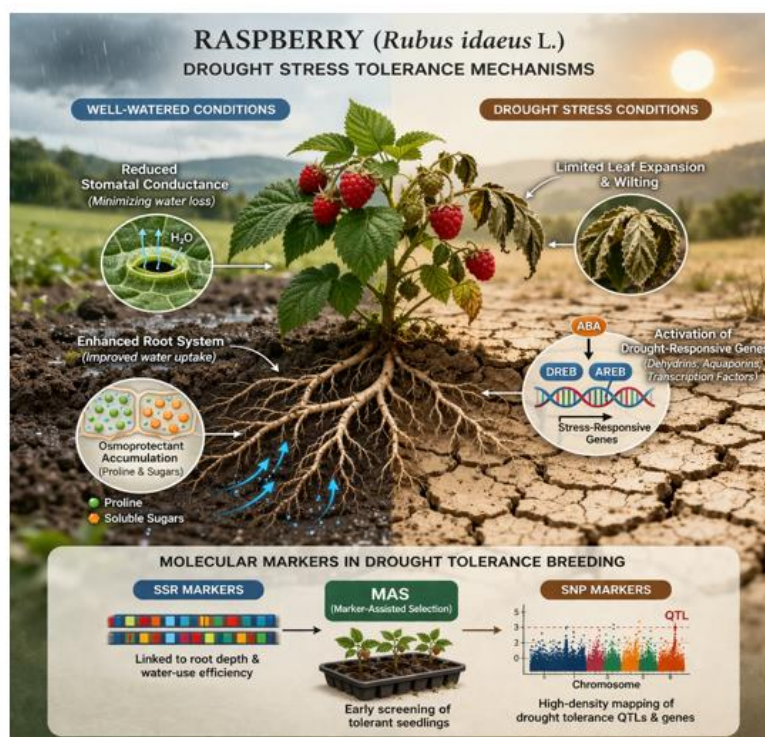


Figure 1. Drought Stress Tolerance Mechanisms in Raspberry (*Rubus idaeus* L.)

Previous studies have demonstrated that SSR-based genotyping can differentiate drought-tolerant and sensitive genotypes by revealing allelic variation in stress-related loci. In addition, SNP-based approaches have enabled the identification of quantitative trait loci (QTLs) associated with drought tolerance traits in perennial fruit crops. These findings highlight the importance of integrating molecular markers with physiological traits to improve selection accuracy [6].

Marker-assisted selection (MAS) provides a powerful approach for early screening of drought-tolerant genotypes at the seedling stage, significantly reducing the time and cost associated with conventional breeding. The combination of molecular data with phenotypic and physiological evaluations enhances the efficiency of breeding programs aimed at developing drought-resilient raspberry cultivars.

SALINITY STRESS TOLERANCE

Salinity stress is one of the major abiotic constraints limiting plant productivity, particularly in irrigated and arid regions where soil salinization is increasing. Excess accumulation of sodium (Na^+) and chloride (Cl^-) ions in the soil leads to osmotic stress, reduces water uptake, and causes ionic toxicity in plant tissues. As a result, essential nutrients such as potassium (K^+) and calcium (Ca^{2+}) are displaced, disrupting cellular homeostasis and metabolic processes[6].

In raspberry (*Rubus idaeus L.*), salinity stress negatively affects vegetative growth, leaf morphology, and fruit development. High salt concentrations reduce chlorophyll content, impair photosynthetic efficiency, and lead to oxidative stress through the overproduction of reactive oxygen species (ROS). This ultimately results in reduced yield and deterioration of fruit quality, including lower sugar accumulation and altered acidity [9].

To cope with salinity stress, raspberry plants activate several physiological and molecular defense mechanisms. These include selective ion transport and compartmentalization, where Na^+ ions are sequestered into vacuoles to prevent cytoplasmic toxicity. Key transporter genes such as NHX (Na^+/H^+ antiporters) and HKT (high-affinity potassium transporters) play an important role in maintaining ion balance. Additionally, plants synthesize compatible solutes such as proline, glycine betaine, and soluble sugars, which help stabilize cellular structures and maintain osmotic balance. Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), are also upregulated to mitigate oxidative damage.

Molecular marker technologies have been extensively used to identify genomic regions associated with salinity tolerance. SSR markers have been applied to evaluate genetic diversity and detect allelic variation linked to salt tolerance traits in berry crops and related species. SNP markers, due to their high resolution, have been used in genome-wide association studies (GWAS) to identify candidate genes and quantitative trait loci (QTLs) involved in ion transport, osmotic regulation, and stress signaling pathways. For example, loci associated with ion homeostasis and Na^+ exclusion mechanisms have been successfully identified using SNP-based approaches in several fruit crops[10].

Previous studies indicate that integrating molecular marker data with physiological parameters such as Na^+/K^+ ratio, chlorophyll content, and osmolyte accumulation improves the accuracy of selecting salt-tolerant genotypes. Marker-assisted selection (MAS) enables early

screening of tolerant individuals, significantly reducing breeding time and increasing selection efficiency.

Overall, the application of molecular markers, particularly SSR and SNP, provides a reliable and efficient approach for identifying salinity-tolerant genotypes and supports the development of improved raspberry cultivars adapted to saline environments.

CONCLUSION

Raspberry (*Rubus idaeus* L.) productivity is significantly affected by abiotic stresses such as drought and salinity, which disrupt physiological and biochemical processes. The plant exhibits adaptive responses including osmotic regulation, ion balance, and activation of stress-related genes. Molecular markers, particularly SSR and SNP, have proven effective in identifying stress-tolerant genotypes and associated QTLs. Marker-assisted selection (MAS) enables rapid and accurate screening of desirable traits. Integrating molecular and physiological approaches enhances breeding efficiency. Overall, molecular tools provide a strong foundation for developing stress-resilient raspberry cultivars.

REFERENCES

1. Graham, J., Smith, K., MacKenzie, K., Jorgenson, L., Hackett, C., & Powell, W. (2004). The construction of a genetic linkage map of red raspberry (*Rubus idaeus* subsp. *idaeus*) based on AFLP, genomic-SSR and EST-SSR markers. *Theoretical and Applied Genetics*, 109, 740–749.
2. Woodhead, M., McCallum, S., Smith, K., Cardle, L., Mazzitelli, L., & Graham, J. (2008). Identification, characterisation and mapping of simple sequence repeat (SSR) markers from raspberry root and bud ESTs. *Molecular Breeding*, 22, 555–563.
3. Fernández-Fernández, F., Antanaviciute, L., Govan, C.L., & Sargent, D.J. (2011). Development of a multiplexed microsatellite set for fingerprinting red raspberry (*Rubus idaeus*) germplasm and its transferability to other *Rubus* species. *Journal of Berry Research*, 1, 177–187.
4. Bassil, N.V., Nyberg, A., Hummer, K.E., Graham, J., Dossett, M., & Finn, C.E. (2012). A universal fingerprinting set for red raspberry. *Acta Horticulturae*, 946, 83–87.
5. Kostamo, K., Toljamo, A., Antonius, K., Kokko, H., & Kärenlampi, S.O. (2013). Morphological and molecular identification to secure cultivar maintenance and management of self-sterile *Rubus arcticus*. *Annals of Botany*, 111, 713–721.
6. Girichev, V., Hanke, M.-V., Peil, A., & Flachowsky, H. (2017). SSR fingerprinting of a German *Rubus* collection and pedigree-based evaluation of trueness-to-type. *Genetic Resources and Crop Evolution*, 64, 189–203.
7. Marulanda, M., López, A.M., & Uribe, M. (2012). Molecular characterization of Andean blackberry (*Rubus glaucus*) using SSR markers. *Genetics and Molecular Research*, 11, 322–331.

8. Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B., & Wrolstad, R.E. (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. *Journal of Agricultural and Food Chemistry*, 50, 519–525.
9. Longhi, S., Giongo, L., Buti, M., Surbanovski, N., Viola, R., Velasco, R., Ward, J.A., & Sargent, D.J. (2014). Molecular genetics and genomics of the Rosoideae: Current status and future perspectives. *Horticulture Research*, 1, 1–10.
10. Graham, J., Hackett, C.A., Smith, K., Woodhead, M., Hein, I., & McCallum, S. (2011). Mapping QTLs for developmental traits in raspberry. *Theoretical and Applied Genetics*, 122, 1143–1156.