Stress induced MAPK genes show distinct pattern of codon usage in *Arabidopsis thaliana*, *Glycine max* and *Oryza sativa*

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Abstract:
Mitogen activated protein kinase (MAPK) genes provide resistance to various biotic and abiotic stresses. Codon usage profiling of the genes reveals the characteristic features of the genes like nucleotide composition, gene expressivity, optimal codons etc. The present study is a comparative analysis of codon usage patterns for different MAPK genes in three organisms, *viz.* *Arabidopsis thaliana*, *Glycine max* (soybean) and *Oryza sativa* (rice). The study has revealed a high AT content in MAPK genes of *Arabidopsis* and soybean whereas in rice a balanced AT-GC content at the third synonymous position of codon. The genes show a low bias in codon usage profile as reflected in the higher values (50.83 to 56.55) of effective number of codons ($N_c$). The prediction of gene expression profile in the MAPK genes revealed that these genes might be under the selective pressure of translational optimization as reflected in the low codon adaptation index (CAI) values ranging from 0.147 to 0.208.

Keywords: MAPK, *Arabidopsis*, synonymous codons, codon usage bias, mutational pressure.

Background:
Many codons in the genetic code are functionally synonymous. A single amino acid is encoded by two to six codons, a phenomenon called synonymous codon usage. All the synonymous codons of an amino acid show a variation in the occurrence in a gene frequency. Some codons show a high frequency indicating that the usage of the particular codon is biased. The study of the codon usage bias may assist to design the DNA primers [1]. The synonymous codons show variation between as well as within the genome of an organism [2, 3]. This variation could be a consequence of natural and/or mutational pressure to determine the accuracy and the efficiency of the translation process of the organisms. Study of the synonymous codon usage patterns of a gene in various organisms could provide an insight into the evolution profile and the level of gene expression as well [4, 5]. In due course of evolution, plants have developed their own mechanisms to combat the stresses- both biotic as well as abiotic which they are continually subject to. Mitogen activated protein kinase (MAPK) genes present in the plants actively respond to various stresses. Abiotic stresses mainly include drought, high salinity, heat, cold, freezing, limited nutrient availability, heavy metals etc. [6, 7]. The MAPK genes are generally classified into three distinct types-MAPKKK, MAPKK and MAPK. The string of reactions in the stress signalling pathways involve these three MAPK families; MAPKKKs phosphorylate the serine/threonine of MAPKKs at their activation loop which in turn double phosphorylate the MAPKs at their T-D-Y motif in the activation loop. The MAPK genes get activated by the stress stimuli and form various cascade which form the metabolic pathways to regulate the stress response. The cascade actions acts downstream of the receptors on extracellular surface or acts as a sensor that’s transduces the extracellular signals into intracellular responses [8].

*Arabidopsis* genome has been reported to harbour 80 MAPKKKs, 10 MAPKKs and 20 MAPKs genes. Whole genome...
analysis of rice (*Oryza sativa*) has revealed 75 MAPKKKs, 8 MAPKKs and 15 MAPKs [9, 10]. *In-silico* studies of the Soybean (*Glycine max*) has identified 38 MAPKs, 11 MAPKKs, and 150 MAPKKKs [11]. Chilling stress is one of the serious problems associated with the production of major crops such as rice and maize [12] in temperate zone. The *Arabidopsis* MAPKs, based on their structural motif and sequence similarities, is classified into four groups (A-D). The group A, B and C possesses a T-E-Y motif whereas the fourth group A possesses a T-D-Y motif [13, 14]. A signalling pathway comprising of MEKK1-MKK2-MPK4/MPK6 is reported to regulate the response to abiotic stresses in *Arabidopsis* [15]. The abiotic stresses induce the production of ROS (reactive oxygen species). Several MAPK signalling pathways are induced only by ROS and in turn regulate the ROS production. Many MAPK pathways in response to abiotic stresses have been studied in rice. Experiment on long term exposure of rice plants to cooling stress revealed the involvement of several MAPKs namely MEK1 (MAPKK) and MAP1 (MAPK) [16]. MAPK5 gene in rice has shown multiple activities, responding to both biotic and abiotic stress. Resistivity to drought in rice plants is provided by an MAPKK of the B3 subgroup named DSM1 [17]. MAPK33 has also shown activity withstanding drought [18].

**Methodology:**

**Coding DNA sequence data**

Coding sequence data (a total of 127 cde) of the different genes of the MAPK families of *Arabidopsis*, soybean and rice were retrieved from NCBI (www.ncbi.nlm.nih.gov). The genes with different accession numbers are listed in Table 1 (see supplementary material). Different analytical parameters for codon usage bias of the MAPK genes with different accession number were estimated and analysed.

**Analysis of the codon usage profile**

Several parameters have been used to characterize the sequence data of the genes. Gene members of each of the three MAPK families are analysed and compared family-wise across the three organisms.

**RSCU**

The relative synonymous codon usage (RSCU) measures the frequencies of optimal codons of each of the synonymous codons encoding an amino acid. It assists in characterising the codons in a genetic sequence, whether it follows unbiased pattern of the codons being used or certain codons are more preferred. Codons with RSCU values greater than 1 are generalised to possess a positive codon usage bias [20]. Bioinformatics tools available online, codonW (mobyle.pasteur.fr/cgi-bin/portal.py#forms::codonW) have been used to estimate the RSCU which is mathematically expressed as: (For equation-> 1 please see supplementary material).

The GC content is a measure of the occurrence of the nucleotide bases guanine (G) and cytosine (C) in the entire genetic sequence [21]. The GC content can be measured partially at the 1st, 2nd and 3rd synonymous codon position throughout the sequence, designated as $G_{1S}$, $G_{2S}$, and $G_{3S}$ respectively [22]. Other indices for nucleotide bias such as $A_{%}$, $T_{%}$, $G_{%}$, and $C_{%}$ are also measured. The nucleotide measure at the third codon positions designated as $A_{3S}$, $T_{3S}$, $G_{3S}$ and $C_{3S}$ are also calculated. Mathematically GC% is expressed as: (For equation-> 2 please see supplementary material).
Effective number of codons ($N_c$)
As proposed by Wright in the year 1990, $N_c$ measures the absolute synonymous codon usage bias [23]. It measures the total number of synonymous codons used for each amino acid. The values of $N_c$ ranges from 20 (when only one codon is used for an amino acid), to 61 (when all the codons are used with equal frequency) [1]. It may be expressed as: (For equation $>$ (3) please see supplementary material). Where, $F_2$ is the probability that two randomly chosen codon for an amino acid, possibly encoded by two distinct codons, are identical, $F_3$ is the probability that three randomly chosen codons for an amino acid with three synonymous codons are identical and so on [1, 23].

Gene expression analysis
The expressivity of a gene is characterized on the basis of CAI value for a gene. It is measured as calculating the geometric means of the relative adaptiveness ($w$) of all the codons in a gene. The CAI value ranges from 0 (when less frequent codons are used) to 1 (when most frequent codons are used) [24]. Highly expressed genes tend to have high CAI values whereas less expressive genes possess low CAI values. CAI is mathematically expressed as: (For equation $>$ (4) please see supplementary material), where, $L$ is the number of codons in the gene and $w_{(k)}$ is the $w$ value for the $k_{th}$ codon in the gene. Relative adaptiveness ($w$) can be calculated as: (For equation $>$ (5) please see supplementary material). Where $X_{ij}$ is the number of occurrence of $j^{th}$ codons in the reference set of highly expressed genes and $X_{max}$ is the maximum $X_{ij}$ for $i^{th}$ amino acid.

Results:
Characteristic patterns of nucleotide composition
The nucleotide composition of the MAPK genes in three organisms shows a clear characteristic pattern of resemblance with a few exceptions in rice (Figure 1). In case of MAPKK family, the overall GC% of Arabidopsis is 35.6%, which is low, revealing that the organism has high overall AT content. The overall GC content in rice and soybean gene sequences are 43.99% and 33.10% respectively. This result suggests that there exists a balance between the AT and GC content in the soybean genes, while in rice the overall AT content slightly exceeds the overall GC content for MAPK genes. From the GC% in all the three gene families across three species, it is evident that the
GC% is markedly suppressed in Arabidopsis and soybean at the third synonymous codon position. The rice genes show an overall consistent pattern from the other two species in respect of the GC3%. The overall GC3 content in all the gene families of rice is slightly lower (40.02%-45.39%) as against the AT3 content (54.61%-59.08%). Thus the comparative study of the MAPKK, MAPKK and the MAP family genes show that Arabidopsis and soybean have resemblance in their pattern of nucleotide usage whereas rice genes deviate from the other two species (Table 1).

![Figure 3: Frequency of estimated CAI values of different genes in Arabidopsis, rice and soybean](image)

### Synonymous codon usage pattern

Codons with RSCU values greater than 1 are considered to have positive codon usage bias [22]. The most preferred codon for each of 18 amino acids bearing the highest RSCU values are shown in Table 2 (see supplementary material). Inspection of the overall RSCU values reveals that the codons TTT, GTT, AAT, and GAT coding for phenylalanine, valine, asparagine and aspartic acid respectively, have got the highest preference in all the three organisms. Besides this, the comparative study between the Arabidopsis and soybean (G. max) reveals significant resemblance in the preference of codons; 11 out of 18 amino acids show the same preferred codon. But rice does not show any significant trend: only two amino acids resemble with soybean, and three with Arabidopsis for preferred codon. The resemblance between Arabidopsis and soybean is further evident from the codon preference in the MAPKK family; 12 out of 18 amino acids show the same preference of codons. Only one amino acid i.e. valine is encoded by the most preferred codon GTT in three MAPK gene families across all the species. The MAPKK family has shown the extreme resemblance for the most preferred codons between Arabidopsis and soybean; 15 out of 18 amino acids have the same preferred codon. But in rice the most preferred codon for most amino acids differs from Arabidopsis and soybean. The comparison of RSCU values for most preferred codon for each amino acid in three organisms is shown in Figure 2 for each gene family. The third codon position of all the preferred codons predominantly possesses the nucleotide T followed by A in all the three gene families indicating that this preference could be due to translational selection or mutation bias.

### Expressivity of genes

Codon adaptation index (CAI), as proposed by Sharp and Li in the year 1987, is a measure to predict the expressivity of genes [24]. The CAI value of each gene belonging to different families was found to be very low, ranging from 0.147 to 0.208 (Table 1), which indicates that the genes are not possibly optimized for high expression (Figure 3). This could be due to the fact that stress induces the MAPK gene expression and that MAPK genes are not house-keeping genes by nature. Hence their CAI values are usually low indicating low expression under non-stress environment.

### Biasness in codon usage

The Nc values of all genes are towards higher side, ranging from 50.83 to 56.55. The higher values of Nc signify that the gene sequences show low biasness in the codon usage profile.

### Discussion:

The comparative study of the codon usage for MAPK genes in three species i.e. Arabidopsis, soybean and rice have shown that the first two organisms share a high degree of similarity between them. The MAPK genes of these two organisms have shown very high resemblance in codon preference in the coding sequences. The gene family wise comparison across the three organisms revealed that Arabidopsis and soybean are compositionally alike. In contrast, rice showed a somewhat different nucleotide composition from the other two species. This could be due to the fact that Arabidopsis and soybean are dicots unlike rice which is a monocot. Arabidopsis and soybean genes are overall AT-rich as well as at the third synonymous codon position. The rice MAPK sequences, on the other hand, are overall GC-rich and at the third synonymous position. Guo and his co-workers (2007) also reported a high GC content in rice genes [25]. The results suggest that the codon usage pattern in all the MAPK genes in the three species used in the study might be influenced by their base compositional properties. The general trend of the Nc values in the three species is consistently higher side for all the genes indicating a low bias in the codon usage pattern in these genes.

The overall perusal of the RSCU values for the three gene families revealed a high similarity between the codon usage in Arabidopsis and G. max. Majority of the preferred codons in the genes of these two species are T-redundant at the silent third codon position. This may be due to the high prevalence of AT content in these genes. However, in case of O. sativa, the preference for the nucleotides at the third synonymous codon position is balanced in MAPK and MAPKK genes, which is evident from the nearly equal distribution of overall GC and AT content in these two gene families. In MAPKK gene family of O. sativa, however, the preferential codons showed the increased tendency of using T at the third position. Valine is encoded by the most preferred codon GTT in three species. Highly expressed genes generally show a tendency of using a limited number of codons which they use preferentially [24]. The level of gene expression as estimated by the CAI values has shown that the MAPK genes are not possibly highly expressed. Since stress induces the expression of MAPK genes, these genes might be evolutionarily so organized as to give low expression under non-stress or normal conditions. CAI values of all the genes have shown a close proximity to 0, suggesting that they have less expressivity [24]. Translational selection might have played a role in this context, rendering the MAPK genes to be optimized for low expression under non-stress environment.

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Conclusion:
This work is the first attempt to gain insight into the codon usage profiles of stress induced MAPK genes across three plant species. Mutational pressure and natural selection have been projected as the main impetus behind the codon usage bias in various organism ranging from small procaryotes to large plants and animals [21, 26, 27]. In the present study, the results indicate that apart from the mutational pressure, translational selection might be playing a pivotal role in order to make these genes optimized for translating efficiently. MAPK families comprise of a huge number of genes interacting with each other in order to combat different environmental stresses. Based on the type of stress and the species involved, the codon usage as well as the expression of genes may vary. Thus, it is necessary to carry out further detailed analysis of the codon usage pattern in MAPK and the associated genes involved in the cascade of actions under biotic and abiotic stress environments in different species.

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Supplementary material:

Methodology:

| Eq.No | Equation | Explanation |
|-------|----------|-------------|
| (1)   | RSCU_ij = \frac{x_{ij}}{1 + \frac{1}{n_j} \sum_{i=1}^{n_j} x_{ij}} | where, x_i is the number of frequency of jth codon for ith amino acid and n_j being the number of alternative synonymous codons available for the ith amino acid. |
| (2)   | GC = \frac{G + C}{A + T + G + C} x 100 | GC content can be measured partially at the 1st, 2nd and 3rd synonymous codon |
| (3)   | N_c = 2 + \frac{9}{F_2} + \frac{1}{F_3} + \frac{5}{F_4} + \frac{3}{F_6} | F_2 is the probability that two randomly chosen codon for an amino acid, possibly encoded by two distinct codons, are identical, F_3 is the probability that three randomly chosen codons for an amino acid with three synonymous codons are identical and so on |
| (4)   | CAI = \exp^\frac{1}{L} \sum_{k=1}^{L} \ln w_c(k) | L is the number of codons in the gene and w_c(k) is the w value for the kth codon in the gene. |
| (5)   | w_j = \frac{X_{ij}}{X_{i,max}} | Where X_ij is the number of occurrence of jth codons in the reference set of highly expressed genes and X_{i,max} is the maximum X_ij for ith amino acid. |

Table 1: Nucleotide composition of the MAPK gene families in A. thaliana, G. max and O. sativa

| Gene family | Organism | A% | T% | G% | C% | A3% | T3% | G3% | C3% |
|-------------|----------|----|----|----|----|-----|-----|-----|-----|
| MAPKK       | A. thaliana | 27.19 | 30.56 | 23.47 | 18.95 | 26.66 | 26.66 | 27.05 | 19.64 |
|             | G. max    | 28.09 | 27.92 | 23.71 | 20.29 | 27.03 | 28.79 | 23.21 | 20.97 |
|             | O. sativa | 22.68 | 24.18 | 26.48 | 26.66 | 18.24 | 26.14 | 25.23 | 30.39 |
| Mean        |           | 25.98 | 27.55 | 24.55 | 21.96 | 23.98 | 27.20 | 25.16 | 23.66 |
| SD          |           | 2.366 | 2.618 | 1.368 | 3.364 | 4.060 | 1.148 | 1.570 | 4.788 |
| MAPKK       | A. thaliana | 27.31 | 31.12 | 21.60 | 19.95 | 25.95 | 34.05 | 20.23 | 19.77 |
|             | G. max    | 29.89 | 33.55 | 19.07 | 17.49 | 29.21 | 31.62 | 19.81 | 19.37 |
|             | O. sativa | 26.79 | 23.87 | 25.69 | 23.65 | 23.65 | 23.01 | 26.86 | 26.48 |
| Mean        |           | 25.33 | 28.01 | 22.12 | 20.36 | 26.27 | 29.56 | 22.50 | 21.87 |
| SD          |           | 1.354 | 4.115 | 2.729 | 2.532 | 2.282 | 4.735 | 3.229 | 3.264 |
| MAPK        | A. thaliana | 27.30 | 31.79 | 22.14 | 18.79 | 26.43 | 31.56 | 22.25 | 19.76 |
|             | G. max    | 27.47 | 31.26 | 22.01 | 19.22 | 28.80 | 33.15 | 18.04 | 20.01 |
|             | O. sativa | 26.48 | 23.87 | 22.80 | 23.43 | 23.08 | 24.90 | 25.79 | 26.22 |
| Mean        |           | 27.08 | 29.81 | 22.32 | 20.79 | 26.10 | 29.87 | 22.03 | 22.00 |
| SD          |           | 0.433 | 2.437 | 0.345 | 2.525 | 2.344 | 3.575 | 3.171 | 2.987 |
| Gene family | Organism | GC% | AT% | GC3% | AT3% | Nc | CAI |
| MAPKK       | A. thaliana | 35.60 | 64.40 | 36.87 | 36.87 | 56.27 | 0.1466 |
|             | G. max    | 43.99 | 56.01 | 34.12 | 34.12 | 54.97 | 0.1807 |
|             | O. sativa | 53.10 | 46.90 | 45.39 | 45.39 | 56.55 | 0.2080 |
| Mean        |           | 44.23 | 55.77 | 38.79 | 38.79 | 55.93 | 0.1785 |
| SD          |           | 7.145 | 7.145 | 4.800 | 4.80 | 0.688 | |
| MAPKK       | A. thaliana | 34.31 | 65.69 | 31.30 | 31.30 | 52.61 | 0.1853 |
|             | G. max    | 36.57 | 63.43 | 29.96 | 29.96 | 51.56 | 0.1627 |
|             | O. sativa | 49.38 | 50.63 | 40.89 | 40.89 | 54.09 | 0.2075 |
| Mean        |           | 40.08 | 59.92 | 34.05 | 34.05 | 52.75 | 0.1852 |
| SD          |           | 6.635 | 6.635 | 4.868 | 4.868 | 1.036 | |
| MAPK        | A. thaliana | 33.46 | 66.54 | 32.19 | 32.19 | 55.29 | 0.1912 |
|             | G. max    | 32.92 | 67.08 | 29.98 | 29.98 | 53.42 | 0.1825 |
|             | O. sativa | 47.14 | 52.86 | 40.02 | 40.02 | 50.83 | 0.2005 |
| Mean        |           | 37.84 | 62.16 | 34.07 | 34.07 | 53.18 | 0.1914 |
| SD          |           | 6.579 | 6.579 | 4.309 | 4.309 | 1.826 | |

*SD stands for standard deviation
Table 2: RSCU values of the most preferred codons for MAPK genes in Arabidopsis, soybean and rice

| MAPKK  | Amino acids | Codons | G. max | Codons | A. thaliana | Codons | O. sativa |
|--------|-------------|--------|--------|--------|-------------|--------|----------|
|        | Phe         | TTT    | 1.08   | TTT    | 1.13        | TTT    | 1.00     |
|        | Leu         | TTG    | 1.50   | TTG    | 1.53        | CTC    | 2.00     |
|        | Ile         | ATT    | 1.18   | ATT    | 1.04        | ATT    | 1.74     |
|        | Val         | GTT    | 1.34   | GTT    | 1.55        | GTT    | 1.55     |
|        | Ser         | TCT    | 1.23   | TCT    | 1.45        | TCC    | 1.32     |
|        | Pro         | CCA    | 1.38   | CCA    | 1.30        | CCA    | 1.74     |
|        | Thr         | ACA    | 1.37   | ACA    | 1.47        | ACC    | 1.38     |
|        | Ala         | GCT    | 1.40   | GCT    | 1.39        | GCG    | 1.21     |
|        | Tyr         | TAT    | 1.25   | TAT    | 1.26        | TAC    | 1.05     |
|        | His         | CAT    | 1.20   | CAT    | 1.16        | CAT    | 1.22     |
|        | Gln         | CAA    | 1.18   | CAA    | 1.09        | CAG    | 1.08     |
|        | Asn         | AAT    | 1.15   | AAT    | 1.19        | AAT    | 1.38     |
|        | Lys         | AAA    | 1.06   | AAA    | 1.03        | AAG    | 1.27     |
|        | Asp         | GAT    | 1.38   | GAT    | 1.27        | GAC    | 1.02     |
|        | Glu         | GAA    | 1.18   | GAG    | 1.11        | GAG    | 1.17     |
|        | Cys         | TGT    | 1.07   | TGT    | 1.16        | TGC    | 1.54     |
|        | Arg         | AGA    | 1.93   | AGA    | 2.09        | AGG    | 1.67     |
|        | Gly         | GGA    | 1.37   | GGT    | 1.40        | GCC    | 1.87     |

| MAPKK  | Amino acid | Codons | G. max | Codons | A. thaliana | Codons | O. sativa |
|--------|-------------|--------|--------|--------|-------------|--------|----------|
|        | Phe         | TTT    | 1.27   | TTT    | 1.25        | TTT    | 1.04     |
|        | Leu         | TTG    | 1.61   | TTT    | 1.54        | CTG    | 1.35     |
|        | Ile         | ATC    | 1.08   | TTT    | 1.18        | ATC    | 1.33     |
|        | Val         | GTT    | 1.31   | GTT    | 1.51        | GTT    | 1.42     |
|        | Ser         | AGT    | 1.52   | TCT    | 1.58        | TCC    | 1.28     |
|        | Pro         | CCA    | 1.71   | CCT    | 1.56        | CCA    | 1.08     |
|        | Thr         | ACA    | 1.58   | ACT    | 1.20        | ACA    | 1.45     |
|        | Ala         | GCT    | 1.68   | GCT    | 1.72        | GCA    | 1.42     |
|        | Tyr         | TAT    | 1.41   | TAT    | 1.20        | TAC    | 1.15     |
|        | His         | CAT    | 1.22   | CAT    | 1.26        | CAT    | 1.01     |
|        | Gln         | CAA    | 1.27   | CAA    | 1.27        | CAG    | 1.08     |
|        | Asn         | AAT    | 1.22   | AAT    | 1.04        | AAG    | 1.06     |
|        | Lys         | AAA    | 1.22   | AAA    | 1.14        | AAG    | 1.12     |
|        | Asp         | GAT    | 1.38   | GAT    | 1.43        | GAT    | 1.04     |
|        | Glu         | GAA    | 1.23   | GAG    | 1.04        | GAG    | 1.17     |
|        | Cys         | TGT    | 1.23   | TGT    | 1.55        | TGT    | 1.02     |
|        | Arg         | AGA    | 2.92   | AGA    | 1.93        | CGC    | 1.26     |
|        | Gly         | GGA    | 1.38   | GGA    | 1.79        | GGT    | 1.18     |

| MAPK   | Amino acid | Codons | G. max | Codons | A. thaliana | Codons | O. sativa |
|--------|-------------|--------|--------|--------|-------------|--------|----------|
|        | Phe         | TTT    | 1.36   | TTT    | 1.23        | TTT    | 1.04     |
|        | Leu         | CTT    | 2.08   | TTT    | 1.38        | CTG    | 1.28     |
|        | Ile         | ATT    | 1.30   | TTT    | 1.09        | ATT    | 1.18     |
|        | Val         | GTT    | 2.21   | GTT    | 1.89        | GTT    | 1.30     |
|        | Ser         | TCT    | 2.48   | TCT    | 1.70        | TCC    | 1.51     |
|        | Pro         | CCC    | 1.39   | CCA    | 1.60        | CCA    | 1.45     |
|        | Thr         | ACA    | 1.33   | ACT    | 1.40        | ACT    | 1.13     |
|        | Ala         | GCA    | 1.69   | GCT    | 1.74        | GCA    | 1.25     |
|        | Tyr         | TAT    | 1.04   | TAT    | 1.34        | TAC    | 1.11     |
|        | His         | CAT    | 1.29   | CAT    | 1.27        | CAT    | 1.01     |
|        | Gln         | CAA    | 1.22   | CAA    | 1.16        | CAG    | 1.06     |
|        | Asn         | AAT    | 1.36   | AAT    | 1.07        | AAT    | 1.07     |
|        | Lys         | AAA    | 1.09   | AAA    | 1.05        | AAG    | 1.21     |
|        | Asp         | GAT    | 1.50   | GAT    | 1.41        | GAT    | 1.17     |
|        | Glu         | GAA    | 1.19   | GAG    | 1.02        | GAG    | 1.14     |
|        | Cys         | TGT    | 1.23   | TGT    | 1.21        | TGC    | 1.28     |
|        | Arg         | AGA    | 2.00   | AGA    | 2.28        | CGC    | 1.21     |
|        | Gly         | GGT    | 1.64   | GGA    | 1.41        | GCC    | 1.40     |