NOTE

Targeting MAT Gene (*LOC_Os11g15410*) polymorphisms involved in tocopherol and tocotrienol conversions in rice

Andrea Irías-Mata[1*], Valery Conejo-López[1], Álvaro Azofeifa-Delgado[1], Andrea Holst-Sanjuán[1], and Luis Barboza-Barquero[1]

Crop Breeding and Applied Biotechnology
26(1): e53722619, 2026
Brazilian Society of Plant Breeding.
Printed in Brazil
http://dx.doi.org/10.1590/1984-70332026v26n1n9

Abstract: *Accumulation of tocopherol and tocotrienol antioxidant compounds in rice seeds depends on enzymes involved in their biosynthetic pathways, including methionine adenosyltransferase (MAT). The aim of this study was to develop molecular markers for MAT gene* LOC_Os11g15410 *and evaluate associations between its polymorphisms and the accumulation of tocopherol and tocotrienol congeners in uncharacterized rice germplasm. Using high-resolution melting (HRM) analysis, we identified two consecutive SNPs (Chr11:8733700 bp and Chr11:8733701 bp) defining two haplotypes (AA and GG), and a novel 49-bp indel spanning from the second exon to the third intron. A panel comprising twelve rice samples and four* Oryza sativa L. *accessions was evaluated. The GG haplotype combined with the indel was associated with higher accumulation of δ and γ congeners, which are intermediates in the biosynthetic pathway. In contrast, the AA haplotype without the indel was associated with β and α congeners, which are end products of the MAT-mediated pathway.*

Keywords: *HRM, molecular markers,* Oryza sativa *L., tocopherols, tocotrienols*

INTRODUCTION

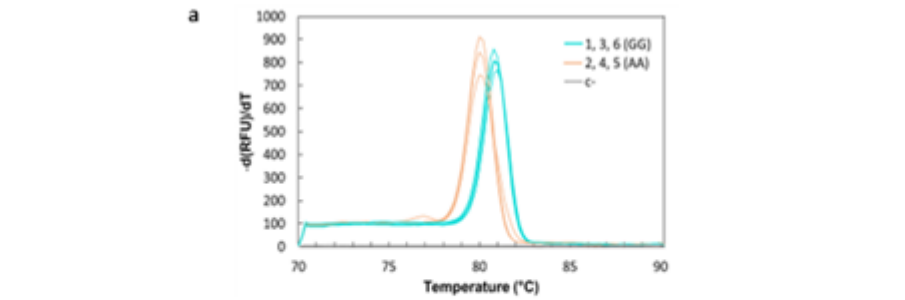
Tocopherols and tocotrienols refer to a group of eight lipid-soluble antioxidants characterized by a chromanol head and a saturated (tocopherols) or unsaturated (tocotrienols) side chain. The congener classification (α-, β-, γ-, δ-) depends on the number and position of methyl groups on the chromanol ring (Figure 1) (Galli et al. 2017). Tocopherol and tocotrienol biosynthesis in plants has been well described, with S-adenosylmethionine (SAM) acting as a methyl donor in key conversion steps from δ to β and from γ to α congeners (Supplementary Information, Figure S1) (Mène-Saffrané 2017). Tocopherols and tocotrienols are synthesized only by photosynthetic organisms, and they accumulate in seeds and oils (Munné-Bosch and Alegre 2002).

Due to the antioxidant capacity of tocopherols and tocotrienols, they have been suggested as a potential biomarker for seed longevity in rice. They act as a protective mechanism against oxidative stress in seeds during storage and contribute to extend seed longevity (Bailly 2004, Sano et al. 2016). However, allelic variations in genes involved in their biosynthesis may affect congener levels and function (Lee et al. 2017, Lee et al. 2019). Notably, SNPs in the gene that codifies the S-adenosylmethionine synthetase (SAM) – also known as methionine adenosyltransferase (MAT; *LOC_Os11g15410*) – the enzyme responsible for the

***Corresponding author:**
E-mail: andrea.iriasmata@ucr.ac.cr

Received: 8 September 2025
Accepted: 8 December 2025
Published: 16 January 2026

[1] University of Costa Rica, Faculty of Agricultural and Food Sciences, Research Center for Seeds and Grains (CIGRAS), 11501-2060, San Pedro, Costa Rica



d

Sample ID	SNPs on Chr11: 8733700 and 8733701 bp
1	CTTCGCCAGATGAGCTGAAACAGCTGGTGCAACGGAGCCA
2	CTTCGCCAGATGAGCTGAAACAGCTGGTGCAACAAAGCCA
3	CTTCGCCAGATGAGCTGAAACAGCTGGTGCAACGGAGCCA
4	CTTCGCCAGATGAGCTGAAACAGCTGGTGCAACAAAGCCA
5	CTTCGCCAGATGAGCTGAAACAGCTGGTGCAACAAAGCCA
6	CTTCGCCAGATGAGCTGAAACAGCTGGTGCAACGGAGCCA
ZS[1]	CTTCGCCAGATGAGCTGAAACAGCTGGTGCAACGGAGCCA
Nip[1]	CTTCGCCAGATGAGCTGAAACAGCTGGTGCAACAAAGCCA

[1] Sequence from NCBI database

Figure 1. HRM analysis of the rice (*Oryza sativa* L.) subspecies *indica*, *aromatic*, and *japonica* using a marker for SNPs at Chr11: 8733700 bp in the MAT gene (*LOC_Os11g15410*). **a.** Derivative melting curves of primer 5-2 showing two genotypes, with mean melting temperatures of 80 °C (orange curve – AA) and 81 °C (light blue curve – GG). **b.** Normalized melting plot and **c.** difference plot showing two clusters (I – 80 °C; II – 81 °C) of the two genotypes. **d.** Sequence alignment with the identified SNPs (orange and light blue font) at Chr11: 8733700 and 8733701 bp, corresponding to the HRM clusters (I – AA; II – GG).

synthesis of the SAM co-substrate, have been linked to altered β/δ-tocopherol ratios and seed longevity. Accessions carrying the G/G haplotype at positions 8732644 and 8733700 (chromosome 11) had β/δ ratios that were approximately 43% of those observed in A/A genotypes, and consequently showed extended seed longevity (Lee et al. 2019).

High-resolution melting (HRM) analysis is a PCR-based, rapid, and sensitive method for detecting SNPs and assessing genetic variation in plant species (Wu et al. 2010, Distefano et al. 2013, Shang et al. 2021, Wang et al. 2022, Khrustaleva et al. 2023). Although HRM has been widely used in plant genetics, it has not yet been applied to polymorphism detection in the MAT gene. Furthermore, evaluation of additional rice germplasm may reveal previously undetected mutations of functional relevance in tocopherol and tocotrienol biosynthesis. All this information is highly relevant for marker-assisted selection in plant breeding programs aiming to improve seed viability. Thus, the objective of this study was to develop HRM markers for the MAT gene associated with tocopherol and tocotrienol biosynthesis, identify novel sequence variants, and quantify these congeners in uncharacterized rice germplasm.

MATERIAL AND METHODS

Twelve commercial and experimental rice samples and four accessions of *Oryza sativa L.* (subspecies *indica*, *aromatic*, and *japonica*) were used in this study. Commercial samples (Senumisa 20 and Lazarroz FL, *indica*) were obtained from Senumisa, Costa Rica. Experimental samples (five *indica*, three *aromatic*, two *japonica*) designated by experimental codes (i.e., UCR-experiment number-year) were provided by the Research Center for Seeds and Grains, University of Costa Rica, and were harvested in December 2020 in Horquetas (lat 10° 21' 0.25" N, long 83° 55' 42.92" W, and alt 68 m asl), Sarapiquí, Costa Rica. The accessions Dom-sufid, Zhenshan, Azucena, and Nipponbare were obtained from the GSOR germplasm bank at the USDA-ARS, USA.

For DNA analysis, seeds were germinated under controlled conditions (30 °C, 100% relative humidity, and a 12-hour light / 12-h dark photoperiod). DNA was extracted from freeze-dried seedling leaves following a previously determined CTAB protocol (Brandfass and Karlovsky 2008), with chloroform-isoamyl alcohol purification, isopropanol precipitation, ethanol washing, and resuspension in TE buffer. DNA concentration and purity were determined via nanophotometry (Implen, CA, USA).

HRM primers (Supplementary Information, Table S1) targeting the SNP at Chr11:8733700 in the MAT gene (*LOC_Os11g15410*) (Lee et al. 2019) were designed using the Nipponbare reference genome (NCBI database) and Primer-BLAST, and were synthesized by Macrogen, Inc (South Korea). Additional primers (1-4 and 1-7) were designed to detect a 49-bp deletion (gap) found during sequencing.

HRM analysis was performed using primers 5-2 on a Bio-Rad CFX real-time PCR system with MeltDoctor HRM Master Mix. The PCR protocol included initial denaturation (95 °C, 10 min), followed by 35 cycles of denaturation (95 °C, 15 s; 62 °C, 30 s; 72 °C, 30 s), and a melting curve from 70 °C to 90 °C with 0.2 °C increments. The HRM analysis was performed using the Bio-Rad Precision Melt Analysis Software. Sequencing of PCR products (Macrogen, Inc, South Korea) confirmed SNP genotypes. Sequence alignment and quality control were performed using ClustalW multiple alignment in the software Bioedit (USA) in comparison with Nipponbare (*Oryza sativa* Japonica group-IRGSP-1.0) and Zhenshan (*Oryza sativa* Indica group-IRGSP-1.0) reference sequences, available in the NCBI database.

The 49-bp deletion (Chr11: 8733111–8733160) between exon 2 and intron 3 (with exon positions confirmed in the NCBI and Phytozome databases) was validated using primers 1-4 and 1-7 by real-time PCR with SYBRGreen and by endpoint PCR, followed by electrophoresis on 1% agarose gel in 1X TBE buffer (100 V, 1.5 h).

The real-time PCR protocol included initial denaturation (95 °C, 10 min), followed by 35 cycles of denaturation (95 °C, 15 s; 62 °C, 40 s; 72 °C, 2 min), and a melting curve from 70 °C to 90 °C with 0.2 °C increments (CFX Real Time PCR System; Bio-Rad, USA). The endpoint PCR protocol began with initial denaturation (95 °C, 3 min), followed by 35 cycles of denaturation (95 °C, 30 s; 62 °C, 40 s; 72 °C, 2 min), plus a final extension step at 72 °C for 10 minutes (Mastercycler Nexus Gradient Flexlid; Eppendorf, Germany).

Tocopherols and tocotrienols were extracted as previously described (Conejo-López et al. 2022) from rice seeds obtained from the field, which were subsequently ground. Samples were saponified, extracted with *n*-hexane, dried under vacuum, and resuspended in 250 µL of methanol. The extracts were membrane filtered (0.20 µm PTFE) and transferred to amber HPLC vials prior to analysis.

Chromatographic separation and analysis of tocopherols and tocotrienols were performed as previously described (Conejo-López et al. 2022) using an ultra-high-performance liquid chromatography (UHPLC) system coupled to a triple quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization source operated in negative mode (APCI(-)-MS, Ultimate 3000, series TQH-E1-0288, TSQ Endura, Thermo Fisher Scientific, Waltham, MA, USA). Compounds were identified and quantified using authentic tocopherol and tocotrienol standards (Sigma-Aldrich, St Louis, MO, USA).

Concentrations of tocopherol and tocotrienol congeners are expressed as means ± standard deviation. Significant differences among means were determined using one-way ANOVA, followed by Tukey's multiple comparison test ($p < 0.05$). Additionally, boxplots and Kruskal–Wallis tests were used to compare congener and total tocopherol and tocotrienol concentrations based on the SNP genotype (Chr11:8733700) in the MAT gene. Statistical analyses were performed using the *multcomp* and *ggbiplot* packages in RStudio (2023.06.0).

RESULTS AND DISCUSSION

High-resolution melting (HRM) analysis using primer set 5-2 successfully discriminated two clusters based on melting temperature: Cluster I (orange curve) at 80 °C and Cluster II (light blue curve) at 81 °C (Fig. 1A). Rice samples 2, 4, and 5, corresponding to two *indica* genotypes (UCR-200-18 and UCR-154-10) and one *japonica* genotype (UCR-205-18), were grouped in Cluster I; while rice samples 1, 3, and 6, corresponding to two *indica* genotypes (UCR-01-08 and Senumisa 20) and one *aromatic* genotype (UCR-167-10), were grouped in Cluster II (Figure 1a, b, c). Sequencing of the amplified region revealed two SNPs at positions Chr11:8733700 and 8733701 bp. Cluster I corresponded to the AA haplotype, and Cluster II to the GG haplotype (Figure 1d). This confirms that HRM is an effective, non-sequencing method to detect SNPs in the MAT gene (*LOC_Os11g15410)*, which encodes the methionine adenosyltransferase (MAT or SAM) enzyme. The technique is effective for marker-assisted selection and rapid genotyping in rice (Li et al. 2011, Li et al. 2018, Grazina et al. 2022), helping identify candidate genes associated with traits such as purple glume tips (Jin et al. 2024). Studies

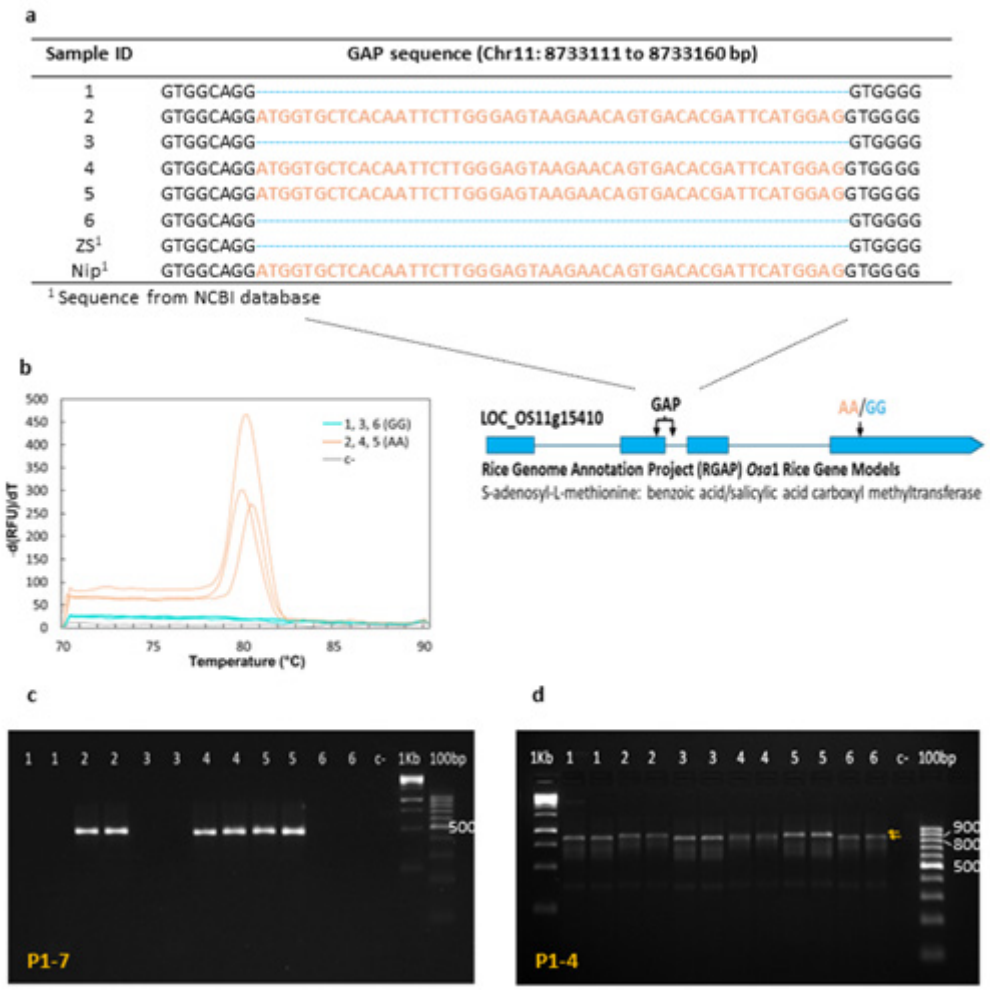


Figure 2. Indel analysis of the rice (*Oryza sativa* L.) subspecies *indica*, *aromatic*, and *japonica* using the markers within the indel at Chr11: 8733111 to 8733160 bp in the MAT gene (*LOC_Os11g15410*). **a.** Sequence alignment showing the presence or absence of the indel (bp in orange font) in the gene relative to the SNP positions. **b.** Derivative melting curves with primers 1-7 showing three rice samples with amplification (without indel, samples 2, 4, and 5) and three rice samples without amplification (with indel, samples 1, 3, and 6). **c.** Electrophoresis of primers 1-7 confirming the absence/presence of the indel seen in the melting curves. **d.** Electrophoresis of primers 1-4 showing a difference of 49 bp between the rice samples without and with the indel.

using ISSR and SRAP markers have also shown significant genetic diversity in Indonesian rice, highlighting the value of exploiting germplasm diversity for rice breeding (Rini et al. 2023).

During SNP screening, a previously unreported 49 bp indel was identified between positions 8733111 and 8733160 bp, spanning from the second exon into the third intron. This indel was confirmed by sequencing using primers 1-4 and validated by PCR using primers 1-7, which only led to amplification in samples without the deletion (Figure 2b, c). Electrophoresis of PCR products with primers 1-4 further confirmed a 49 bp size difference between haplotypes (Figure 2d). A gene model (Figure 2a) illustrated the spatial relationship between the SNPs and the indel, which were found to co-occur consistently across all tested genotypes.

Translation of the gapped sequence using ExPASy (Swiss Institute of Bioinformatics; https://www.expasy.org/) revealed a frameshift mutation leading to the loss of seven amino acids: methionine, valine, leucine, threonine, isoleucine, leucine, and glycine (MVLTILG) and the introduction of a premature stop codon, likely truncating the MAT protein. As the SNPs and the indel are physically close, they appear to be inherited as a linked haplotype block (Lübberstedt et al. 2023) This structural mutation may impair MAT function, thereby affecting the methylation steps of tocopherol and tocotrienol biosynthesis.

A strong correlation was observed between the SNP haplotypes and the indel. The AA haplotype (Cluster I) was always associated with the intact sequence, whereas the GG haplotype (Cluster II) always included the indel. This association was consistent in six additional rice genotypes and four reference accessions (Dom-Sufid, Zhenshan, Azucena, and Nipponbare), regardless of subspecies (Table 1). The stability of this association reinforces the conclusion that the SNPs and the indel are part of a shared haplotype.

Seven tocopherol and tocotrienol congeners were detected across all samples, with γT3 being the most abundant (Table S2). Rice genotypes with the GG haplotype showed significantly higher levels of γT3, γT, and δT, whereas αT3 was more abundant in AA haplotype genotypes. Total tocopherol and tocotrienol content was significantly greater in GG genotypes ($p \leq 0.05$), and the β/δ-tocopherol ratio was higher in AA genotypes (Figure 3).

This pattern suggests that the intact MAT gene in AA genotypes allows for efficient methylation of δ to β congeners and γ to α forms, consistent with the established role of SAM in these reactions (Mène-Saffrané 2017). In contrast, the indel and resulting frameshift in GG genotypes may disrupt MAT activity, reducing conversion efficiency and causing accumulation of intermediate isomers.

Table 1. Association between SNP nucleotides classified by HRM into two clusters based on mean melting temperatures and indel presence on Chr11 of the MAT gene (*LOC_Os11g15410*) in twelve rice samples and four accessions (*Oryza sativa* L.)

Sample ID	Sample name	Subspecies	SNP haplotype	Mean melting temperature (°C)	HRM cluster	Indel presence
1	UCR-01-08	*indica*	GG	80.8 (81)	II	Yes
2	UCR-200-18	*indica*	AA	79.8 (80)	I	No
3	UCR-167-10	*aromatic*	GG	80.6 (81)	II	Yes
4	UCR-154-10	*indica*	AA	79.8 (80)	I	No
5	UCR-205-18	*japonica*	AA	79.8 (80)	I	No
6	Senumisa 20	*indica*	GG	80.6 (81)	II	Yes
7	UCR-170-15	*aromatic*	AA	80.3 (80)	I	No
8	UCR-203-18	*indica*	GG	81.5 (82)	II	Yes
9	UCR-03-08	*indica*	GG	81.4 (81)	II	Yes
10	UCR-162-10	*aromatic*	GG	81.5 (82)	II	Yes
11	Lazarroz FL	*indica*	GG	81.5 (82)	II	Yes
12	UCR-208-18	*japonica*	GG	81.5 (82)	II	Yes
DS	Dom-sufid	*aromatic*	AA	80.3 (80)	I	No
ZS	Zhenshan	*indica*	GG	81.5 (82)	II	Yes
AZ	Azucena	*japonica*	AA	80.4 (80)	I	No
Nip	Nipponbare	*japonica*	AA	80.6 (81)	I	No

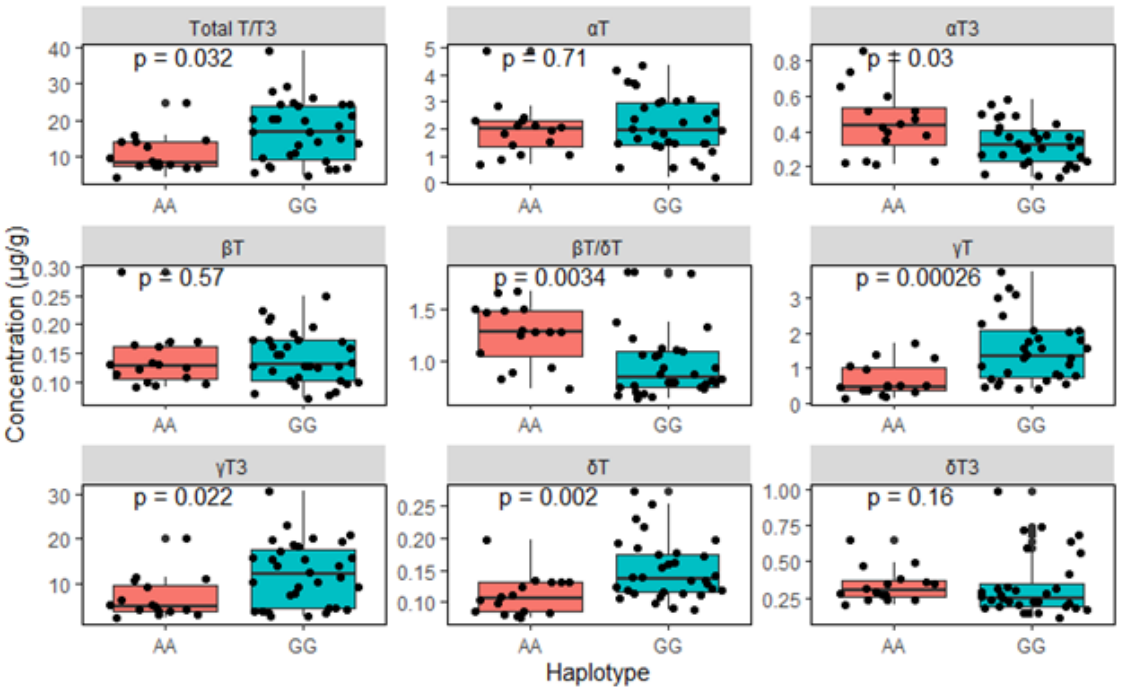


Figure 3. Boxplots comparing mean concentrations of each tocopherol and tocotrienol congener (α, β, γ, and δ tocopherols and tocotrienols), the β/γ tocopherol ratio, and total tocopherols (T) and tocotrienols (T3) with the nucleotides identified in the SNPs (Chr11: 8733700 bp and 8733701 bp) in the MAT gene (*LOC_Os11g15410*) of the twelve rice (*Oryza sativa* L.) samples. A P value < 0.05 indicates significant differences between mean concentrations.

A previous study (Lee et al. 2019) linked non-synonymous mutations in *LOC_Os11g15410* to changes in congener ratios. Our results support these findings and add the discovery of the 49 bp indel. Our results further suggest that the newly described indel may impair full protein translation, thereby contributing to altered congener profiles. However, functional validation should be performed to confirm this effect. Nevertheless, since all congeners are present to some extent, other MAT genes may partially compensate, as six congeners have been reported in rice (Lee et al. 2019). Future studies should investigate the functional redundancy among these MAT genes or apply gene-editing tools to manipulate tocopherol and tocotrienol profiles in rice breeding lines.

CONCLUSION

We identified a molecular marker based on HRM that distinguishes two SNPs in the MAT gene (*LOC_Os11g15410*) on Chr11 and detected a novel 49 bp indel that may affect MAT protein translation. Rice genotypes with the GG haplotype and the indel accumulated intermediate tocopherol and tocotrienol congeners, whereas AA haplotype genotypes without the indel favored end-product isomers, suggesting a role of MAT in the final step of biosynthesis. Although this study used a representative rice panel with the most common subspecies of *O. sativa*, the observed genotype-phenotype associations between MAT gene variations and tocopherols and tocotrienols require validation in larger and more genetically diverse populations. Further research should investigate the relationship between tocopherols and tocotrienols and seed longevity, as these compounds are considered potential biomarkers for this trait and could be a useful tool in breeding programs.

ACKNOWLEDGEMENTS

We gratefully acknowledge SENUMISA Company for supplying the commercial rice materials for this research. We would like to thank the Genetic Stocks Oryza (GSOR) germplasm bank of the Dale Bumpers National Rice Research

Center of the USDA Agricultural Research Service, Arkansas, USA for proving the cited germplasm. This research was performed with the financial support of the Vice Rector's Office for Research of the University of Costa Rica (Project number 734-C0-213).

DATA AVAILABILITY

All data generated or analyzed during this study are included in the article or in its supplementary information files, which are available from the corresponding author upon request.

REFERENCES

Bailly C (2004) Active oxygen species and antioxidants in seed biology. **Seed Science Research 14**: 93-107.

Brandfass C and Karlovsky P (2008) Upscaled CTAB-based DNA extraction and real-time PCR assays for *Fusarium culmorum* and *F. graminearum* DNA in plant material with reduced sampling error. **International Journal of Molecular Sciences 9**: 2306-2321.

Conejo-López V, Barboza-Barquero L, Azofeifa-Delgado Á, Vargas-Ramírez E and Irías-Mata A (2022) Perfil de vitamina E en semillas de variedades de arroz (*Oryza sativa* L.) cultivadas y comercializadas en Costa Rica. **Agronomía Mesoamericana** 51283-51283.

Distefano G, La Malfa S, Gentile A and Wu SB (2013) EST-SNP genotyping of citrus species using high-resolution melting curve analysis. **Tree Genetics & Genomes 9**: 1271-1281.

Galli F, Azzi A, Birringer M, Cook-Mills JM, Eggersdorfer M, Frank J, Cruciani G, Lorkowski S and Özer NK (2017) Vitamin E: Emerging aspects and new directions. **Free Radical Biology and Medicine 102**: 16-36.

Grazina L, Costa J, Amaral JS, Garino C, Arlorio M and Mafra I (2022) Authentication of carnaroli rice by HRM analysis targeting nucleotide polymorphisms in the Alk and Waxy genes. **Food Control 135**: 108829.

Jin G, Cai Z, Chen Y, Ling Y, Wang L and Mo D (2024) Genetic analysis and gene mapping of the purple glume tip trait in rice (*Oryza sativa*). **Crop Breeding and Applied Biotechnology 24**: e47842431.

Khrustaleva L, Nzeha M, Ermolaev A, Nikitina E and Romanov V (2023) Two-step identification of N-, S-, R- and T-cytoplasm types in onion breeding lines using high-resolution melting (HRM)-based markers. **International Journal of Molecular Sciences 24**: 1605.

Lee J-S, Kwak J, Cho J-H, Chebotarov D, Yoon M-R, Lee Jeom-Sig, Hamilton NRS and Hay FR (2019) A high proportion of beta-tocopherol in vitamin E is associated with poor seed longevity in rice produced under temperate conditions. **Plant Genetic Resources 17**: 375-378.

Lee J-S, Kwak J, Yoon M-R, Lee Jeom-Sig and Hay FR (2017) Contrasting tocol ratios associated with seed longevity in rice variety groups. **Seed Science Research 27**: 273-280.

Li J, Wang X, Dong R, Yang Y, Zhou J, Yu C, Cheng Y, Yan C and Chen J (2011) Evaluation of high-resolution melting for gene mapping in rice. **Plant Molecular Biology Reporter 29**: 979-985.

Li S, Liu S, Fu H, Huang J and Shu Q (2018) High-resolution melting-based TILLING of γ ray-induced mutations in rice. **Journal of Zhejiang University-SCIENCE B 19**: 620-629.

Lübberstedt T, Campbell A, Muenchrath D, Merrick L and Fei S (2023) Chapter 5: Linkage. In Suza W and Lamkey K (eds) **Crop genetics**. Iowa State University Digital Press, Iowa, 313p.

Mène-Saffrané L (2017) Vitamin E biosynthesis and its regulation in plants. **Antioxidants 7**: 2.

Munné-Bosch S and Alegre L (2002) The function of tocopherols and tocotrienols in plants. **Critical Reviews in Plant Sciences 21**: 31-57.

Rini DS, Budiyanti Y, Valentine M and Permana R (2023) ISSR and SRAP for assessing genetic variability of Indonesian local rice genotypes (*Oryza sativa* L.). **Crop Breeding and Applied Biotechnology 23**: e448923411.

Sano N, Rajjou L, North HM, Debeaujon I, Marion-Poll A and Seo M (2016) Staying alive: Molecular aspects of seed longevity. **Plant and Cell Physiology 57**: 660-674.

Shang Z, Zhu Y, Guo X and Zhao M (2021) Identification of polymorphic markers by high-resolution melting (HRM) assay for high-throughput SNP genotyping in maize. **Phyton 90**: 1711-1725.

Wang Y, Song L, Zhao L, Yu W and Zhao T (2022) Development of a gene-based high resolution melting (HRM) marker for selecting the gene ty-5 conferring resistance to tomato yellow leaf curl virus. **Horticulturae 8**: 112.

Wu S-B, Franks TK, Hunt P, Wirthensohn MG, Gibson JP and Sedgley M (2010) Discrimination of SNP genotypes associated with complex haplotypes by high resolution melting analysis in almond: implications for improved marker efficiencies. **Molecular Breeding 25**: 351-357.