

Short Communication

A high proportion of beta-tocopherol in vitamin E is associated with poor seed longevity in rice produced under temperate conditions

Jae-Sung Lee¹, Jieun Kwak², Jun-Hyeon Cho², Dmytro Chebotarov¹, Mi-Ra Yoon², Jeom-Sig Lee², N. Ruairaidh Sackville Hamilton¹ and Fiona R. Hay^{1,3*}

¹International Rice Research Institute, Los Baños, College, Laguna 4031, Philippines, ²National Institute of Crop Science, Rural Development Administration, Jeonju, Jeollabuk-do, Republic of Korea and ³Department of Agroecology, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark

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Abstract

Seeds of 15 diverse rice accessions, representing *aus*, *indica*, *temperate japonica* and *tropical japonica* subpopulations, were produced under temperate climate conditions in Korea and used for vitamin E analysis and seed storage experiments at 45°C and 10.9% seed moisture content. High γ -tocotrienol was significantly positively correlated with seed longevity. In addition, a high β -tocopherol proportion relative to δ -tocopherol was significantly negatively correlated with seed longevity. Using high-density single-nucleotide polymorphism marker data, DNA haplotype analysis showed clear allelic variations in the region of two S-adenosylmethionine synthetase genes: LOC_Os04g42095 and LOC_Os11g15410, which regulate the conversion of δ -tocopherol into β -tocopherol. Four *indica* accessions with rare and subpopulation-specific alleles showed a 2.3-fold lower β -/ δ -tocopherol ratio compared with accessions from other subpopulations.

Keywords: β -tocopherol, rice, seed longevity, vitamin E

Introduction

During seed storage, lipid peroxy radicals cause membrane breakdown and cell ageing. Vitamin E, a group of eight homologues (four tocopherols and four tocotrienols), is a strong antioxidant that scavenges lipid peroxy radicals, hence they play a role in seed longevity (Sattler *et al.*, 2004; Chen *et al.*, 2015; Lee *et al.*, 2017). Our previous study using a diverse rice panel grown in the Philippines

indicated that the ratio of different vitamin E homologues rather than total vitamin E content is crucial in extending seed longevity (Lee *et al.*, 2017). In particular, a high proportion of γ -tocotrienol was correlated with high seed longevity. A better understanding of the molecular basis of seed longevity may help improve longevity and/or identify varieties that are short-lived in storage. We were therefore interested in understanding whether the relationship between vitamin E homologue ratios and seed longevity is still apparent when seeds are produced under different environmental conditions. In this study, we used seeds produced under temperate conditions to assess the correlation between seed longevity and vitamin E.

*Corresponding author. E-mail: fiona.hay@agro.au.dk

Table 1. Correlation coefficients (*r*) between seed longevity parameters and proportions of vitamin E homologues for 15 diverse rice accessions.

Seed longevity parameter	Tocopherol				Tocotrienol				β -/ δ -tocopherol ratio
	α -T	β -T	γ -T	δ -T	α -T ₃	β -T ₃	γ -T ₃	δ -T ₃	
K_i	-0.387 ^{ns}	-0.533*	0.358 ^{ns}	0.220 ^{ns}	-0.110 ^{ns}	0.097 ^{ns}	0.214 ^{ns}	-0.036 ^{ns}	-0.557*
Slope	-0.131 ^{ns}	-0.046 ^{ns}	0.209 ^{ns}	0.425 ^{ns}	0.163 ^{ns}	0.384 ^{ns}	-0.162 ^{ns}	0.201 ^{ns}	-0.279 ^{ns}
p_{50}	-0.477 ^{ns}	-0.740**	0.384 ^{ns}	-0.020 ^{ns}	-0.406 ^{ns}	-0.152 ^{ns}	0.516*	-0.061 ^{ns}	-0.567*

* and **, significant at $P < 0.05$ and 0.01 , respectively. ns, not significant ($P > 0.05$).

Experimental

Seeds of 20 *Oryza sativa* accessions held in the T.T. Chang Genetic Resources Center at the International Rice Research Institute (IRRI), representing the five subpopulations of Indica and Japonica Variety Groups (Supplementary Table S1; IRRI, 2006a–q, 2007a–c) were grown in the experimental field of the Rural Development Administration (RDA), Republic of Korea (N35° latitude and E128° longitude) in 2016. During seed production, mean temperature and rainfall per day were 23.7°C and 6.3 mm, respectively. After dormancy-breaking (50°C, 5 d), seeds were germinated in seedling boxes and 30-d-old seedlings transplanted on 5 June 2016, with 300 × 150 mm² spacing. Fertilizer, insect, disease and weed control were according to standard procedures. Five accessions did not flower. Seeds of the other 15 accessions were harvested between 50 and 56 d after heading and dried at 25°C, 30–40% relative humidity (RH). A sample of 300 g seeds of each accession were sent to IRRI for seed storage experiments; remaining seeds were used for vitamin E analysis. Eight vitamin E homologues (α -, β -, γ -, δ -tocopherol/tocotrienol) in lipid extracts of brown rice were analysed by ultra-performance liquid chromatography (H-Class system, Waters, USA; Ko *et al.*, 2003). Individual homologues were determined at 298 nm excitation and 325 nm emission with a Lichrospher Si-60 column (250 × 4.6 mm i.d.; Merck Co. Germany) and quantified based on retention time and amounts of standards (Darmstadt, Germany; Helios, Singapore). Seed longevity parameters were determined at IRRI through standard storage experiments (Hay *et al.*, 2015, 2018). After moisture content (MC) equilibration at 20°C, 60% RH, seeds were heat-sealed inside aluminium foil bags and placed at 45°C. Samples were taken at 3-d intervals and tested for the ability to germinate on two layers of Whatman No. 1 filter paper in 90 mm-diameter Petri dishes. Additional samples were used for monitoring MC: ground seeds were weighed before and after 2 h oven-drying at 130°C (ISTA, 2018). The mean MC was 10.3% (mean of all accessions and sample times). Germination data were analysed by probit analysis in GenStat v. 18 (VSN International Ltd., Hemel

Hempstead, UK). Correlations between seed longevity parameters and proportion of vitamin E homologues were determined using STAR v2.0.1 (IRRI). Genomic information in the region of vitamin E-related genes were extracted from the 700k high-density single-nucleotide polymorphism (SNP) marker data (McCouch *et al.*, 2016).

The estimate for K_i (initial viability in normal equivalent deviates (NED)) ranged between 1.10 and 10.90 NED (mean 5.66 NED; Supplementary Table S1). The slope of the survival curves, $-1/\sigma$, ranged between 0.18 and 0.44 NED/d (mean 0.16 NED/d). Time for viability to fall to 50%, p_{50} , ranged between 5.73 and 34.58 d (mean 18.75 d). Total vitamin E content varied between 9.53 and 21.66 mg/kg brown rice (mean 15.80 mg/kg). The proportion of γ -tocotrienol was significantly positively correlated with p_{50} ($r = 0.516$; $P < 0.05$) (Table 1). In contrast, the proportion of β -tocopherol was significantly negatively correlated with K_i ($r = -0.533$; $P < 0.05$) and p_{50} ($r = -0.740$; $P < 0.01$). There was no significant correlation between $-1/\sigma$ and any of the vitamin E homologues. Using the public rice genomes database (Mansueto *et al.*, 2017; Rice SNP-Seek Database: <http://snp-seek.irri.org/>, latest access on 26 January 2019), we determined the DNA haplotype of 12 accessions in the region of six known S-adenosylmethionine synthetase (SAM) genes which regulate the conversion of δ -tocopherol into β -tocopherol (Sattler *et al.*, 2004). The association between haplotype and β -/ δ -tocopherol ratio was assessed. Clear allelic variations were observed in the region of two SAM genes: LOC_Os04g42095 and LOC_Os11g15410. Four accessions belonging to the indica subpopulation with the favourable haplotype (shaded green in Fig. 1) showed a 2.3-fold lower β -/ δ -tocopherol ratio (mean 0.49) compared with the eight accessions having the unfavourable haplotype (shaded grey) (mean 1.11).

Discussion

Consistent with Lee *et al.* (2017), the proportion of γ -tocotrienol relative to other vitamin E homologues in the caryopses was significantly positively correlated with seed

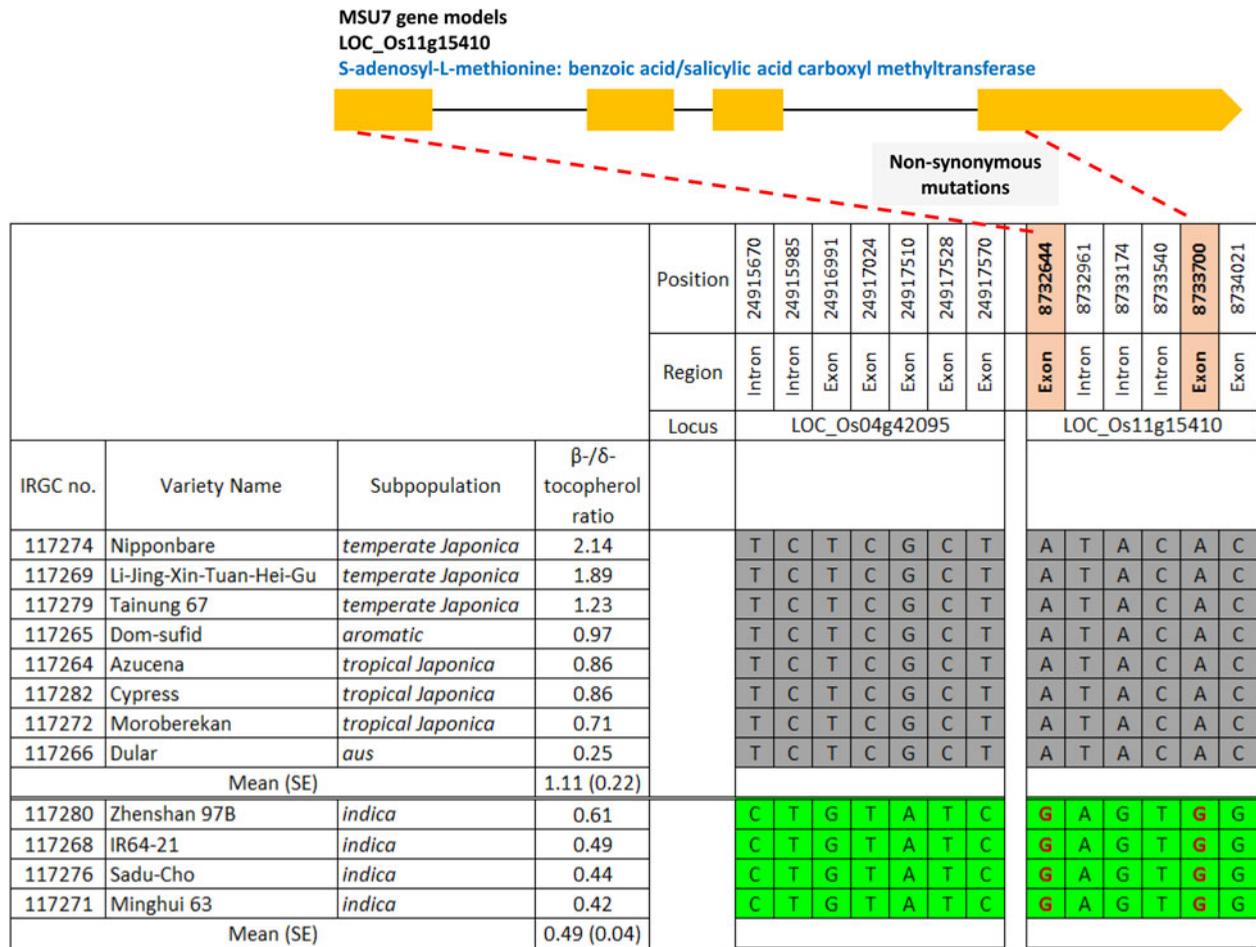


Fig. 1. Haplotype analysis on two S-adenosylmethionine synthetase genes regulating δ -tocopherol conversion into β -tocopherol for 12 rice accessions (IRRI, 2006a-c, e-f, h-i, k, m, p-q; 2007, b). Green shading indicates the favourable haplotype, grey shading the unfavourable haplotype; non-synonymous mutations are shown in red font. Three accessions, IRGC 117270, 117273 and 117277 (IRRI 2006g, j, n), were not included in the high-density SNP data set.

longevity (p_{50}). Further, a high proportion of β -tocopherol relative to δ -tocopherol was significantly negatively correlated with K_i and p_{50} . Vitamin E α -/ β - homologues have inefficient molecular structures for scavenging free radicals compared with γ -/ δ - homologues (Jiang, 2014; Kim 2014; Lee *et al.*, 2017). LOC_Os11g15410 is annotated to S-adenosyl-L-methionine: benzoic acid/salicylic acid carboxyl methyltransferase, but its network function has not been validated. In four *indica* accessions with high seed longevity, there were two non-synonymous mutations (positions 8,732,644 and 8,733,700). An insertion at position 8,733,744 and a deletion at position 8,733,747 may be linked to the mutation at position 8,733,700 (Rice SNP-Seek Database: <http://snp-seek.irri.org/>, last accessed 26 January 2019). These functional changes might be responsible for inhibition of δ -/ β -tocopherol conversion resulting in less β -tocopherol and greater seed longevity. This hypothesis needs validating since the favourable haplotype was specific to the *indica* subpopulation and all

other groups have the unfavourable haplotype. Further research such as transgenic experiments could be used to validate gene function. A better understanding of the molecular basis for seed longevity will facilitate screening of genebank accessions for this trait and ultimately, rationalization of retest intervals.

Supplementary material

The supplementary material for this article can be found at <https://doi.org/10.1017/S147926211900008X>.

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References

- Chen D, Li Y, Fang T, Shi X and Chen X (2015) Specific roles of tocopherols and tocotrienols in seed longevity and germination tolerance to abiotic stress in transgenic rice. *Plant Science* 244: 31–39.
- Hay FR, Timple S and van Duijn B (2015) Can chlorophyll fluorescence be used to determine the optimal time to harvest rice seeds for long-term genebank storage? *Seed Science Research* 25: 321–334.
- Hay FR, Valdez R, Lee J-S and Sta Cruz PC (2018) Seed longevity phenotyping: recommendations on research methodology. *Journal of Experimental Botany* 70: 425–434.
- IRRI (2006a) Data on IRGC 117264. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZFFQ>.
- IRRI (2006b) Data on IRGC 117265. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/1PG6J>.
- IRRI (2006c) Data on IRGC 117266. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZGR>.
- IRRI (2006d) Data on IRGC 117267. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZHS>.
- IRRI (2006e) Data on IRGC 117268. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZJT>.
- IRRI (2006f) Data on IRGC 117269. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZKV>.
- IRRI (2006g) Data on IRGC 117270. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZMW>.
- IRRI (2006h) Data on IRGC 117271. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZNX>.
- IRRI (2006i) Data on IRGC 117272. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZPY>.
- IRRI (2006j) Data on IRGC 117273. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZQZ>.
- IRRI (2006k) Data on IRGC 117274. Global Information System of the International Treaty on Plant Genetic Resources. https://doi.org/10.17830/4WZR*.
- IRRI (2006l) Data on IRGC 117275. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZS~>.
- IRRI (2006m) Data on IRGC 117276. Global Information System of the International Treaty on Plant Genetic Resources. [https://doi.org/10.17830/4WZT\\$](https://doi.org/10.17830/4WZT$).
- IRRI (2006n) Data on IRGC 117277. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZV=>.
- IRRI (2006o) Data on IRGC 117278. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZWU>.
- IRRI (2006p) Data on IRGC 117279. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZXO>.
- IRRI (2006q) Data on IRGC 117280. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/4WZY1>.
- IRRI (2007a) Data on IRGC 117281. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/5CMYC>.
- IRRI (2007b) Data on IRGC 117282. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/5CMZD>.
- IRRI (2007c) Data on IRGC 117283. Global Information System of the International Treaty on Plant Genetic Resources. <https://doi.org/10.17830/5CN0E>.
- ISTA (2018) International rules for seed testing 2018. The International Seed Testing Association, Bassersdorf, Switzerland.
- Jiang Q (2014) Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radical Biology and Medicine* 72: 76–90.
- Kim HJ (2014) Effect of α -, β -, γ -, and δ -Tocotrienol on the oxidative stability of lard. *Journal of the American Oil Chemists' Society* 91: 777–782.
- Ko SN, Kim CJ, Kim H, Kim CT, Chung SH, Tae BS and Kim IH (2003) Tocol levels in milling fractions of some cereal grains and soybean. *Journal of the American Oil Chemists' Society* 80: 585–589.
- Lee J-S, Kwak J, Yoon M-R, Lee J-S and Hay FR (2017) Contrasting tocol ratios associated with seed longevity in rice sub-populations. *Seed Science Research* 27: 273–280.
- Mansueto L, Fuentes RR, Borja FN, Detras J, Abriol-Santos JM, Chebotarov D, Sanciango M, Palis K, Copetti D, Poliakov A, Dubchak I, Solovyev V, Wing RA, Hamilton RS, Mauleon R, McNally KL and Alexandrov N. (2017) Rice SNP-seek database update: new SNPs, indels, and queries. *Nucleic Acids Research* 45: D1075–D1081.
- McCouch SR, Wright MH, Tung CW, Maron LG, McNally KL, Fitzgerald M, Singh N, DeClerck G, Perez FA, Korniliev P, Greenberg AJ, Naredo MEB, Mercado SMQ, Harrington SE, Shi YX, Branchini DA, Kuser-Falcao PR, Leung H, Ebana K, Yano M, Eizenga G, McClung A and Mezey J (2016). Open access resources for genome-wide association mapping in rice. *Nature Communications* 7: 11346.
- Sattler SE, Gilliland LU, Magallanes-Lundback M, Pollard M and DellaPenna D (2004) Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. *The Plant Cell* 16: 1419–1432.