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Meshal M. Almutairi* * and Saad M. Alzahrani

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The Molecular Mass and Isoelectric Point of Cytokinin Riboside 5'-Monophosphate Phosphoribohydrolase Sequences for Wheat Genome

Meshal M. Almutairi * and Saad M. Alzahrani

Advanced Agricultural & Food technology Institute, King Abdulaziz City for Science and Technology (KACST), P.O. Box 6086, Riyadh, 11442, Saudi Arabia

* Correspondence: malmetairy@kacst.gov.sa. Meshal M. Almutairi. ORCID: https://orcid.org/0000-0002-2255-3226.

Abstract: Cytokinin is play strongly role and implicated in wheat breeding in terms of flowering and yield. The aims here was to explain the wheat cytokinin riboside 5'-monophosphate phosphoribohydrolase sequences from two different databases through the using relative synonymous codon usage (RSCU), molecular weight (g/mol), theoretical isoelectric point, instability index, aliphatic index, and hydrophobicity. The RSCU divided values into two crops. First crop, significant overrepresentation were values above 1.6 that as in phenylalanine (TTC) located in 5B chromosomes, Leucine (TTA) located in 5A, and 1D chromosomes, leucine (TTG) located in 5B chromosome, leucine (CTC) located in 5D, 7D, and 7B. Leucine (CTA) located in 5D and second crop, underrepresentation were values below 0.6 as in leucine (CTA) located in 3A, 5D, 1B, 5B, and 7B. Valine (GTT) located in 4D, 5B, 4A, and 5B. In addition, theoretical isoelectric point (PI) ranged from 4.81 to 6.6 in chromosomes 3A and 4D respectively that were instability index was 34.3 and 38.16 respectively. The high instability was found at 1D and 5D with 54.16 and 50.36 respectively with decreasing in their stability as shown in aliphatic index at 92.98 and 88.91. Principal component analysis (PCA) of RSCU were explained main of variation is assigned to PCA1 with total variation about 72.11% and these amino acids are Isoleucine, Leucine, Lysine, Aspartic Acid, and Serine while the PCA2 and PCA3 had total variation, 17.04% and 10.85. While PCA of the theoretical isoelectric point results explained main of variation was assigned to PCA1 with total variation about 58.88 % and these chromosomes are 5D, 4D, 1A, 4B, 3D. While the PCA2 and PCA3 had total variation, 27.52% and 13.6% respectively assigned with 1D, 3B, 3A, 2D, 2A and 2B. The future objective from these results would be benefit for in nutrition and industrial and aid in breeding programs.

Keywords: wheat; chromosome sequences; amino acids; relative codon bias strength (RCBS); and molecular weight (g/mol); theoretical isoelectric point.

1. Introduction

As one of most important plant hormone group, cytokinin is play strongly role and implicated in plant breeding of wheat in terms of flowering and yield [1,2] and including other role such as environmental stress (biotic and abiotic stresses) [3]. The metabolic cycling of cytokinin involves initial by isopentenyl transferase enzymes in order to formation of cytokinin nucleotides and thus activation into active free-base forms by lonely guy enzymes (LOG) [4]. The pathway of signaling cytokinins have multi-step systems of phosphorylation by involving histidine kinases, histidine phosphotransfer proteins, and regulators response [5]. In wheat, cytokinins occur during grain development especially in rapid endosperm nuclear divisions and can help to delaying senescence by stimulating sucrose by stay-green phonotypes in late stage of development. In addition, cytokinin can manipulate amino acid metabolism by distribution of nutrient inside plan and enhance the expression of transported and mobilization [6].

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A common wheat (*Triticum aestivum*) is allohexaploid that has three genomes, which has been driven from ancestral species (AABBDD, A genome from Triticum Urartu, B genome from Aegilops speltoides, and D genome: from Aegilops tauschii). This genome reflects complex instruction of genetic wheat, which has six sets of chromosomes as hexa-ploid [7]. The benefit of this complexity of wheat genome leads to genetic diversity and adaptability to grown and survive in a variety of environmental conditions. However, wheat under drought stress has been classified based on its ability by different mechanisms such as mange water or reduced transpiration, or improved root depth [6]. Cytokine influence stomatal closure to reduce water loss and can develop and elongation of root growth to access water deeper from soil. Under water limitation, wheat species were significantly noted by reduction in traits such as photosynthesis rate (14% and 10% compared to 24% and 12%), stomatal conduction, total sugar, and total starch content [1–8]. The metabolism of cytokinin in wheat regulates by active cytokinin level by dephoshorylation and deribosylation during development processes. This processes included shoot apical meristem, flower, and vascular developments, and retaining sink activity to inhibit nitrogen remobilization, and maintaining proteins synthesis [9]. The objective of study wheat's cytokinin expression through amine acid is crucial for development wheat stage. The aims here was to explain the wheat genomes (mRNA and proteins) for its properties and function from two different databases through the cytokinin riboside 5'-monophosphate phosphoribohydrolase sequences using relative synonymous codon usage (RSCU), molecular weight (g/mol), theoretical isoelectric point, instability index, aliphatic index, and hydrophobicity. The future objective from these results would be benefit for in nutrition and industrial applications such as digestibility and nutritional supplements that can aid in breeding programs [10].

2. Results

2.2. The Number of Chromosomes for Cytokinin Riboside 5'-Monophosphate Phosphoribohydrolase Genes Through NCBI and Ensemble Databases

NCBI had 35 genes in different chromosomes while ensemble had 38 genes and both databases had able to show these genes in different chromosomes. Figure 1 shows that A, B, and D genomes with different numbers of chromosomes in each one. 2A, 2B, and 2D was zero number from NCBI databases comparing with ensemble databases that shows 1 genes in both genomes 2A and 2D and 2 genes in 2B genome. Some genomes had more genes than other genes. For example, genome 4A and 5B had five genes from NCBI databases while 4 genes shown in genomes 1B, 1D, 4D, and 5D from ensemble databases. This gene differences came from key factors such as accumulation over times of genetic differences, repeated sequences due to transposable elements (TEs) that could insert into different locations, gene loss as shown in several reports by 10 to 16 thousand genes losses, and hybridization of wheat from breeding programs. In addition, the Cytokinin riboside 5'-monophosphate phosphoribohydrolase has multiple members from 1 to 11 reflect the functional redundancy for cytokinin metabolism roles across developmental stages. Gene expression of cytokinin riboside 5'-monophosphate phosphoribohydrolase has different elements of cis-regulatory.

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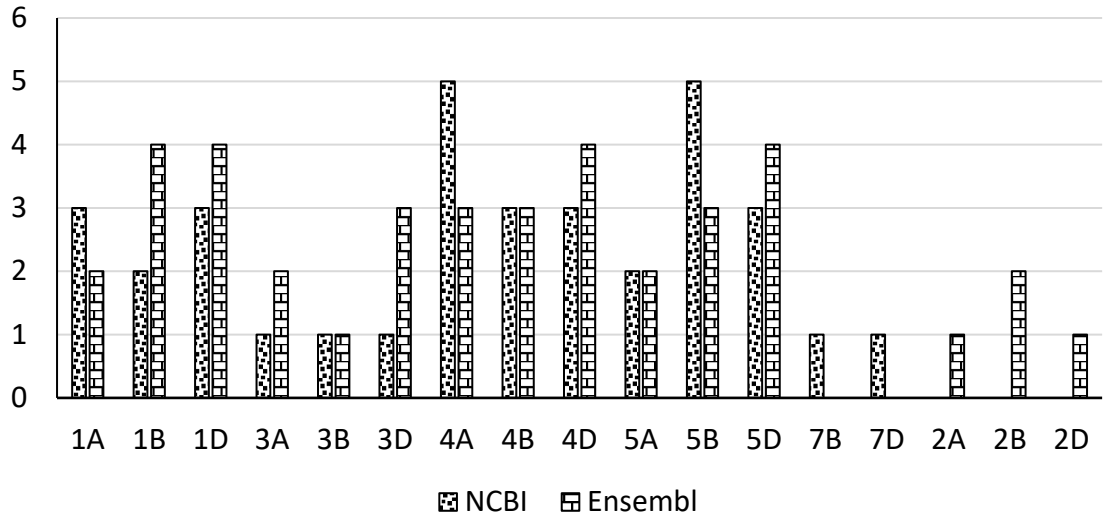

Figure 1. The number of study sequences in each chromosomes between NCBI and ensemble plants databases.

2.3. The Relative Synonymous Codon Usage (RSCU)

The results of RSCU values (data not shown due to large table) above 1.6 reflect significant overrepresentation such as phenylalanine (TTC) located in 5B chromosomes, Leucine (TTA) located in 5A, and 1D chromosomes, leucine (TTG) located in 5B chromosome, leucine (CTC) located in 5D, 7D, and 7B. Leucine (CTA) located in 5D. Isoleucine (ATT) located in 5A, and 1D. Valine (GTC) located in 5D, and 4A. Serine (TCT) located in 5D, 1B, and 4A. Proline (CCT) located in 4B. Threonine (ACG) located in 4D, 5B, 5A, and 4B. Arginine (CGC) located in 5B, 5D, and 1B. Glycine (GGC) 5D, 4A, 5B, 3D, 7B, and 5D. However, RSCU values below 0.6 suggest underrepresentation such as leucine (CTA) located in 3A, 5D, 1B, 5B, and 7B. Valine (GTT) located in 4D, 5B, 4A, and 5B. Proline (CCC) located in 5B, 5D, 5A, 4B, 1B, and 4A. Threonine (ACC) located in 3A, 4D, 5B, 5A, and 4B. Glutamic Acid (GAA) located in 5B, 5D, 5A, 4A, 1D, 5B, and 7B. Arginine (CGT) located in 3A, 5B, 1B, 5B, and 7D. Serine (AGT) located in 4D, 5B, 5A, 4D, 4B, and 7B. Glycine (GGT) located in 3A, 5B, 5D, 7D, and 7B. Figure 2 is data of principal component analysis that shown amino acid patterns. From this Figure 2, amino acids that explained main of variation is assigned to PCA1 with total variation about 72.11% and these amino acids are Isoleucine, Leucine, Lysine, Aspartic Acid, and Serine and more shown in Figure 2. While the PCA2 and PCA3 had total variation, 17.04% and 10.85 respectively assigned with arginine, glutamic acid and threonine for PCA2 and assigned with aspartic Acid.

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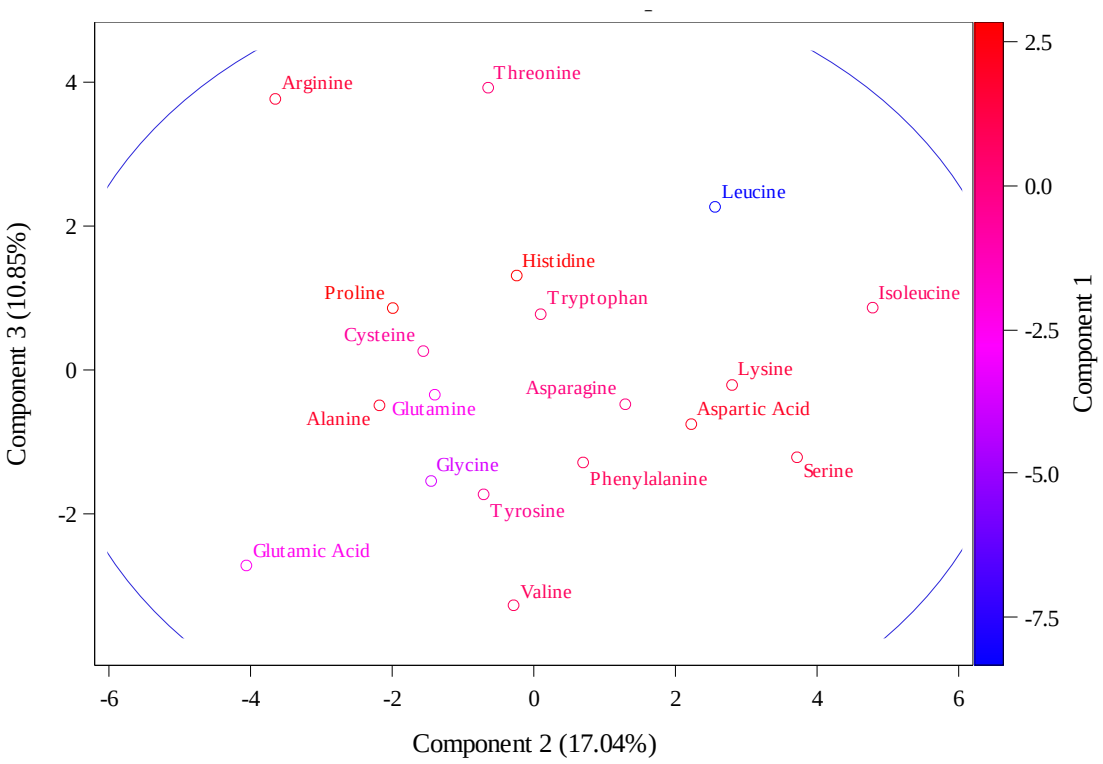

Figure 2. The principal component analysis (PCA) for the relative synonymous codon usage (RSCU) for 35 gene sequences retrieved from NCBI.

The physiochemical characterization:

Tables 1 shows the results values of molecular weight (g/mol), theoretical isoelectric point, instability index, aliphatic index, and hydrophobicity. Theoretical isoelectric point (PI) is the pH that ranged from 4.81 to 6.6 in chromosomes 3A and 4D respectively (average 5.37 ± 0.297 Figure 3). However, the instability index showed that these two chromosomes (3A and 4D) were at 34.3 and 38.16 respectively. In addition, these two chromosomes (3A, 4D) had 88.69 and 89.59 of aliphatic index indicate increasing of their stability and their hydrophobicity were lower 0.04 and -0.04 respectively. The high instability was found at 1D and 5D with 54.16 and 50.36 respectively with decreasing in their stability as shown in aliphatic index at 92.98 and 88.91. Figure 4 is data of principal component analysis that shown amino acid patterns. From this Figure 4, amino acids that explained main of variation is assigned to PCA1 with total variation about 58.88 % and these chromosomes are 5D, 4D, 1A, 4B, 3D and more shown in Figure 4. While the PCA2 and PCA3 had total variation, 27.52% and 13.6% respectively assigned with 1D, 3B, 3A, 2D, 2A and 2B.

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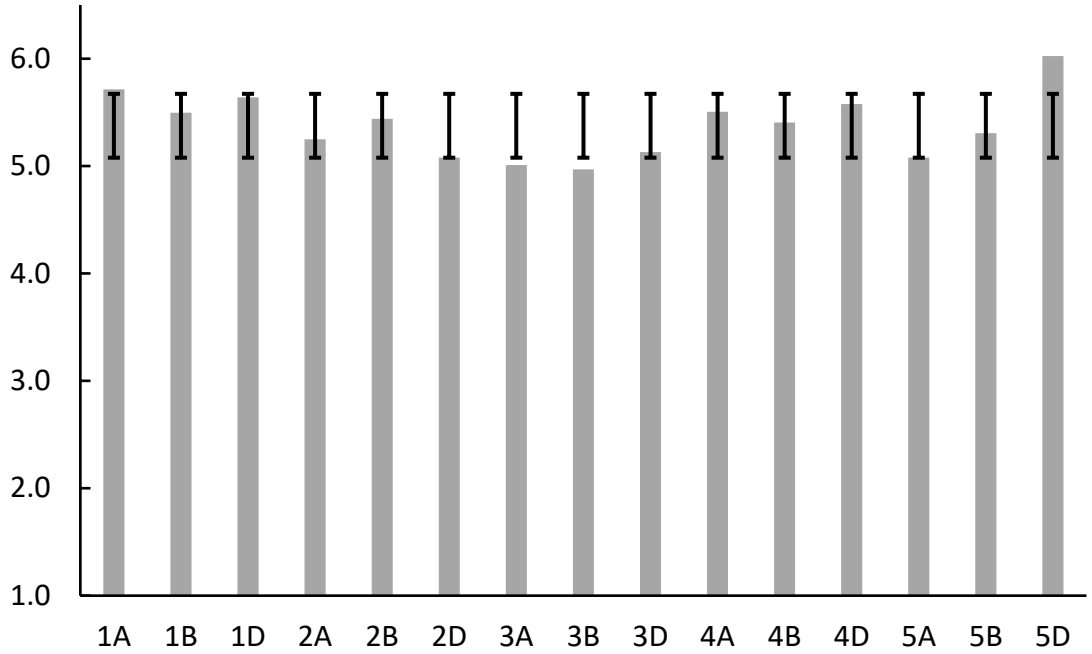

Figure 3. The average theoretical isoelectric point (pI) of each wheat chromosomes with standard deviation.

Table 1. shows the results values of molecular weight (g/mol), theoretical isoelectric point, instability index, aliphatic index, and hydrophobicity. .

Location	bp	molecular weight (g/mol)	theoretical isoelectric point (pI)	instability index (II),	aliphatic index (AI)	hydrophobicity (HY)
4A	218	22991.1	5.38	38.33	93.53	-0.105
2A	243	26618.74	5.25	40.31	91.44	-0.096
4B	205	22513.91	5.78	41.69	94.63	0.008
3A	234	25265.85	5.21	34.3	95	-0.017
1D	215	23152.61	5.77	54.16	92.98	0.035
3D	241	25737.38	5.21	33.91	93.49	0.01
1B	268	28401.58	5.06	38.27	96.12	0.071
1D	239	26037.71	5.66	43.3	84.1	-0.38
1A	208	22818.19	6.13	35.3	97.5	-0.045
2B	280	30712.62	5.71	39.74	95.71	-0.03
2B	246	26897.94	5.17	38.94	93.09	-0.118
5D	235	24482.96	6.22	42.68	88.04	0.008
3A	229	24220.69	4.81	29.84	88.69	0.04
4D	245	25850.59	6.6	38.16	89.59	-0.049
5D	248	26288.03	6.23	50.36	88.91	-0.108
5D	247	26070.71	6.17	41.8	86.52	-0.082
5A	246	26030.62	5.23	38.94	88.82	-0.063
1B	208	22819.17	5.98	36.23	97.5	-0.045
5B	241	25631.11	5.15	35.92	92.24	-0.012
1D	273	28817.09	5.15	36.73	39.99	0.067
2D	246	26898.99	5.08	43.64	94.31	-0.085
1A	215	23217.63	5.3	50	92.05	0.02
5B	241	25631.11	5.15	35.92	92.24	-0.012
4A	206	22487.78	5.15	37.95	95.58	0.052
4D	225	23599.69	5.26	36.46	93.24	-0.106
1B	215	23221.66	5.45	48	91.12	-0.003

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5B	246	26101.79	5.62	41.79	91.99	-0.002
4D	250	26176.7	5.28	36.82	87.8	-0.004
3D	226	24032.55	4.97	28.66	88.98	0.023
5A	245	26310.95	4.93	39.99	95.51	-0.001
1D	208	22819.17	5.98	36.23	97.5	-0.045
3B	226	24032.55	4.97	27.99	88.98	0.023
4A	248	26111.76	5.99	48.45	88.06	-0.105
5D	249	26428.14	5.48	43.07	90.48	-0.032
3D	239	25637.3	5.21	34.82	94.64	0.014
4B	205	22282.61	5.29	34.03	97.51	0.107
4D	205	22315.99	5.17	36.73	96.05	0.08
4B	226	23572.58	5.15	37.49	91.15	-0.125
Mean	233.947	25058.883	5.455	39.130	90.923	-0.029
StD	19.385	1982.593	0.439	5.748	9.140	0.084

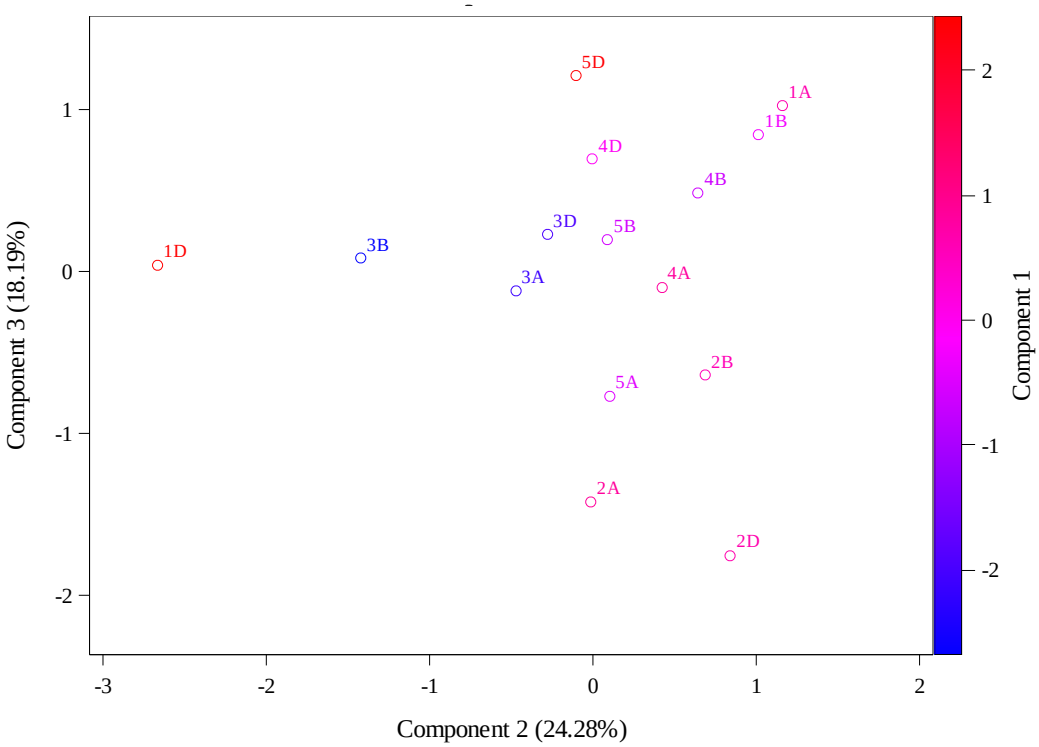

Figure 4. The principal component analysis (PCA) of Molecular weight (g/mol), theoretical isoelectric point, instability index, aliphatic index, and hydrophobicity values using ensemble plants databases.

3. Discussion

These cytokinins are important phytohormones encoded by LOG family of genes in wheat and other crops. It is play roles in several stage of plant development such as regulating growth and affecting of yield component of wheat [11]. Its biosynthesis occurs in the plant cell and. It also showed about 11 to 14 genes family allocated in three chromosomes of wheat. In barely, report showed that the pattern of expression of these genes reflects role in growth and reproductive development. In addition, the results showed the possibility of selection natural variation in gene expression for incorporation into wheat genotypes breeding material. In Arabidopsis, this cytokinin acted at specific level with redundantly at vascular tissues of developing flowers and leaves [12,13].

The important of the relative synonymous codon usage (RSCU) in plant breeding is to help breeders for understanding the mechanisms and functional wheat genomes and thus wheat genomes can be build for environmental adaptations. Selection optimal codon helps for transcription termination of mRNA expression especially in binding sites by either generating or depleting this site

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of gene expression. RSCU values above 1.6 is strong positive codon in several codons such as TTC, TTA, TTG, CTC, CTA, ATT, and GTC these codons are hold T codon. In addition, codons TCT, CCT, ACG have C codon while codons CGC and GGC have G codon. Thus, these codons can be used for optimizing gene expressions in order to modifying endogenous of some genes to increase protein productions [14]. The important of principal component analysis here is to identify important amino acid for the Cytokinin riboside 5'-monophosphate phosphoribohydrolase to make best decision about selection in plant breeding programs. From PCA1 that explain total variation as largest loading has highlights most important amino acids that are associated to variability of this enzyme and thus more attention in breeding program to these amino acids [15,16]. Thus, the beneficial of these results would be developed wheat in nutrition and industrial applications such as increasing essential amino acids that related to improve proteins quality for valuable nutrition and to address starch for better wheat digestible and thus enhance human consumption [17].

4. Materials and Methods

The DNA and proteins sequences of cytokinin riboside 5'-monophosphate phosphori-bohydrolase have been retrieved from two different databases names the National Center for Biotechnology Information (NCBI) and ensemble plants websites as shown in Table 2. All of the studied sequences were annotated such as genes, regulatory elements and location of genes. From NCBI, the relative synonymous codon usage (RSCU) were calculated individually as the ration between the observed frequency and expected frequency for the particular codon by using website such as https://jamiemcgowan.ie/bioinf/index.html#. Some of codons were excluded from the calculation such as methionine (ATG), and tryptophas (TGG), and (TAA, TAG, TGA). The formula of calculation RSCU for codon j of amino acid I is by using formula as below: RSCU= (n_(i x_(i,j)))/(∑_(j=1)^(n_i)▒〖xi,j〗) where n_i is the number of codon that code amino acid i, and the x_(i,j)is the number of occurrences of codon j [18]. The interpretation for RSCU values equals to 1 indicated no codon preferences (no bias) while values less than 1 indicated notable negative bias in codon usage. Values above 1.6 reflects significant overrepresentation while values below 0.6 suggest underrepresentation. Once, the RSCU values were determined, all values were analysis through SAS for more analysis by using principal component.

Table 2. The DNA and proteins sequences of cytokinin riboside 5'-monophosphate phosphori-bohydrolase from the National Center for Biotechnology Information (NCBI) and ensemble plants databases.

	NCBI		Ensembl	
	Gene symbol ID	Chro.	Gene symbol ID	Chro.
1	LOC123045576	1A	TraesCS4A02G277200	4A
2	LOC123045860	1A	TraesCS2A02G380600	2A
3	LOC123061754	3A	TraesCS4B02G250400	4B
4	LOC123065219	1A	TraesCS3A02G211100	3A
5	LOC123070388	3B	TraesCS1D02G367400	1D
6	LOC123078846	3D	TraesCS3D02G213900	3D
7	LOC123080662	1B	TraesCS1B02G471300	1B
8	LOC123082392	4A	TraesCS1D02G003500	1D
9	LOC123083722	4A	TraesCS1A02G156100	1A
10	LOC123087480	4A	TraesCS2B02G397600	2B
11	LOC123087481	4A	TraesCS5D02G568400	5D
12	LOC123088143	4A	TraesCS3A02G251500	3A
13	LOC123093512	4B	TraesCS4A02G317900	4A
14	LOC123094293	4B	TraesCS5D02G568500	5D
15	LOC123095076	4B	TraesCS5B02G561400	5B
16	LOC123097019	4D	TraesCS5A02G347400	5A
17	LOC123098787	4D	TraesCS1B02G173200	1B

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18	LOC123099424	4D	TraesCS5B02G348600	5B
19	LOC123107636	5A	TraesCS1D02G444500	1D
21	LOC123107637	5A	TraesCS2D02G376900	2D
22	LOC123112988	5B	TraesCS1A02G362500	1A
23	LOC123112989	5B	TraesCS5B02G561300	5B
24	LOC123114455	5B	TraesCS4A02G413200	4A
25	LOC123116784	5B	TraesCS4D02G033800	4D
26	LOC123117873	5B	TraesCS1B02G379700	1B
27	LOC123121452	1B	TraesCS5B02G348400	5B
28	LOC123123922	5D	TraesCS5B02G348300	5B
29	LOC123125354	5D	TraesCS3D02G251900	3D
30	LOC123126254	5D	TraesCS5A02G347500	5A
31	LOC123157422	7B	TraesCS1D02G154700	1D
32	LOC123160435	1D	TraesCS3B02G281000	3B
33	LOC123161849	1D	TraesCS4A02G318100	4A
34	LOC123165646	7D	TraesCS5D02G353600	5D
35	LOC123181086	1D	TraesCS3B02G241600	3B
36			TraesCS4B02G313800	4B
37			TraesCS4D02G310800	4D
38			TraesCS4B02G035700	4B

The physiochemical characterization

From ensemble plants were used to analysis some of chromosomes characterization such as molecular weight (g/mol), theoretical isoelectric point, instability index, aliphatic index, and hydrophobicity. These characterizations were contacted through web.expasy.org. The theoretical isoelectric point (PI) was the pH value that can be calculated through the formula pI = (pKa1 + pKa2) / 2 where pKa1 refers to dissociation constant for group of carboxylic acid and pKa2 refers to amino group. Both molecular weight and isoelectric point help to understand of biochemical and functional chromosomes of wheat breeding programs. The theoretical isoelectric point helps to know the stability of protein charge. The instability index refers to stability of protein if the values less than 40 reflects stable proteins while above 40 showed instability protein. This instability index can be calculated through the sequences length and the weighted sum of dipeptides. The aliphatic index refers to relative volume through using mole percent of amino acid (alanine, valine, isoleucine, and leucine). Lastly, the hydrophobicity refers to tendency of amino acid [19].

Principal component analysis

Principal component analysis of the RSCU and physiochemical characterization values and the physiochemical characterization were used through SAS software version 9.4 by using excel file format. The PCA plot can help to analyze values for identify patterns in amino acid or codons that were favored or disfavored in specific or major trends with reducing complexity of datasets. In general, PCA were analytical method for enhances our understanding of protein characteristics and for gene expression and evolutionary biology.

Contribution statement: Meshal M. Almutairi: Conceptualization, Writing-original draft, Data curation, Formal analysis, methodology. ???Conceptualization, writing editing, Data curation.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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