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THE RELATIONSHIP OF SYNONYMOUS CODON USAGE BIAS ANALYSES OF STRESS RESISTANT AND REFERENCE GENES IN SIGNIFICANT SPECIES OF PLANTS

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Abstract

The Synonymous Codon Usage Bias (SCUB) corresponds to the difference in the expression level of a gene that interrupts the quantity of proteins and varies the expression level of a protein. Stress associated with dehydration, cold, heat, salt, heavy metals, oxidative and drought also lessen the yield of significant plants. This research is centered upon the CUB and RNA structures (RS) analyses of abiotic stress resistant and reference genetic material in significant species of dicot (*Arabidopsis thaliana, Populous trichocarpa*) and monocot (*Oryza sativa* and *Triticum aestivum*) plants. Precisely, 11 stress resistant and 3 reference genes were subjected to SCUB and RS analysis. SCUB analysis revealed that *O. sativa* presented 82%, *P. trichocarpa* 91% and *T. aestivum* with about 55% similarity with *A. thaliana* in the utilization of synonymous codons for abiotic stress resistant genes. The Reference genes for *O. sativa* and *P. trichocarpa* each manifest 100% similarities with *A. thaliana* except for the *T. aestivum* that exhibit 67% similarities in the usage of codons. Mfold algorithms were implemented for the prediction of RNA Secondary Structures (RSS) of abiotic stress resistant and Reference genes, the Dicot species expressed a significant difference in the MFE with the Monocot species; however, there were no significant difference in the number of stems and loops. The Reference genes indicated a random behavior for MFE, number of stems and loops. Most preferred codons were calculated for stress resistant and Reference genes via gene infinity software. SCUB and RSS results will be supportive to encounter numerous stress resistant crops across codon optimization.

Key words: Stresses; Synonymous Codon Usage Bias; Reference genes; RNA secondary structures.

Introduction

In the presence of variable codons, CUB (codons usage bias) corresponds to the possibility of a coding to code for an amino acid. Certain identical codons are employed as optimal codons whereas the non-optimal codons are characterised being less frequently used. Non-optimal codons frequently correspond to less abundant tRNA compared to optimal codons (Akashi, 2001). In *Arabidopsis thaliana* and *Oryza sativa*, Sadia *et al.,* 2018 also analysed salt stress resistant and housekeeping genes. Code immorality consents to the implication of several amino acids by numerous codons with codon coining being correlated to mutation or natural selection (Novoa & Pouplana, 2012). Preferences for codon are retained by the stability of natural selection and mutation-based biases for optimizing translation, whereas either natural selection acts at the speed or accuracy level of mRNA translation remains uncertain (Ermolaeva, 2001). Synonymous based mutation in plants is believed to exist as a consequence of co-adaptation between usage of codon and transfer large quantity of RNA that forefront to optimize the yield of protein production (Marais & Duret, 2001). CUB differs from one organism to other as well as gene to gene in a genome, while it also varies from place to place inside a genetic material. Codons responsible for the effective translation of transgenes could alter an endogenous gene from the active codons (Plotkin & Kudla, 2011). In multicellular organism's codon are used to increase the stability of protein production (Marais & Duret, 2001). Biotic and abiotic stress (environmental aspect) influences the use of codon in species (i.e., drought, cold, freezing, water, heat, metal, osmotic, oxidative and salt stress change the coding region's appearance level). Several plants via stress resistance genes manifest resistance to different stresses and also genes, that are positively classified as stress-resistant genes are referred to as stress-resistant genes that may code a transcript aspect which regulates the presence of stress-acceptant genes responding directly to stress, additionally due to hormones (ethylene jasmonic acid, salicylic acid and abscisic acid), plants may also react to environmental conditions (Vandenbroucke, 2008). Owing to simple make-up, *Arabidopsis thaliana* is considered as modal plant for molecular heredities hence a comprehensive analysis of stress tolerant genes is carried out (Salt, 2004). Nearly 44.5% of entire stress accepting (stress tolerant) genes are related to Arabidopsis genes, whereas around 50% of all stress tolerant genes have been distinguished by practising Arabidopsis as the transgenic types (Vandenbroucke, 2008).

Usage of codon affects the gene appearance route (expression), still the course of action for controlling the arrangement of codons is not yet clear. There are numerous particulars that effect the choice of codons, like coregulation of gene, arrangement of codon, codon autocorrelation and abundance of degeneracy of RNAs. Modification of tRNA monitors the rate of translation of codons and consequently expression level of specific gene subgroups. Current aspects suggest that alteration of transfer RNA has a key role in genes regulation by stimulating the genes expression ranks, elaborate in cellular responses. It shows the creation of mature cellular transfer RNA, which makes it possible to recognize a special regulatory (control) system (Novoa & Pouplana, 2012).

When a plant is exposed to the abiotic (environmental) factor, its genes allow improvements in the frequency of expression to persuade resistance against the stress's negative effects. Now it's obvious that increase in protection includes a multi-dimensional governing linkage that intermediates biochemical, morphological, molecular and physiological variations. It is significant to recognize these types of variations regarding existence to abiotic (environmental) stress in plant breeding (Vandenbroucke, 2008).

The current research is focused on Codon usage bias analysis in terms of the similar use of codon and its interaction with environmental factor and housekeeping genes in the important monocot and dicot plant species. The major focus of this study is to discover the most desired codons (amino acid coding), stress-resistant genes and housekeeping genes. The genes that show resemblance by more than 50% in the usage of codon will be focused for the codon optimization so that to find a sequence that react to multiple stresses.

Materials and Methods

Identification of the genes: The 11 abiotic stress (PHYA, PDR^{12}, ATM^{3}, SZF^{1}, SZF^{1}, NHX1, GI-^{3}, LTL^{1}, COR1^{5}a, ALDH^{3}I^{3}, ABO^{1}, VTC^{1},) and 3 reference genes (sand family, expressed protein, ubiquitin transferees) were showed through literature review in *A. thaliana* and their homologues were shown by consuming the BLASTn in *P. trichocarpa*, *O. sativa* and *T. aestivum*.

Sequence retrieval: The FASTA Sequence of the environmental factor and Reference genes in *A. thaliana* were received from nucleotide database of NCBI database and their homologous were found in *P. trichocarpa*, *O. sativa* and *T. aestivum*. The FASTA formats were saved for further search.

Analysis of synonymous codon usage bias: The FASTA formats of the genes were exposed to gene infinity software (Stothard, 2000). The candidate genes' Synonymous codon use Bias was explored through the codon use analysis method. This program predicted the usage value of synonymous codons for each amino acid, of which the greatest value is chosen and compared for both monocot and dicot species (Barozai & Din, 2014; Shah *et al.*, 2016).

The following formula was used for the calculation of comparative synonymous codon usage (RSCU).

$$\text{RSCU}=\frac{\text{Number. of used codon}}{\text{Total number of codon}}\text{Number of amino acids}$$

Analysis of RNA secondary structure: For the prediction of RNA's secondary structures of candidate genes in monocot and Dicot plants, the FASTA format of 11 abiotic stress resistant and 3 Reference genes was submitted to Mfold algorithm (Zuker, 2003). The Mfold's parameters were comparable with what Barozai, 2012 presumed. The abiotic and Reference gene secondary structures were counted and held in terms of minimum free energy (MFE). The structures of such RNA were manually examined for MFE, no of stems and loops.

Results

Abiotic stress resistant genes: 11 abiotic stress (GI-^{3}, PHYA, COR^{15}a, SZF^{1}, VTC^{1}, LTL^{1}, ABO^{1}, ALDH^{3}I^{3}, ATM^{3}, PDR^{12}, NHX^{1}) and 3 Reference genes (Sand family, expressed protein, ubiquitin transferase) were determined through literature survey in *A. thaliana* and their homologues from BLASTn in *P. trichocarpa*, *O. sativa* and *T. aestivum* (Tables 1 & 2).

Table 1. Classification of abiotic stress resistant genes in monocot and dicot plants in terms of their MF; molecular functions; accession number and ST; stress types.

S. No.	Gene name	Acc. No.	MF	ST
1.	NHX^{1}	AT5G^{27150}	Na^{+}/H^{+} antiporter	S*
2.	ALDH^{3I3}	AT4G^{34240}	Aldehyde dehydrogenase	D*, S*, Ox*, M*
3.	GI-^{3}	AT1G^{22770}	Gigantea	Ox*
4.	VTC^{1}	AT2G^{39770}	Vitamin C defective 1, encodes mannose-1- pyrophosphatase	H*
5.	LTL^{1}	AT3G^{04290}	GDSL-type lipase	S*
6.	COR^{15}a	AT2G^{42540}	LEA	C*/F*
7.	ABO^{1}/ELO^{2}	AT5G^{13680}	Transcription Elongator complex subunit	D*, Ox*
8.	SZF^{1}		Transcription factor CCCH-type zinc finger proteins	S*
9.	ATM^{3}	AT5G^{58270}	ABC transporter	M*
10.	PDR^{12}	AT1G^{15520}	ABC transporter	M*
11.	PHYA (ars4 ars5)	AT1G^{09570}	Cytoplasmic red/far-red light photoreceptor	M*

Abbreviations: c*: cold, f*: freezing, d*: drought, h*: heat stress, ox*: oxidative stress, s*: salt stress, c*/f*: cold / freezing stress, m*: metal stress

Table 2. Classification of reference genes in monocot and dicot plants in terms of their MF; molecular functions & ACC. no; accession number.

S. No.	Gene name	Accession No.	Molecular function
4.	Sand family	AT2G^{28390}	Expressed through 14 growth phases
5.	Expressed protein	AT2G^{32170}	Growth phases, plant structures,
7.	Ubiquitin transferase	AT3G^{53090}	Encodes a ubiquitin-protein ligase containing a HECT domain

Analysis of synonymous codon usage bias: The CUB examined 11 stress resistant genes and 3 reference genes in the significant species of monocot and Dicot plants showed notable results. The commonly used synonymous codons were calculated and summarized for 11 abiotic (environmental) stress resistant and three Reference genes in significant species of plants for each of the stop codons and twenty amino acids.

The CUB analyses were accepted for abiotic stress resistant genes in four significant species of monocot and dicot plants. The *Arabidopsis thaliana* displayed correspondence in the usage of codons with *Oryza sativa* by 82% i.e., nine abiotic stress resistant genes out of eleven showed likeness by more than 50% while two genes disclosed likeness by less than 50%. The *Arabidopsis thaliana* showed similarity with *populous trichocarpa* by 91% i.e., ten genes disclosed similarity by greater and one by less than 50% and that of *Triticum aestivum* showed similarity in the usage of codon with *Arabidopsis thaliana* by 55% i.e., five gene showed similarity by greater and six gene by less than 50%. While the *Oryza sativa* showed similarity with *populous trichocarpa* by 82 i.e., nine genes out of eleven disclosed likeness by more than 50% and two genes exposed resemblance by less than 50%. The *Oryza sativa* revealed likeness with *Triticum aestivum* by 73% i.e., eight genes exposed likeness by more than 50% and three genes disclosed likeness by less than 50%. The *Populous trichocarpa* indicated likeness in the usage of codons by 27% with *Triticum aestivum* i.e., three genes out of eleven presented likeness by greater than 50% and eight genes exhibited match by less than 50%, So in the usage of codons less than 50% similarity is only observed between *populous trichocarpa* and *Triticum aestivum*. On the other hand, the CUB analysis of Reference genes revealed an interested result i.e., all species disclosed likeness in the usage of codons by more than 50%. The *Arabidopsis thaliana* presented similarity in the usage of codon by 100% with *Oryza sativa* and *populous trichocarpa*. The *Oryza sativa* and *populous trichocarpa* also presented 100% similarity in the usage of codons for Reference genes, while that of *Triticum aestivum* presented 67% similarity with *Arabidopsis thaliana*, *Oryza sativa* and *populous trichocarpa*, i.e., only one Reference genes out of three resulted in equivalence in the usage of codons by less than 50% respectively.

The preferred codons were found for 11 abiotic (environmental) stresses and 3 reference genes (Figs. 1 and 2). This indicates the relationship of different codons related to abiotic stress and the reference genes displayed in Table 3. The constant utilization of a single codon was observed for Methionine, Tryptophan and Asparagine amino acids i.e., ATG, TGG and GAT respectively. The same result is reported by Mukhopadhyay *et al*., 2008, for reference and tissue specific genes in *O. sativa* and *A. thaliana*. The abiotic stress related and reference genes association displayed an amazing result for Monocot and Dicot plants. The use of identical codons in CUB relationships is 71 percent whereas that of the dissimilar codons is 29 percent for Comparison and stress genes in Dicot plants, but in Monocot plants the ratio of identical and dissimilar codons is 38 percent and 62 percent respectively in relation to codon use biased analysis of household and stress-resistant genes. Dicot and Monocot's reference genes showed similarity and variability in codon use by 67 percent and 33 percent respectively. The general assessment of stress and reference genes disclosed likeness and variance in the usage of codons by 43% and 57% respectively, which indorse the non-uniform application of codon for abiotic (environmental) stress tolerant genes between four types of plants.

Analysis of RNA secondary structure: The secondary structure of all abiotic stress-resistant and Monocot and Dicot plant reference genes was examined in MFE relationships, number of stems, and loops (Tables 4 & 5). The Monocot and Dicot plants showed significant variation in terms of MFE for abiotic stress resistant genes i.e., the average value of MFE were -275.01 and -194.92 respectively while there was no more significant variation for reference genes i.e., the average value of MFE for Monocot and Dicot were -167.93 and -199.20 respectively, so the average difference in MFE of Monocot and dicot plants for abiotic stress resistant and reference genes is -80.09 and -31.27 respectively.

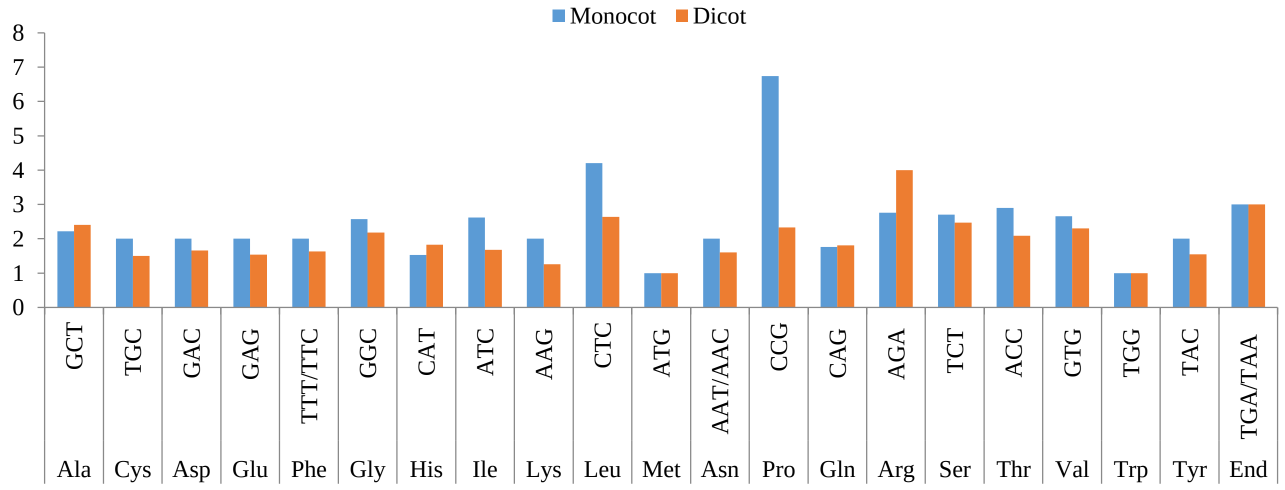

Fig. 1. Most favoured codon for each amino acid of abiotic stress resistant genes in monocot & dicot species of plants.

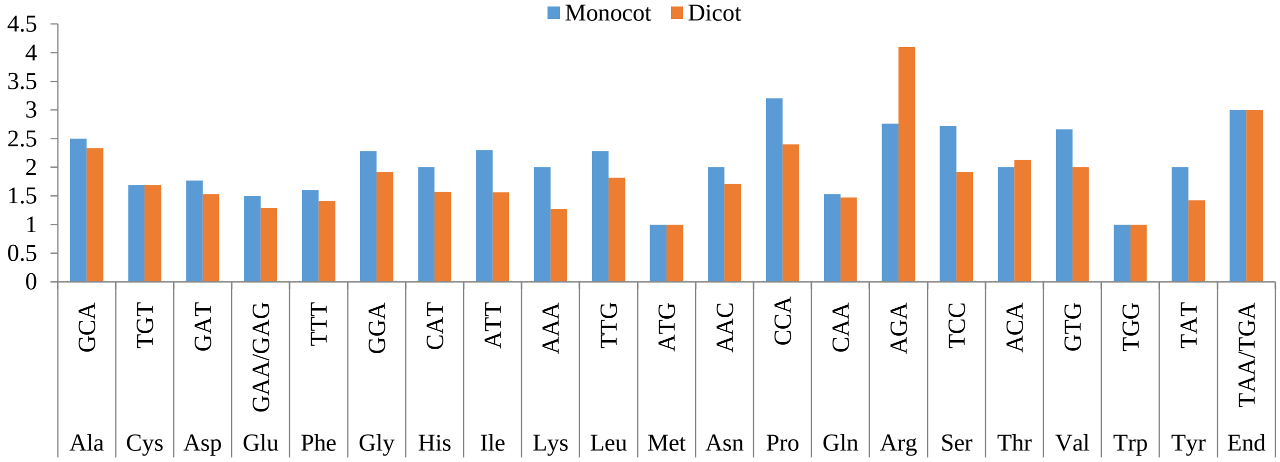

Fig. 2. Most favoured codon for each amino acid of reference genes in monocot & dicot species of plants.

Table 3. The comparison of most ideal codons calculated in term of RSCU for 11 abiotic stress resistant & 3 reference genes of monocot and dicot plants.

Amino acids	Monocot abiotic stress genes	Dicot abiotic stress genes	Monocot reference genes	Dicot reference genes
Ala	GCC 2.22	GCT 2.40	GCA 2.50	GCT 2.33
Cys	TGC 2.00	TGT 1.50	TGT 1.69	TGT 1.69
Asp	GAC 2.00	GAT 1.66	GAT1.77	GAT 1.53
Glu	GAG 2.00	GAA 1.54	GAA/GAG 1.50	GAA 1.29
Phe	TTT/TTC 2.00	TTT 1.63	TTT 1.60	TTT 1.41
Gly	GGC 2.57	GGA 2.18	GGA 2.28	GGA 1.92
His	CAC 1.53	CAT 1.83	CAT 2.00	CAT 1.57
Ile	ATC 2.62	ATT 1.68	ATT 2.30	ATC 1.56
Lys	AAG 2.00	AAA 1.26	AAA 2.00	AAG 1.27
Leu	CTC 4.20	TTG 2.64	TTG 2.28	TTG 1.82
Met	ATG 1.00	ATG 1.00	ATG 1.00	ATG 1.00
Asn	AAT/AAC 2.00	AAT 1.60	AAC 2.00	AAT 1.71
Pro	CCG 6.74	CCA 2.33	CCA 3.20	CCT 2.40
Gln	CAG 1.76	CAA 1.81	CAA 1.53	CAA 1.47
Arg	AGA/CGC 2.76	AGA 4.00	AGA 2.76	AGA 4.10
Ser	TCG 2.70	TCT 2.47	TCC 2.72	TCA 1.92
Thr	ACC 2.90	ACT 2.09	ACA/ACC 2.00	ACA 2.13
Val	GCT/GTC 2.66	GTT 2.30	GTG 2.66	GTG 2.00
Trp	TGG 1.00	TGG 1.00	TGG 1.00	TGG 1.00
Tyr	TAC 2.00	TAT 1.55	TAT 2.00	TAT 1.42
End	TGA/TAG 3.00	TGA/TAA 3.00	TGA 3.00	TAA 3.00

Table 4. RSS analysis of abiotic stress resistant genes in Dicot & Monocot species of plants.

S.No.	Genes	Dicot						Monocot						Difference		
		A. thaliana			*P. trichocarpa*			*O. sativa*			*T. aestivum*					
		MFE	NS	NL	MFE	NS	NL	MFE	NS	NL	MFE	NS	NL	MFE	Stem	Loop
1.	NHX^{1}	-167.21	26	16	-186.18	29	29	-261.21	30	23	-254.72	25	26	-81.27	00	2.00
2.	ALDH^{3I3}	-187.09	35	29	-199.66	34	35	-340.07	32	24	-302.00	27	34	-127.66	5.00	3.00
3.	GI-^{3}	-189.84	31	25	-209.68	28	29	-259.00	30	22	-206.05	29	31	-32.66	00	0.5
4.	VTC^{1}	-223.29	29	26	-211.69	36	37	-283.15	31	24	-292.41	35	34	-70.29	0.5	2.5
5.	LTL^{1}	-187.52	26	27	-176.11	28	30	-374.90	35	29	-249.90	29	32	-130.59	5.00	2.00
6.	COR^{15}a	-169.23	27	26	-189.89	33	32	-313.47	33	25	-221.98	34	33	-88.16	3.5	00
7.	SZF^{1}	-210.99	35	36	-162.72	34	37	-218.46	36	25	-327.57	27	29	-86.16	2.00	9.5
8.	ABO^{1}/ELO^{2}	-213.00	29	30	-169.92	26	28	-288.35	35	28	-212.24	28	32	-58.83	5.5	1.00
9.	ATM^{3}	-170.00	30	31	-206.76	32	32	-399.62	31	21	-187.85	13	19	-105.35	9.00	11.5
10.	PDR^{12}	-206.10	36	37	-193.82	30	30	-270.57	30	20	-309.51	36	37	-90.08	00	5.00
11.	PHYA (ars4 ars5)	-231.55	28	29	-225.78	30	29	-200.77	35	28	-276.40	32	32	-9.92	4.5	1.5

Table 5. RSS analysis of reference genes in dicot & monocot species of plants.

S.No.	Genes	*Dicot*						*Monocot*						Difference		
		A. thaliana			*P. trichocarpa*			*O. sativa*			*T. aestivum*					
		MFE	NS	NL	MFE	NS	NL	MFE	NS	NL	MFE	NS	NL	MFE	Stem	Loop
1.	Sand family	-233.90	36	36	-182.69	32	33	-186.68	29	30	-233.65	32	34	-1.87	4.5	2.5
2.	Expressed protein	-192.01	34	32	-183.99	33	33	-108.68	21	23	-242.92	30	30	-12.2	8.0	7.0
3.	Ubiquitin transferase	-203.77	34	33	-198.87	27	27	-94.79	19	19	-140.89	21	19	-83.48	10.5	11.0

Discussion

In this study 11 abiotic stress genes and 3 housekeeping genes (HKG) have been identified. Similar research was performed by Barozai *et al.,* 2012 for five salt-resistant genes and three housekeeping genes in *Arabidopsis thaliana (ATH)* and their homologues in *Oryza sativa (OSA)*. Likewise, Barozai and Din, 2014 also indicated six cold resistant and three HKG in *ATH* and *OSA* along with their homologues. The Biotic stress resistant genes and housekeeping genes were studied for the same criteria by Barozai *et al.,* 2014.

The Codon use bias (CUB) tested for 11 stress resistant genes and 3 reference genes showed notable results in the significant species of monocot and Dicot plants. The similarity in CUB is estimated to be more than 50 per cent significant. The CUB similarity of ATH (*Arabidopsis thaliana)* and OSA (*Oryza sativa)* is 82%, ATH and PTC (*populous trichocarpa)* is 91% and that of TAE (*Triticum aestivum*) with ATH is 55% while the OSA showed resemblance with PTC by 82% and OSA revealed likeness with TAE by 73%. Only PTC and TAE showed 27% similarity. The CUB analysis of Reference genes revealed an interested result i.e., all species disclosed likeness in the usage of codons by more than 50%. Barozai *et al.,* 2012 applied same idea on 5 salt resistant and 3 HKG and obtained uniform result i.e., 4 genes disclosed more than 50% (71%, 57%, 67% and 57% respectively) CUB similarity while 5th gene displayed 29% similarity for ATH and OSA. The three RG showed more than 50% comparable codon usage bias for ATH and OSA.

Barozai and Din., 2014 studied CUB in 6 cold resistant genes, 3 of which (MYB15, DREB1B, and RCI) showed similarity by more than 50% (66%, 61% and 52% respectively) while other 3 genes (ZAT12, ICE1 and MPK3) showed less than 50% (47%, 38% and 38% respectively) similarity for ATH and OSA. Their 3 RG (Ubiquitin, Actin and Tubulin) showed 76% CUB comparable for ATH and OSA. Mukhopadhyay *et al.,* 2008 also described comparable CUB for tissue precise genes and HKG in OSA and ATH and recommend that there is a clear unimodal spreading of codon usage patterns for the salt resistant genes in plants (Whittle *et al*., 2007; Wang & Hickey, 2007; Mukhopadhyay *et al*., 2008).

The ideal codons were found for 11 abiotic (environmental) Stress and 3 reference genes. Three amino acids (Methionine, Tryptophan and Asparagine) used single codons i.e., ATG, TGG and GAT respectively. The usage of homogeneous codons in relations of CUB is 71% while that of the dissimilar codons is 29% for Reference and stress genes in Dicot plants while in case of Monocot plants the ratio of alike and unalike codons is 38% and 62% individually in relation of codon usage biased study of RG and stress resistant genes. The reference genes of Dicot and Monocot displayed likeness and variation in the usage of codons by 67% and 33% respectively. The general assessment of stress and reference genes disclosed likeness and variance in the usage of codons by 43% and 57% respectively, which indorse the non-uniform application of codon for abiotic (environmental) stress tolerant genes between four types of plants. The abiotic stress and HKG that displayed likeness by more than 50% in the exploitation of codon proposes the straight association between the SCUB of abiotic stress and SCUB of Reference genes, regardless of the plant species. These outcomes signify that plant might be strategic for stress resistance by the procedure of optimizing of codons, although the genes resemblance less than 50%, suggests the independency of the stress genes and species of plants, similarly inferences were also detailed in several other species of plants (Whittle *et al.*, 2007; Wang & Hickey, 2007). The likeness of codon usage bias by less than 50% recommends identical configurations of codon usage in ordinary and some kind of stress circumstances. These outcomes basically indicate the reliance of codon usage in plant types in usual and abiotic stress conditions and also these outcomes imitate the earlier stated investigation (Whittle *et al.*, 2007; Wang & Hickey, 2007; Mukhopadhyay *et al*., 2008). The outcomes of this study are the platform for the new findings by using bioinformatics (Barozai *et al*., 2012a; 2012b; Barozai & Wahid, 2012; Barozai, 2012c; Shah *et al.*, 2016).

The Monocot and Dicot plants showed significant variation in terms of MFE for stress resistant and RG. The average value of MFE for Stress genes are -275.01 and -194.92 while -167.93 and -199.20 respectively for RG. Sadia *et al*., 2018 also analysed salt stress genes in ATH and OSA with average MFE of 434.06 and 395.39 respectively. Barozai *et al*, 2014 also studied the secondary structures of virus resistant and RG in ATH and OSA and found mixed behaviour in terms of MFE, no. of stems and loops.

Conclusions

This study shows the multi-dimensional associations between the abiotic stress resistant and codon usage bias and reference genes for the important species of plants. A uniform interrelation with Codon Usage Bias is observed for dicot and monocot plants in term of housekeeping genes. Plants can improve stress tolerance ability by marvels of codon optimization.

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