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Research Article

Molecular evolution of the *ent*-kaurenoic acid oxidase gene in Oryzeae

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Abstract

We surveyed the substitution patterns in the *ent*-kaurenoic acid oxidase (KAO) gene in 11 species of Oryzeae with an outgroup in the Ehrhartoidaea. The synonymous and non-synonymous substitution rates showed a high positive correlation with each other, but were negatively correlated with codon usage bias and GC content at third codon positions. The substitution rate was heterogenous among lineages. Likelihood-ratio tests showed that the non-synonymous/synonymous rate ratio changed significantly among lineages. Site-specific models provided no evidence for positive selection of particular amino acid sites in any codon of the *KAO* gene. This finding suggested that the significant rate heterogeneity among some lineages may have been caused by variability in the relaxation of the selective constraint among lineages or by neutral processes.

Key words: codon usage bias, *ent*-kaurenoic acid oxidase (KAO), positive selection, rate heterogeneity, substitution rate.

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Introduction

Gibberellins (GAs) are an important class of plant hormones involved in the regulation of various growth and developmental processes in higher plants (Appleford *et al.*, 2006). The absence of GAs results in dwarfism in some plant species. *ent*-kaurenoic acid oxidase (KAO), a member of the CYP88A subfamily of cytochrome P450 enzymes, catalyzes a three-step reaction in the gibberellin biosynthetic pathway from *ent*-kaurenoic acid to GA12 (Helliwell *et al.*, 2001). A primary goal of molecular evolutionary studies is to estimate the rate of DNA mutation and elucidate the mechanisms of molecular evolution. Such studies frequently involve a comparison of orthologous DNA fragments among species to determine evolutionary rates and an assessment of the evolutionary processes involved, *e.g.*, natural selection, rate heterogeneity of lineages and mutational biases. Analysis of the molecular evolutionary patterns of different genes provides understanding of the evolutionary processes and pressures experienced by particular lineages.

The tribe Oryzeae (Poaceae) includes approximately 12 genera and more than 70 species distributed throughout tropical and temperate regions of the world (Clayton and Renvoize, 1986; Vaughan, 1994). In the genus *Oryza*, the Asian cultivated rice (*Oryza sativa* L.) is one of the world's most important crops and a primary food source for more than one-half of the world's population (Chandler and Wessler, 2001). This species has become a model monocotyledon in scientific research and its entire genome has been sequenced. Other members of the Oryzeae are also of economic importance, including wild species of *Oryza* that can be used in the genetic improvement of rice.

Analysis of the substitution patterns in the *KAO* gene can provide insights into the driving forces that have led to evolutionary change in this gene in Oryzeae. In addition, the identification of patterns of molecular evolution in the *KAO* gene can improve our understanding of the evolutionary history of some Oryzeae species. In this work, we examined the heterogeneity of the substitution rate in the *KAO* gene among various genera and species of Oryzeae and sought to identify the possible causes of such heterogeneity. We also sought for evidence of natural selection in the exon regions of the *KAO* gene.

Materials and Methods

Plant material

A portion of the *KAO* gene was isolated and sequenced from members of the rice tribe (Oryzeae) (Table 1). Eleven diploid species were selected to represent the major phylogenetic lineages of Oryzeae (Figure S1, Supplementary Material) (Guo and Ge, 2005). These consisted of seven *Oryza* species representing six diploid genome types, namely, *Oryza sativa* (AA), *O. meridionalis* (AA), *O. punctata* (BB), *O. officinalis* (CC), *O. australiensis* (EE), *O. brachyantha* (FF), *O. granulata* (GG), and one

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Table 1 - Species used in this study.

Species	Genome	Accession[a]	Country
Oryza sativa	A	*japonica*	GenBank
O. meridionalis	A	105282	Australia
O. punctata	B	103903	Tanzania
O. officinalis	C	104972	China
O. australiensis	E	105263	Australia-PNAS
O. brachyantha	F	105151	Sierra Leone-PNAS
O. granulata	G	M8-15	Ledong, Hainan
Leersia tisserantti	—	105610	Cameroon
Chikusichloa aquatica	—	106186	Japan
Rhynchoryza subulata	—	100913	Argentina
Luziola leiocarpa	—	82043	Argentina
Ehrharta erecta	—	218290	South Africa

[a]All accessions were obtained from the International Rice Research Institute at Los Banos, Philippines.

species from each of four other genera in the tribe Oryzeae (*Leersia tisserantti*, *Chikusichloa aquatica*, *Luziola leiocarpa*, and *Rhynchoryza subulat*a) (Table 1). *Ehrharta erecta*, a species in the tribe Ehrhartoideae, which is a sister tribe to the Oryzeae, was used as an outgroup (GPWG, 2001; Guo and Ge, 2005). Plastid, mitochondrial and nuclear gene sequences have been used to establish the phylogeny of the Oryzeae (Ge *et al.*, 1999; Guo and Ge, 2005; Tang *et al.*, 2010) and have provided an important framework for the study of molecular evolution in this group (Figure S1, Supplementary Material).

DNA extraction, amplification and sequencing

Total DNA was isolated from silica-gel dried or fresh leaves as described by Ge *et al.* (1999). A 1-2 kb fragment of the *KAO* gene containing several exons and introns was obtained by using the polymerase chain reaction (PCR) in conjunction with the forward primer KAOF (5'-CAGGA CGTTCATGTTCAGCAG-3') and the reverse primers KAOR1 (5'-TCGTCGCCAAGCAGTTGTC-3') and KAOR2 (5'-GCCAAGCAGTTGTCCAC-3') (Figure 1). The PCR was done in a total volume of 25 μL that contained 5-50 ng of template DNA, 0.2 μM of each primer, 200 μM of each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ and 0.75 U of Ex*Taq* DNA polymerase (TaKaRa, Shiga, Japan). Amplifications were done in a T gradient 96 U thermocycler (Biometra, Göttingen, Germany) as follows: 3 min at 94 °C, followed by 33 cycles of 30 s at 94 °C, 30 s at 56 °C, 2.5 min at 72 °C and a final extension at 72 °C for 10 min. Further internal primers used for sequencing were: KAO707F 5'-ACCGTCTTCCTCC AGGAGAAC-3' (Tm = 61.9 °C), KAO931F 5'-GATGCACTTCCTCTCACAG-3' (Tm = 57.6 °C) and KAO1478F 5'-CGTCAACATCTCCTTCGTGTC-3' (Tm = 60 °C) (Yang *et al.*, 2009). All of the sequences were deposited in GenBank under accession numbers EF577665-EF577670 and EU179429-EU179435 (Table 2).

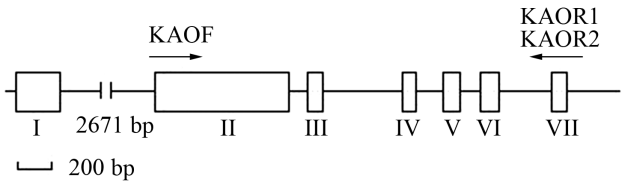

Figure 1 - Schematic diagram of the *KAO* gene and the regions sequenced in this study. Boxes and lines indicate exons and introns, respectively. Exon numbers are labeled with the roman numbers. Locations of primers are shown above the diagram.

Sequence analysis

Sequences were aligned using ClustalX v.1.81 (Thompson *et al.*, 1997) and refined by manual adjustment based on the predicted amino acid sequence. The amino acid sequences (excluding introns) were sufficiently conserved across the 12 species to provide unambiguous alignments. We examined the possibility of sequence saturation using DAMBE v.4.5.45 (Xia and Xie, 2001). Pairwise synonymous and non-synonymous substitutions per site (d_S and d_N) among the 11 species were estimated for the coding regions of the *KAO* gene.

The extent of codon usage bias often reflects the degree of selective constraint in a gene (Sharp, 1991; Sharp *et al.*, 1986). To measure the extent of codon usage bias, we estimated the effective number of codons (ENC) and codon bias index (CBI) using DnaSP v.4.10.9 (Rozas and Rozas, 1999). The ENC values range from 20 (only one codon is used for each amino acid, *i.e.*, the codon bias is maximal) to 61 (all synonymous codons for each amino acid are equally used, *i.e.*, there is no codon bias) (Wright, 1990). The CBI values range from 0 (uniform use of synonymous codons) to 1 (maximum codon bias) (Morton, 1993). Variation in the rate of synonymous substitution among genes may be related to codon use (Sharp, 1991). Therefore, several parameters related to codon usage bias, such as the GC content at the first and second codon positions (GC1, 2), as well as third codon positions (GC3), were also estimated using DnaSP v.4.10.9 (Rozas and Rozas, 1999).

Detecting rate heterogeneity among lineages

The relative-rate test based on the method of Muse and Gaut (1994), as implemented in Hyphy (Pond *et al.*, 2005), was used to detect variation in the synonymous and non-synonymous substitution rates along different lineages, with *Ehrharta erecta* as the reference sequence. This method examines substitution rates between two lineages with reference to a third outgroup lineage. In the first model, the two related taxa from the most recent common ancestor are constrained to have the same substitution rate. In the second model, the two lineages may have different substitution rates. A likelihood ratio test is used to test

Table 2 - Information for the *KAO* gene sampled in this study.

Species	Length sequenced (bp)		Coding					Noncoding	Accession number
	Total	Coding	ENC[a]	CBI	GC	GC1,2	GC3	GC	
Oryza sativa	2231	1053	35.02	0.693	0.600	0.492	0.818	0.307	AP004572[b]
O. meridionalis	1819	1053	35.60	0.678	0.598	0.492	0.818	0.334	EU179429
O. punctata	1833	1053	35.13	0.685	0.597	0.486	0.820	0.333	EF577665
O. officinalis	1844	1053	39.10	0.641	0.600	0.493	0.815	0.328	EF577666
O. australiensis	1867	1053	39.27	0.637	0.602	0.495	0.818	0.334	EF577667
O. brachyantha	2626	1053	39.20	0.642	0.606	0.498	0.823	0.334	EF577668
O. granulata	1808	1053	37.72	0.662	0.612	0.501	0.832	0.336	EF577669
Leersia tisserantti	1775	1053	48.56	0.405	0.565	0.489	0.718	0.327	EF577670
Luziola leiocarpa	1826	1050	38.67	0.636	0.612	0.503	0.831	0.336	EU179408
Chikusichloa aquatica	1772	1047	42.48	0.568	0.598	0.490	0.814	0.338	EU179409
Rhynchoryza subulata	1790	1047	42.02	0.569	0.595	0.490	0.805	0.328	EU179410
Ehrharta erecta	2363	1026	53.65	0.390	0.541	0.451	0.723	0.324	EU179411
Mean ± SE[c]	1962.83 ± 81.51	1049.50 ± 2.24	40.54 ± 1.61	0.601 ± 0.030	0.594 ± 0.006	0.490 ± 0.004	0.803 ± 0.011	0.330 ± 0.002	

[a]ENC – effective number of codons (Wright, 1990), CBI – codon bias index, GC1, 2 is G+C content at the first and second codon positions. [b]Sequences downloaded from GenBank. [c]Average for 11 species of Oryzeae.

which of the models best explains the data (Muse and Gaut, 1994).

Detection of positive selection

The ratio ω (d_N/d_S) provides an effective means of detecting selection or selective pressure on a gene or gene region, with ω < 1, = 1 and > 1 indicating negative selection, neutral evolution and positive selection, respectively (Yang, 2006). We ran likelihood-based analyses using the CODEML program of PAML 4 (Yang, 2007) to explore the selective processes acting on the *KAO* gene. First, we used the branch models to examine whether the evolutionary rates differed among lineages within the gene tree. The one ratio model (M0) assumes a single ω for all branches and all sites. However, the free ratio model (Mf) postulates an independent ω ratio for each branch of the tree. A likelihood ratio test (LRT) was used to decide whether there was a significant difference between M0 and Mf. The model with the higher likelihood value was assumed to be the better model (Bielawski and Yang, 2003; Yang and Nielsen, 1998).

We next used site-specific models to detect whether particular amino acid residues were subject to positive selection (Yang, 2006). The neutral model (M1a) classifies all of the sites into two categories, *i.e.*, strict constraint (0 < ω < 1) (purifying selection) and neutral (ω = 1). Based on M1a, the positive selection model (M2a) assumes a third category under positive selection (ω > 1). The beta model (M7) assumes a beta distribution for the ω ratios over sites, and the beta and ω model (M8) increases the independent ratio estimated by the data. M8 and M2a assume positive selection and are compared with M7 and M1a, respectively. If the LRT is significant and there is a site with ω > 1 then positive selection is invoked for the gene (Bielawski and Yang, 2003; Yang, 2006).

Results and Discussion

Previous studies showed that the *KAO* gene was a single-copy gene (Helliwell *et al.*, 2001; Sakamoto *et al.*, 2004; Yamaguchi, 2008) and the loss-of-function mutant exhibits a typical phenotype, indicating the functional importance of this enzyme in GA biosynthesis (Sakamoto *et al.*, 2004). In view of the importance of comparing orthologous rather than paralogous genes when estimating substitution rates, we initially examined this issue and found that the *KAO* gene was orthologous in all of the species analyzed. The similarity of the aligned coding regions ranged from 87.5% to 99.5% (Figure S2, Supplementary Material). Sequences of the *KAO* gene were isolated from all of the Oryzeae species and from the outgroup, *Ehrharta erecta*. The sequenced regions ranged in size from 1772 bp to 2626 bp and their aligned coding regions varied from 1047 bp to 1053 bp (Table 2). The total GC content and the GC content of the third position of the codons (GC3) were

similar across species. Table 2 summarizes the sequence data for this gene.

Codon usage bias and its correlation with GC3 and substitution rates

Codon usage bias has been important in studies of molecular evolution because it provides examples of weak selection at the molecular level. CBI and ENC were calculated to measure the degree of codon usage bias. CBI showed a marked negative correlation with ENC ($r^2 = 0.958$, $p < 0.0001$) (Figure 2A) such that both CBI and ENC could be used to measure the degree of codon usage bias. In this study, ENC was used to measure the degree of codon usage bias.

To determine the relative effects of mutation pressure versus natural selection on codon composition, we examined the relationship between the GC content at third codon positions (GC3) and the GC content at the first and second codon positions (GC1,2). The GC content of GC1,2 ranged from 48.9% to 50.3%, which there was a tendency of positive correlation with GC3 ($r^2 = 0.227$) but this was not significant ($p = 0.139$) (Figure 2F). This pattern of base composition suggests that the GC content is most likely the result of mutation pressure since natural selection acts differently on different codon positions (Shackelton *et al.*, 2006). Interestingly, after excluding *L. tisserantti*, GC1,2 showed a significant positive correlation with GC3 ($r^2 = 0.604$, $p < 0.05$) (data not shown), which further confirmed that these changes were most likely the result of mutation pressure. d_S was positively correlated with d_N ($r^2 = 0.498$, $p < 0.05$) (Figure 2D), as also observed in other organisms (Bielawski *et al.*, 2000; Dunn *et al.*, 2001; Hurst and Williams, 2000; Kusumi *et al.*, 2002), and negatively correlated with codon bias ($r^2 = 0.713$, $p < 0.05$) (Figure 2B) and GC3 ($r^2 = 0.796$, $p < 0.001$) (Figure 2E). The negative correlation between d_S and codon usage bias may be explained by natural selection (Bielawski *et al.*, 2000; Smith and Eyre-Walker, 2001; Urrutia and Hurst, 2001) since codon usage bias is a primary factor in d_S variation among genes and is thought to be under natural selection, perhaps because of the need to maintain accuracy or speed in translation (Yang and Gaut, 2011). There was also a tendency for d_N being negatively correlated with codon usage bias ($r^2 = 0.348$) but this was not significant ($p = 0.056$) (Figure 2C). The latter would be consistent with sites that are functionally constrained and consequently conserved at the amino acid level. Such sites are also likely to experience stronger selection for translation accuracy and hence have a higher codon bias (Akashi, 2003). This might explain the negative correlation between d_N and codon bias observed here (though not significant), and by others in enteric bacteria (Rocha, 2004; Sharp, 1991), *Drosophila* (Betancourt and Presgraves, 2002), yeast (Drummond *et al.*, 2005), and viruses (Duffy *et al.*, 2008). The fact that d_N is correlated to codon bias suggests that codon bias might be used as a measure of the level of constraint upon a site or gene (Plotkin *et al.*, 2004, 2006; Stoletzki and Eyre-Walker, 2007).

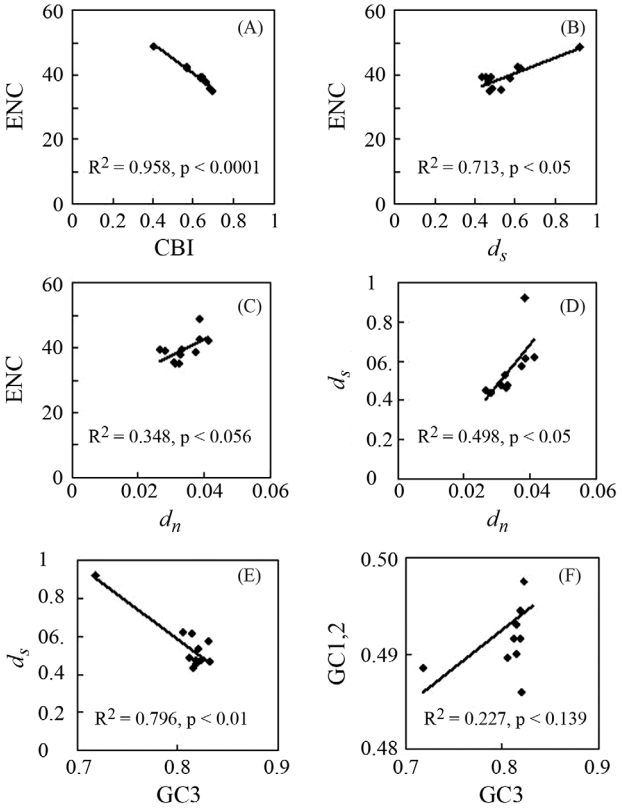

Figure 2 - The relationships between effective number of codons (ENC) and codon bias index (CBI) (A), synonymous substitution rates (d_S) (B), and non-synonymous substitution rates (d_N) (C), between d_S and d_N (D) and third codon positions (GC3) (E), and between the first and second codon positions (GC1, 2) and GC3 (F).

The driving forces governing evolution of the *KAO* gene in Oryzeae

A codon-based approach showed that the free ratio model (Mf) had significantly higher likelihood scores (ln4103.38) than the one ratio model (M0) (ln4124.44) ($p < 0.001$) (Table 3). Although the d_N/d_S ratios varied across lineages from 0.0001 to 0.358 (with one of the 21 lineages showing no predicted synonymous substitutions, *i.e.*, the d_N/d_S ratio was equal to 999.000), the estimated d_N/d_S ratio for each lineage was less than 1. The ω values were estimated to be 0.079 under the M0 model, suggesting that purifying selection or selection constraint best explained the molecular evolution of the *KAO* gene, in agreement with the studies on anthocyanin pathway genes (Lu and Rausher, 2003; Rausher *et al.*, 2008).

The branch model test is a very conservative test of positive selection because it averages the ratio across all sites. We therefore used site-specific codon models to examine whether there was positive selection on codon sites. The M2a and M8 models, which assume positive selection, were not significantly better than the null models M1a and

Table 3 - Log likelihood values, ω ratios and parameter estimates for the *KAO* gene in models with variable ω ratios among codon sites.

Model	p^a	ln	Parameter estimates[b]	Models compared	2ΔL	p-value
Mf	31	-4103.38	ω = 0.0001 ~ 999.000, tree length[c] = 2.140, kappa(ts/tv) = 1.103	M0-Mf	42.12	< 0.001
M0	23	-4124.44	ω = 0.079, tree length = 2.181, kappa (ts/tv) = 1.082			
M1a	24	-4067.60	ω_0 = 0.049, p_0 = 0.921; ω_1 = 1.000, p_1 = 0.079	M1a-M2a	0	1
M2a	26	-4067.60	p_0 = 0.921, p_1 = 0.053, p_2 = 0.026, ω_2 = 1.000			
M7	24	-4061.19	p = 0.282, q = 2.548	M7-M8	0	1
M8	26	-4061.19	p_0 = 1.000, p = 0.282, q = 2.548; p_1 = 0.000, ω = 8.931			

[a]p – number of parameters, ln – log-likelihood values of the data in each model. [b]Parameter estimates in different models.
[c]Tree length is the sum of branch lengths.

M7 (for M1a *vs.* M2a, 2ΔL = 0, p = 1.0; for M7 *vs.* M8, 2Δ = 0, p = 1.0) (Table 3). These results indicate that the *KAO* gene is under strong selective constraint, thus ruling out the possibility of past episodes of positive selection on this gene. Previous studies have shown that variation in the evolutionary rate among nucleotide sites may be attributed to differences in the frequency of positive selection (Yang *et al.*, 2000; Gaut *et al.*, 2011) or in the magnitude of selective constraints (Li, 1997; Rausher *et al.*, 1999, 2008).

In this study, the branch and codon models failed to detect any sign of positive selection for any lineage and codon of the *KAO* gene, suggesting that the significant heterogeneity of some lineages was attributable mainly to the relaxed constraint among lineages or neutral processes rather than positive selection. However, the power to detect positive selection using the methods mentioned above may be low, especially when adaptive substitutions are spread across many amino acid sites (Pond *et al.*, 2005; Rausher *et al.*, 2008). Further investigations with alternative tests on intraspecific changes (Olsen *et al.*, 2002; Whitt *et al.*, 2002; Flowers *et al.*, 2007; Rausher *et al.*, 2008) would be necessary to detect evidence of positive selection.

Rate variation among lineages

There was significant heterogeneity in the synonymous and non-synonymous substitution rates of the *KAO* gene among lineages of the rice tribe (Table 4), especially in *C. aquatica* and *L. leiocarpa*. Among 55 relative-rate tests for synonymous substitutions, 11 comparisons were significant at the 5% or 1% level. At the same time, among 55 relative-rate tests for non-synonymous substitutions, the null hypothesis of rate homogeneity was rejected for 18 comparisons. In *C. aquatica* and *L. leiocarpa* d_N appeared to be decelerated, and did d_S in *C. aquatica*. The significant slowdown in the rate of synonymous and non-synonymous substitutions in *C. aquatica* and *L. leiocarpa* lineages may reflect differences in the intensity of selection, *i.e.*, the *KAO* gene may be under different functional constraints in different lineages.

Several mechanisms could explain the observed rate heterogeneity, including life history traits such as genera-

Table 4 - Results of 110 relative-rate tests for d_S (lower triangle) and d_N (upper triangle). Rejection of rate equality is indicated by * at the 0.05 level, ** at the 0.01 level, or *** at the 0.001 level. *Ehrharta erecta* was used as the outgroup in all comparisons. Species names that were inferred to have evolved more quickly in each pairwise comparison are indicated in the table by the first letter of the genus name and the first three letters of the species name.

	Osat	Omer	Opun	Ooff	Oaus	Obra	Ogra	Ltis	Llei	Caqu	Rsub
Osat	-								***Osat	**Osat	
Omer		-				*Omer	*Ogra		***Omer	***Omer	
Opun			-			*Opun			***Opun	**Opun	
Ooff				-					**Ooff	**Ooff	
Oaus					-				**Oaus	*Oaus	
Obra						-			*Obra		
Ogra							-		*Ogra		
Ltis								-	**Ltis	*Ltis	
Llei									-		
Caqu	***Osat	***Omer	***Opun	**Ooff	**Oaus	**Obra	***Ogra	***Ltis	**Llei	-	* Rsub
Rsub							*Ogra			*Rsub	-

Caqu – *Chikusichloa aquatica*, Llei – *Luziola leiocarpa*, Ltis – *Leersia tisserantti*, Oaus – *O. australiensis*, Obra – *O. brachyantha*, Ogra – *O. granulata*, Omer – *O. meridionalis*, Ooff – *O. officinalis*, Opun – *O. punctata*, Osat – *O. sativa* and Rsub – *Rhynchoryza subulata*.

tion time, biochemical features such as efficiency of DNA repair machinery, and environmental variables such as energy and temperature (Eyre-Walker and Gaut, 1997; Li, 1997; Brown *et al.*, 2005; Soria-Hernanz *et al.*, 2008). Rate heterogeneity may also result from differences in population size since variation in population size can alter evolutionary rates within a lineage (Eyre-Walker and Gaut, 1997; Lynch and Conery, 2003) and vice versa. Variation in the nucleotide substitution rates of the *KAO* gene significantly changed the ω ratios of the respective lineages. These features of the *KAO* gene in Oryzeae resulted from the influence of various factors that affected the evolution of these species and their ancestors. A detailed knowledge of these factors will help us to understand the evolutionary history of Oryzeae species.

Conclusions

The results of this study showed that codon usage bias was negatively correlated with synonymous and nonsynonymous substitution rates, a finding consistent with the importance of codon usage. CBI was positively correlated with ENC, thus confirming the similarity of CBI and ENC as parameters for measuring the degree of codon usage bias. There was considerable heterogeneity in the nucleotide substitution rates of the *KAO* gene and this significantly affected the ω ratios of the respective lineages. There was no positive selection and no positively selected codons in this gene, a finding indicative of substantial selective constraint. These features of nucleotide substitutions in the *KAO* gene reflected the influence of various factors on the evolution of many Oryzeae species and their ancestors.

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Supplementary Material

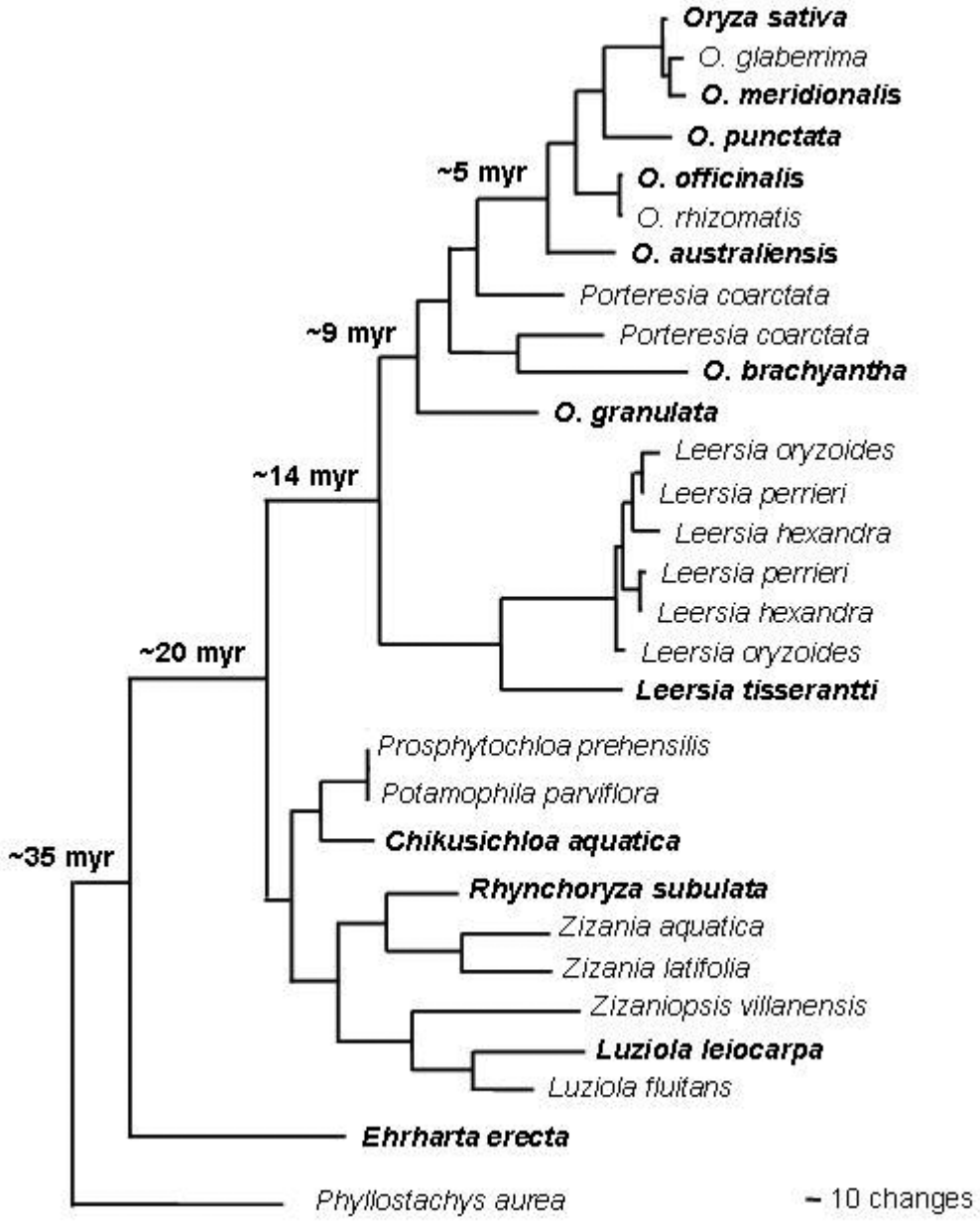
The following online material is available for this article:

Figure S1 - Phylogeny of the rice tribe (Oryzeae) obtained from the combined *Adh2* and *GPA1* sequences by Bayesian inference using the TrN+G model (Guo and Ge, 2005).

Figure S2 - Alignment of coding sequences of the *KAO* gene in 12 species.

This material is available as part of the online article from http://www.scielo.br/gmb.

Associate Editor: Adriana S. Hemerly


Oryza sativa
O. glaberrima
O. meridionalis
O. punctata
~5 myr
O. officinalis
O. rhizomatis
O. australiensis
Porteresia coarctata
~9 myr
Porteresia coarctata
O. brachyantha
O. granulata
Leersia oryzoides
~14 myr
Leersia perrieri
Leersia hexandra
Leersia perrieri
Leersia hexandra
~20 myr
Leersia oryzoides
Leersia tisserantti
Prosphytochloa prehensilis
Potamophila parviflora
Chikusichloa aquatica
~35 myr
Rhynchoryza subulata
Zizania aquatica
Zizania latifolia
Zizaniopsis villanensis
Luziola leiocarpa
Luziola fluitans
Ehrharta erecta
Phyllostachys aurea
– 10 changes

```
                    10        20        30        40        50        60
               ....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
GGCTGGCCCAAGGCCACCGTCACCCTCATCGGCCCCAAATCCTTCGTCAACATGTCCT
AC
O.meridionalis_kao
GGCTGGCCCAAGGCCACCGTCACCCTCATCGGCCCCAAATCCTTCGTCAACATGTCCT
AC
O.punctata_kao
GGCTGGCCCAAGGCCACCGTCACCCTCATCGGCCCCAAATCCTTCGTCAACATGTCCT
AC
O.officinalis_kao
GGCTGGCCCAAGGCCACCGTCACCCTCATCGGCCCCAAATCCTTCGTCAACATGTCCT
AC
O.australiensis_kao
GGCTGGCCCAAGGCCACCGTCACCCTCATCGGCCCCAAATCCTTCGTCAACATGCCCT
AC
O.brachyantha_kao
GGCTGGCCCAAGGCGACCATCACTCTCATCGGCCCCAAGTCCTTCGTCAACATGCCCT
AC
O.granulata_kao
GGATGGCCCAAGGCCACCGTCACCCTCATCGGCCCCAAGTCCTTCGTCAGCATGCCCT
AC
L.tisserantti_kao
GGATGGCCGAAAGCGACGGTGACACTGATCGGGCCAAAATCGTTCGTGAGCATGCCGT
AC
C.aquatica_kao
GGCTGGCCCAAGGCAACGGTCACGCTCATCGGCCGCAAGTCGTTCGTGAGCATGCCG
TAC
R.subulata_kao
GGCTGGCCCAAGGCCACCGTCAATCTCATCGGCCGCAAGTCGTTCGTGAGCATGCCAT
AC
L.leiocarpa_kao
GGCTGGCCCAAGGCCACCGTCACACTCATCGGCCCCAAGTCCTTCGTCAGCATGCCGC
AC
E.erecta_kao
GGCTGGCCCAAGTCGACGGTGACGCTCCTCGGCAACAACTCATTCATAAGCTTGCCCC
AC
Clustal Consensus  ** ***** ** * ** * * ** **** ** ** *** * * * ** * **

                    70        80        90        100       110       120
               ....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
GACGACCACCGCCGCATCCGCAAGCTCACCGCCGCCCCCATCAACGGCTTCGACGCC
CTC
O.meridionalis_kao
```

GACGACCACCGCCGCATCCGCAAGCTCACCGCCGCCCCCATCAACGGCTTCGACGCC
CTC
O.punctata_kao
GACGACCACCGCCGCATCCGCAAGCTCACCGCCGCCCCCATCAACGGCTTCGACGCC
CTC
O.officinalis_kao
GACGACCACCGCCGCATCCGCAAGCTCACCGCCGCCCCCATCAACGGCTTCGACGCG
CTC
O.australiensis_kao
GACGACCACCGCCGCATCCGCAAGCTCACGGCGGCGCCCATCAACGGCTTCGACGCC
CTC
O.brachyantha_kao
GACGACCACCGCCGCCTGCGCAAGCTCACGGCCGCGCCCATCAACGGCTTCGACGCG
CTC
O.granulata_kao
GACGACCACCGCCGCCTCCGCAAGCTCACCGCCGCGCCCATCAACGGCTTCGACGCG
CTC
L.tisserantti_kao
GACGATCACCGGCGAATCCGCAAGCTGACGGCGGCGCCGATCAACGGCTTCGACGCA
CTC
C.aquatica_kao
GAGGAACACCGGCGCTTGCGCAAGCTCACGGCGGCGCCCATCAACGGCTTCGAGGCG
CTC
R.subulata_kao
GAGGATCACCGGCGGCTGCGCAAGCTCACGGCGGCTCCCATCAACGGCTTCGAGGCG
CTC
L.leiocarpa_kao
GACGACCACCGCCGGCTCCGCAAGCTCACGGCGGCGCCCATCAACGGCTTCGACGCG
CTC
E.erecta_kao
GACGACCACCGGCGTCTGCGCAAGCTGACCGCGGCGCCAATCAACGGCTTTGATTCAC
TG
Clustal Consensus ** ** ***** ** * ******** ** ** ** ** ********** ** * **

130 140 150 160 170 180
....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
ACCACCTACCTCTCCTTCATCGACCAGACCGTCGTCGCCTCCCTCCGCCGCTGGTCCT
CG
O.meridionalis_kao
ACCACCTACCTCTCCTTCATCGACCAGACCGTCGTCGCCTCCCTCCGCCGCTGGTCCT
CG
O.punctata_kao
ACCACCTACCTCTCCTTCATCGACCACACCGTCGTCTCCACGCTCCGCCGCTGGTCCT
CC
O.officinalis_kao

AACACCTACCTCGCCTTCATCGACCAAACCGTCGTCGCCACTCTCCGCCGCTGGTCCT
CG
O.australiensis_kao
ACCACCTACCTCGCCTTCATCGACCAGACCGTCGTCGCTACGCTCCGCCGCTGGTCGT
CG
O.brachyantha_kao
ACCACCTACCTCGGCTTCATCGACCGCACCGTCGTCGACACGCTCCGCCGCTGGTCGT
CG
O.granulata_kao
ACCACCTACCTCGGCTTCATCGACCACACCGTCGTCGCCTCGCTCCGCCGCTGGTCGG
AG
L.tisserantti_kao
ACAACCTACCTCTCCTTCATCGACCAAACCGTCGTCGCCACGCTCCGCCGCTGGTCGG
AA
C.aquatica_kao
ACCACCTACCTGGGCTTCATCGACCAGACCGTTGTGTCCACGCTGCGCCGCTGGTCGG
AG
R.subulata_kao
ACCACCTACCTGGGCTTCATCGACCAGACCGTCGTGGCCACGCTGCGCCGCTGGTCG
GAG
L.leiocarpa_kao
ACCACCTACCTCGCCTTCATCGACCAGACCGTCGTCTCCACGCTCCGCCGCTGGTCCG
AC
E.erecta_kao
ACCACGTACCTCGGATTCATCGACAAGACCGTCGTGTCGACGCTGAGTCGGTGGTCGG
AC
Clustal Consensus * ** ***** ********* ***** ** * ** * ** *****

190 200 210 220 230 240
....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
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TC
O.meridionalis_kao
CCGGAGTCCGGCCAGGTCGAGTTCCTCACCGAGCTCAGGCGCATGACCTTCAAGATCA
TC
O.punctata_kao
CCGGACTCCGGCGAGTTCGAGTTCCTCACCGAGCTCCGACGTATGACCTTCAAGATCA
TC
O.officinalis_kao
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O.australiensis_kao
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ATC
O.brachyantha_kao

GAGCCAGCCGCCGAGGTCGAGTTCCTCACCGAGCTGCGCCGGATGACCTTCAAGATC
ATC
O.granulata_kao
CCCGGCGCAGGCGAGGTCGAGTTCCTCACCGAGCTCCGCCGCATGACGTTCAAGATC
ATC
L.tisserantti_kao TCC---
TCCGGCGAGATCAAATTCCTCACCGAGCTCCGCCGCATGACGTTCAAGATCATC
C.aquatica_kao ------
GCCGGCGAGGTGGAGTTCCTCACGGAGCTGCGGCGGATGACCTTCAAGATCATT
R.subulata_kao ------
GCCGGCGAGGTGGAGTTCCTCACCGAGCTGCGGCGGATGACGTTCAAGATCATC
L.leiocarpa_kao ---
GGCGCCGGCGAGGTCGAGTTCCTCACCGAGCTCCGCCGCATGACCTTCAAGATCATC
E.erecta_kao
GGGGGGAAGGAGATGGAGTTGATCCTCACGGAGATGCGGCGGATGAACTTCAAGGTC
ATC
Clustal Consensus * * ******* *** * * ** **** ****** ****

250 260 270 280 290 300
....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
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O.meridionalis_kao
GTCCAGATCTTCATGAGCGGCGCCGACGACGCCACCATGGAGGCCCTGGAGCGGAGC
TAC
O.punctata_kao
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TAC
O.officinalis_kao
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O.granulata_kao
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L.tisserantti_kao
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TAC

R.subulata_kao

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L.leiocarpa_kao

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TAC

E.erecta_kao

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Clustal Consensus *** *********** ************** * ****** * * ** * ******

310 320 330 340 350 360

....|....|....|....|....|....|....|....|....|....|....|....|

O.sativa_kao

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AC

O.meridionalis_kao

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AC

O.punctata_kao

ACCGACCTCAACTACGGCATGCGCGCCATGGCCATCAACCTCCCGGGCTTCGCCTACC
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O.officinalis_kao

ACCGACCTCAACTACGGGATGCGCGCCATGGCCATCAACCTCCCAGGGTTCGCCTACC
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O.brachyantha_kao

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AC

O.granulata_kao

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AC

L.tisserantti_kao

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AT

C.aquatica_kao

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R.subulata_kao

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L.leiocarpa_kao

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E.erecta_kao
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AC
Clustal Consensus ** ** ******** ** **** ******** ****** **** ** ********* *

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CAGG
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CAGG
L.tisserantti_kao
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C.aquatica_kao
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R.subulata_kao
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AGG
L.leiocarpa_kao
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CCGG
E.erecta_kao
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GG
Clustal Consensus * ** ** ***** * *** ** * * ****** * ** ** **

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O.meridionalis_kao  AGGGCCGCCGCCGCC---
AAGGGCTTCAAACGCTCCGGGGCCATGGACATGATGGACCGT
O.punctata_kao      AGGGCCGCCGCCGCC---
AAGGGCTTCACACGCTCCACCGCCATGGACATGATGGACCGC
O.officinalis_kao   AGGGCGGCCGCCGCC---
AATGGCTTCACACGCTCGGGCGCCATGGACATGATGGACCGC
O.australiensis_kao AGGGCGGCCGCCGCC---
AAGGGCTTCACCCGCTCGGGCGTCATGGACATGATGGACCGC
O.brachyantha_kao   AGGGCCGCCACGGCC---
AAAGGCTTCACCCGGCCGACCACCATGGACATGATGGACCGC
O.granulata_kao     AGGGCGGCCACCACC---
AAGGGCTTCACCCGCTCCAGCGCCATGGACATGATGGACCGC
L.tisserantti_kao
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AGG
C.aquatica_kao      AGGGCGGCGACGGGG---
AAAGGGTTCACCCGGTCTAGCAGCATGGACATGATGGACCGG
R.subulata_kao      AGGGCCGCGACGGCC---
AAAGGGTTCACCCGGTCGAGCAGCAGGGACATGATGGACCGG
L.leiocarpa_kao     AGGGCGGCGACGGCC---
AAGGGCTTCACCCGGTCCAGCAGGATGGACATGATGGACCGG
E.erecta_kao        AGGACGGCGACGGCG---
AAAGGATTCACCAGGTCGTCGGCCATGGACATGATGGACAGG
Clustal Consensus   *** * **  *     ** ** ** *   *  *      * ************  *

                    490       500       510       520       530       540
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O.meridionalis_kao
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GTC
O.punctata_kao
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GTC
O.officinalis_kao
CTCATCGACGCCGAGGACGAGCGCGGCCGCCGCCTCGCCGACGACGAGATCATCGAC
GTC
O.australiensis_kao
CTCATCGAGGCCGAGGACGAGCGCGGCCGCCGCCTCGCCGACGACGAGATCATCGAC
GTC
```

O.brachyantha_kao
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GTC
O.granulata_kao
CTCATCGAGGCCGAGGACGACCGCGGCCGCCGCCTCGCCGACGACGAGATCATCGAC
GTC
L.tisserantti_kao
CTTATCGAGGCTGAGGATGAGCGTGGACGGCGGCTCGCCGACGACGAGATCATCGAT
GTG
C.aquatica_kao
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CGTC
R.subulata_kao
CTGATCGAGGCCGAGGACGAGCGCGGCCGGCGGCTCGGCGACGACGAGATCATCGA
CGTC
L.leiocarpa_kao
CTGATCGAGGCGGAGGACGAGCGCGGCCGCCGCCTCGCCGACGACGAGATCATCGA
CGTC
E.erecta_kao
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TC
Clustal Consensus

```
                    550       560       570       580       590       600
               ....|....|....|....|....|....|....|....|....|....|....|....|
```

O.sativa_kao
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CC
O.meridionalis_kao
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CC
O.punctata_kao
CTCATCATGTACCTCAACGCCGGCCACGAGTCCTCCGGCCACATCACCATGTGGGCCA
CC
O.officinalis_kao
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CC
O.australiensis_kao
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CC
O.brachyantha_kao
CTCATCATGTACCTCAACGCCGGCCACGAGTCCTCCGGCCACATCACCATGTGGGCCA
CC
O.granulata_kao
CTCATCATGTACCTCAACGCCGGCCACGAGTCCTCCGGCCACATCACCATGTGGGCCA
CC

L.tisserantti_kao
CTCATCATGTACCTCAACGCCGGCCATGAATCTTCCGGCCATATCACCATGTGGGCCAC
C
C.aquatica_kao
CTCATCATGTACCTCAACGCCGGCCACGAGTCCTCCGGCCACATCACCATGTGGGCCA
CC
R.subulata_kao
CTCGTCATGTACCTCAACGCCGGCCATGAGTCCTCCGGCCACATCACCATGTGGGCCA
CC
L.leiocarpa_kao
CTCATCATGTACCTCAACGCCGGCCACGAGTCCTCCGGCCACATCACCATGTGGGCCA
CC
E.erecta_kao
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T
Clustal Consensus *** ******* ************* ** ** *** ** ************** **

610 620 630 640 650 660
....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
GTCTTCCTCCAGGAGAACCCCGACATCTTCGCAAGAGCAAAGGCTGAGCAAGAGGAGA
TC
O.meridionalis_kao
GTCTTCCTCCAGGAGAACCCCGACATCTTCGCAAGAGCAAAGGCTGAGCAAGAGGAGA
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O.punctata_kao
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TC
O.officinalis_kao
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ATC
L.tisserantti_kao
GTCTTCCTCCAGGAGAATCCCGACATCTTAGCAAGGGCAAAGGCTGAGCAAGAGGAGA
TC
C.aquatica_kao
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TC

R.subulata_kao
GTCTTCCTCCAGGAGAACCCCGAAATCTTCGCAAGGGCAAAGGCCGAGCAAGAGGAAA
TC
L.leiocarpa_kao
GTCTTCCTGCAGGAGAACCCCGACATCTTCGCGAGGGCAAAGGCCGAGCAAGAAGAG
ATC
E.erecta_kao
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TC
Clustal Consensus * ***** ** ***** ** ** * ** ** * ** ** ** * ***** ** ***

670 680 690 700 710 720
....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
ATGAGAAGCATTCCAGCAACGCAGAACGGATTAACCCTCAGGGACTTCAAGAAGATGC
AC
O.meridionalis_kao
ATGAGAAGCATTCCAGCAACGCAGAACGGATTAACCCTCAGGGACTTCAAGAAGATGC
AC
O.punctata_kao
ATGAGAAGCATACCAGCAACGCAGAAGGGATTAACGCTCAGGGACTTCAAGAAGATGC
AT
O.officinalis_kao
ATGAGAAGCATACCAGCAACGCAGAAGGGATTAACGCTCAGGGACTTCAAGAAGATGC
AC
O.australiensis_kao
ATGAGAAGCATACCAGCAACGCAGAAGGGATTAACCCTCAGGGACTTCAAGAAGATGC
GC
O.brachyantha_kao
ATGAGAAGCATACCCGCAACGCAGAAGGGACTGACACTCAGGGACTTCAAGAAGATGC
AG
O.granulata_kao
ATGAGAAGCATACCACCAACGCAGAAGGGACTCAACCTCAGGGACTTCAAGAAGATGC
AG
L.tisserantti_kao
ATGAGAAGCATACCACCAACGCAGAAGGGACTTACCCTTAGGGACTTCAAGAAGATGC
AC
C.aquatica_kao
ATGAGAAGCATACCACCAACACAGAAGGGACTGAACCTCAGGGACTTCAAGAAGATGC
AG
R.subulata_kao
ATGAGAAACATACCATCAACACAGAAGGGACTGAACCTCAGGGACTTCAAGAAGATGCA
T
L.leiocarpa_kao
ATGAGAAGCATACCACCAACACAGAAGGGGCTGAGCCTCAGGGACTTCAAGAAGATGC
AG

```
E.erecta_kao       ATGAGAAGC----------CTGAGGGATTTCAGGAAGATGGAG
Clustal Consensus  ******** *          ** ***** **** *******

                      730       740       750       760       770       780
                 ....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
TTCCTCTCACAGGTTGTCGACGAGACACTTCGCTGCGTCAACATCTCCTTCGTGTCCTT
C
O.meridionalis_kao
TTCCTCTCACAGGTTGTCGACGAGACACTTCGCTGCGTCAACATCTCCTTCGTGTCCTT
C
O.punctata_kao
TTCCTCTCACAGGTTGTCGACGAGACACTTCGCTGCGTCAACATCTCCTTCGTGTCCTT
C
O.officinalis_kao
TTCCTCTCACAGGTTGTCGACGAGACACTTCGCTGCGTCAACATCTCCTTCGTGTCCTT
C
O.australiensis_kao
TTCCTCTCACAGGTTATCGACGAGACACTTCGCTGCGTCAACATCTCCTTCGTGTCCTT
C
O.brachyantha_kao
TTCCTCTCCCAGGTTATCGATGAGACACTTCGCTGCGTCAACATCTCGTTCGTGTCATT
C
O.granulata_kao
TTCCTCTCGCAGGTTATCGACGAGACGCTTCGCTGCGTCAACATCTCGTTTGTGTCCTT
C
L.tisserantti_kao
TTCCTCCAGCAGGTTGTCGACGAGACACTTCGCTGCGTCAACATCTCCTTCGTGTCCTT
C
C.aquatica_kao
TACCTCTCACAGGTTATCGACGAGACACTTCGCTGCGTCAACATCTCCTTTGTGTCCTT
C
R.subulata_kao
TACCTCTCACAGGTTGTCGACGAGACACTTCGCTGCGTCAACATCTCGTTTGTGTCCTT
C
L.leiocarpa_kao
TACCTCTCACAGGTTATCGACGAGACGCTTCGCTTTGTCAACATCTCGTTTGTGTCCTTC
E.erecta_kao
TACCTCTCACATGTTATCGACGAGACACTTCGCTTCGTCAACATGTCCTTTTTGTCGTTC
Clustal Consensus  * *****  ** *** **** ***** *******  ******** ** **  **** ***

                      790       800       810       820       830       840
                 ....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
CGTCAGGCCACAAGAGACATCTTTGTGAACGGTTATCTTATCCCCAAGGGGTGGAAGG
TT
```

O.meridionalis_kao
CGTCAGGCCACAAGAGACATCTATGTGAACGGTTATCTGATCCCCAAGGGGTGGAAGG
TT
O.punctata_kao
CGTCAGGCCACAAGAGACATCTATGTGAACGGCTATCTGATCCCCAAGGGGTGGAAGG
TC
O.officinalis_kao
CGTCAGGCCACAAGAGACATCTATGTGAACGGCTATCTGATCCCCAAGGGGTGGAAGG
TT
O.australiensis_kao
CGCCAGGCCACAAGAGACATCTATGTGAACGGCTATCTGATACCCAAGGGCTGGAAGG
TT
O.brachyantha_kao
CGTCAGGCGACCAGAGACGTCTATGTGAACGGCTATCTGATACCCAAGGGCTGGAAGG
TT
O.granulata_kao
CGTCGGGCGACAAGAGACGTCTATGTGAACGGTTATCTGATACCCAAGGGTTGGAAGG
TT
L.tisserantti_kao
CGTCAGGCGACAAAAGACGTCTATGTGAACGGCTATCTGATACCCAAGGGCTGGAAGG
TT
C.aquatica_kao
CGCCAAGCGACACGAGACGTCTTTGTGAACGGCTATCTGATACCAAAGGGCTGGAAGG
TT
R.subulata_kao
CGTCAGGCGACCCGAGACGCCTTCGTGAACGGCTATCTGATACCAAAGGGCTGGAAG
GTT
L.leiocarpa_kao
CGTCAGGCAACACGCGACGTCTATGTGAACGGTTATCTGATACCAAAGGGCTGGAAGG
TT
E.erecta_kao
CGCCAGGCAACAAAAGACGTCTTTGTGAATGGCTATCTGATACCAAAGGGTTGGAAGGT
G
Clustal Consensus ** * ** ** *** ** ***** ** ***** ** ** ***** ********

850 860 870 880 890 900
....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
CAGCTGTGGTACAGAAGTGTGCACATGGATGACCAAGTTTATCCTGACCCCAAAATGTT
C
O.meridionalis_kao
CAGCTGTGGTACAGAAGTGTGCACATGGATGACCAAGTTTATCCTGACCCAAAAATGTT
C
O.punctata_kao
CAGCTGTGGTACAGAAGTGTGCACATGGATGACCAAGTTTATCCTGACCCCAAAATGTT
T

O.officinalis_kao
CAGCTGTGGTACAGAAGTGTGCACATGGATGACCAAGTTTATCCTGACCCCAAAATGTT
C
O.australiensis_kao
CAGCTGTGGTATAGAAGTGTTCACATGGATGACCAAGTTTATCCTGACCCCAAAATGTT
C
O.brachyantha_kao
CAGCTGTGGTACAGAAGTGTACACATGGATGACCAAGTTTATCCTGACCCCAAAATGTT
C
O.granulata_kao
CAGCTGTGGTACAGAAGCGTGCACATGGATGACCAAGTTTATCCTGACCCCAAAGTATT
C
L.tisserantti_kao
CAGTTGTGGTACAGAAGTGTGCACATGGATGACCAAGTTTATCCTGACCCCAAAACGTT
C
C.aquatica_kao
CAGCTGTGGTACAGAAGTGTGCACATGGATCCTCAAGTTTACCCTGACCCCAACAAGTT
C
R.subulata_kao
CAGCTGTGGTACAGAAGCGTGCACATGGATTCTCAAGTTTACCCTGATCCCAAAAAGTT
C
L.leiocarpa_kao
CAGCTGTGGTACAGAAGTGTGCACATGGATCCTCAAGTTTATCCTGACCCCTACAAGTT
C
E.erecta_kao
CAGCTGTGGTTCAGAAATGTGCATATGGATCCTCAGGTTTATTCAGATCCCAGCAAGTT
C
Clustal Consensus *** ****** **** ** ** ****** ** ***** * ** ** ** **

910 920 930 940 950 960
....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
AACCCTTCAAGATGGGAGGGACCCCCTCCGAAAGCCGGAACATTCCTTCCATTTGGAC
TG
O.meridionalis_kao
AACCCTTCAAGATGGGAGGGACCCCCTCCGAAAGCCGGAACATTCCTTCCATTTGGAC
TG
O.punctata_kao
AACCCTTCAAGATGGGAGGGCCCCCCTCCGAAAGCCGGAACATTCCTTCCATTTGGAC
TG
O.officinalis_kao
AACCCTTCAAGATGGGAGGGGCCCCCTCCGAAAGCCGGAACATTCCTTCCATTTGGAC
TG
O.australiensis_kao
AACCCTTCAAGATGGGAGGGTCCCCCTCCGAAAGCCGGAACATTCCTTCCATTTGGACT
G

O.brachyantha_kao
AATCCATCAAGATGGGAGGGTCCCCCGCCGAGAGCCGGAACATTCCTTCCATTTGGAC
TG
O.granulata_kao
AACCCATCAAGATGGGAGGGTCCCCCGCCGAGAGCTGGAACATTCCTTCCATTTGGAC
TG
L.tisserantti_kao
AACCCATCAAGATGGGAGGGTCCTCCGCCGAGAGCCGGAACATTCCTTCCATTTGGAC
TG
C.aquatica_kao
AACCCTTCAAGATGGGAGGGTCCCCCGCCGAGAGCCGGAACATTCCTTCCATTTGGAC
TG
R.subulata_kao
AACCCTTCAAGATGGGAGGGTCCCCCGCCGAGAGCCGGAACATTCCTTCCATTTGGAC
TG
L.leiocarpa_kao
AACCCTTCAAGATGGGAGGGTCCCCCGCCAAGAGCCGGAACATTCCTCCCATTTGGAC
TG
E.erecta_kao
AAGCCTTCAAGATGGGATGGTTCTTCACCGAGAGCCGGAACGTTCCTTCCATTCGGACT
G
Clustal Consensus ** ** ********** ** * * ** * *** ***** ***** ***** ******

970 980 990 1000 1010 1020
....|....|....|....|....|....|....|....|....|....|....|....|
O.sativa_kao
GGAGCAAGACTGTGCCCTGGAAATGATCTTGCAAAGCTGGAGATCTCTGTCTTCCTCCA
T
O.meridionalis_kao
GGAGCAAGACTGTGCCCTGGAAATGATCTTGCAAAGCTGGAGATCTCTGTCTTCCTCCA
T
O.punctata_kao
GGATCGAGACTGTGCCCTGGAAATGATCTTGCAAAGCTGGAGATCTCTGTCTTCCTCCA
T
O.officinalis_kao
GGAGCAAGACTGTGCCCTGGAAATGATCTTGCAAAGCTGGAGATCTCTGTCTTCCTCCA
T
O.australiensis_kao
GGAGCGAGGCTGTGCCCTGGAAATGATCTTGCAAAGCTGGAGATCTCTGTCTTCCTCC
AT
O.brachyantha_kao
GGAGCGAGACTGTGCCCTGGAAATGATCTTGCAAAGCTGGAGATCTCTGTCTTTCTCCA
T
O.granulata_kao
GGAGCGAGACTATGCCCTGGAAATGATCTTGCAAAGCTGGAGATCTCTGTCTTCCTCCA
T

```
L.tisserantti_kao
GGATCGAGACTGTGCCCTGGAAATGATCTTGCAAAGCTCGAGATCTCTGTCTTCCTCCA
T
C.aquatica_kao
GGAGCGAGACTCTGCCCTGGAAATGATCTTGCAAAGCTGGAGATCTCTGTCTTCCTCCA
T
R.subulata_kao
GGATCGAGACTCTGCCCTGGAAATGATCTTGCAAAGTTGGAGATCTCTGTCTTCCTCCA
T
L.leiocarpa_kao
GGAGCAAGGCTCTGCCCTGGAAATGATCTTGCAAAGCTGGAGATCTCTGTCTTCCTCCA
T
E.erecta_kao
GGTGCAAGACTGTGCCCTGGAAATGATCTTGCAAAGCTGGAGATCTCTGTCTTCCTCCA
C
Clustal Consensus  ** * ** ** ********************** * ************** *****

                    1030      1040      1050
               ....|....|....|....|....|....|....|.
O.sativa_kao        CATTTTCTCCTGGGTTACAAGCTGAAGAGGGCAAAT
O.meridionalis_kao  CATTTTCTCCTGGGTTACAAGCTGAAGAGGGCAAAT
O.punctata_kao      CATTTTCTCCTAGGTTACAAGCTGACGAGGACAAAT
O.officinalis_kao   CATTTTCTCCTAGGTTACAAGCTGACGAGGACAAAT
O.australiensis_kao CATTTTCTCCTAGGTTACAAGCTGACGAGGACAAAT
O.brachyantha_kao   CATTTTCTCCTAGGTTACAAGCTGACGAGGACAAAT
O.granulata_kao     CATTTTCTCCTAGGTTACAAGCTGACGAGGACAAAT
L.tisserantti_kao   CATTTTCTCCTGGGTTACAAGCTGACGAGGACAAAT
C.aquatica_kao      CATTTTCTCCTAGGTTACAAGCTGACGAGGACAAAT
R.subulata_kao      CATTTTCTCCTAGGTTACAAGCTGACGAGGACAAAT
L.leiocarpa_kao     CACTTTCTCCTAGGTTACAAGCTGACGAGGACAAAT
E.erecta_kao        CATTTCATCCTAGGTTACAAGCTTACAAGGACAAAT
Clustal Consensus   ** **  **** *********** *  *** *****
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Figure S2 Alignment of coding sequences of *KAO* gene in twelve species. Highly conserved sites are indicated with asterisks in the bottom.