Construction of Anti-codon Table of the Plant Kingdom and Evolution of tRNA Selenocysteine (tRNA\textsc{Sec})

Tapan Kumar Kumar Mohanta (nostoc.tapan@gmail.com)
Yeungnam University

Awdhesh Kumar Mishra
Yeungnam University

Abeer Hashem
King Saud University

Elsayed Fathi Abd_Allah
King Saud University

Abdul Latif Khan
University of Nizwa

Ahmed Al-Harrasi
University of Nizwa

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Abstract

**Background** The tRNAs act as a bridge between the coding mRNA and incoming amino acids during protein translation. The anti-codon of tRNA recognizes the codon of the mRNA and deliver the amino acid into the protein translation chain. However, we did not know about the exact abundance of anti-codons in the genome and whether the frequency of abundance remains same across the plant lineage or not.

**Results** Therefore, we analysed the tRNAnome of 128 species and reported an anti-codon table of the plant kingdom. We found that CAU anti-codon of tRNA\textsuperscript{Met} has highest (5.039%) whereas GCG anti-codon of tRNA\textsuperscript{Arg} has lowest (0.004%) abundance. However, when we compared the anti-codon frequencies according to the tRNA isotypes, we found tRNA\textsuperscript{Leu} (7.808%) has highest abundance followed by tRNA\textsuperscript{Ser} (7.668%) and tRNA\textsuperscript{Gly} (7.523%). Similarly, suppressor tRNA (0.036%) has lowest abundance followed by tRNA\textsuperscript{Sec} (0.066%) and tRNA\textsuperscript{His} (2.109). The genome of Ipomoea nil, Papaver somniferum, and Zea mays encoded the highest number of anti-codons at 59 each whereas the genome of Ostreococcus tauri was found to encode only 18 isoacceptors. The tRNA\textsuperscript{Sec} genes undergone losses more frequently than duplication and we found that tRNA\textsuperscript{Sec} showed anti-codon switch during the course of evolution.

**Conclusion** The anti-codon table of the plant tRNA will enable us to understand the synonymous codon usage of the plant kingdom and can be very helpful to understand which codon is preferred over other during the translation.

**Background**

The proteins present in cells are the product of the blueprint prescribed by the genes [1–3]. Collectively, all of the genes (including coding and non-coding) present in a cell represent the genome of an organism [4,5]. The construction of a protein from a gene is a complex procedure and requires the involvement of transfer RNA (tRNA), messenger RNA (mRNA), ribosomes, amino acids, and other molecules [6–9]. This process is commonly known as translation which is a fundamental parameter of living cells [6–9]. The functional apparatus involved in gene translation is highly conserved across the tree of life [10]. mRNA conveys the blueprint information as triplet codons composed of nucleotides and tRNA are able to perceive the cognate codons [11,12]. Although mRNA and ribosomes represent the two major parts of the machinery responsible for translation, transfer RNAs (tRNAs) are the fundamental units of this translation machinery [13–15]. The anti-codon of a tRNA links to the codon of the mRNA and supplies the corresponding amino acid into the protein translation chain [3,8,15,16]. Two or more different tRNAs can bind an amino acid and transfer it to the ribosome [17–20]. There are 22 different amino acids encoded by 63 codons (including UGA and UAG codons for selenocysteine and pyrolysine, respectively) as several of the amino acids are encoded by more than one codon and hence its corresponding anti-codon [21–25]. Therefore, it is possible to encode more than one tRNA molecule with different anti-codons to transfer a particular amino acid [21,26–28]. Although codon selection for a corresponding anti-codon is the primary unit of the translation machinery, mutational bias, selection, drift, and codon usage bias also shape the prescribed translation [29–32]. Although there are critical steps for the efficient and proper functioning of the translation machinery, other synonymous codons can also serve as an alternative choice [32–34]. The differential use of codons also reflects their natural demand in the protein translation machinery [35,36]. tRNAs are classified into various gene families based on their isoacceptor anti-codons [17,19,20]. The available tRNA pool is maintained at a level that can accommodate the transcript levels present in a cell, thus ensuring efficient and accurate translation. Highly-expressed genes, however, exhibit codon usage bias that reflects the copy number of the corresponding tRNA [37–39]. Translational selection acts to maintain the balance between codon usage and tRNA availability [40–42]. There is always selection pressure, however, to increase the production of the codons used in highly-expressed genes [32,43,44].
Over the course of evolution, the earth has undergone enormous changes and the Plant Kingdom has been subjected to numerous stresses [45–47]. All living organisms had to adapt to a changing environment, which resulted in the increased importance of some protein-coding genes while others became less important [48–50]. Accordingly, there was a need to alter the relative number and type of available tRNAs to fulfil the translational requirements of the new and/or modified protein-coding genes [51,52]. Changes in the relative number and type of tRNA molecules are also associated with a change in the number and type of anti-codons [53,54]. The role of selection pressure brought about by translational demand and its role in maintaining tRNA pools has not been adequately addressed. Furthermore, the selection pressure that determines the maintenance of low copy tRNA families and anti-codons also remains unclear. Whether translational selection pressure favours optimal codons in particular cases and keeps other codons as non-optimal, and hence in low supply, is unknown. It is also unknown if the amino acid requirements of proteins impact the need to provide specific tRNAs having the required anti-codons, as well as the genes that encode those tRNAs. In the present study, an attempt was made to determine the frequency of anti-codons in the tRNAome of the Plant Kingdom to better understand the presence of codons and anti-codon frequency. Our objective was to provide information on the link between the presence of codons and their corresponding anti-codons, tRNAs, and the number of amino acids utilized in plant proteomes. Therefore, we analysed the frequency of anti-codons in the tRNA of plant genomes and constructed an anti-codon table of the Plant Kingdom.

**Material And Methods**

**Sequence Retrieval**

The annotated RNA sequence files of all 128 plant species were downloaded from the National Center for Biotechnology Information (NCBI) using the Ensemble genome browser. The downloaded sequence files were scanned for the presence of tRNAs using tRNAscan-SE software on a Linux-based platform. The resulting tRNAscan files were used for further analysis. After the completion of the scanning of individual files, all files were merged to obtain a complete plant tRNAome file. The frequency of each individual anti-codon was obtained from the tRNAome file and presented as a number and percentage (%). In the course of the analysis, several tRNA$^{\text{Sec}}$ were identified in different plant genomes and were kept separately for further study.

**Sequence alignment**

Multiple sequence alignment of tRNA$^{\text{Sec}}$ genes was conducted using multalin software with default parameters. To construct the phylogenetic tree, a multiple sequence alignment of tRNAs and tRNA$^{\text{Sec}}$ were conducted using the MUSCLE program in MEGA7 software [55,56]. The resulting alignment was saved in a MEGA file format. The alignment file was subsequently used to construct a phylogenetic tree using MEGA7 software. Prior to the construction of the phylogenetic tree, a model selection was carried out using the following statistical parameters; statistical method, maximum likelihood substitution type, nucleotides, gaps/missing data treatment, complete deletion. Based on the lowest BIC score, a phylogenetic tree of tRNAs and tRNA$^{\text{Sec}}$ was constructed. The statistical parameters used to construct the phylogenetic tree were: statistical method (maximum likelihood), test of phylogeny (bootstrap method), no. of bootstrap replicates (1000), substitution type (nucleotides), model/method (Kimura-2-parameter model), rates among sites (gamma distributed), no. of discrete gamma parameters (5), gaps/missing data treatment (partial deletion), site coverage cut-off (95 %), ML Heuristic method (nearest-neighbour-interchange), and branch swap filter (very strong). A separate phylogenetic tree was constructed using all of the tRNA$^{\text{Sec}}$ sequences and the same statistical approaches as mentioned above to determine deletion and duplication events. The constructed phylogenetic tree of tRNA$^{\text{Sec}}$ genes was exported in a Newick file format. Subsequently, a species tree was constructed using all of the 128 species in the taxonomy browser of NCBI. To determine RNA$^{\text{Sec}}$ deletion and duplication events, the phylogenetic tree
of tRNA$^{\text{Sec}}$ was reconciled with the species tree using Notung software, version 2.9. The reconciled gene and species tree revealed deletion, duplication, and co-divergence events that occurred in tRNA$^{\text{Sec}}$ genes. The resultant phylogenetic tree of tRNAs (with tRNA$^{\text{Sec}}$) and the phylogenetic tree of tRNA$^{\text{Sec}}$ were analysed by using Icy Tree to identify recombination events.

**Cluster based grouping of the anti-codons**

Anti-codons were grouped based on their percentage frequency in the tRNAnome. To cluster them, the percent frequency of anti-codons was used against each anti-codon. A classical clustering approach was used to cluster the anti-codons using a paired group UPGMA algorithm and Euclidean similarity index with 1000 bootstrap replicates.

**Statistical analysis**

The probability plot linear regression analysis of tRNA gene number per genome and frequency of anti-codons were statistically analysed and a value of p<0.05 was considered to be significant. To investigate anti-codon numbers in different lineages and their statistical significance, a t-test was conducted comparing anti-codon number in eudicot vs. monocot, eudicot vs. algae, and monocot vs. algae. Differences were deemed significant at p < 0.05. All of the statistical analyses were conducting using Past3 software.

**Results**

**Genome size is not proportional to the number of tRNA genes**

A genome-wide analysis of fully-annotated whole genome sequences of 128 species was conducted to identify tRNA genes and to construct an anti-codon table of the Plant Kingdom (Table 1). The species included in the study varied in the size of their respective genomes (Table 2). A regression analysis was conducted to determine the correlation between genome size and the number of tRNA genes encoded per genome. Results indicated that plant genome size was not correlated ($r = 0.5471$, $y=0.17892x+619.76$) with the number of the tRNA genes per genome (Figure 1). *Ipomoea nil*, with a genome size of genome size of 735.23 Mb, possesses 6,475 tRNA genes which was the highest number of tRNA encoding genes identified in the species of plants that were analysed. Other species with a high number of tRNA genes in their genome were *Cucurbita moschata* (4,062), *Cucurbita pepo* (3,228), *Cucurbita maxima* (3,036), *Papaver somniferum* (2,571), *Brassica napus* (2,180), and *Ipomoea triloba* (2,180). Among the 128 analysed plant species, 22 (16.92%) species possessed more than one thousand tRNA genes in their genome. In contrast, *Ostreococcus tauri* and *Phaedactylum tricornutum* only encoded 41 tRNA genes in their genome, which was the lowest number of tRNA encoding genes identified in the species of plants that were analysed. Other species encoding lower number of tRNA genes were *Raphidocelis subcapitata* (43), *Monoraphidium neglectum* (48), and *Bathycoccus prasinus* (57). The genome size of *O. tauri*, *P. tricornutum*, *R. subcapitata*, and *M. neglectum* was 14.76, 27.4, 51.16, and 69.71 Mb, respectively. These genome sizes are relatively smaller than the genome of most of the other plant species that were analysed.

**CAU (Met) was the most abundant and GCG (Arg) was the least abundant encoded Anti-codons in the Plant Kingdom**

The occurrence of each of the anti-codons were separately analysed to determine the frequency of anti-codons in the genomes of the Plant Kingdom. Results indicated that CAU (Met) was the most abundant (5.033%) anti-codon in the Plant Kingdom, followed by GUC (Asp, 4.274%), GUU (Asn, 4.020%), and GCC (Gly, 3.811%) (Table 1, Supplementary File 1). In contrast, GCG (Arg) was identified as the least abundant (0.004%) anti-codon in the Plant Kingdom, followed by GAG (Leu, 0.009%), CUA (Sup, 0.011%), and ACU (Ser, 0.019%) (Table 1, Supplementary File 1). The lowest-abundant anti-codon (GCG) was only present in *Ipomea nil*, *Nicotiana attenuata*, *Papaver somniferum*, and *Ziziphus*.
When the anti-codon frequency of different tRNA isoacceptor was considered, however, tRNA<sub>Leu</sub> was found to be the most abundant tRNA isoacceptor (Table 1). Approximately 7.808% of all anti-codons in the Plant Kingdom were found to be encoded by tRNA<sub>Leu</sub> (Table 1). The abundance of tRNA<sub>Leu</sub>, was followed by tRNA<sub>Ser</sub> (7.668%), tRNA<sub>Gly</sub> (7.523%), and tRNA<sub>Arg</sub> (7.284%) (Table 1). tRNA<sub>Leuc</sub>, tRNA<sub>Ser</sub>, and tRNA<sub>Arg</sub> encode six different isoacceptors which might be the reason for their higher abundance in the plant genomes. Suppressor tRNA (0.036%) was found to be the least abundant tRNA isoacceptor in the plant genomes, followed by tRNA<sub>Sec</sub> (0.066%), tRNA<sub>His</sub> (2.109%), and tRNA<sub>Cys</sub> (2.547%) (Table 1). Suppressor tRNA (CUA) anti-codon was only found in *Ectocarpus siliculosus*, *Nicotiana sylvestris*, and *Zea mays* (Supplementary File 1).

**Anti-codons can be classified into five groups based on their frequency of occurrence in plant genomes**

A clustering analysis based on the frequency of abundance of the anti-codons in the Plant Kingdom was conducted using the paired group (UPGMA) algorithm and Euclidean similarity index with 1000 bootstrap replicates. The analysis revealed five distinct groups of anti-codons and were named as group A, B, C, D, and E (Figure 2). The anti-codons in the different groups were: Group A - CAU, GCC, GUU, and GUC; Group B - CUU, GAA, AAA, AGA, UCC, GCA, GCU, UCC, AAC, CCA, GUA, UUU, UGG, AGC, UUC, and UAC; Group C - UGA, UGU, UAG, UUG, UCU, CAC, AGU, GUG, AAG, AGG, UGC, CAA, and ACC; Group D - CGG, CGU, CGA, CGG, CAG, UAA, AGG, UAG, UCC, CCC, UAC, CCU, and CUG; and Group E - GGU, GGA, AUU, GUA, GAC, AUC, AUG, AAA, ACA, UCA, GGG, ACU, UUA, GCC, ACC, AUA, GAG, CUA, and GCG (Figure 2). The anti-codon groupings are based on their abundance in plant genomes, from highest (Group A) to lowest (Group E).

**Plant genomes encode 18 to 59 isoacceptors (anti-codons)**

The genome-wide analysis of the Plant Kingdom revealed the diversity in the number of anti-codons present in the genomes of individual species, which ranged from 18–59 (Table 2). *Ostreococcus tauri* was found to encode only 18 isoacceptors while *Micromonas commoda* encodes only 26 isoacceptors (Table 2). *Ipomoea nil*, *Papaver somniferum*, and *Zea mays* encoded the highest number of anti-codons at 59 each. At least 51 (39.53%) species were found to encode 50 or more anti-codons in their genome. On average, plant genomes encode 48.25 anti-codons per genome. A paired two tailed t-test was conducted to statistically analyse the frequency of anti-codons present in algae, eudicot, and monocot species. The comparison between eudicot and monocot species indicated that the frequency of tRNA anti-codons in these two groups was not significantly different (P < 0.05) at 1.2691 < 1.984 (t-test result 1.2691, critical value $T_{1.984}$), respectively (Table 3). In contrast, a significant difference in tRNA frequency was observed between eudicots and algae (10.3939 > 1.987), and between monocots and algae (6.2914 > 2.037) (Table 3). Notably, the variance in tRNA frequency in the monocot lineage was much lower than it was in the eudicots and algae.

**Only a few species have lost tRNA genes**

Our analysis revealed that a few species have lost the presence of specific tRNA genes (tRNA isotype) in their genome. These species include *Coccomyxa subellipsoidea* (tRNA<sub>Tyr</sub>), *Corchorus capsularis* (tRNA<sub>Leu</sub>, tRNA<sub>Thr</sub>, tRNA<sub>Lys</sub>), *Corchorus olitorius* (tRNA<sub>Tyr</sub>), *Klebsormidium nitens* (tRNA<sub>Tyr</sub>, tRNA<sub>Ser</sub>), *Monoraphidium neglectum* (tRNA<sub>Thr</sub>), *Ostreococcus tauri* (tRNA<sub>Phe</sub>, tRNA<sub>Gln</sub>), *Picea glauca* (tRNA<sub>Ser</sub>), *Phaedactylum tricornutum* (tRNA<sub>Cys</sub>), and *Raphidocelis subcapitata* (tRNA<sub>Tyr</sub>) (Table 2). These species were found to lost the mentioned gene(s) in their genome. Understanding the loss of tRNA genes and its functional implication in protein translation is very crucial.
Some plant species encode tRNA\textsuperscript{Sec} in their genomes

Several plant species were found to encode tRNA genes for selenocysteine amino acids. More specifically, 22 (17.187%) species were found to encode a tRNA\textsuperscript{Sec} gene in their genome. These species were Aegilops tauschii, Beta vulgaris, Brassica rapa, Cucumis sativus, Cucurbita maxima, Cucurbita moschata, Cucurbita pepo, Ectocarpus siliculosus, Ipomoea nil, Ipomoea triloba, Lactuca sativa, Momordica charantia, Medicago truncatula, Monoraphidium neglectum, Nicotiana tabacum, Papaver somniferum, Picea glauca, Populus euphratica, Salvia splendens, Tarenaya hassleriana, Triticum urartu, and Zea mays (Table 2). The length of tRNA\textsuperscript{Sec} encoding genes was ranged from 70 to 90 nucleotides with average length being 72.93 nucleotides per tRNA. A multiple sequence alignment of tRNA\textsuperscript{Sec} genes indicated the presence of a conserved G-x-C nucleotide at the 30\textsuperscript{th} and 32\textsuperscript{nd} positions and a conserved U-C-A at 34\textsuperscript{th}, 35\textsuperscript{th}, and 36\textsuperscript{th} positions (Supplementary Figure 1). The pseudo-uridine loop was also found to contain a conserved G-U-U-x\textsuperscript{2}-A-x\textsuperscript{2}-C nucleotide consensus sequence (Supplementary Figure 1). The tRNA\textsuperscript{Sec} in C. maxima (NW_019272053.1), however, was found to encode a C-U-U nucleotide sequence instead of a G-U-U conserved consensus sequence in its pseudo-uridine loop (Supplementary Figure 1).

Loss of tRNA\textsuperscript{Sec} occurred to a greater extent than duplication

A phylogenetic tree was constructed to investigate the evolution of tRNA\textsuperscript{Sec} genes by considering the nucleotide sequences of all the 20 tRNA genes along with tRNA\textsuperscript{Sec} genes. The phylogenetic tree revealed the 28 major tRNA groups (Figure 3). The tRNA\textsuperscript{Sec} genes were clustered in the middle of the phylogenetic tree and tRNA\textsuperscript{Sec} was found to be present in at least six different clusters (Figure 3). A few tRNA\textsuperscript{Sec} genes were grouped with tRNA\textsuperscript{Lys} (CUU), tRNA\textsuperscript{Asn} (GUU), tRNA\textsuperscript{Arg} (UCG, CCG), tRNA\textsuperscript{Gly} (UCC), and tRNA\textsuperscript{Trp} (CCA) (Figure 3). The analysis indicates that tRNA\textsuperscript{Sec} is distributed in different clusters in the phylogenetic tree. This explains the role of duplication events in the evolution of tRNA\textsuperscript{Sec} genes. Therefore, an analysis was conducted to investigate the deletion/duplication events related to tRNA\textsuperscript{Sec} genes. As a result, we found that tRNA\textsuperscript{Sec} deletion events occurred more frequently than duplication events. A total of 45 duplications, 119 deletions, and 9 co-divergent events were identified within 68 tRNA\textsuperscript{Sec} genes found in 22 species (Supplementary Figure 2). The role of recombination in the evolution of tRNA\textsuperscript{Sec} was further analysed. Results indicated that tRNA\textsuperscript{Sec} genes had undergone recombination events, as did other tRNA genes (Figure 4). The role of recombination and duplication of tRNA\textsuperscript{Sec} genes resulted in the sharing of its genetic sequence with other tRNAs genes which may perhaps explain why tRNA\textsuperscript{Sec} was present in different clusters within the phylogenetic tree. A recombination analysis of tRNA\textsuperscript{Sec} genes indicated the role of recombination events within the tRNA\textsuperscript{Sec} itself (Figure 5). A time tree analysis revealed that the divergence time of tRNA\textsuperscript{Sec} genes in plant species occurred at least 2,466.30 million years ago (MYA) (Supplementary Figure 3) and less than a MYA in the case of the tRNA\textsuperscript{Sec} in P. somniferum. The tRNA\textsuperscript{Sec} in P. somniferum was found to arise from a duplication event. The recent divergence time for the tRNA\textsuperscript{Sec} in P. somniferum indicates that this gene has undergone a recent duplication event.

tRNA\textsuperscript{Sec} underwent a switch in anti-codons during evolution

tRNA genes undergo rapid changes during the course of their evolution to meet translational demand. Therefore, an attempt was made to better understand the role of tRNA\textsuperscript{Sec} genes in plant evolution. It is well known that the tRNA\textsuperscript{Sec} gene is encoded by a UCA anticyodon and that this gene was found in different clusters in the phylogenetic tree of tRNAs. An anti-codon switch occurs more frequently with a nucleotide sequence of a tRNA gene with a different anti-codon than with a gene with a similar anti-codon [51]. Therefore, the possibility of anti-codon switch in tRNA\textsuperscript{Sec} gene was examined. tRNA\textsuperscript{Sec} grouped with tRNA\textsuperscript{Lys} (CUU), tRNA\textsuperscript{Asn} (GUU), tRNA\textsuperscript{Arg} (UCG, CCG), tRNA\textsuperscript{Gly} (UCC), and tRNA\textsuperscript{Trp}
(CCA). The UCA anti-codon of tRNA^{Sec} was replaced by CUU in tRNA^{Lys} and in tRNA^{Asn} it was replaced by GUU where the 2\textsuperscript{nd} and 3\textsuperscript{rd} nucleotide of the anti-codons were constant. In tRNA^{Arg} and tRNA^{Gly}, the UCA anti-codon of tRNA^{Sec} was replaced by UCG and UCC where the 1\textsuperscript{st} nucleotide of the anti-codons remained constant and the 2\textsuperscript{nd} and 3\textsuperscript{rd} anti-codons were variable. For the CCG anti-codon of tRNA^{Arg} and the CCA anti-codon of tRNA^{Trp}, the 1\textsuperscript{st} nucleotide of U(CA) of tRNA^{Sec} was replaced with a C nucleotide and the 3\textsuperscript{rd} nucleotide remained variable.

**Statistical Analysis**

The varied number and frequency of anti-codons led us to understand whether or not a dataset is approximately normally distributed. Therefore, we conducted normal probability plot study of anti-codon numbers (Figure 6). The normal probability plot correlation coefficient was 0.9632. the correlation co-efficient and an approximately straight line indicate that normal distribution was good for the dataset (Figure 6). Ordinary linear fit least square regression model of anti-codon numbers was conducted to find the best fit for a set of data by minimizing the sum of the offsets or residuals of points from the plotted curve and to understand the behaviour of dependent variables (Supplementary Figure 4). The method estimates the relationship by minimizing the sum of the squares in the difference between the observed and predicted values of dependent variable configured as a straight line. At 95% significance and intercept at zero, the slope was found to be 34.621 (Supplementary Figure 4). The statistical result of the ordinary least square regression was; \( t=10.728 \), standard error \( \sigma=3.227 \), and \( p(\text{slope})=6.161E-16 \). For 95% bootstrap confidence interval (\( N=1999 \)); correlation \( r=0.00916 \), \( r^2=8.3917E-05 \), \( t=0.072713 \), \( p(\text{uncorr})=0.94226 \), and permutation \( p=0.9404 \). the residual standard error of estimate was 147.

**Discussion**

tRNA is an adaptor molecule that becomes charged when it binds an amino acid and subsequently donates it to an elongating peptide chain as determined by a codon-anti-codon recognition system. Each tRNA contain a characteristics anti-codon sequence which dictates the translation of a mRNA sequence into a protein. In some cases, the same codon can get decoded by different tRNA species and the same tRNA species can also become decoded by different codons due to wobble interactions (Watson-Crick base pairing) at the first position of an anti-codon and third position of the codon [26–28]. In our analysis of 128 species of the plants, none were found to encode all 64 anti-codons, which suggests that wobble base pairing exists in all plant species. The wobble interaction occurs at the G:U (guanine-uracil) base pairing and modifications in anti-codons that change the specificity of a codon [57–59]. Due to this redundancy, it is not necessary for a plant genome to encode all of existing anti-codons and utilize different tRNAs according to the requirement. The presence of only 29 anti-codons in the genome of *Klebsormidium nitens* and 31 anti-codons in *Bathycoccus prasinos*, however, are somewhat very interesting. Species *K. nitens* and *B. prasinos* belonged to the phylum algae and the genome sizes of these species are much smaller than the genome sizes found in gymnosperm and angiosperms. The absence of a greater number of anti-codons in these species suggests that the rate of wobble base-pairing might be quite high in these species. Mohanta et al., (2020) reported that species of cyanobacteria possessed 32 to 43 anti-codons per genome [20]. Cyanobacterial genomes are smaller than genomes of alae and higher plants [60]. The absence of a greater number of anti-codons in species with smaller genome is directly related to a higher frequency of wobble base-pairing. *Ipomea nil* (59), *Ipomea triloba* (58), *Papaver somniferum* (59), *Cucurbita pepo* (56), and *Zea mays* (59) possess a high number of anti-codons and so the occurrence of wobble base pairing may be quite minimal in these species. It will be interesting to determine the factors responsible for the occurrence of high and low frequencies of wobble base-pairing. Zhang et al., (2013) reported that the presence of high concentration of amino acids in the nutrient media led to higher rate of mismatch incorporation of amino acids into the translating protein chain [61]. They also reported that wobble codon position is less stringent in base pair
mismatch and base change in 3rd position explained additional 25% misincorporation either by favourable G\textsuperscript{mRNA}/U\textsuperscript{tRNA} mismatch or wobble position mismatch [61]. The G/U mismatch was predominant during the codon recognition and which is commonly found in the nucleic acid secondary structures as well [62–64].

The abundance of the CAU anti-codon that encodes tRNA\textsuperscript{Met} was the greatest among all of the anti-codons (Supplementary File 1). Methionine is used to initiate the start of a polypeptide chain, and as a result, almost all proteins require a methionine amino acid. Therefore, the abundance of an anti-codon for tRNA\textsuperscript{Met} was found to be the highest. Additionally, tRNA\textsuperscript{Met} (CAU) was found to have evolved earlier than other tRNAs during the course of evolution [18,19]. If the abundance of isoacceptors is considered, tRNA\textsuperscript{Leu}, which contain six isoacceptors (GGA, AGA, CGA, UGA, ACU, GCU), has the highest abundance (7.808% of the collective plant species). Similarly, tRNA\textsuperscript{Ser}, and tRNA\textsuperscript{Arg}, both with six isoacceptors, have a high percentage of anti-codon abundance. This finding led us to conclude that, the higher the number of isoacceptors for tRNA isotypes, the greater the level of anti-codon sharing in a genome. The study also reveals that plant genomes encode tRNA\textsuperscript{Leu}, tRNA\textsuperscript{Ser}, and tRNA\textsuperscript{Arg} more frequently than other tRNAs. A proteome-wide analysis by Mohanta et al., (2019) reported a higher abundance of Leu amino acids in the proteomes of the Plant Kingdom [65]. This observation directly corroborates that the number and abundance of tRNA\textsuperscript{Leu} genes in genome is directly proportional to the number of Leu amino acids in the proteome. In contrast, a few anti-codons, including GCG, GAG, GGG, GCC, ACU, ACC, UCA (Sec) (group E) of different tRNA isotypes were found to have a low abundance (Figure 2). Yona et al., (2013) reported that multiple copies of rare tRNAs are deleterious to a cell [51]. They also stated that the effective gene copy number of each tRNA anti-codon set can undergo changes during evolution that may be due to the changes in demand-to-supply [51]. A single point mutation in an anti-codon can change one tRNA to another. The lowest encoding anti-codon GCG of tRNA\textsuperscript{Arg} may have undergone a point mutation resulting in tRNA\textsuperscript{Arg} with ACG, CCG, and UCG, which avoids the deleterious effect of the GCG anti-codon. Previous studies have also noted that rare tRNAs may be essential for co-translational folding as low abundance could provide a pause in translation [44,66].

When plants grow in a multitude of environmental conditions, environmental stress can induce the expression of genes needed for stress adaptation, which may affect codon usage by the transcriptome. This leads to a demand for a different pool of tRNAs to support the change in codon usage and avoid a translational imbalance [52,67]. If the altered environmental conditions persist, the tRNAs have to undergo changes in their level of expression to meet and respond to the environmental stress-induced changes in gene expression. If the changes in supply-demand continue, it may lead to changes in the genetic pool of the tRNAs that are beneficial and favoured by selection pressures. These novel translational demands can be maintained by shifting nucleotides in the anti-codons rather than by the duplication of genes. The tRNA pool can evolve to maintain the translational requirement by adjusting the number and/or ratio of tRNA isotypes encoding the same amino acid. An anti-codon switch, however, can also dramatically change the ratios of tRNA isoacceptor within a tRNA pool. This can be done by increasing the copy number of one isoacceptor at the expense of others. The high sequence similarity of different anti-codons (anti-codon switch) can be the result of purifying selection that maintains sequence similarity. Sequence similarity, however, can result from concerted evolution that maintains sequence similarity through frequent recombination among members of the same gene family [68,69]. The presence of a high level of recombination in tRNAs indicates that the evolution of plant tRNAs for anti-codon switch and sequence similarity may be due to concerted evolution. A single point mutation in an anti-codon can result in the encoding of a different tRNA family. It would be interesting to understand the evolutionary constraints that lead to the generation of more members while others have fewer members. It has been previously reported that tRNA\textsuperscript{Leu} encodes a higher number of tRNA genes in the genome, a feature that is directly related to the higher number of tRNA isoacceptors in tRNA\textsuperscript{Leu} [17–20]. The question remains if purifying selection plays a role in maintaining a low level of certain tRNAs, such as tRNA\textsuperscript{Sec}, tRNA\textsuperscript{His}, tRNA\textsuperscript{Trp}, and tRNA\textsuperscript{Tyr}. It is plausible that this purifying selection might be responsible for maintaining the anti-codons of these tRNAs at non-optimal levels. A
previous study reported that increasing the copy number of a low copy tRNA gene family in a cell results in proteotoxic stress due to problems in protein folding [51]. In addressing the need for environmental adaptation, tRNA isotypes provide evolutionary plasticity to changes in translational demand due to their presence as a multi-member gene family. A few species have lost tRNA genes for particular tRNA isotypes and anti-codon switch/point mutations of anti-codons may be a factor that contributes to maintaining the function of a genome in the complete absence of a particular gene family.

Selenocysteine (a selenium containing cysteine analog) is co-translationally inserted in a small fraction of proteins (selenoproteins) and is driven by a tRNAsSec gene. Although Sec is found in all three domains of life, it is not universal. Approximately 20% of the prokaryotic genome contains selenoproteins, while in eukaryotes selenoproteins are reported to be more concentrated in the metazoan lineage [70–73]. The absence of selenoproteins in fungi and land plants has also been reported previously [74] and results from a lack of a tRNAsec gene in their genomes. tRNAsec is encoded by a UGA anti-codon which also encodes a stop codon. A highly sensitive and efficient method of tRNA identification is needed to find tRNAsec. The lack of suitable identification techniques may be the main reason for stating the absence of tRNAsec genes in fungal and plant genomes. Using current technology, however, we were able to identify tRNAsec, as well as tRNAsec genes in a few of the genomes of the analysed plant species.

**Conclusion**

The repertoire of tRNA has a significant impact on the fitness of an organism. The frequency (abundance) of anti-codons that explains synonymous codon usage in coding genes, however, has remained unexplored. Anti-codon frequency can be directly attributed to the frequency of synonymous codon usage and an anti-codon table of the Plant Kingdom, along with the percent abundance of each anti-codon, can be very helpful for understanding the relationship between codon and anti-codon frequency in the genome. The 21st amino acid, selenocysteine, encoded by tRNAsec has undergone a duplication event along with an anti-codon switch. Understanding the mechanisms involved in the loss of tRNA genes in a few species may be crucial to deciphering the translation mechanism in these species. The frequency of the anti-codons GCG (Arg), GAG (Leu), ACU (Ser), GGG (Pro) were very low in abundance and appear to be the rarest form of anti-codons in the Plant Kingdom. Yona et al., (2013) reported that multiple copies of rare tRNAs are deleterious to a cell [51], which suggests that large copy numbers of CGC, GAG, ACU, and GGG anti-codons may be deleterious to plant cells. Therefore, a very low number of these anti-codons are encoded in the plant genome. A few species have completely lost specific tRNA isotype genes in their genome. Additionally, a previous also reported the loss of tRNA genes in some plant genomes [75].

**Declarations**

**Ethic approval and consent to participate**

Not applicable

**Consent for publication**

All authors agree and have consent for publication

**Availability of data material**

All the studied data were taken from publicly available databases and data associated with the manuscript is provided in supplementary file.
Competing of interest

There is no competing of interest to declare

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Author contribution

TKM: conceived the idea, collected and annotated the genome sequences, analysed and interpreted the data and drafted the manuscript, AKM: analysed the data; AH and EFA: drafted and revised the manuscript, ALK: revised the manuscript, AA: revised the manuscript

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Tables

Table 1

Anti-codon table of the plant kingdom with frequency of anti-codons.
| tRNA Isotypes | Isoacceptors | Total no of Anti-codons (%) |
|---------------|-------------|-----------------------------|
| Asparagine    | AUU (155) GUU (3972) | 4.176 |
| Cysteine      | GCA (2454) ACA (64) | 2.547 |
| Glutamine     | CUG (1321) UUG (1775) | 3.133 |
| Glycine       | ACC (31) GCC (3766) CCC (1105) UCC (2532) | 7.523 |
| Serine        | GGA (378) AGA (2332) CGA (820) UGA (1506) ACU (19) GCU (2522) | 7.668 |
| Threonine     | AGU (1946) GGU (366) CGU (729) UGU (1619) | 4.716 |
| Tyrosine      | AUA (38) GUA (2825) | 2.897 |
| Alanine       | AGC (2897) GGC (25) CGC (1060) UGC (2125) | 6.180 |
| Isoleucine    | AAU (3200) GAU (188) UAU (1069) CAU (0) | 4.510 |
| Leucine       | AAG (2065) GAG (9) CAG (859) UAG (1625) CAA (2145) UAA (1012) | 7.808 |
| Methionine    | CAU (4979) | 5.039 |
| Phenylalanine | AAA (55) GAA (3330) | 3.425 |
| Proline       | AGG (2070) GGG (22) CGG (845) UGG (2861) | 5.868 |
| Tryptophan    | CCA (2736) | 2.769 |
| Valine        | AAC (2700) GAC (205) CAC (1934) UAC (1267) | 6.179 |
| Arginine      | ACG (2178) GCG (4) CCG (705) UCG (1084) CCU (1311) UCU (1915) | 7.284 |
| Histidine     | AUG (53) GUG (2031) | 2.109 |
| Lysine        | CUU (3387) UUU (2862) | 6.324 |
| Aspartic acid | GUC (4223) AUC (52) | 4.326 |
| Glutamic acid | CUC (2909) UUC (2370) | 5.342 |
|Suppressor     | CUA (11) UUA (25) | 0.036 |
| Selenocysteine| UCA (66) | 0.066 |
Table 2

Genomic details of plant anti-codons.

| Not determined | ? (59) | 0.059 |
|----------------|--------|-------|

| Species Name          | Classification | Total No of tRNAs | Total No of anticodons | Missing anticodons | Amino Acids of Missing anticodons | Missing tRNA Genes | Encoding Sec Amino Acids |
|-----------------------|----------------|-------------------|------------------------|--------------------|-----------------------------------|--------------------|--------------------------|
| Abrus precatorius     | Eudicot        | 702               | 49                     | ACA, AUG, AUA, ACC, GGC, UUA, GGG, ACU, CUA, GAG, GCG | His, Tyr, Cys, Val, Ala, Sup, Pro, Ser, Sup, Leu |
| Aegilops tauschii     | Monocot        | 1701              | 53                     | ACA, ACC, ACU, AUA, AUG, CUA, GAG, GCG, GGC, GGG, UUA | Cys, Gly, Ser, Tyr, His, Sup, Leu, Arg, Ala, Pro, Sup |
| Amborella trichopoda  | Amborella      | 321               | 49                     | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAG, GCG, GGC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| Ananas comosus        | Monocot        | 446               | 49                     | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAG, GCG, GGC, GGG, UCA, UUA | Phe, Cys, Gly, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| Arabidopsis thaliana  | Eudicot        | 678               | 49                     | AAA, ACA, ACC, AUA, AUG, AUC, AUG | Phe, Cys, Gly, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| Species                        | Phylum | Length (nt) | Stop codon |
|-------------------------------|--------|-------------|------------|
| *Arachis duranensis*         | Eudicot| 579         | 51         |
| *Arachis hypogaea*            | Eudicot| 1250        | 56         |
| *Arachis ipaensis*           | Eudicot| 562         | 52         |
| *Arabidopsis lyrata*          | Eudicot| 567         | 50         |
| *Asparagus officinalis*       | Monocot| 493         | 45         |
| Species                  | Kingdom | References | Start | Stop | Codons | Amino Acids                              |
|-------------------------|---------|------------|-------|------|--------|------------------------------------------|
| Bathycoccus prasinos    | Algae   | 57         | 31    |      | AAA, AAG, AAU, ACA, ACC, ACU, AGA, AU, AUG, AU, CAC, CCC, CCG, CU, CGA, CGC, CGU, CUA, CUC, CUG, GAC, GCC, GCG, GGA, GCC, GGG, GGU, UAU, UCA, UCG, UGU, UUA | Phe, Leu, Ile, Cys, Gly, Ser, Ser, Tyr, Asp, His, Asn, Val, Gly, Arg, Arg, Ser, Ala, Thr, Sup, Glu, Gln, Val, Gly, Arg, Ser, Ala, Pro, Thr, Ile, Sup, Arg, Thr, Sup |
| Beta vulgaris           | Eudicot | 942        | 53    |      | AAA, ACA, ACC, ACU, AUA, AUG, AU, CAC, CCC, CCG, CU, CGA, CGC, CGU, CUA, CUC, CUG, GAC, GCC, GCG, GGA, GCC, GGG, GGU, UAU, UCA, UCG, UGU, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Leu, Arg, Sup |
| Brachypodium distachyon | Monocot  | 563        | 49    |      | AAA, ACA, ACC, ACU, AUA, AUG, AU, CAC, CCC, CCG, CU, CGA, CGC, CGU, CUA, CUC, CUG, GAC, GCC, GCG, GGA, GCC, GGG, GGU, UAU, UCA, UCG, UGU, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Ser, Arg, Ala, Pro, Sup, Sup | Yes |
| Species            | Taxonomy   | Length | Score | TAA | Amino Acids                  | Note |
|--------------------|------------|--------|-------|-----|------------------------------|------|
| *Brassica napus*    | Eudicot    | 2180   | 53    | GCG, GGC, GGG, UCA, UUA      |      |
| *Brassica oleracea* | Eudicot    | 993    | 48    | AAA, ACA, ACC, ACU, AUA, AUI, CUA, GCG, GGG, UCA, UUA | Cys, Gly, Ser, Tyr, Asn, Sup, Arg, Pro, Sup, Sup, Phe |
| *Brassica rapa*     | Eudicot    | 1047   | 50    | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUI, CUA, GAC, GAG, GAU, GCG, GGC, GGG, GGU, UCA, UUA | Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ser, Pro, Sup, Phe |
| Yes                |            |        |       |        |                              |      |
| *Cajanus cajan*     | Eudicot    | 694    | 49    | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUI, CUA, GAG, GCG, GGA, GGG, GGU, UCA, UUA | Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup, Phe |
| *Camelina sativa*   | Eudicot    | 1652   | 51    | ACA, Gly, Ser,              |      |

Page 21/55
| Species                  | Domain | Accession | Length | Coding Sites |
|-------------------------|--------|-----------|--------|--------------|
| *Camellia sinensis*     | Eudicot | 612       | 51     | AAA, ACC, ACU, AUA, AUC, AUG, CUA, GAG, GAC, GCU, GGG, UCA, UUA | Gly, Ser, Tyr, Asn, Sup, Val, Leu, Ile, Arg, Ala, Pro, Sup, Cys |
| *Cannabis sativa*       | Eudicot | 490       | 46     | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUC, AUA, CUA, GAC, GAG, GCU, GGG, UCA, UUA | Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Val, Leu, Ile, Arg, Ser, Ala, Pro, Sup, Sup, Phe |
| *Capsella rubella*      | Eudicot | 557       | 47     | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUA, CUA, GAC, GAG, GAU, GCG, GGA, GGC, GGG, UCA, UUA | Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Val, Leu, Ile, Arg, Ala, Pro, Sup, Sup, Phe |
| *Capsicum annuum*       | Eudicot | 794       | 53     | ACA, ACC, ACU, GAC, GAG, GAU, GCG, GGC, GGG, UCA, UUA | Cys, Gly, Ser, Tyr, Asp, Sup, |
| Species               | Domain | Accession | Length | Codon Usage | Amino Acid Composition |
|-----------------------|--------|-----------|--------|-------------|------------------------|
| Carica papaya         | Eudicot| 378       | 51     | ACA, ACC, ACU, AUA, AUG, AUC, AUA | Leu, Arg, Ala, Sup, Sup |
| Chenopodium quinoa    | Eudicot| 1017      | 51     | AAA, ACC, ACU, AUG, ACU, AUG, AUA | Cys, Gly, Ser, Tyr, Asp, His, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| Chlamydomonas reinhardtii | Algae  | 87        | 45     | AAA, ACA, ACC, ACU, AUA, AUG, AUC, AUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Val, Leu, Ile, Arg, Ser, Ala, Pro, Thr, Sup, Sup |
| Cicer arietinum       | Eudicot| 665       | 48     | AAA, ACA, ACC, ACU, AUA, AUG, AUC, AUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Thr, Sup, Sup |
| Organism                     | Domain | Species | Accession | ORF 1 | ORF 2 | Codons | Amino Acids |
|------------------------------|--------|---------|-----------|-------|-------|--------|-------------|
| *Citrus clementina*          | Eudicot| 428     | 47        | AAA,  | ACA,  | GAG,   | Phe, Cys,   |
|                             |        |         |           | ACC,  | ACU,  | GAU,   | Gly, Ser,   |
|                             |        |         |           | UUA,  | AUG,  | UUG,   | Tyr, Asp,   |
|                             |        |         |           | AUG,  | AUU,  | AUU,   | His, Asn,   |
|                             |        |         |           | CUA,  | GAC,  | GAC,   | Sup, Val,   |
|                             |        |         |           | GAG,  | GGU,  | GGU,   | Leu, Ile,   |
|                             |        |         |           | GAU,  | GUA,  | GUA,   | Arg, Ala,   |
|                             |        |         |           | AUG,  | GUA,  | GUA,   | Pro, Sup,   |
|                             |        |         |           | AUU,  | GUA,  | UUA,   | Sup          |

| *Citrus sinensis*            | Eudicot| 417     | 48        | AAA,  | ACA,  | GAG,   | Phe, Cys,   |
|                             |        |         |           | ACC,  | ACU,  | GAU,   | Gly, Ser,   |
|                             |        |         |           | UUA,  | AUG,  | UUG,   | Tyr, Asp,   |
|                             |        |         |           | AUG,  | AUU,  | AUU,   | His, Asn,   |
|                             |        |         |           | CUA,  | GAC,  | GAC,   | Sup, Val,   |
|                             |        |         |           | GAG,  | GGU,  | GGU,   | Leu, Ile,   |
|                             |        |         |           | GAU,  | GUA,  | GUA,   | Arg, Ser,   |
|                             |        |         |           | AUG,  | AUU,  | AUU,   | Ala, Pro,   |
|                             |        |         |           | CUA,  | GAC,  | GAC,   | Thr, Tyr,   |
|                             |        |         |           | GAG,  | GGU,  | GUA,   | Sup, Sup    |

| *Coccomyxa subellipsoidea*   | Algae  | 77      | 44        | AAA,  | ACA,  | GAG,   | Phe, Cys,   |
|                             |        |         |           | ACC,  | ACU,  | GAU,   | Gly, Ser,   |
|                             |        |         |           | UUA,  | AUG,  | UUG,   | Tyr, Asp,   |
|                             |        |         |           | AUG,  | AUU,  | AUU,   | His, Asn,   |
|                             |        |         |           | CUA,  | GAC,  | GAC,   | Sup, Val,   |
|                             |        |         |           | GAG,  | GGU,  | GGU,   | Leu, Ile,   |
|                             |        |         |           | GAU,  | GUA,  | GUA,   | Arg, Ser,   |
|                             |        |         |           | AUG,  | AUU,  | AUU,   | Ala, Pro,   |
|                             |        |         |           | CUA,  | GAC,  | GAC,   | Thr, Tyr,   |
|                             |        |         |           | GAG,  | GGU,  | GUA,   | Sup, Sup    |

| *Coffea arabica*             | Eudicot| 747     | 50        | AAA,  | ACA,  | GAG,   | Phe, Cys,   |
|                             |        |         |           | ACC,  | ACU,  | GAU,   | Ser, Tyr,   |
|                             |        |         |           | UUA,  | AUG,  | UUG,   | Asp, His,   |
|                             |        |         |           | AUG,  | AUU,  | AUU,   | Sup, Leu,   |
|                             |        |         |           | CUA,  | GAC,  | GAC,   | Ile, Arg    |

Page 24/55
| Species               | Kingdom   | Genes | Codons | Amino Acids              | Additional Information |
|----------------------|-----------|-------|--------|--------------------------|------------------------|
| Coffea eugenioides   | Eudicot   | 529   | 49     | Ala, Pro, Sup, Sup       |                        |
| Corchorus capsularis | Eudicot   | 200   | 38     | Phe, Cys, Ser, Tyr, Asp, His, Sup, Val, Leu, Ile, Arg, Ala, Pro, Sup, Sup |                        |
| Corchorus olitorius  | Eudicot   | 473   | 49     | Phe, Ile, Cys, Gly, Arg, Ala, Pro, Tyr, Asp, His, Asn, Leu, Ser, Ala, Sup, Lys, Val, Leu, Ile, Arg, Ala, Pro, Tyr, Sup, Sup, Lys | Lys, Tyr |
| Species                  | Kingdom   | Length | Codons |
|-------------------------|-----------|--------|--------|
| *Cucumis melo*          | Eudicot   | 598    | 49     |
|                         |           |        | AAA,   |
|                         |           |        | ACA,   |
|                         |           |        | ACC,   |
|                         |           |        | ACU,   |
|                         |           |        | AUA,   |
|                         |           |        | AUG,   |
|                         |           |        | AUU,   |
|                         |           |        | CUA,   |
|                         |           |        | GAG,   |
|                         |           |        | GCG,   |
|                         |           |        | GGC,   |
|                         |           |        | GGG,   |
|                         |           |        | UCA,   |
|                         |           |        | UUA    |
|                         |           |        | Phe, Cys, |
|                         |           |        | Gly, Ser, |
|                         |           |        | Tyr, Asp, |
|                         |           |        | His, Asn, |
|                         |           |        | Sup, Leu, |
|                         |           |        | Arg, Ala, |
|                         |           |        | Pro, Sup, |
|                         |           |        | Sup     |
| *Cucumis sativus*       | Eudicot   | 640    | 52     |
|                         |           |        | AAA,   |
|                         |           |        | ACC,   |
|                         |           |        | ACU,   |
|                         |           |        | AUG,   |
|                         |           |        | AUU,   |
|                         |           |        | CUA,   |
|                         |           |        | GAG,   |
|                         |           |        | GCG,   |
|                         |           |        | GGC,   |
|                         |           |        | GGG,   |
|                         |           |        | UUA    |
|                         |           |        | Phe, Gly, |
|                         |           |        | Ser, Tyr, |
|                         |           |        | His, Asn, |
|                         |           |        | Sup, Leu, |
|                         |           |        | Arg, Ala, |
|                         |           |        | Pro, Sup  |
| *Cucurbita maxima*      | Eudicot   | 3036   | 55     |
|                         |           |        | ACU,   |
|                         |           |        | AUG,   |
|                         |           |        | AUU,   |
|                         |           |        | CUA,   |
|                         |           |        | GAG,   |
|                         |           |        | GCG,   |
|                         |           |        | GGA,   |
|                         |           |        | GGC,   |
|                         |           |        | UUA    |
|                         |           |        | Ser, Tyr, |
|                         |           |        | His, Sup, |
|                         |           |        | Leu, Arg, |
|                         |           |        | Ser, Ala, |
|                         |           |        | Sup     |
| *Cucurbita moschata*    | Eudicot   | 4062   | 57     |
|                         |           |        | CUA,   |
|                         |           |        | GAC,   |
|                         |           |        | GAG,   |
|                         |           |        | GCG,   |
|                         |           |        | GGC,   |
|                         |           |        | GGG,   |
|                         |           |        | UUA    |
|                         |           |        | Sup, Val, |
|                         |           |        | Leu, Arg, |
|                         |           |        | Ala, Pro, |
|                         |           |        | Sup     |
| *Cucurbita pepo*        | Eudicot   | 3228   | 56     |
|                         |           |        | ACC,   |
|                         |           |        | AUC,   |
|                         |           |        | AUG,   |
|                         |           |        | CUA,   |
|                         |           |        | GAG,   |
|                         |           |        | GCG,   |
|                         |           |        | GGC,   |
|                         |           |        | GGG,   |
|                         |           |        | UUA    |
|                         |           |        | Gly, Asp, |
|                         |           |        | His, Sup, |
|                         |           |        | Leu, Arg, |
|                         |           |        | Pro, Sup  |
| *Cynara cardunculus*    | Eudicot   | 586    | 47     |
|                         |           |        | AAA,   |
|                         |           |        | ACA,   |
|                         |           |        | ACC,   |
|                         |           |        | ACU,   |
|                         |           |        | AUG,   |
|                         |           |        | AUU,   |
|                         |           |        | CUA,   |
|                         |           |        | GAC,   |
|                         |           |        | GAG,   |
|                         |           |        | GAU,   |
|                         |           |        | Phe, Cys, |
|                         |           |        | Gly, Ser, |
|                         |           |        | Tyr, Asp, |
|                         |           |        | Asn, Sup, |
|                         |           |        | Val, Leu, |
|                         |           |        | Ile, Arg, |
|                         |           |        | Ala, Pro, |
|                         |           |        | Thr, Val, |
|                         |           |        | Sup     |
| Species                  | Taxonomy | Codons | Amino Acids |
|-------------------------|----------|--------|-------------|
| *Cyanophoraparadoxa*    | Algae    | 68     | AAA, ACA, ACC, ACU, AUU, AUA, AUC, AUG, AUJ, CUA, GCA, GGA, GGC, GGU, UCA, UGA, UUG, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Arg, Ser, Ala, Pro, Thr, Sup |
| *Daucus carota*         | Eudicot  | 494    | AAA, ACA, ACC, ACU, AUU, AUA, AUC, AUG, AUU, CUA, GCA, GGA, GGC, GGU, UCA, UGA, UUG, UUA | Phe, Cys, Gly, Ser, Asp, His, Asn, Sup, Leu, Ile, Arg, Ala, Pro, Sup, Sup |
| *Dendrobium catenatum*  | Monocot   | 254    | AAA, ACA, ACC, ACU, AUU, AUA, AUC, AUG, AUU, CUA, GCA, GGA, GGC, GGU, UCA, UGA, UUG, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| *Dendrobium officinale* | Orchid   | 254    | AAA, ACA, ACC, ACU, AUU, AUA, AUC, AUG, AUU, CUA, GCA, GGA, GGC, GGU, UCA, UGA, UUG, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| Species           | Class      | Length | Reading Frame | AA          | Other Amino Acids |
|-------------------|------------|--------|---------------|-------------|------------------|
| *Duriozibethinus* | Eudicot    | 604    | 48            | AAA, ACA,   | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Val, Leu, Ile, Arg, Pro, Sup, Sup |
|                   |            |        |               | ACC, ACU,   |                  |
|                   |            |        |               | AUA, AUC,   |                  |
|                   |            |        |               | AUG, AUU,   |                  |
|                   |            |        |               | CUA, GAC,   |                  |
|                   |            |        |               | GAG, GAU,   |                  |
|                   |            |        |               | GCG, GGG,   |                  |
|                   |            |        |               | GGU, UCA,   |                  |
|                   |            |        |               | UUA         |                  |
| *Ectocarpussiliculosus* | Algae | 116    | 48            | AAA, AAC,   | Phe, Val, Ile, Cys, Gly, Ser, Tyr, Asp, His, Asn, Leu, Arg, Ala, Pro, Thr |
|                   |            |        |               | AAU, ACA,   |                  |
|                   |            |        |               | ACC, ACU,   |                  |
|                   |            |        |               | AUA, AUC,   |                  |
|                   |            |        |               | AUG, AUU,   |                  |
|                   |            |        |               | CUA, GAG,   |                  |
|                   |            |        |               | GGC, GGU,   |                  |
|                   |            |        |               | GGU, UCA,   |                  |
|                   |            |        |               | UUA         |                  |
| *Elaeisguineensis* | Monocot    | 436    | 49            | AAA, ACA,   | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
|                   |            |        |               | ACC, ACU,   |                  |
|                   |            |        |               | AUA, AUC,   |                  |
|                   |            |        |               | AUG, AUU,   |                  |
|                   |            |        |               | CUA, GAG,   |                  |
|                   |            |        |               | GGC, GGG,   |                  |
|                   |            |        |               | UCA, UUA    |                  |
| *Erythranthegeuttata* | Eudicot | 729    | 49            | AAA, ACA,   | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
|                   |            |        |               | ACC, ACU,   |                  |
|                   |            |        |               | AUA, AUC,   |                  |
|                   |            |        |               | AUG, AUU,   |                  |
|                   |            |        |               | CUA, GAG,   |                  |
|                   |            |        |               | GGC, GGG,   |                  |
|                   |            |        |               | UCA, UUA    |                  |
| Organism                | Domain | Length | Identifier | Codons | Amino Acids          |
|------------------------|--------|--------|------------|--------|---------------------|
| *Eucalyptus grandis*   | Eudicot| 463    | 50         | AAA,   | Phe, Gly, Ser, Tyr, |
|                        |        |        |            | ACC,   | Asp, His, Sup, Ser, |
|                        |        |        |            | ACU,   | Arg, Ser, Ala, Pro, |
|                        |        |        |            | AUA,   |                     |
|                        |        |        |            | AUC,   |                     |
|                        |        |        |            | AUG,   |                     |
|                        |        |        |            | CUA,   |                     |
|                        |        |        |            | GAG,   |                     |
|                        |        |        |            | GCG,   |                     |
|                        |        |        |            | GGA,   |                     |
|                        |        |        |            | GGC,   |                     |
|                        |        |        |            | GGG,   |                     |
|                        |        |        |            | UCA,   |                     |
|                        |        |        |            | UUA    |                     |
| *Eutrema salignum*     | Eudicot| 468    | 46         | AAA,   | Phe, Cys, Gly, Ser, |
|                        |        |        |            | ACA,   | Tyr, Asp, His, Asn, |
|                        |        |        |            | ACC,   | Sup, Val, Leu, Ile, |
|                        |        |        |            | ACU,   | Arg, Ala, Pro, Thr, |
|                        |        |        |            | AUA,   |                     |
|                        |        |        |            | AUC,   |                     |
|                        |        |        |            | AUG,   |                     |
|                        |        |        |            | AUU,   |                     |
|                        |        |        |            | CUA,   |                     |
|                        |        |        |            | GAC,   |                     |
|                        |        |        |            | GAG,   |                     |
|                        |        |        |            | GAU,   |                     |
|                        |        |        |            | GCG,   |                     |
|                        |        |        |            | GGC,   |                     |
|                        |        |        |            | GGG,   |                     |
|                        |        |        |            | GGU,   |                     |
|                        |        |        |            | UCA,   |                     |
|                        |        |        |            | UUA    |                     |
| *Fragaria vesca*       | Eudicot| 507    | 49         | AAA,   | Phe, Cys, Gly, Ser, |
|                        |        |        |            | ACA,   | Tyr, Asp, His, Asn, |
|                        |        |        |            | ACC,   | Sup, Leu, Arg, Ala, |
|                        |        |        |            | ACU,   | Pro, Thr, Sup,      |
|                        |        |        |            | AUA,   |                     |
|                        |        |        |            | AUC,   |                     |
|                        |        |        |            | AUG,   |                     |
|                        |        |        |            | AUU,   |                     |
|                        |        |        |            | CUA,   |                     |
|                        |        |        |            | GAC,   |                     |
|                        |        |        |            | GAG,   |                     |
|                        |        |        |            | GAU,   |                     |
|                        |        |        |            | GCG,   |                     |
|                        |        |        |            | GGC,   |                     |
|                        |        |        |            | GGG,   |                     |
|                        |        |        |            | UCA,   |                     |
|                        |        |        |            | UUA    |                     |
| *Glycine max*          | Eudicot| 747    | 50         | AAA,   | Phe, Cys, Gly, Ser, |
|                        |        |        |            | ACA,   | Tyr, Asp, His, Sup, |
|                        |        |        |            | ACC,   | Leu, Arg, Ala, Pro, |
|                        |        |        |            | ACU,   |                     |
|                        |        |        |            | AUA,   |                     |
|                        |        |        |            | AUC,   |                     |
|                        |        |        |            | AUG,   |                     |
|                        |        |        |            | CUA,   |                     |
|                        |        |        |            | GAC,   |                     |
|                        |        |        |            | GAG,   |                     |
|                        |        |        |            | GAU,   |                     |
|                        |        |        |            | GCG,   |                     |
|                        |        |        |            | GGC,   |                     |
|                        |        |        |            | GGG,   |                     |
|                        |        |        |            | UCA,   |                     |
|                        |        |        |            | UUA    |                     |
| **Gossypium arboreum** | Eudicot | 906 | 50 | AAA, ACA, ACC, ACU, AUA, AUC, AUG, CUA, GAG, GCG, GGC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Sup, Leu, Arg, Ala, Pro, Sup |  |
|------------------------|----------|-----|----|---------------------|----------------------|---|
| **Gossypium hirsutum**  | Eudicot  | 1436 | 55 | ACC, ACU, AUA, CUA, GAG, GCG, GGC, GGG, UCA, UUA | Gly, Ser, Tyr, Sup, Leu, Arg, Ala, Sup, Sup |  |
| **Gossypium raimondii** | Eudicot  | 798  | 51 | AAA, ACA, ACC, ACU, AUA, AUG, CUA, GAG, GCG, GGC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, His, Sup, Leu, Arg, Ala, Pro, Sup, Sup |  |
| **Helianthus annuus**   | Eudicot  | 1262 | 49 | AAA, ACA, ACC, ACU, AUC, AUG, CUA, GAG, GAU, GCG, GGC, GGG, GGU, UCA, UUA | Phe, Cys, Gly, Ser, Asp, His, Sup, Leu, Ile, Arg, Ala, Pro, Thr, Sup, Sup |  |
| **Herrania umbratica**  | Eudicot  | 325  | 45 | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAC, GAG, GAU | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Val, Leu, Ile, Arg, Ser, Ala, Pro, Thr, Sup, Sup |  |
| Species                  | Class     | Genes | Codons | Amino Acids          |
|-------------------------|-----------|-------|--------|----------------------|
| *Hevea brasiliensis*    | Eudicot   | 592   | 49     | AAA, ACA, ACC, ACU, AUA, AUG, AUU, GUA, GCAG, GCG, GGC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup |
| *Ipomoea nil*           | Eudicot   | 6475  | 59     | CUA, GAG, GCG, GGC, GGG, UUA | Sup, Leu, Ala, Pro, Sup |
| *Ipomoea triloba*       | Eudicot   | 2180  | 58     | ACU, CUA, GAG, GCG, GGG, UUA | Ser, Sup, Leu, Arg, Pro, Sup |
| *Jatropha curcas*       | Eudicot   | 471   | 49     | AAA, ACA, ACC, ACU, AUA, AUG, AUU, CUA, GAG, GCG, GGC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Thr, Sup |
| *Juglans regia*         | Eudicot   | 572   | 45     | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAC, GAG, GAU, GCG, GGA, | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Val, Leu, Ile, Arg, Ser, Ala, Pro, Thr, Sup |
| Species                  | Kingdom   | Accession | GCs | GCs | Amino Acids | Codon Usage |
|-------------------------|-----------|-----------|-----|-----|-------------|-------------|
| Klebsormidium nitens    | Algae     | 62        | 29  |     | Phe, Val,   | UGA, UUA    |
|                         |           |           |     |     | Leu, Ile,   |             |
|                         |           |           |     |     | Cys, Gly,   |             |
|                         |           |           |     |     | Ser, Ser,   |             |
|                         |           |           |     |     | Ala, Pro,   |             |
|                         |           |           |     |     | Thr, Tyr,   |             |
|                         |           |           |     |     | Asp, His,   |             |
|                         |           |           |     |     | Asn, Val,   |             |
|                         |           |           |     |     | Leu, Gly,   |             |
|                         |           |           |     |     | Arg, Ala,   |             |
|                         |           |           |     |     | Pro, Thr,   |             |
|                         |           |           |     |     | Sup, Glu,   |             |
|                         |           |           |     |     | Gln, Lys,   |             |
|                         |           |           |     |     | Val, Leu,   |             |
|                         |           |           |     |     | Arg, Ala,   |             |
|                         |           |           |     |     | Sup, Thr,   |             |
|                         |           |           |     |     | Sup         |             |
| Lactuca sativa          | Eudicot   | 903       | 53  |     | Phe, Gly,   | UGA, UUA    |
|                         |           |           |     |     | Ser, Tyr,   |             |
|                         |           |           |     |     | His, Sup,   |             |
|                         |           |           |     |     | Leu, Arg,   |             |
|                         |           |           |     |     | Ala, Pro,   |             |
|                         |           |           |     |     | Sup         |             |
| Lupinus angustifolius   | Eudicot   | 900       | 47  |     | Phe, Cys,   | UGA, UUA    |
|                         |           |           |     |     | Ser, Tyr,   |             |
|                         |           |           |     |     | Asp, His,   |             |
|                         |           |           |     |     | Asn, Sup,   |             |
|                         |           |           |     |     | Val, Leu,   |             |
|                         |           |           |     |     | Ile, Arg,   |             |
|                         |           |           |     |     | Ala, Pro,   |             |
|                         |           |           |     |     | Thr, Sup,   |             |
|                         |           |           |     |     | Sup         |             |
| Genus                      | Kingdom     | Species Number | Codon Count | Codon Position | Codon Count | Codon Position | Codon Count | Codon Position |
|---------------------------|-------------|----------------|-------------|----------------|-------------|----------------|-------------|----------------|
| Malus domestica           | Eudicot     | 761            | 47          | AAA, ACA, ACC, ACU, AUA, AUC, AUG, CU, GAC, GAG, GGA, GGU, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Sup, Val, Leu, Ile, Arg, Ala, Thr, Sup, Sup |
| Manihot esculenta         | Eudicot     | 815            | 50          | ACA, ACC, ACU, AUA, AUC, AUG, AU, CUA, GAG, GCG, GGU, UCA, UUA | Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Thr, Leu, Sup |
| Medicago truncatula       | Eudicot     | 898            | 55          | ACU, AUC, AUG, CU, GAG, GCG, GGC, GGU, UCA, UUA | Cys, Asp, His, Leu, Arg, Ala, Pro |
| Micromonas commoda        | Algae       | 60             | 26          | AAA, AAC, AAG, AAU, ACA, ACC, ACU, AGA, AGC, AGG, AGU, AUA, AUC, AUG, AU, CAA, CAC, CAG, CCC, CGG, CGU | Phe, Val, Leu, Ile, Cys, Gly, Ser, Ser, Ala, Pro, Thr, Tyr, Asp, His, Asn, Leu, Val, Leu, Gly, Arg, Arg, Ser, Ala, Pro, Thr, Sup, Glu, Ala, Lys, Val, Arg, Ser, Ala, Pro, Ile, Sup, Arg, Sup |
|                      | Family     | Taxon      | Loci | Peptides   | Codons |
|----------------------|------------|------------|------|------------|--------|
| **Momordica charantia** | Eudicot    | 667        | 49   | Cys, Ser,  | ACA,   |
|                      |            |            |      | Tyr, Asp,  | ACU,   |
|                      |            |            |      | His, Sup,  | AUA,   |
|                      |            |            |      | Val, Leu,  | AUG,   |
|                      |            |            |      | Ile, Arg,  | AUG,   |
|                      |            |            |      | Ser, Thr,  | UUA,   |
|                      |            |            |      | Ala, Pro,  | CUA,   |
|                      |            |            |      | Thr, Sup   | GUA,   |
|                      |            |            |      |            | GCG,   |
|                      |            |            |      |            | GGA,   |
|                      |            |            |      |            | GGC,   |
|                      |            |            |      |            | GGG,   |
|                      |            |            |      |            | GGU,   |
|                      |            |            |      |            | UUA    |
|                      |            |            |      |            |        |
| **Monoraphidium neglectum** | Algae      | 48         | 30   | Phe, Val,  | AAA,   |
|                      |            |            |      | Leu, Ile,  | AAC,   |
|                      |            |            |      | Cys, Gly,  | AAG,   |
|                      |            |            |      | Ser, Ser,  | AAU,   |
|                      |            |            |      | Thr, Tyr,  | ACA,   |
|                      |            |            |      | Asp, His,  | ACC,   |
|                      |            |            |      | Asn, Leu,  | ACU,   |
|                      |            |            |      | Gly, Arg,  | AGA,   |
|                      |            |            |      | Arg, Ser,  | AGU,   |
|                      |            |            |      | Pro, Thr,  | AAU,   |
|                      |            |            |      | Sup, Glu,  | AUG,   |
|                      |            |            |      | Val, Leu,  | AUC,   |
|                      |            |            |      | Arg, Ala,  | AUG,   |
|                      |            |            |      | Pro, Thr,  | UAA,   |
|                      |            |            |      | Leu, Sup,  | UCA,   |
|                      |            |            |      | Arg, Ser,  | UCG,   |
|                      |            |            |      | Thr, Sup   | UGA,   |
|                      |            |            |      |            | UGU,   |
|                      |            |            |      |            | UUA    |
| Species                  | Class     | Accession | PIs | Translation Products |
|-------------------------|-----------|-----------|-----|----------------------|
| *Morus notabilis*       | Eudicot   | 392       | 47  | AAA, ACA, ACC, ACU, AUA, AUC, AUU, CUA, GAC, GAG, GAU, GCG, GGA, GGC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, Asn, Sup, Val, Leu, Ile, Arg, Ser, Ala, Pro, Sup, Sup |
| *Musa acuminata*        | Monocot   | 710       | 48  | ACA, ACC, ACU, AUA, AUC, AUG, CUA, GAC, GAG, GAU, GCG, GGA, GGG, GGU, UCA, UUA | Cys, Gly, Ser, Tyr, Asp, His, Sup, Val, Leu, Ile, Arg, Ser, Pro, Thr, Sup, Sup |
| *Nelumbo nucifera*      | Eudicot   | 980       | 53  | ACC, AUA, AUC, AUG, CUA, GAC, GAG, GCG, GGC, GGG, UCA, UUA | Gly, Tyr, Asp, His, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| *Nicotiana attenuata*   | Eudicot   | 1086      | 53  | ACC, ACU, AUA, CUA, GAC, GAG, GAU, GCG, GGC, GGG, UCA, UUA | Gly, Ser, Tyr, Sup, Val, Leu, Ile, Ala, Pro, Sup, Sup |
| *Nicotiana sylvestris*  | Eudicot   | 809       | 52  | AAA, ACC, ACU, AUA, AUC, AUU, GAG, GCG, GUA, GCG | Phe, Gly, Ser, Tyr, Asp, Asn, Leu, Arg, Ala, Pro, Sup, Sup |
| Species                          | Class      | Accession No | Exon | Codons |
|---------------------------------|------------|--------------|------|--------|-------------------------------------------------|
| *Nicotiana tabacum*             | Eudicot    | 1504         | 55   | AAA,   | Phe, Ser, Tyr, Sup, Leu, Arg, Ala, Pro, Sup    |
|                                 |            |              |      | ACU,   |                                                 |
|                                 |            |              |      | AUA,   |                                                 |
|                                 |            |              |      | CUA,   |                                                 |
|                                 |            |              |      | GAG,   |                                                 |
|                                 |            |              |      | GCG,   |                                                 |
|                                 |            |              |      | GGC,   |                                                 |
|                                 |            |              |      | GGG,   |                                                 |
|                                 |            |              |      | UUA,   |                                                 |
| *Nicotiana tomentosiformis*     | Eudicot    | 798          | 52   | AAA,   | Phe, Gly, Ser, Asp, His, Sup, Leu, Arg, Ala, Pro, Sup |
|                                 |            |              |      | ACC,   |                                                 |
|                                 |            |              |      | ACU,   |                                                 |
|                                 |            |              |      | AUC,   |                                                 |
|                                 |            |              |      | AUG,   |                                                 |
|                                 |            |              |      | CUA,   |                                                 |
|                                 |            |              |      | GAG,   |                                                 |
|                                 |            |              |      | GCG,   |                                                 |
|                                 |            |              |      | GGC,   |                                                 |
|                                 |            |              |      | GGG,   |                                                 |
|                                 |            |              |      | UCA,   |                                                 |
|                                 |            |              |      | UUA,   |                                                 |
| *Olea europaea*                 | Eudicot    | 557          | 52   | AAA,   | Phe, Gly, Asp, His, Sup, Leu, Arg, Ala, Pro, Sup |
|                                 |            |              |      | ACC,   |                                                 |
|                                 |            |              |      | AUC,   |                                                 |
|                                 |            |              |      | AUG,   |                                                 |
|                                 |            |              |      | CUA,   |                                                 |
|                                 |            |              |      | GAG,   |                                                 |
|                                 |            |              |      | GCG,   |                                                 |
|                                 |            |              |      | GGA,   |                                                 |
|                                 |            |              |      | GGC,   |                                                 |
|                                 |            |              |      | GGG,   |                                                 |
|                                 |            |              |      | UCA,   |                                                 |
|                                 |            |              |      | UUA,   |                                                 |
| *Oryza barthii*                 | Monocot    | 455          | 48   | AAA,   | Phe, Cys, Gly, Ser, Tyr, His, Asn, Sup, Val, Ile, Arg, Ala, Pro, Thr, Sup |
|                                 |            |              |      | ACA,   |                                                 |
|                                 |            |              |      | ACC,   |                                                 |
|                                 |            |              |      | ACU,   |                                                 |
|                                 |            |              |      | AUA,   |                                                 |
|                                 |            |              |      | AUC,   |                                                 |
|                                 |            |              |      | AUG,   |                                                 |
|                                 |            |              |      | AUB,   |                                                 |
|                                 |            |              |      | CUA,   |                                                 |
|                                 |            |              |      | GAG,   |                                                 |
|                                 |            |              |      | GAC,   |                                                 |
|                                 |            |              |      | GUA,   |                                                 |
|                                 |            |              |      | GCG,   |                                                 |
|                                 |            |              |      | GGG,   |                                                 |
|                                 |            |              |      | GGU,   |                                                 |
|                                 |            |              |      | UCA,   |                                                 |
|                                 |            |              |      | UUA,   |                                                 |
| *Oryza brachyantha*             | Monocot    | 476          | 47   | AAA,   | Phe, Cys, Gly, Ser, Tyr, His, Asn, Sup, Val, Ile, Arg, Ala, Pro, Thr, Sup |
|                                 |            |              |      | ACA,   |                                                 |
|                                 |            |              |      | ACC,   |                                                 |
|                                 |            |              |      | ACU,   |                                                 |
|                                 |            |              |      | AUA,   |                                                 |
|                                 |            |              |      | AUC,   |                                                 |
|                                 |            |              |      | AUG,   |                                                 |
|                                 |            |              |      | AUB,   |                                                 |
|                                 |            |              |      | CUA,   |                                                 |
|                                 |            |              |      | GAC,   |                                                 |
| Species                | Classification | Length | Stop Codons | Start Codons | Translation Products |
|------------------------|----------------|--------|-------------|--------------|----------------------|
| Oryza glaberrima       | Monocot        | 788    | 48          | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAG, GAU, GCG, GCC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Ile, Arg, Ala, Pro, Sup, Sup |
| Oryza glumaepatula     | Monocot        | 554    | 50          | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAG, GAU, GCG, GCC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Asp, His, Asn, Sup, Leu, Ile, Arg, Ala, Pro, Sup, Sup |
| Oryza meridionalis     | Monocot        | 552    | 48          | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAG, GAU, GCG, GCC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Ile, Arg, Ala, Pro, Sup, Sup |
| Oryza nivara           | Monocot        | 290    | 48          | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAG, GAU, GCG, GCC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Asp, His, Asn, Sup, Leu, Arg, Ser, Ala, Pro, Thr, Sup, Sup |
| Organism             | Kingdom | Length | Start | Stop |
|----------------------|---------|--------|-------|------|
| Oryza punctata       | Monocot | 195    | 43    |      |
| Oryza rufipogon      | Monocot | 582    | 50    |      |
| Oryza sativa         | Monocot | 668    | 51    |      |
| Ostreococcus tauri   | Algae   | 41     | 18    |      |

**Codon Usage**

- **Oryza punctata**: AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CU, GAC, GAG, GAU, GAG, GCG, GCA, GGA, GCC, GGG, UAA, UAC, UAU, UCA, UUA
- **Oryza rufipogon**: AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAC, GAG, GAU, GCG, GGA, GCC, GGG, UAA, UAC, UAU, UCA, UUA
- **Oryza sativa**: AAA, ACC, ACU, AUA, AUG, AUA, AUA, CUA, GAC, GAG, GCG, GCC, GGG, UAA, UAC, UAU, UCA, UUA
- **Ostreococcus tauri**: AAA, AAG, AAU, ACA, ACC, ACG, AUC, AGG, AGU, AUA, AUA, AUA, UAA, UAA, UAA

**Amino Acid Translation**

- **Oryza punctata**: Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Val, Leu, Ile, Arg, Ser, Ala, Pro, Leu, Val, Ile, Sup, Sup
- **Oryza rufipogon**: Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Val, Leu, Ile, Arg, Pro, Sup, Sup
- **Oryza sativa**: Phe, Gly, Ser, Tyr, His, Asn, Sup, Ser, Arg, Ala, Pro, Sup, Sup
- **Ostreococcus tauri**: Phe, Leu, Ile, Cys, Gly, Arg, Ser, Pro, Thr, Tyr, Asp, His, Asn, Leu, Val, Gly, Arg, Arg, Ser, Ala, Phe, Gln
| Species                        | Kingdom   | Taxonomy | Count | Codons |
|-------------------------------|-----------|----------|-------|--------|
| Panicum hallii                | Monocot   |          | 541   | AAA,   |
|                               |           |          |       | ACA,   |
|                               |           |          |       | ACC,   |
|                               |           |          |       | ACU,   |
|                               |           |          |       | AUA,   |
|                               |           |          |       | AUC,   |
|                               |           |          |       | AAA,   |
|                               |           |          |       | ACA,   |
|                               |           |          |       | ACC,   |
|                               |           |          |       | ACU,   |
|                               |           |          |       | AUA,   |
|                               |           |          |       | AUC,   |
|                               |           |          |       | AAA,   |
|                               |           |          |       | ACA,   |
|                               |           |          |       | ACC,   |
|                               |           |          |       | ACU,   |
|                               |           |          |       | AUA,   |
|                               |           |          |       | AUC,   |

| Papaver somniferum           | Eudicot   |          | 2571  | AAA,   | Phe, Ser |
|                              |           |          |       | ACU,   | Sup, Leu |
|                              |           |          |       | CUA,   | Sup, Sup |
|                              |           |          |       | GAG,   |
|                              |           |          |       | GGC,   |
|                              |           |          |       | GGU,   |
|                              |           |          |       | UAA,   |       |
|                              |           |          |       | UAC,   |
|                              |           |          |       | UAG,   |
|                              |           |          |       | UCA,   |
|                              |           |          |       | UCC,   |
|                              |           |          |       | UCU,   |
|                              |           |          |       | UGA,   |
|                              |           |          |       | UGC,   |
|                              |           |          |       | UGU,   |

| Picea glauca                 | Gymnosperm|          | 57    | AAA,   | Phe, Val |
|                              |           |          |       | AAC,   | Leu, Ile |
|                              |           |          |       | AAG,   | Cys, Gly |
|                              |           |          |       | AAU,   | Ser, Ser |
|                              |           |          |       | ACA,   | Ala, Pro |
|                              |           |          |       | ACC,   | Thr, Tyr |
|                              |           |          |       | ACU,   | Asp, His |
|                              |           |          |       | AGA,   | Asn, Val |
| Phaeodactyllum tricornutum | Alage | 41  | 38 | AAA, ACA, ACC, ACG, ACU, AUG, AUA, AUC, UAA, UAU, UUA | Phe, Cys, Gly, Arg, Ser, Tyr, Asp, His, Asn, Pro, Leu, Ile, Cys, Arg, Ser, Ala, Pro, Thr, Ile, Sup, Arg, Pro, Leu |
|---------------------------|-------|-----|----|-------------------------------------------------|-------------------------------------------------|
| Phalaenopsis equestris    | Monocot | 236 | 48 | AAA, ACA, ACC, ACG, ACU, AUG, AUA, AUC, UAA, UCA, UCU, UGG, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Ile, Arg, Ala, Pro, Sup, Sup |
| Species                  | Domain  | Length | Codons | Amino Acids |
|-------------------------|---------|--------|--------|-------------|
| *Phoenix dactylifera*   | Monocot | 470    | 50     | AAA, ACC, ACU, AUA, AUG, AUG, AUU, CUA, GAG, GCG, GGC, GGG, UCA, UUA | Phe, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| *Physcomitrella patens* | Bryophyte | 60    | 29     | AAA, AAC, AAG, AAU, ACA, ACC, ACU, AGA, AGC, AGU, AUA, AUC, AUG, AUU, CAC, CAG, CCC, CCU, CGA, CGC, CGG, CGU, CUA, CUC, CUG, CUU, GAG, GCG, GCC, GGG, UAA, UCA, UCG, UUA | Phe, Val, Leu, Ile, Cys, Gly, Ser, Ser, Ala, Pro, Thr, Tyr, Asp, His, Asn, Val, Leu, Gly, Arg, Ser, Ala, Pro, Thr, Sup, Glu, Gln, Lys, Leu, Arg, Ala, Pro, Ile, Sup, Arg, Sup |
| *Populus trichocarpa*   | Eudicot | 623   | 49     | AAA, ACA, ACC, ACU, AUA, AUG, AUG, AUU, CUA, GAG, GCG, GCC, GGG, UAA, UCA, UCG, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| Species               | Clade   | Genes | ORFs | Codons | Standard Amino Acids | Stop Codon(s) |
|----------------------|---------|-------|------|--------|----------------------|---------------|
| Populus euphratica   | Eudicot | 662   | 54   | AAA,   | Phe, Gly, Ser, Tyr,  | Yes/Sup       |
|                      |         |       |      | ACC,   | His, Asn, Leu, Arg,  |               |
|                      |         |       |      | ACU,   | Pro                  |               |
|                      |         |       |      | AUA,   |                      |               |
|                      |         |       |      | AUG,   |                      |               |
|                      |         |       |      | AUU,   |                      |               |
|                      |         |       |      | CUA,   |                      |               |
|                      |         |       |      | GAG,   |                      |               |
|                      |         |       |      | GCG,   |                      |               |
|                      |         |       |      | GGG,   |                      |               |
|                      |         |       |      | UCA,   |                      |               |
|                      |         |       |      | UUA    |                      |               |
| Prosopis alba        | Eudicot | 728   | 47   | AAA,   | Phe, Cys, Gly, Ser,  |              |
|                      |         |       |      | ACA,   | Tyr, Asp, His, Asn,  |              |
|                      |         |       |      | ACC,   | Sup, Val, Leu, Ile,  |              |
|                      |         |       |      | ACU,   | Arg, Ala, Pro, Sup,  |              |
|                      |         |       |      | AUA,   | Sup                  |               |
|                      |         |       |      | AUG,   |                      |               |
|                      |         |       |      | AUU,   |                      |               |
|                      |         |       |      | CUA,   |                      |               |
|                      |         |       |      | GAC,   |                      |               |
|                      |         |       |      | GAG,   |                      |               |
|                      |         |       |      | GAU,   |                      |               |
|                      |         |       |      | GCG,   |                      |               |
|                      |         |       |      | GGC,   |                      |               |
|                      |         |       |      | GGG,   |                      |               |
|                      |         |       |      | GGU,   |                      |               |
|                      |         |       |      | UCA,   |                      |               |
|                      |         |       |      | UUA    |                      |               |
| Prunus avium         | Eudicot | 419   | 46   | AAA,   | Phe, Cys, Gly, Ser,  |              |
|                      |         |       |      | ACA,   | Tyr, His, Asn, Sup,  |              |
|                      |         |       |      | ACC,   | Val, Leu, Ile, Arg,  |              |
|                      |         |       |      | ACU,   | Ser, Ala, Pro, Thr,  |              |
|                      |         |       |      | AUA,   | Sup                  |               |
|                      |         |       |      | AUG,   |                      |               |
|                      |         |       |      | AUU,   |                      |               |
|                      |         |       |      | CUA,   |                      |               |
|                      |         |       |      | GAC,   |                      |               |
|                      |         |       |      | GAG,   |                      |               |
|                      |         |       |      | GAU,   |                      |               |
|                      |         |       |      | GCG,   |                      |               |
|                      |         |       |      | GGC,   |                      |               |
|                      |         |       |      | GGG,   |                      |               |
|                      |         |       |      | GGU,   |                      |               |
|                      |         |       |      | UCA,   |                      |               |
|                      |         |       |      | UUA    |                      |               |
| Prunus dulcis        | Eudicot | 1208  | 49   | AAA,   | Phe, Cys, Gly, Ser,  |              |
|                      |         |       |      | ACA,   | His, Asn, Sup, Leu,  |              |
|                      |         |       |      | ACC,   | Ile, Arg, Ala, Pro,  |              |
|                      |         |       |      | ACU,   | Sup                  |               |
|                      |         |       |      | AUC,   |                      |               |
|                      |         |       |      | AUG,   |                      |               |
|                      |         |       |      | AUU,   |                      |               |
|                      |         |       |      | CUA,   |                      |               |
|                      |         |       |      | GAC,   |                      |               |
|                      |         |       |      | GAG,   |                      |               |
|                      |         |       |      | GAU,   |                      |               |
|                      |         |       |      | GCG,   |                      |               |
|                      |         |       |      | GGC,   |                      |               |
|                      |         |       |      | GGG,   |                      |               |
|                      |         |       |      | GGU,   |                      |               |
|                      |         |       |      | UCA,   |                      |               |
|                      |         |       |      | UUA    |                      |               |
| Species                | Classification | Bases | Identity | Codons | Amino Acids |
|-----------------------|----------------|-------|----------|--------|-------------|
| Prunus mume           | Eudicot        | 466   | 49       | AAA,   | Phe, Cys,   |
|                       |                |       |          | ACA,   | Gly, Ser,   |
|                       |                |       |          | ACC,   | Asp, His,   |
|                       |                |       |          | ACU,   | Asn, Sup,   |
|                       |                |       |          | AUU,   | Leu, Arg,   |
|                       |                |       |          | AUC,   | Ala, Pro,   |
|                       |                |       |          | AUG,   | Sup, Sup    |
|                       |                |       |          | AUU,   |             |
|                       |                |       |          | CUA,   |             |
|                       |                |       |          | GAG,   |             |
|                       |                |       |          | GCG,   |             |
|                       |                |       |          | GGC,   |             |
|                       |                |       |          | GGG,   |             |
|                       |                |       |          | UCA,   |             |
|                       |                |       |          | UUA    |             |
| Prunus persica        | Eudicot        | 452   | 49       | AAA,   | Phe, Cys,   |
|                       |                |       |          | ACA,   | Gly, Ser,   |
|                       |                |       |          | ACC,   | Tyr, Asp,   |
|                       |                |       |          | ACU,   | His, Asn,   |
|                       |                |       |          | AUU,   | Sup, Leu,   |
|                       |                |       |          | CUA,   | Arg, Ala,   |
|                       |                |       |          | AUG,   | Pro, Sup,   |
|                       |                |       |          | AUU,   | Sup         |
|                       |                |       |          | CUA,   |             |
|                       |                |       |          | GAG,   |             |
|                       |                |       |          | GCG,   |             |
|                       |                |       |          | GGC,   |             |
|                       |                |       |          | GGG,   |             |
|                       |                |       |          | UCA,   |             |
|                       |                |       |          | UUA    |             |
| Punicagranatum        | Eudicot        | 430   | 49       | AAA,   | Phe, Cys,   |
|                       |                |       |          | ACA,   | Gly, Ser,   |
|                       |                |       |          | ACC,   | Tyr, Asp,   |
|                       |                |       |          | ACU,   | His, Asn,   |
|                       |                |       |          | AUU,   | Sup, Leu,   |
|                       |                |       |          | CUA,   | Arg, Ala,   |
|                       |                |       |          | AUG,   | Pro, Sup,   |
|                       |                |       |          | AUU,   | Sup         |
|                       |                |       |          | CUA,   |             |
|                       |                |       |          | GAG,   |             |
|                       |                |       |          | GCG,   |             |
|                       |                |       |          | GGC,   |             |
|                       |                |       |          | GGG,   |             |
|                       |                |       |          | UCA,   |             |
|                       |                |       |          | UUA    |             |
| Pyrus bretschneideri   | Eudicot        | 553   | 46       | AAA,   | Phe, Cys,   |
|                       |                |       |          | ACA,   | Gly, Ser,   |
|                       |                |       |          | ACC,   | Tyr, Asp,   |
|                       |                |       |          | ACU,   | His, Asn,   |
|                       |                |       |          | AUU,   | Sup, Leu,   |
|                       |                |       |          | CUA,   | Ile, Arg,   |
|                       |                |       |          | AUG,   | Ser, Ala,   |
|                       |                |       |          | AUU,   | Pro         |
|                       |                |       |          | CUA,   | Thr, Sup,   |
|                       |                |       |          | GAG,   |             |
|                       |                |       |          | GAU,   |             |
|                       |                |       |          | GCG,   |             |
|                       |                |       |          | GGC,   |             |
|                       |                |       |          | GGG,   |             |
|                       |                |       |          | GUU,   |             |
|                       |                |       |          | UCA,   |             |
|                       |                |       |          | UUA    |             |
| Quercus suber         | Eudicot        | 746   | 46       | AAA,   | Phe, Cys,   |

Page 43/55
| Species                  | Kingdom   | Length | Start | Codons | Amino Acids                  |
|-------------------------|-----------|--------|-------|--------|----------------------------|
| *Raphanus sativus*      | Eudicot   | 1383   | 49    | AAA,   | Phe, Cys, Gly, Ser, Tyr, Asp, |
|                         |           |        |       | ACA,   | His, Asn, Sup, Val, Leu, Ile, |
|                         |           |        |       | ACC,   | Sup, Leu, Arg, Ala, Pro, Thr, |
|                         |           |        |       | ACU,   | Sup, Sup                      |
|                         |           |        |       | AUA,   |                                              |
|                         |           |        |       | AUC,   |                                              |
|                         |           |        |       | AUG,   |                                              |
|                         |           |        |       | AUU,   |                                              |
|                         |           |        |       | CUA,   |                                              |
|                         |           |        |       | GAC,   |                                              |
|                         |           |        |       | GAG,   |                                              |
|                         |           |        |       | GAU,   |                                              |
|                         |           |        |       | GCG,   |                                              |
|                         |           |        |       | GGC,   |                                              |
|                         |           |        |       | GGU,   |                                              |
|                         |           |        |       | UCA,   |                                              |
|                         |           |        |       | UUA    |                                              |
| *Raphidocelissubcapitata* | Algae     | 43     | 32    | AAA,   | Phe, Leu, Ile, Cys, Gly, Ser, |
|                         |           |        |       | AAG,   | Tyr, Asp, |
|                         |           |        |       | AAU,   | His, Asn, |
|                         |           |        |       | ACA,   | Sup, Val, |
|                         |           |        |       | ACC,   | Leu, Ile, |
|                         |           |        |       | ACU,   | Arg, Ser, Ala, Pro, |
|                         |           |        |       | AUA,   | Thr, Tyr, Leu, Val, |
|                         |           |        |       | AUC,   | Leu, Sup, Ser, Ala, |
|                         |           |        |       | AUG,   | Pro, Thr, Sup, Glu, |
|                         |           |        |       | AUU,   | Gln, Lys |
|                         |           |        |       | CUA,   |                                              |
|                         |           |        |       | GAC,   |                                              |
|                         |           |        |       | GAG,   |                                              |
|                         |           |        |       | GAU,   |                                              |
|                         |           |        |       | GCG,   |                                              |
|                         |           |        |       | GGA,   |                                              |
|                         |           |        |       | GGC,   |                                              |
|                         |           |        |       | GGU,   |                                              |
|                         |           |        |       | GUA,   |                                              |
|                         |           |        |       | UAA,   |                                              |
|                         |           |        |       | UAC,   |                                              |
|                         |           |        |       | UAG,   |                                              |
|                         |           |        |       | UCA,   |                                              |
|                         |           |        |       | UGA,   |                                              |
|                         |           |        |       | UGC,   |                                              |
|                         |           |        |       | UGG,   |                                              |
|                         |           |        |       | UGU,   |                                              |
|                         |           |        |       | UUA,   |                                              |
|                         |           |        |       | UUC,   |                                              |
|                         |           |        |       | UUG,   |                                              |
|                         |           |        |       | UUU    |                                              |
| *Rhodamniaargentea*     | Eudicot   | 628    | 48    | AAA.   | Phe, Cys, |

Page 44/55
| Species                  | Kingdom      | Length | Orthologs | Amino Acids       |
|-------------------------|--------------|--------|-----------|------------------|
| *Ricinus communis*      | Eudicot      | 539    | 50        | Gly, Ser, Tyr, Asp, His, Sup, Val, Leu, Ile, Arg, Ala, Pro, Sup, Sup |
| *Rosa chinensis*        | Eudicot      | 486    | 48        | Phe, Cys, Gly, Ser, Tyr, Asp, His, Sup, Leu, Arg, Ala, Pro, Thr, Sup, Sup |
| *Salvia splendens*      | Eudicot      | 1501   | 55        | Gly, Ser, Tyr, His, Asn, Sup, Ile, Arg, Sup |
| *Selaginella moellendorfii* | Pteridophyte | 1054   | 48        | Phe, Gly, Tyr, Asp, His, Asn, Sup, Val, Leu, Arg, Ser, Ala, Pro, Thr, Sup, Sup |
| Species                  | Kingdom | Code | Size | Codon | Amino Acids |
|-------------------------|---------|------|------|-------|-------------|
| Sesamum indicum         | Eudicot | 824  | 50   | AAA,  | Phe, Cys,   |
|                         |         |      |      | ACA,  | Gly, Ser,   |
|                         |         |      |      | ACC,  | Tyr, Asp,   |
|                         |         |      |      | ACU,  | His, Sup,   |
|                         |         |      |      | AUU,  | Leu, Arg,   |
|                         |         |      |      | UUA,  | Ala, Pro,   |
|                         |         |      |      | UCA,  | Sup, Sup    |
| Setaria italica         | Monocot  | 576  | 49   | AAA,  | Phe, Cys,   |
|                         |         |      |      | ACA,  | Gly, Ser,   |
|                         |         |      |      | ACC,  | Tyr, Asp,   |
|                         |         |      |      | ACU,  | His, Asn,   |
|                         |         |      |      | AUU,  | Sup, Leu,   |
|                         |         |      |      | UUA,  | Arg, Ala,   |
|                         |         |      |      | UCA,  | Pro, Sup,   |
|                         |         |      |      | UUA,  | Sup         |
| Solanum lycopersicum    | Eudicot  | 793  | 48   | AAA,  | Phe, Cys,   |
|                         |         |      |      | ACA,  | Gly, Ser,   |
|                         |         |      |      | ACC,  | Tyr, Asp,   |
|                         |         |      |      | ACU,  | His, Asn,   |
|                         |         |      |      | AUU,  | Sup, Leu,   |
|                         |         |      |      | UUA,  | Ile, Arg,   |
|                         |         |      |      | UCA,  | Ala, Pro,   |
|                         |         |      |      | AUU,  | Sup, Sup    |
|                         |         |      |      | UUA,  |             |
| Solanum pennellii       | Eudicot  | 823  | 49   | AAA,  | Phe, Cys,   |
|                         |         |      |      | ACA,  | Gly, Tyr,   |
|                         |         |      |      | ACC,  | Asp, Asn,   |
|                         |         |      |      | AUU,  | Sup, Val,   |
|                         |         |      |      | UUA,  | Leu, Ile,   |
|                         |         |      |      | UUA,  | Arg, Ala,   |
|                         |         |      |      | UUA,  | Pro, Sup,   |
|                         |         |      |      | UUA,  | Sup         |
| Species              | Class   | Genes | Codons | Amino Acids | Backstop Codon |
|---------------------|---------|-------|--------|-------------|----------------|
| *Solanum tuberosum* | Eudicot | 799   | 50     | Phe, Cys, Gly, Ser, Tyr, Asp, Sup, Leu, Ile, Arg, Ala, Pro, Sup | UCA, UUA |
| *Sorghum bicolor*   | Monocot | 550   | 49     | Phe, Cys, Gly, Ser, Tyr, Asp, Asn, Sup, Leu, Ile, Arg, Ala, Pro, Sup | UCA, UUA |
| *Spinacia oleracea* | Eudicot | 798   | 50     | Phe, Cys, Gly, Ser, Tyr, Asp, His, Sup, Leu, Arg, Ala, Pro, Sup | UCA, UUA |
| *Syzygium oleosum*  | Eudicot | 445   | 47     | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Val, Leu, Ile, Arg, Ala, Pro, Sup | UCA, UUA |
| Species                  | Kingdom | Genus | Bloom | Fdnr | Amino Acids                                                                 |
|-------------------------|---------|-------|-------|------|----------------------------------------------------------------------------|
| Tarenayahassleriana     | Eudicot |       | 595   | 46   | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAC, GAG, GAG, GCU, GGC, GGG, GGU, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Val, Leu, Arg, Ala, Pro, Thr, Sup |
| Theobroma cacao         | Eudicot |       | 415   | 49   | AAA, ACA, ACC, ACU, AUA, AUC, AUG, AUU, CUA, GAC, GAG, GCU, GGC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup, Arg, Ala, Pro, Sec, Sup |
| Triticum urartu        | Monocot |       | 412   | 51   | AAA, ACA, ACC, ACU, AUA, AUC, AUG, CCA, CUA, GAC, GCG, GGC, GGG, UAU, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Gly, Sup, Arg, Ala, Ile, Sup |
| Vigna angularis        | Eudicot |       | 610   | 50   | AAA, ACA, ACC, ACU, AUA, AUC, AUG, CUA, GAG, GCG, GGC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, Asp, His, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| Vigna radiata          | Eudicot |       | 614   | 50   | ACA, ACC, ACU, AUA | Cys, Gly, Ser, Tyr, Asp, His, Asn, Sup |
### Table 3

AUC, AUG, AUU, CUA, GAG, GCG, GGC, GGG, UCA, UUA

Leu, Arg, Ala, Pro, Sup, Sup

| Species            | Type     | Code | Count | Count | Code       | Count | Type     | Code       | Count | Type     | Code       | Count |
|--------------------|----------|------|-------|-------|------------|-------|----------|------------|-------|----------|------------|-------|
| Vitis vinifera     | Eudicot  | 448  | 50    | AAA,  | ACA, ACC,  | ACU,  | AUG,     | AUU, CUA,  | GAG,  | GCG, GGC, GGG, UCA, UUA | Phe, Cys, Gly, Ser, Tyr, His, Asn, Sup, Leu, Arg, Ala, Pro, Sup, Sup |
| Zea mays           | Monocot  | 1884 | 59    | ACC,  | GAG, GAU,  | GCG,  | GGC      | ACC, ACU, AUA, AUC, AUG, CUA, GAC, GAG, GCC, GGG, UCA, UUA | Gly, Leu, Ile, Arg, Ala |
| Ziziphus jujuba    | Eudicot  | 566  | 52    | ACC,  | ACU, AUC,  | AUG,  | CUA,     | GAC, GAG, GCC, GGG, UCA, UUA | Gly, Ser, Tyr, Asp, His, Sup, Val, Leu, Ala, Pro, Sup, Sup |

(A) t-test (two tailed) between eudicot and monocot anti-codon numbers. The t-value is smaller than critical value (1.2691 < 1.984). So, the mean was not significantly different ($p < 0.05$). (B) t-test (two tailed) between eudicot and algae anti-codon numbers. The t-test result was greater than critical value (10.3939 > 1.987). So, the mean is significantly different ($p < 0.05$). (C) t-test (two tailed) between Eudicot and algae anti-codon numbers. The t-test result was greater than critical value (6.2914 > 2.037). So, the mean is significantly different ($p < 0.05$).
| Statistical parameters | Eudicot | Monocot | t     | Degree of freedom | Critical value (T) |
|------------------------|---------|---------|-------|------------------|-------------------|
| Mean                   | 50.0562 | 49.087  | 1.2691| 110              | 1.984             |
| Variance               | 11.1673 | 8.6285  |       |                  |                   |
| Stand. Dev             | 3.3418  | 2.9374  |       |                  |                   |
| n                      | 89      | 23      |       |                  |                   |

(B)

| Statistical parameters | Monocot | Algae   | t     | Degree of freedom | Critical value (T) |
|------------------------|---------|---------|-------|------------------|-------------------|
| Mean                   | 49.087  | 35.2727 | 6.2914| 32               | 2.037             |
| Variance               | 8.6285  | 95.8182 |       |                  |                   |
| Stand. Dev             | 2.9374  | 9.7878  |       |                  |                   |
| n                      | 23      | 11      |       |                  |                   |

(C)

| Statistical parameters | Monocot | Algae   | t     | Degree of freedom | Critical value (T) |
|------------------------|---------|---------|-------|------------------|-------------------|
| Mean                   | 49.087  | 35.2727 | 6.2914| 32               | 2.037             |
| Variance               | 8.6285  | 95.8182 |       |                  |                   |
| Stand. Dev             | 2.9374  | 9.7878  |       |                  |                   |
| n                      | 23      | 11      |       |                  |                   |

Figures
Figure 1

Regression analysis of tRNA gene number with plant genome. The analysis indicated that the number of tRNAs were not significantly correlated with plant genome size.
Figure 2

Grouping of plant anti-codons. The clustering was conducted based on the frequency (percentage) of the anti-codons found in the collective plant genomes of 128 plant species. The grouping A, B, C, D, and E were made based on the decreasing order of anti-codon frequency. The clustering was conducted using the UPGMA algorithm and Euclidean distance matrix with 1000 bootstrap replicates in Past3 software.
Figure 3

Phylogenetic tree of tRNAsSec and other tRNA isotypes. The phylogenetic tree with 21 tRNA isotypes revealed at least 28 major phylogenetic groups where tRNAsSec (red) was placed with different tRNA isotypes. The phylogenetic tree indicates that tRNA has most likely evolved from multiple common ancestors and has also undergone duplication. The evolutionary history was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood (-7466.51) is illustrated. The percentage of the branches in which the associated taxa cluster together is shown next to the branches. Initial tree(s) for the heuristic search were automatically obtained applying the Neighbor-Join and BIONJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with a superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among the sites [5 categories (+G, parameter = 2.6875)]. The tree is drawn to scale, with branch lengths representing the number of substitutions per site. The analysis utilized 702 nucleotide sequences. All positions with less than 95% site coverage were eliminated. Fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. Evolutionary analyses were conducted in MEGA7 [2].
Figure 4

Recombination events in tRNA isotypes. Results indicated that tRNAs have undergone dynamic recombination events during the course of evolution. The recombination study was conducted using IcyTree software using a nwk file format obtained from the phylogenetic tree.

Figure 5

Recombination events in tRNAsec genes. Results indicate that tRNAsec have undergone recombination events within themselves as well during evolution. The recombination study was conducted using IcyTree using the nwk file format of the phylogenetic tree of the tRNAsec.
Figure 6

Normal probability plot of anti-codon numbers of the plant kingdom with correlation coefficient 0.9636 suggesting the datasets are normally distributed.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile1.xlsx
- SupplementaryFigure4.tif
- SupplementaryFigure3.PDF
- SupplementaryFigure2.pdf
- SupplementaryFigure1.pdf