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In Silico Characterization of a Cyclin Dependent Kinase -A (CDKA) and its Coding Gene in some *Oryza* Species

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Abstract:

Rice (*Oryza sativa*) is a fundamental food for the majority of world population. Cyclin Dependent Kinase -A (CDKA) accelerates transition through different stages of cell cycle and contributes in gametes formation. In the present investigation, a CDKA encoding gene along with the corresponding protein were characterized in *O. sativa* Indica Group, *O. glaberrima*, *O. barthii*, *O. brachyantha*, *O. glumipatula*, *O. longistaminata*, *O. meridionalis*, *O. nivara*, *O. punctata* and *O. rufipogon* using *in silico* analyses. The results reflected little variation in most species except *O. longistaminata* and *O. brachyantha*. Compared with the remaining species, *O. longistaminata* lacked a negative regulatory binding site and had a modified cyclin binding site (PSTAICE instead of PSTAIRE) that may lead to future characterization of a new distinct subclass of CDKAs. *O. brachyantha* had a modified SUC/CKS (suppressor of CDC2/cyclin dependent-kinase regulatory subunit)-binding motif. The observed variations can be exploited through traditional breeding or molecular approaches to manipulate cell division and growth of cultivated *Oryza* species.

Key words: CDKA, Genomes, Rice, Wild relatives.

Introduction:

Rice is an important strategic world crop stands second among the cultivated cereal crops with annual yield of 770 million ton from about 2.38 million acre (1). Possessing balanced contents of carbohydrates, lipids and proteins put rice as the predominant food for more than two thirds of the world population (2, 3). The global climate change in addition to the growing world population necessitates breeding for new cultivars with better quantitative and qualitative traits (3, 4).

Rice breeding suffers from the limited genetic variations of cultivated *Oryza* species (*O. glaberrima* and *O. sativa*) that do not exceed 20% of those recorded in wild *Oryza* species (5, 6). Thus efforts should be continued to demonstrate inter- and intraspecific genetic variations in wild *Oryza* species to compensate shortage in variations observed in cultivated rice (5). Fortunately, the full sequence genomes of many wild *Oryza* species are now available and ready to be used for mining of valuable genetic information.

The *in silico* analyses provide precious genetic information for rice breeding including identification of important sequences in wild *Oryza* species such as regulatory elements for pathogenesis-related proteins (7), cyclin dependent kinase-B coding gene (5) and a gene encoding a Pathogenesis-Related Protein-10 (6).

Growth and development are regulated through strict control of cell cycle (8, 9). All eukaryotic cells possess a group of Ser/Thr protein kinases known as cyclin dependent protein kinases (CDKs); that form complexes with cyclin then phosphorylate proteins crucial for cell cycle progression (10). All eukaryotes have a class of Ser/Thr protein kinases, known as CDKs that control progression through cell cycle checkpoints (7). CDKs contain a cyclin-binding domain, one or more phospho-regulatory sites in addition to an ATP binding site (11). Based on cyclin-binding domains, CDKs are clustered into seven classes (CDKA to CDKG) in addition to CDK-like kinases (CKLs) (8).

CDKAs constitute the biggest group of plant CDKs, distinguished with conserved PSTAIRE motif devoted for binding to cyclins (12). Through the constitutive expression of their coding genes (13), CDKAs are produced in plant cells to

control plant growth through accelerating transition through different stages of cell cycle (14), phosphorylation of phosphatidic acid phosphohydrolase1 (15) and development of cytoskeleton and cell walls (16). Also, CDKAs contribute in gametes formation through a meiotic role recognized through preventing premature meiotic exit (17) and controlling chromosome axis assembly (18).

CDKA and its gene were characterized in rice (*O. sativa* Japonica group) for the first time by Hashimoto *et al.* (19). Thus, the present study aims at *in silico* characterizing of CDKA and its coding gene in cultivated and some wild *Oryza* species.

Material and Methods

NCBI (http://www.ncbi.nlm.nih.gov) database was employed to download the amino acid sequence of CDKA (CAA42923.1) identified in *O. sativa* Japonica Group. The sequence was targeted in cultivated (*O. glaberrima*, *O. sativa* Indica Group and *O. sativa* Japonica Group) and eight wild (*O. barthii*, *O. brachyantha*, *O. glumipatula*, *O. longistaminata*, *O. meridionalis*, *O. nivara*, *O. punctata* and *O. rufipogon*) *Oryza* species genomes published in EnsemblPlants database (http://www.http://plants.ensembl.org) utilizing BLASTP search tool to demonstrate candidate genes and coding sequences.

Exon-intron structures of the retrieved genes were built up using coding and genomic sequences with the aid of Gene Structure Display Server website (http://gsds.cbi.pku.edu.cn/). The mined CDKA genes and the corresponding ones in the closest Gramineae species available in Gene Bank were aligned using Clustal W. The aligned sequences were employed to establish a phylogenetic tree based on Maximum Likelihood (ML) method in MEGA v. 6 (20) according to Hasegawa-Kishino-Yano model (21) with gamma distribution. 1000 replica-Bootstrap was utilized to judge significance of grouping patterns support (22).

The predicted amino acid sequences were aligned employing (multalin) (23, http://multalin.toulouse.inra.fr/multalin) to highlight the functionally important domains. The important physico-chemical characteristics of the mined proteins including molecular mass and isoelectric points were resolved utilizing Expasy Protparm server (24, http://us.expasy.org/tools/protparam.html).

Subcellular distribution of the mined proteins was anticipated with the aid of the CELLO2GO server (25). Finally, secondary structure and 3-D models were established using Phyre2 server (26, http://www.sbg.bio.ic.ac.uk/phyre2) and Z-score values for the predicted 3-D models were computed using ProSA-web server (27, https://prosa.services.came.sbg.ac.at/prosa.php). Docking was implemented between the predicted proteins and cyclin D that binds to CDKA in many CDKA-related functions (7) using Frodock server (28, 29, http://frodock.chaconlab.org).

Results and Discussion:

The mined data of this investigation reflected open reading frames (ORFs) of 819 bp in *O. longistaminata*, 873 bp in *O. sativa* Indica gp, *O. glumipatula*, *O. rufipogon*, and *O. glaberrima* while other species exhibited an ORF of 876 bp for CDKA coding gene. However, all mined genes shared the same general exon-intron structure of 7 exons spaced with 6 introns (Fig. 1). The same exon-intron structure for CDKA coding gene was observed in walnut hybrid (30) and *Physcomitrella patens* (31). On the other hand, Gao *et al.* (32) recorded 8 exons spaced with 7 introns studying the same gene in *Arabidopsis thaliana* and *Gossypium hirsutum*. Such species-dependent exon-intron arrangement for CDKA coding gene was also recorded upon studying a gene encoding class B of these kinases (5).

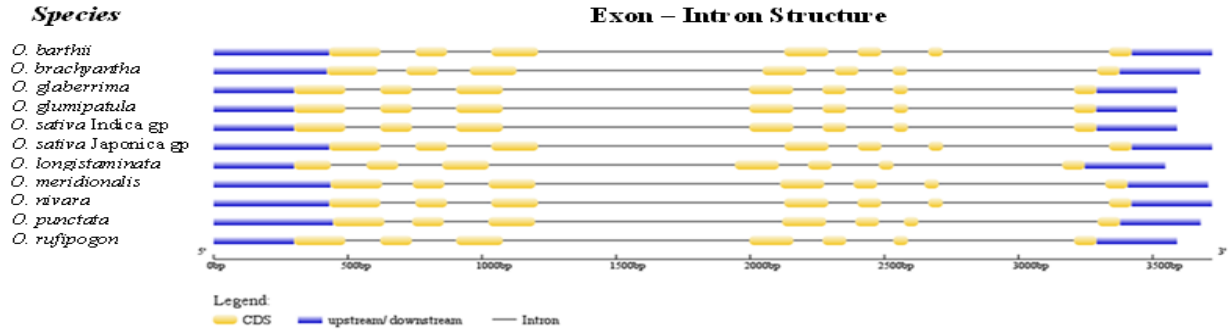

Figure 1. Exon-intron arrangement of a CDKA coding gene in some *Oryza* species.

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Phylogenetic tree (Fig. 2) declared gathering of all examined *Oryza* species in a large clade greatly backed with maximum bootstrap value without any of the taxonomically close Gramineae taxa suggesting CDKA encoding gene as a powerful taxonomic tool. The tree showed that *O. nivara* and *O. rufipogon* are the closest wild *Oryza* species to the Asian cultivated one (*O. sativa*), while *O. barthii* is the closest wild taxon to the African cultivated species (*O. glaberrima*). Compared with cultivated species, genetic variation in CDKA encoding gene reached its maximum in *O. brachyantha* and *O. punctata* that appeared as outgroup for all the studied *Oryza* species. Similar general phylogenetic relationships appeared among wild and cultivated

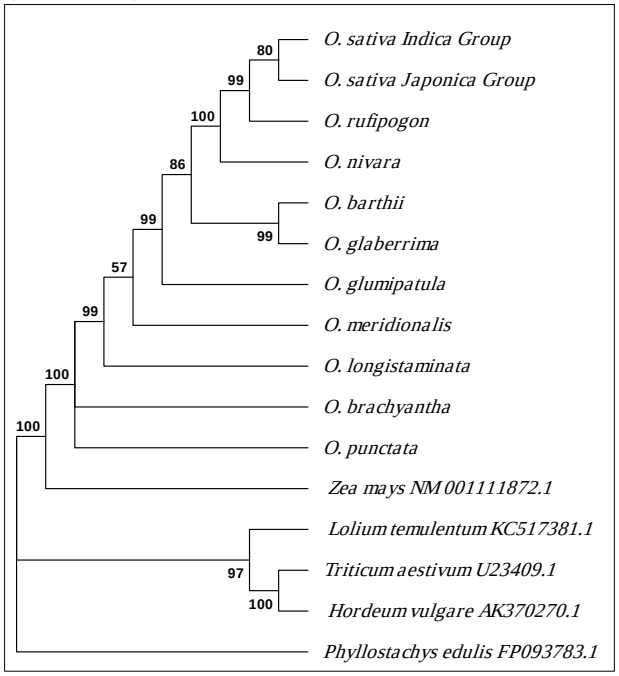

Figure 2. Phylogenetic tree for CDKA coding gene predicted in some *Oryza* species along with some of the taxonomically closest monocot Graminae taxa (*Lolium temulentum, Zea mays, Hordeum vulgare* and *Triticum aestivum*) based on Maximum Likelihood method using the Hasegawa-Kishino-Yano model. Based on 1000 replicates, Bootstrap values are presented as percentages at branching points.

Oryza species upon employing different sequences including a supermatrix of more than 4600 nuclear gene (33), sequences of centromeres in addition to centromere-linked genes (34), CDKB1 coding gene (5) and PR-10 coding gene (6).

Supported with instability index value lower than 40 (Table 1), all the retrieved CDKAs exhibited in vitro stability (35). Subcellular location analysis reflected cytoplasmic and nuclear distribution of the predicted CDKA protein (Table 1) which is suitable for roles recorded to be played by such protein. In nucleus, CDKA is involved in DNA replication (36) and formation of synaptonemal complex at the beginning of meiosis (18). In cytoplasm, CDKA is associated with spindle (37, 38) and phragmoplast formation (39).

Table 1. Subcellular localization and physiochemical characters of the predicted CDKA in some *Oryza* species.

Species	Subcellular location	Molecular mass (KDa)	Formula:	No. of amino acids	No. of negatively charged residues	No. of positively charged residues	PI*	Instability index
O. sativa Japonica gp	Cytoplasm / Nucleus	33692.90	$C_{1525}H_{2398}N_{410}O_{431}S_{10}$	292	40	39	6.87	30.87
O. rufipogon		33561.71	$C_{1520}H_{2389}N_{409}O_{430}S_{9}$	291	40	39	6.97	30.94
O. nivara		33692.90	$C_{1525}H_{2398}N_{410}O_{431}S_{10}$	292	40	39	6.87	30.87
O. sativa Indica gp		33561.71	$C_{1520}H_{2389}N_{409}O_{430}S_{9}$	291	40	39	6.97	30.94
O. meridionalis		33664.89	$C_{1525}H_{2398}N_{408}O_{431}S_{10}$	292	40	39	6.87	30.21
O. longistaminata		31288.10	$C_{1414}H_{2224}N_{378}O_{403}S_{10}$	273	37	33	6.22	34.04
O. glumipatula		33561.71	$C_{1520}H_{2389}N_{409}O_{430}S_{9}$	291	40	39	6.97	30.94
O. glaberrima		33533.66	$C_{1518}H_{2385}N_{409}O_{430}S_{9}$	291	40	39	6.97	30.94
O. barthii		33664.85	$C_{1523}H_{2394}N_{410}O_{431}S_{10}$	292	40	39	6.87	30.87
O. punctate		33603.72	$C_{1518}H_{2387}N_{407}O_{434}S_{10}$	292	41	38	6.38	31.24
O. brachyantha		33658.84	$C_{1522}H_{2396}N_{408}O_{433}S_{10}$	292	41	39	6.56	28.21

*Isoelectronic point

Analyses of the amino acid sequences of CDKA mined from the studied *Oryza* genomes showed 291-292 amino acid length in all species except *O. longistaminata* that appeared as a shorter amino acid chain of 273 residues (Fig. 3). Working on *O. sativa*, Hashimoto *et al.* (19) recorded a 292 amino acid long CDKA. Generally, protein kinases share a similar feature of having a 250–300 amino acid residue domain for the phospho-transfer reaction (40).

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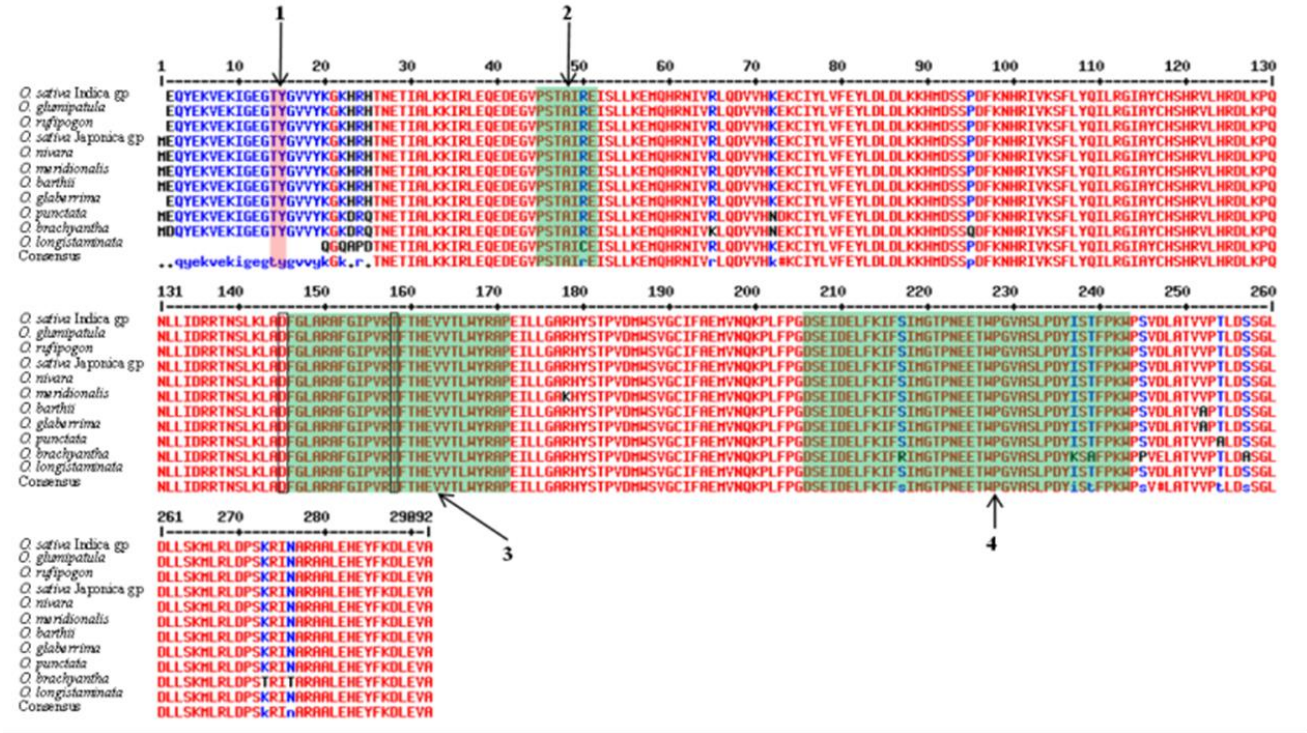


Figure 3. Multalin-based amino acid sequence alignment of a CDKA in some *Oryza* species showing: 1. threonine (T) and tyrosine (Y) residues, 2. PSTAIRE motif, 3. T-loop preceded with asparagine (D) and 4. SUC/CKS -binding motif

Regarding the functionally important binding sites, the retrieved CDKAs showed the same sites appeared in CDKA characterized in *O. sativa* Japonica Group by Hashimoto *et al.* (19) with few but important exceptions (Fig. 3). Except *O. longistaminata*, all CDKAs showed threonine (T) and tyrosine (Y) residues whose phosphorylation blocks enzymatic activity. Absence of this binding site in *O. longistaminata* indicates a new mechanism for negative regulation that may be beneficial for breeding of cultivated *Oryza*. A second interesting variation in functionally important sites was also demonstrated in *O. longistaminata* where PSTAIRE motif, specialized for cyclin binding, was modified to PSTAICE. Docking with cyclin D reflected absolute energy score of 3276 to 3479 kcal/mol in species having PSTAIRE motif (Table 2). Within this range, *O. longistaminata* having PSTAICE motif showed 3342 kcal/mol absolute energy score for the same docking process strongly highlighting insignificant effect for difference between the two motifs on binding to cyclin.

Though PSTAIRE was known to be evolutionarily conserved signature for CDKA, it was modified to PSTALRE in diatoms (41) and sea lettuce (42) that adds to the importance of our finding in *O. longistaminata* and necessitates wet lab-based future investigations to characterize this CDKA that may lead to a distinct subclass of these important kinases.

The third functionally important area was identical in all *Oryza* species of the present study; it consists of asparagine (D) and adjacent T-loop (Fig. 3). Asparagine is required for positioning of the bound ATP essential for kinase activity. The T-loop consists of 27 residue centered around threonine (T) whose phosphorylation stabilizes the cyclin-binding (43).

With one exception observed in *O. brachyantha*, SUC/CKS (suppressor of CDC2/cyclin dependent-kinase regulatory subunit)-binding motif showed complete matching in all studied species. Three substitutions were recorded in where serine, isoleucine and threonine in the consensus sequence where replaced with arginine,

Table 2. Characteristic features of the predicted secondary structures and 3-D models of CDKA in some *Oryza* species.

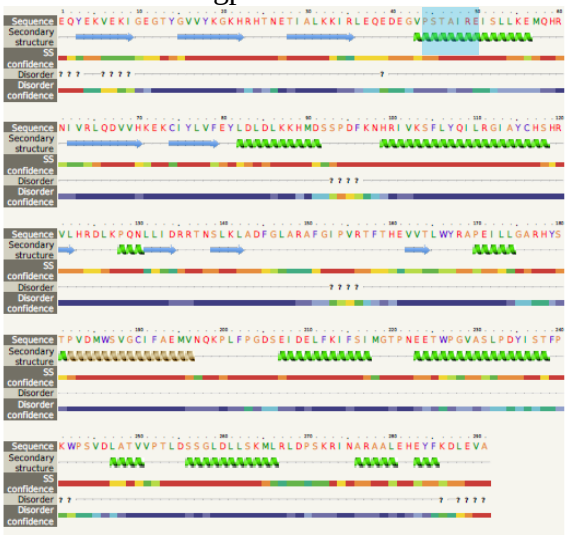
Species	Secondary Structure		Dimensions (Å)			Z-Score	Absolute Energy Score for Docking with Cyclin D (kcal/mol)
	α helix (%)	β strand (%)	X	Y	Z		
O. sativa Indica gp	41	18	55.728	50.854	51.340	-6.58	3388
O. rufipogon	41	18	55.728	50.854	51.340	-6.58	3283
O. nivara	40	17	55.728	51.490	51.340	-6.54	3388
O. sativa Japonica gp	40	17	55.728	51.490	51.340	-6.54	3283
O. meridionalis	41	17	55.728	51.490	51.340	-6.61	3480
O. longistaminata	45	13	56.321	59.238	42.077	-4.98	3342
O. glumipatula	41	18	55.728	50.854	51.340	-6.58	3283
O. glaberrima	41	18	55.728	50.854	51.340	-6.64	3314
O. barthii	41	18	55.728	51.490	51.340	-6.55	3382
O. punctate	41	18	55.560	50.854	51.340	-6.54	3356
O. brachyantha	41	18	55.728	51.564	51.201	-6.69	3277

Lysine and alanine. The same substitutions were also recorded in Jerusalem artichoke (44), coconut palm (45), *Dendrobium candidum* (46) and *Lolium temulentum* (47).

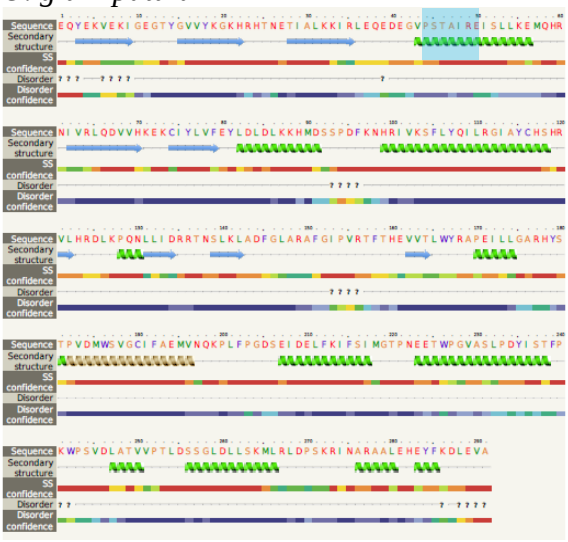
Except for *O. longistaminata* with molecular weight of 31.29 KDa and isoelecteric point of 6.22 (Table 1), physiochemical characteristics of the mined CDKAs in other taxa showed narrow ranges of molecular weights (33.56 - 33.69 KDa) and isoelecteric points (6.38 - 6.97). CDKAs having similar characteristics were identified in *Physcomitrella patens* (31), *Dendrobium candidum* (46), maize endosperm (48) and *Arabidopsis thaliana* (49).

Secondary structures (Fig. 4 and Table 2) of the retrieved CDKAs showed PSTAIRE motif in the first α-helix as described by Sorrell *et al.* (50). 3-D models (Fig. 5 and Table 4) supported with negative Z-score also showed the pattern described for such kinases consisting of joined couple of α-helices and β-strands (46).

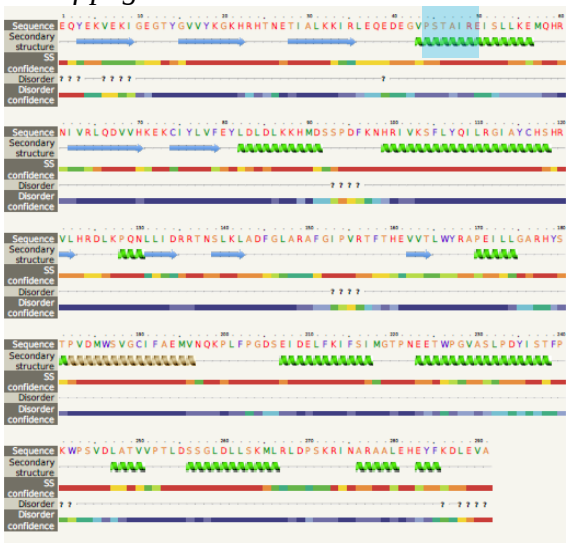
O. sativa Indica gp



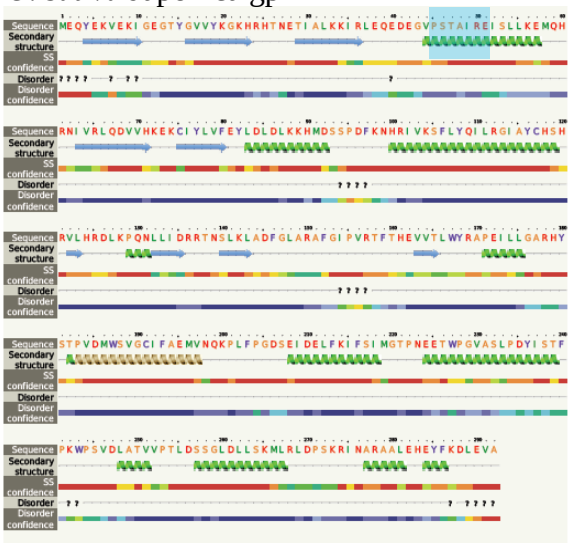
O. glumipatula



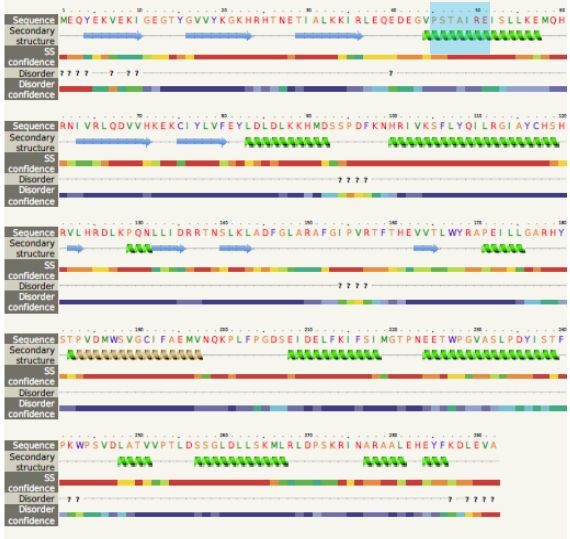
O. rufipogon



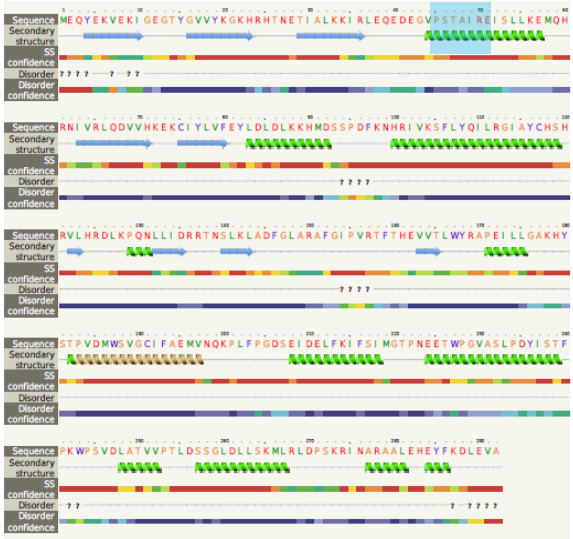
O. sativa Japonica gp



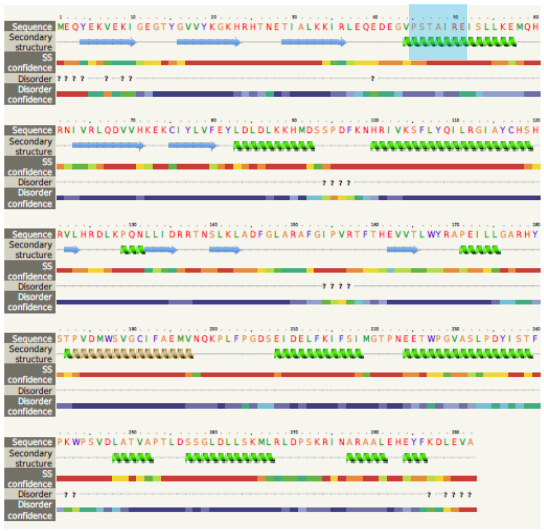
O. nivara



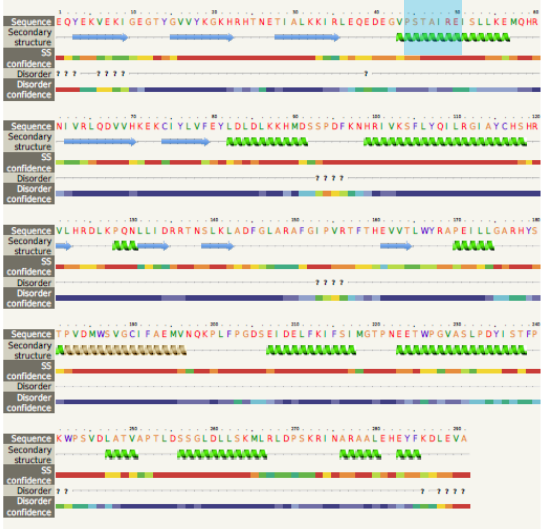
O. meridionalis



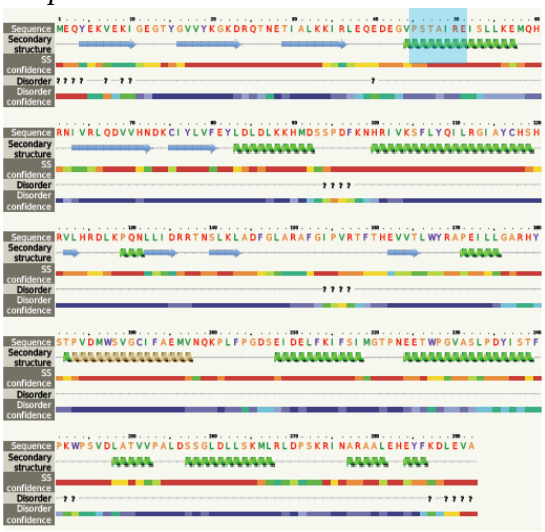
O. barthii



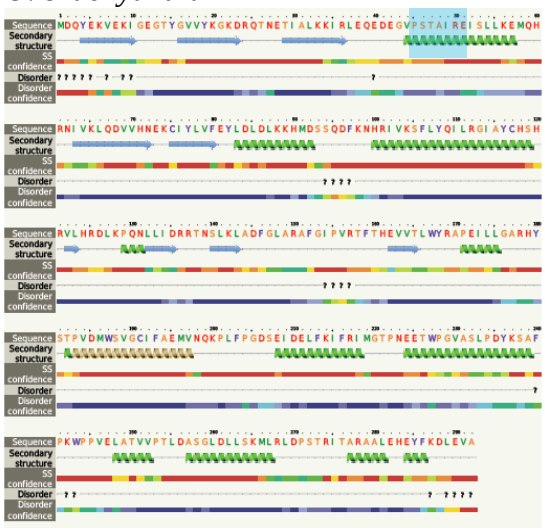
O. glaberrima



O. punctata



O. brachyantha



O. longistaminata

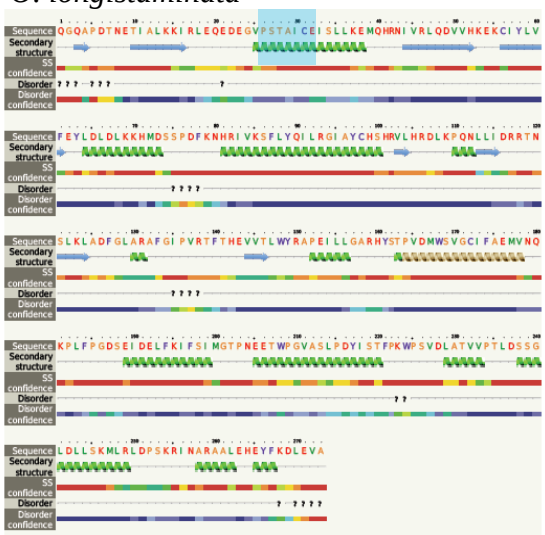


Figure 4. Secondary structures of the retrieved CDKAs in some *Oryza* spesies showing PSTAIRE motif in the first α-helix.

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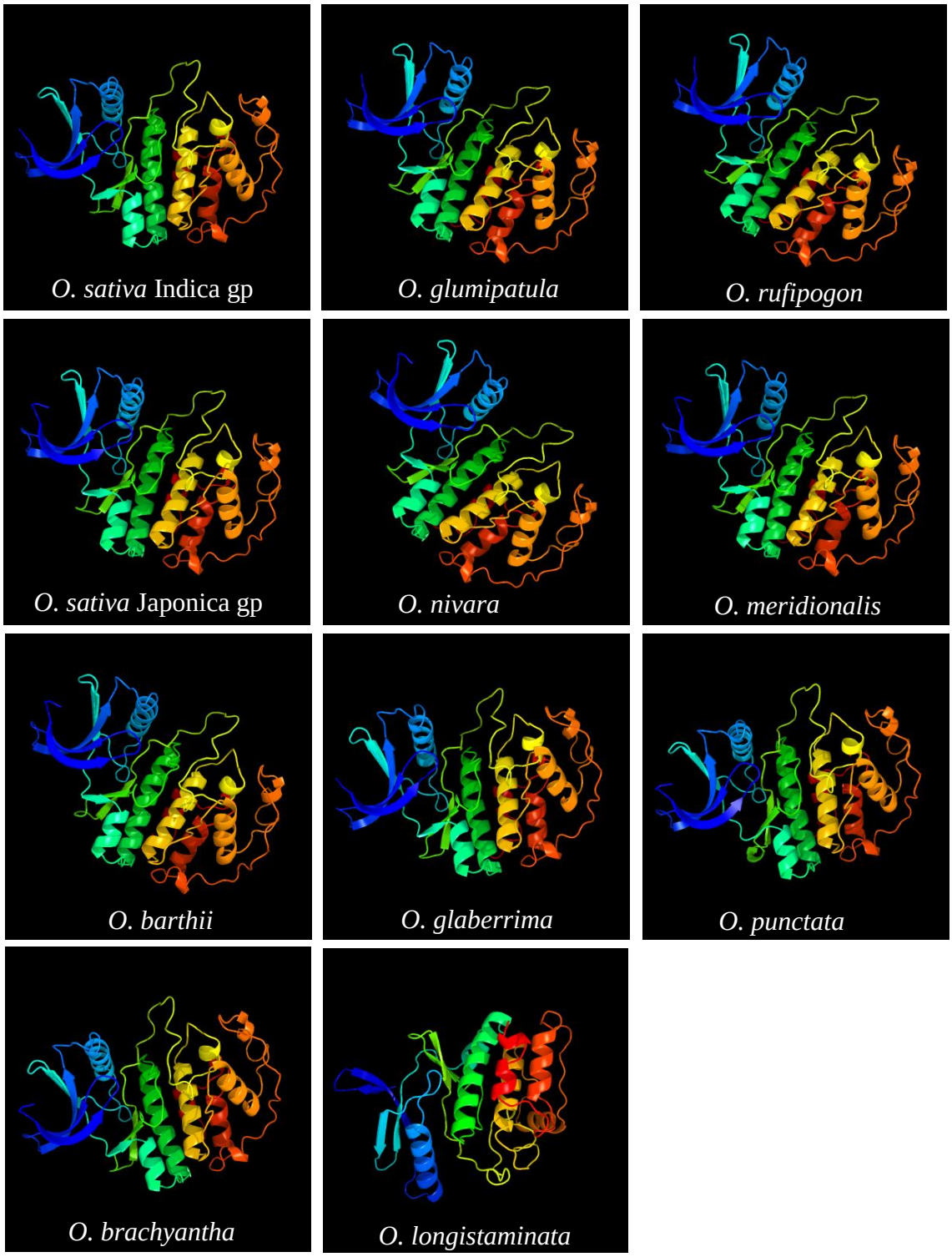

Figure 5. The predicted 3-D models for the mined CDKA in some *Oryza* species.

In conclusion, *in silico* analyses provided a time and cost effective tool to highlight valuable genetic variations in wild relatives of rice. The unique CDKA predicted in *O. longistaminata* lacking the negative regulatory binding site observed in other species may be exploited to accelerate growth in cultivated species through traditional breeding or molecular approaches. Similarly, polymorphism in SUC/CKS - binding motif recorded in *O. brachyantha*, can be employed in cultivated species to make benefit of such variation in manipulating cell division.

Authors' declaration:

- Conflicts of Interest: None.

- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides,

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توصيف أحد بروتينات الفسفرة المعتمدة على السيكلين من المجموعة A و الجين الخاص به فى بعض أنواع جنس *Oryza* بإستخدام التقنيات الحاسوبية

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الخلاصة:

يعد الأرز طعاما أساسيا لمعظم سكان العالم. تساهم بروتينات الفسفرة المعتمدة على السيكلين من المجموعة A (CDKA) فى الإنتقال عبر المراحل المختلفة لدورة الخلية و كذلك تساهم فى تخليق الأمشاج. و قد هدفت الدراسة الحالية إلى توصيف أحد هذه البروتينات و الجين المسئول عنها فى Indica Group *O. sativa* ,*O. glaberrima* ,*O. barthii* ,*O. glumipatula* ,*O.* ,*O. nivara* *O. meridionalis* ,*O. longistaminata* ,*O. punctata* و*O. rufipogon* بإستخدام التقنيات الحاسوبية. كشفت النتائج إختلافات فى بعض تتابعات الأحماض الأمينية التى تنظم عمل البروتين محل الدراسة فى كلا من *O. longistaminata* و *O. brachyantha* و ذلك مقارنة بالبروتين ذاته فى باقى الأنواع. إفتقد البروتين محل الدراسة فى *O. longistaminata* أحد تتابعات الأحماض الأمينية المسئولة عن تثبيط عمل البروتين كما لوحظ إختلاف فى تتابع الأحماض الأمينية المسئولة عن الإرتباط بالسيكلين (PSTAICE بدلا من PSTAIRE) مما قد يسفر عن توصيف لمجموعة فرعية جديدة متفردة من هذه البروتينات. فى *O. brachyanth* تم تسجيل إختلاف فى تتابع الأحماض الأمينية بالموضع المسئول عن النشاط المرتبط بلسيكلين. توصى الدراسة بالأستفادة من الإختلافات سابقة الذكر فى التحكم فى الإنقسام الخلوى و النمو فى الأنواع المزروعة من جنس *Oryza* بإستخدام طرق التربية التقليدية او الطرائق الجزيئية.

الكلمات المفتاحية: CDKA، الجينومات، الأرز، الأقارب البرية.