A large deletion within intron 20 sequence of single-copy *PolA1* gene as a useful marker for the speciation in *Oryza* AA-genome species

Aung Htut Htet¹, So Makabe², Hiroko Takahashi³, Poku Aduse Samuel¹, Yo-ichiro Sato³ and Ikuo Nakamura*¹

¹) Graduate School of Horticulture, Chiba University, Matsudo, Chiba 271-8510, Japan  
²) BEX Co. Ltd., Itabashi-ku, Tokyo 173-0004, Japan  
³) Kyoto Washoku Institute, Kyoto Prefectural University, Kyoto 606-8522, Japan

*Oryza* AA-genome complex comprises five wild species, *O. rufipogon*, *O. barthii*, *O. longistaminata*, *O. glumaepatula*, and *O. meridionalis*. Evolutionary relationships among these five wild species have remained contentious and inconclusive. We found that intron 20 of *PolA1*, a single-copy nuclear gene, was short (S-type: 141–142 bp) in *O. rufipogon*, *O. barthii*, and *O. glumaepatula*, while long (L-type: ca. 1.5 kb) introns were apparent in *O. longistaminata* and *O. meridionalis*. Because *Oryza* species containing BB, CC, EE, FF, and GG genome showed L-type introns, the S-type intron was probably derived from the L-type intron by the deletion of a 1.4 kb fragment through intramolecular homologous recombination between two tandem TTTTGC repeats. Excluding the large deletion sequence, intron 20 sequence of *O. barthii* was identical to that of *O. longistaminata*. As more than 3,470 accessions of *O. rufipogon* and *O. sativa* also contained the same intron 20 sequence with *O. longistaminata* except for single T-nucleotide deletion, which was shared with *O. glumaepatula*, the deletion of the T-nucleotide probably occurred in the L-type intron 20 of *O. logistaminata*. Deletions of a large 1.4 kb fragment and single T-nucleotide within the intron 20 of *PolA1* gene were considered as useful DNA markers to study the evolutionary relationships among *Oryza* AA-genome species.

**Key Words:** AA-genome species, deletion, *Oryza*, RNA polymerase I largest subunit, single-copy nuclear gene, speciation.

---

**Introduction**

The genus *Oryza* (2n = 24 to 48) comprises 24 wild species representing 11 genomes: AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, HHKK and KLLL. It has two cultivated species, *O. sativa* L. and *O. glaberrima* Steud, while the other five species: *O. rufipogon* (including *O. nivara*), *O. barthii*, *O. longistaminata*, *O. glumaepatula* and *O. meridionalis* are regarded as wild species in the AA-genome in *Oryza sativa* complex (Ge et al. 1999, Vaughan 1994). The wild species of the AA-genome have been recognized as genetic resources for various kinds of useful genes to improve cultivated rice (Jena 2010).

Two cultivated rice species, *O. sativa* in Asia and *O. glaberrima* in Africa, are thought to be originated from *O. rufipogon* and *O. barthii*, respectively (Morishima et al. 1992, Oka 1988). Previous study by Second (1985) pointed out that *O. longistaminata* and *O. meridionalis* represented two distinct species. On the other hand, *O. rufipogon*, *O. barthii*, and *O. glumaepatula* were closely related based on isozyme analysis. Many phylogenetic studies of *Oryza* species have been performed with several DNA molecular markers, such as RFLP (Wang et al. 1992), RAPD (Ishii et al. 1996), AFLP (Aggarwal et al. 1999), rDNA spacer (Cordesse et al. 1992), transposon (Kanazawa et al. 2000), catalase gene (Iwamoto 2000), and genome wide sequence analysis (Zhu and Ge 2005); the detailed phylogenetic relationships of AA-genome species are still inconsistent among many studies. Guo and Ge (2005) reported that monophyly in Oryzeae was strongly supported by either individual or combined analyses of both cytoplasmic and nuclear sequence in tribe level. Reconstruction of a phylogenetic tree based on combinations of sequence data from different sources such as plastid, mitochondrial and nuclear DNA produced complicated phylogenetic relationships among wild AA-genome species (Duan et al. 2007).

We are interested in the involvement of *PolA1* gene with speciation because it encodes species-specific protein tag
sequence not only in plants but also animals, fungi, and protists (Nakamura 2016). The PolA1, single-copy nuclear gene, encodes the largest subunit of RNA polymerase I that plays an essential role in 45S rRNA transcription (Seither et al. 1997). PolA1 consists of 21 exons and spans approximately 15.0 kb on chromosome 6 in Oryza sativa subsp. japonica ‘Nipponbare’ (LOC_Os06g40950 and The Rice Annotation Project Database: Os06g0612200 in Rice Genome Annotation Project, Kawahara et al. 2013). Recently, particular DNA sequences from exons 19 to 21 of PolA1 gene have been useful to elucidate the phylogenetic relationships of Petunia (Zhang et al. 2008), Oryza (Takahashi et al. 2009), Triticum (Takahashi et al. 2010), Brassica (Fareed et al. 2016) and Triticum-Aegilops (Nakamura et al. 2009), and Triticum-Aegilops and Hordeum (Rai et al. 2012).

In this study, we found that the intron 20 sequences of PolA1 gene were differentiated in length into S-type (141–142 bp) or L-type (ca. 1.5 kb) in Oryza AA-genome species. As Oryza species outside the AA-genome had the L-type intron, the S-type intron was probably originated from the L-type intron by the deletion of 1.4 kb DNA fragment. This result suggested good evidence for the evolutionary relationships among Oryza AA-genome species.

### Materials and Methods

#### Plant materials and DNA extraction

Almost all accessions of Oryza species used in this study were provided by the National Institute of Genetics, Japan. Two accessions of O. longistaminata were obtained from the Genebank of the International Rice Research Institute (IRRI). Of the 30 accessions, listed in Table 1, of 13 Oryza diploid species containing AA, BB, CC, EE, FF, and GG genome were analyzed for the intron 20 sequence of PolA1 gene. Within AA-genome species, the length of intron 20 was analyzed by PCR in 35 accessions of O. rufipogon, 18 accessions of O. barthii, 21 accessions of O. longistaminata, 17 accessions of O. glumaepatula, and 16 accessions of O. meridionalis (Supplemental Table 1).

Young leaves (ca. 100 mg) of seedlings were frozen in 2-ml plastic tubes with liquid nitrogen and crushed into fine

### Table 1. Materials to analyze the intron 20 sequence of PolA1 gene in Oryza species

| Species          | Accession   | Description     | Intron 20 | Genome |
|------------------|-------------|-----------------|-----------|--------|
| O. sativa        | ‘Nipponbare’| Temperate Japonica | 141 bp    | AA     |
|                  | Ac221       | Tropical Japonica | 141 bp    | AA     |
|                  | Ac130       | Indica          | 141 bp    | AA     |
| O. rufipogon     | W0106       | Annual, India   | 141 bp    | AA     |
|                  | W0107       | Annual, India   | 141 bp    | AA     |
|                  | W1724       | Perennial, India| 141 bp    | AA     |
|                  | W1956       | Perennial, China| 141 bp    | AA     |
| O. barthii       | W0652       | Annual, Sierra Leone | 142 bp  | AA     |
|                  | W1416       | Annual, Sierra Leone | 142 bp  | AA     |
| O. longistaminata| W0643       | Perennial, Gambia| 1499 bp   | AA     |
|                  | W0708       | Perennial, Guinea| n.d.      | AA     |
|                  | W1232       | Perennial, Tanganyika | 1523 bp | AA     |
| O. meridionalis  | W1297       | Annual, Australia| 1519 bp   | AA     |
|                  | W1631       | Annual, Australia| 1523 bp   | AA     |
| O. glumaepatula  | W1169       | Perennial, Cuba | 141 bp    | AA     |
|                  | W1185       | Perennial, Suriname | 141 bp | AA     |
| O. punctata      | W1514       | Kenya           | 1485 bp   | BB     |
|                  | W1577       | Nigeria         | n.d.      | BB     |
| O. officinalis   | W0002       | Thailand        | 1764 bp   | CC     |
|                  | W0614       | Burma           | n.d.      | CC     |
| O. eichingeri    | W1521       | Uganda          | 1767 bp   | CC     |
|                  | W1527       | Uganda          | n.d.      | CC     |
| O. rhizomatis    | W1805       | Sri Lanka       | 1766 bp   | CC     |
|                  | W1808       | Sri Lanka       | n.d.      | CC     |
| O. australiensis | W0008       | Australia       | 1103 bp   | EE     |
|                  | W1296       | Australia       | 1100 bp   | EE     |
| O. brachyantha   | W0656       | Guinea          | 2643 bp   | FF     |
|                  | W1401       | Sierra Leone    | n.d.      | FF     |
| O. granulata     | W0003       | India           | 1634 bp   | GG     |
|                  | W0004       | India           | 1634 bp   | GG     |

n.d.: sequence was not determined because of sequence heterogeneity.
powder using a multi-beads shocker (Yasui Kikai Co., Kyoto, Japan). Total genomic DNA was extracted by CTAB method (Doyle and Doyle 1987) and used for PCR and sequence analyses.

**PCR amplification and direct sequencing**

As shown in Figs. 1A and 2A, DNA fragments containing intron 20 (S-type and L-type) were amplified by PCR using two different pairs of primers a and b, and e and f, which were located on the exon 20 and exon 21 of PolA1 gene, respectively. The primers, listed in Supplemental Table 2, were designed based on the sequence of rice PolA1 gene (NC_029261, DDBJ). Subsequently, PCR amplification was performed with ExTaq DNA polymerase (TaKaRa, Shiga, Japan) according to manufacturer’s instruction. The PCR conditions were 40 cycles of 94°C for 1 min, 58°C for 1 min for annealing, and 72°C for 2 min for elongation in a PTC200 thermocycler (MJ Research, Waltham, MA, USA).

The amplified PCR products were subjected to 1.0–1.5% agarose gel electrophoresis and purified using a PCR purification kit (QIAquick; Qiagen, CA, USA). DNA sequences of the purified PCR products were determined by direct sequencing with the same primer as used for PCR amplification in an automated DNA sequencer ABI310 (Applied Biosystems, CA, USA). Sequences of the L-type intron 20 were determined by using primers c, d1, and d2 as a sequencing primer (Supplemental Table 2). The determined intron 20 sequences of PolA1 genes in *Oryza* species were registered in the DDBJ as accession nos. (LC638415–LC638446).

**Data analysis**

Sequences of PCR products read by direct sequencing were analyzed to determine the positions for donor and acceptor sites of the intron 20 in the PolA1 gene using NCBI web-based Blast sever (Altschul et al. 1990). The sequences were aligned by using CLUSTAW (Thompson et al. 1994) and the alignment was then manually adjusted using Genetyx Software ver. 6.0 (Software Development Co., Tokyo, Japan). The phylogenetic tree of intron 20 sequences was constructed using Neighbor-joining method with bootstrap estimate from 1,000 replicates in the MEGA6 software (Tamura et al. 2011). We analyzed SNPs in intron 20 of PolA1 gene between 24412641–24426383 on chromosome 6 of 3,024 accessions of *O. sativa* (http://iric.irri.org/resources/3000-genomes-project) from the International
Rice Research Institute (IRRI) and 446 accessions of *O. rufipogon* (http://viewer.shigen.info/oryzagenome21/detail/index.xhtml) from the National Institute of Genetics (NIG) to confirm sharing of the same one base T-nucleotide deletion in the S-type intron 20 sequences.

**Results and Discussion**

Over the past half century, the utility and potential of various molecular approaches have been effectively used to solve the controversies of evolution and biosystematics that had remained unresolved despite many efforts made through conventional approaches (Avise 1995). Although it is difficult to infer the direction of speciation by comparing association of SNPs and DNA markers, large insertion/deletion inside single-copy conserved gene, such as PolA1, are thought to be good markers for determining evolutionary relationships. Previous study by Takahashi et al. (2009) reported that PCR products containing intron 19 sequence of *PolA1* gene were differentiated in length among AA, EE, FF and GG genome species in the genus *Oryza* while the amplicon sizes were identical between AA, BB, and CC genome species.

In this study, using a pair of primers a and b (Fig. 1A), amplified DNA fragments containing intron 20 sequences of *PolA1* gene were differentiatied into two types, long type (L-type) and short type (S-type), in *Oryza* species. As shown in Fig. 1B, the L-type intron 20 (ca. 1.1–2.6 kb) was observed in BB genome species (*O. punctata* W1514, W1577), CC (*O. officinalis* W0002, *O. eichingeri* W1521, *O. rhizomatis* W1808), EE (*O. australiensis* W1628, W1632), FF (*O. brachyantha* W0656, W1706), and GG (*O. granulata* W0003, W0004) (Table 1). Two AA-genome species, *O. sativa* Ac221 showed S-type (141 bp) while *O. longistaminata* W0708 contained both S- and L-type. This result suggested that L-type introns were ancestral to S-type introns and large deletion within the intron 20 happened after the AA-genome species originated.

Within AA-genome species, using a different pair of primers e and f (Fig. 2A), all accessions of *O. rufipogon* showed the S-type intron 20 except two accessions W1235 and W1239 (Fig. 2B, Supplemental Table 1), As Sotowa et al. (2013) reported that two New Guinea accessions (W1235 and W1239) shared the same deletions in nuclear genome with *O. meridionalis*, these two accessions were misclassified as *O. rufipogon* (Lam et al. 2020). Furthermore, the S-type was also observed in all accessions of *O. barthii* and *O. glumaepatula*. In contrast, all accessions of *O. meridionalis* showed L-type (ca. 1.5 kb). In case of *O. longistaminata*, 15 accessions showed L type and one accession W1573 contained S-type. Five accessions had both S- and L-type introns. This result indicated that *O. longistaminata* and *O. meridionalis* predominantly had the L-type whereas *O. rufipogon*, *O. barthii*, and *O. glumaepatula* contained the S-type. Five accessions of *O. longistaminata* having both L-type and S-type were probably hybrids between *O. longistaminata* and *O. barthii*, which shared the same habitat in West Africa.

Although L-type intron 20 sequences of several accessions could not be determined because of sequence heterogeneity (Table 1), Neighbor-joining phylogenetic tree of the L-type sequences in *Oryza* species was constructed (Fig. 1C). Two AA-genome species, *O. longistaminata* and *O. meridionalis*, had closely related L-type sequences. *Oryza punctata* (BB-genome) and *O. officinalis*, *O. eichingeri*, *O. rhizomatis* (CC-genome species) formed a single clade, which was closely related to that of AA-genome species. *Oryza australiensis* (EE), *O. brachyantha* (FF), and *O. granulata* (GG) formed paraphyletic groups those were distantly related to AA-genome species.

The phylogenetic analyses based on the L-type intron 20 sequence (Fig. 1C) was consistent with those based on nuclear ribosomal DNA sequence (Kim et al. 2015) and multiple SINE inserts (Cheng et al. 2002), which supported the position of *O. longistaminata* as the basal AA-genome species. The ancestor of the Asian *Oryza* AA-genome species was diverged from ancestor of *O. longistaminata* in Africa involving the changes from perennial to annual and sympatric speciation during the course of evolution (Cheng et al. 2002, Iwamoto et al. 1999, Ohtsubo et al. 2004).

We found that a large DNA fragment (ca. 1.4 kb) was probably deleted between two tandem TTTTGC repeats in the L-type intron, which resulted in the S-type intron (141–142 bp) (Fig. 2C). Also, the identical two tandem repeats were present at the same positions in the L-type intron of *O. officinalis* W0002 (Fig. 3A). Excluding the large 1.4 kb sequence, sequences of S- and L-type introns were highly homologous (Fig. 3A). In detail, intron 20 sequence of *O. barthii* W0652 and W1416 was identical to that of *O. longistaminata* W1232. And both annual W0106 (*O. nivara*) and perennial W1956 accessions of *O. rufipogon* as well as template *japonica* ‘Nipponbare’, tropical *japonica* Ac221, and indica Ac130 of *O. sativa* contained the same intron 20 sequence with *O. longistaminata* W1232 except for single T-nucleotide deletion (Fig. 3A). Interestingly, the same single T-nucleotide deletion was shared with two accessions (W1169, W1185) of *O. glumaepatula*.

The evolutionary relationships among *O. rufipogon*, *O. barthii*, *O. longistaminata*, *O. glumaepatula* and *O. meridionalis* have long been a subject of controversy (Vaughan et al. 2005, Wang et al. 1992, Zhu and Ge 2005). Based on whole genome sequencing of 3,024 accessions of *O. sativa* (IRRI 3K SNP project) and 446 accessions of *O. rufipogon* (Oryza base, NIG), *O. rufipogon* and *O. sativa* shared the same one base T-deletion in the S-type intron 20 sequences (Fig. 3A), because there was no SNP in the intron 20 between columns BF and BG in Supplemental Table 3 (O. sativa) and columns FJ and FL in Supplemental Table 4 (O. rufipogon). While *O. longistaminata* and *O. barthii* shared the non T-deletion in their intron 20.

These data proposed two scenarios of evolutionary relationships among *Oryza* AA-genome species. Scenario 1: a
large deletion of 1.4 kb fragment in the intron 20 first happened in an accession of *O. longistaminata* when *O. barthii* originated. Then, single T-nucleotide deletion occurred in the intron 20 (blue triangle) when common ancestral species of *O. rufipogon* and *O. glumaepatula* originated. *O. meridionalis* probably originated from *O. longistaminata*.

Scenario 2: single T-nucleotide deletion first happened in an accession of *O. longistaminata* which was common ancestral to *O. rufipogon* and *O. glumaepatula*. Deletions of the same 1.4 kb fragment independently happened in the intron 20 during the speciation from *O. longistaminata* to *O. barthii* and from *O. longistaminata*-like species to *O. rufipogon* and *O. meridionalis*. Extensive sequence analysis of the intron 20 in *PolA1* gene will be necessary in *AA*-genome species except for *O. rufipogon* and *O. sativa* to reveal which of two scenario will be correct.

A previous study by Akimoto et al. (1997) presumed that *O. glumaepatula* was associated both of *O. rufipogon* and *O. longistaminata*. In this study, as *O. glumaepatula* shared the S-type intron 20 sequence containing single T-nucleotide deletion with *O. rufipogon* (Fig. 3A), it might be originated with relation to *O. rufipogon* (Vaughan et al. 2005, Yin et al. 2015). Two cultivated species, *O. sativa* and *O. glaberrima*, were independently originated from *O. rufipogon* in South-east Asia and *O. barthii* in West Africa, respectively. The origins of two cultigens have been supported by many researches (Aggarwal et al. 1999, Chang 1976, Zhu and Ge 2005). In this study, we found that intron 20 sequences of the *PolA1* gene differed by an order of magnitude in length between two L-type (ca. 1.5 kb) species *O. longistaminata* and *O. meridionalis* which was common ancestral to *O. rufipogon* and *O. glumaepatula* (Fig. 3C). Then, deletions of the same 1.4 kb fragment independently arose during the speciation from *O. longistaminata* to *O. barthii* and from a *O. longistaminata*-like species to *O. rufipogon* and *O. meridionalis*. Extensive sequence analysis of the intron 20 in *PolA1* gene will be necessary in AA-genome species except for *O. rufipogon* and *O. sativa* to reveal which of two scenario will be correct.

Fig. 3. Alignment of intron 20 sequences of *PolA1* gene in *Oryza AA*-genome species. A: The intron 20 sequences excluding 1.4 kb fragments at TTTTGC (green letter) were shown in *O. longistaminata*, *O. meridionalis*, and *O. officinalis* (CC genome). And intron 20 sequences containing single copy of TTTTGC (blue) were shown in *O. rufipogon*, *O. barthii*, and *O. glumaepatula*. Single nucleotide polymorphism (SNP) and donor/acceptor sites at both ends of intron 20 were shown in red letter. Insertion/deletion of T-nucleotide was shown by blue triangle. B: A proposed evolutionary relationships among *Oryza AA*-genome species. Scenario 1: deletion of 1.4 kb fragment in the intron 20 (red triangle) first happened in *O. longistaminata* when *O. barthii* originated. Then, single T-nucleotide deletion occurred in the intron 20 (blue triangle) when common ancestral species of *O. rufipogon* and *O. glumaepatula* originated. *O. meridionalis* probably originated from *O. longistaminata*. C: Scenario 2: single T-nucleotide deletion first happened in an accession of *O. longistaminata* which was common ancestral to *O. rufipogon* and *O. glumaepatula*. Deletions of the same 1.4 kb fragment independently happened in the intron 20 during the speciation from *O. longistaminata* to *O. barthii* and from *O. longistaminata*-like species to *O. rufipogon* and *O. meridionalis*. Extensive sequence analysis of the intron 20 in *PolA1* gene will be necessary in AA-genome species except for *O. rufipogon* and *O. sativa* to reveal which of two scenario will be correct.
to distribute in the past not only in Africa but also in Asia and Australia.

Author Contribution Statement

IN and YS planned this study. HAH, SM, HT and PS performed molecular and phylogenetic analyses. HAH and IN wrote manuscript.

Acknowledgments

We are grateful to Dr. Takato Koba, Chiba University, for his encouragement and helpful advice on this research. The authors thank Sakura Science Exchange Program of Japanese governmental scholarship for the valuable supports of the first author.

Literature Cited

Aggarwal, R.K., D.S. Brar, S. Nandi, N. Huang and G.S. Khush (1999) Phylogenetic relationships among Oryza species revealed by AFLP markers. Theor Appl Genet 98: 1320–1328.

Akimoto, M., Y. Shimanoto and H. Morishima (1997) Genetic differentiation in Oryza glumaepatula and its phylogenetic relationships with other AA genome species. Rice Genet News 14: 37–39.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman (1990) Basic local alignment search tool. J Mol Biol 215: 403–410.

Avise, J.C. (1995) Molecular Markers, Natural History and Evolution. Chapman & Hall, NY, pp. 1–511.

Chang, T.T. (1976) The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. Euphytica 25: 425–441.

Cheng, C., S. Tsuchimoto, H. Ohtsubo and E. Ohtsubo (2002) Evolutionary relationship among rice species with AA genome based on SINE insertion analysis. Genes Genet Syst 77: 323–334.

Cordesse, F., F. Greillet, A.S. Reddy and M. Delseny (1992) Genome specificity of rDNA spacer fragments from Oryza sativa L. Theor Appl Genet 83: 864–870.

Doyle, J.J. and J.L. Doyle (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Duan, S., B. Lu, Z. Li, J. Tong, J. Kong, W. Yao, S. Li and Y. Zhu (2007) Phylogenetic analysis of AA-genome Oryza species (Poaceae) based on chloroplast, mitochondrial, and nuclear DNA sequences. Biochem Genet 45: 113–129.

Fareed, A., H. Shindo, H. Takahashi and I. Nakamura (2016) Phylogeny of PolA1 gene consistent with the relationships of U’s triangle in Brassica. Hort J 85: 55–62.

Ge, S., T. Sang, B.R. Lu and D.Y. Hong (1999) Phylogeny of rice genomes with emphasis on origins of allotetraploid species. Proc Natl Acad Sci USA 96: 14400–14405.

Guo, Y.-L. and S. Ge (2005) Molecular phylogeny of Oryzae (Poaceae) based on DNA sequences from chloroplast, mitochondrial, and nuclear genomes. Am J Bot 92: 1548–1558.

Ishii, T., T. Nakano, H. Maeda and O. Kamijima (1996) Phylogenetic relationships in A-genome species of rice as revealed by RAPD analysis. Genes Genet Syst 71: 195–210.

Iwamoto, M., H. Nagashima, T. Nagamine, H. Higo and K. Higo (1999) p-SINE1-like intron of the CatA catalase homologs and phylogenetic relationships among AA-genome Oryza and related species. Theor Appl Genet 98: 853–861.

Jena, K.K. (2010) The species of the genus Oryza and transfer of useful genes from wild species into cultivated rice, O. sativa. Breed Sci 60: 518–523.

Kanazawa, A., M. Akimoto, H. Morishima and Y. Shimamato (2000) Inter- and intra-specific distribution of Stowaway transposable elements in AA-genome species of wild rice. Theor Appl Genet 101: 327–335.

Kawahara, Y., M. de la Bastide, J.P. Hamilton, H. Kamamori, W.R. Mccombie, S. Ouyang, D.C. Schwartz, T. Tanaka, J. Wu, S. Zhou et al. (2013) Improvement of the Oryza sativa Nipponbare reference genome using next generation sequence and optical map data. Rice (N Y) 6: 3–10.

Kim, K., S.-C. Lee, J. Lee, Y. Yu, K. Yang, B.-S. Choi, H.-J. Koh, N.E. Waminal, H.-I. Choi, N.-H. Kim et al. (2015) Complete chloroplast and ribosomal sequences for 30 accessions elucidate evolution of Oryza AA genome species. Sci Rep 5: 15655.

Lam, D.T., K. Ichitani, R.J. Henry and R. Ishikawa (2020) Molecular and morphological divergence of Australien wild rice. Plants 9: 224.

Morishima, H., Y. Sano and H.I. Oka (1992) Evolutionary studies in cultivated rice and its wild relatives. Oxford Surveys in Evolutionary Biology 8: 135–184.

Motohashi, R., K. Mochizuki, H. Ohtsubo and E. Ohtsubo (1997) Structures and distribution of p-SINE1 members in rice genomes. Theor Appl Genet 95: 359–368.

Nakamura, I. (2016) Method of identifying eukaryotic species. JP 2016129518-A 358.

Nakamura, I., B. Rai, H. Takahashi, K. Kato, Y. Sato and T. Komatsuda (2009) Aegilops section Sitopsis species contains the introgressive PolA1 gene with a closer relationship to that of Hordeum than Triticum–Aegilops species. Breed Sci 59: 602–610.

Ohtsubo, H., C. Cheng, I. Ohsawa, S. Tsuchimoto and E. Ohtsubo (2004) Rice retroposon p-SINE1 and origin of cultivated rice. Breed Sci 54: 1–11.

Oka, H.I. (1988) The genus Oryza. In: Oka, H.I. (ed.) Origin of cultivated rice. Japan Scientific Societies Press Tokyo, Elsevier, Amsterdam, pp. 1–14.

Rai, B., H. Takahashi, K. Kato, Y.I. Sato and I. Nakamura (2012) Single-copy nuclear PolA1 gene sheds light on the origin of S genome with relationships to B and G genomes of polyploid wheat species. Genet Resour Crop Evol 59: 1713–1726.

Second, G. (1985) Evolutionary relationships in the Sativa group of Oryza based on isozyme data. Genet Sel Evol 17: 89–114.

Seither, P., J.F. Croy, A. Pouska and I. Grummt (1997) Molecular cloning and characterization of the cDNA encoding the largest subunit of mouse RNA polymerase I. Mol Gen Genet 255: 180–186.

Sotowa, M., K. Ootsuka, Y. Kobayashi, Y. Hao, K. Tanaka, K. Ichitani, J.M. Flowers, M.D. Purugganan, I. Nakamura, Y.I. Sato et al. (2013) Molecular relationships between Australian annual wild rice, Oryza meridionalis, and two related perennial forms. Rice (N Y) 6: 26.

Takahashi, H., T. Sato, Y.I. Sato and I. Nakamura (2009) Genome-type-specific variation of the 19th intron sequence within the RNA polymerase I largest subunit gene in the genus Oryza. Plant Syst Evol 282: 21–29.

Takahashi, H., B. Rai, K. Kato and I. Nakamura (2010) Divergent evolution of wild and cultivated subspecies of Triticum
timopheevii as revealed by the study of PolA1 gene. Genet Resour Crop Evol 57: 101–109.
Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731–2739.
Thompson, J.D., D.G. Higgins and T.J. Gibson (1994) CLUSTALW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680.
Vaughan, D.A. (1994) The wild relatives of rice: A genetic resources handbook. IRRI, Los Banos, Manila, Philippines. p. 137.
Vaughan, D.A., K. Kadowaki, A. Kaga and N. Tomooka (2005) On the phylogeny and biogeography of the genus Oryza. Breed Sci 55: 113–122.

Wang, Z.Y., G. Second and S.D. Tanksley (1992) Polymorphism and phylogenetic relationships among species in the genus Oryza as determined by analysis of nuclear RFLPs. Theor Appl Genet 83: 565–581.
Yin, H., M. Akimoto, R. Kaewcheenchai, M. Sotowa, T. Ishii and R. Ishikawa (2015) Inconsistent diversities between nuclear and plastid genomes of AA genome species in the genus Oryza. Genes Genet Syst 90: 269–281.
Zhang, X., H. Takahashi, I. Nakamura and M. Mii (2008) Molecular discrimination among taxa of Petunia axillaris complex and P. integrifolia complex based on PolA1 sequence analysis. Breed Sci 58: 71–75.
Zhu, Q. and S. Ge (2005) Phylogenetic relationships among A-genome species of the genus Oryza revealed by intron sequences of four nuclear genes. New Phytol 167: 249–265.