

A Comprehensive RRM Domains Sequence Analysis from Nucleolin Proteins in Plants

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Abstract

In contrast to yeast and animal cells, plants contain two genes which is encoding to nucleolin proteins. A comprehensive protein sequence analysis revealed that the two RNA recognition motifs (RRM) domains are highly conserved and suggesting that the mechanisms and functions related to RNA contained RRM are highly conserved in both plant nucleolin proteins sequences. Whereas Ribonucleoprotein 1 (RNP1) and Ribonucleoprotein 2 (RNP2) that are highly conserved sequences in RRM from yeast and animal nucleolin, also my study confirmed that RNP1 and RNP2 sequences are conserved in plants. Nucleolin could use the same manner in the different species of plants and animals to achieve its function. Study of evolutionary relationships among nine different plant species revealed that monocots and dicots form two separated monophyletic group meaning that they composed of a collection of organisms, share a common evolutionary history and including the most recent common ancestor of all those organisms.

Keywords: *Nucleolin proteins, evolutionary relationships, RNA recognition motifs, Ribonucleoprotein.*

Introduction

The most copious protein in nucleolus is nucleolin which might play a major role in the multiple functions of the nucleolus. Moreover, its function in the cytoplasm and in the nucleoplasm, nucleolin has been participated in other processes, including repair, replication and recombination of DNA (Tuteja *et al.*, 1995; Gregório *et al.*, 2018 and Carvalho *et al.*, 2021), pathogens internalization, -replication, -resistance (Daniely and Borowiec, 2000; Nisole *et al.*, 2002; Masiuk, 2010; Jiang *et al.*, 2010), telomerase nucleolar localization (Khurts *et al.*, 2004), growth and cell proliferation (Srivastava and Pollard, 1999), apoptosis (Zhang *et al.*, 2010) and remodeling of chromatin (Angelov *et al.*, 2006).

In plants, nucleolin proteins were described in alfalfa (Bogre *et*

al., 1996), rice (Udomchalothorn *et al.*, 2017) and pea (Tong *et al.*, 1997). In alfalfa (*Medicago sativa*), seven cDNA clones were isolated from cDNA libraries and the sequencing of these clones indicated that at least three class encode to *nucMs* (nucleolin of *Medicago sativa*) genes. It appears to be the various transcripts encoded by different alleles, as a result of alfalfa is an autotetraploid species (Bogre *et al.*, 1996). In pea, Southern blot analysis show only one copy gene of nucleolin protein, and northern blot analysis indicated that the labelled cDNA binds to unique RNA band, nearly the selfsame size (2.3Kb) of the cDNA (Tong *et al.*, 1997).

Most of the studies directed to understand nucleolin function in plants come from crucifer plant species. The first functional description

of nucleolin protein came from studies in cauliflower (*B. oleracea* var. botrytis). Nucleolin from cauliflower that participated in processing of 45S pre-rRNA, was joined to a large complex named ribonucleoprotein (Saez-Vasquez *et al.*, 2004a, 2004b). In the other hand, it was shown that the genome of *Arabidopsis thaliana* (Col0) plants contains two genes encoding for nucleolin proteins: AtNUC-L1 and AtNUC-L2, located on chromosomes 1 and 3 respectively (Pontvianne *et al.*, 2007 and Durut *et al.*, 2014). During evolution, the nucleolin structure is highly conserved; it consists of three domains with same arrangement in animal and yeast: acidic domain, RNA recognition motifs domain (RRMs) and glycine-arginine rich domain (GAR). RRM domain was discovered in the late 1980s in the pre-mRNA splicing protein hnRNP (heterogeneous nuclear ribonucleoprotein) (Maris *et al.*, 2005 and Carvalho *et al.*, 2021).

The number of RRMs is similar in nucleolin from yeast, which possess two, compared to animals that contain four RRMs: Four in human (Srivastava *et al.*, 1989); chicken (Maridor and Nigg, 1990); rat (Bourbon and Amalric, 1990); hamster (Lapeyre *et al.*, 1985); mouse (Bourbon *et al.*, 1988); and frog (Rankin *et al.*, 1993); two RRMs in *S. cerevisiae* (Lee *et al.*, 1992) and *S. pombe* (Gulli *et al.*, 1995) and two in alfalfa (Bogre *et al.*, 1996), *Arabidopsis* (Pontvianne *et al.*, 2007) and pea (Tong *et al.*, 1997). Interestingly, within the same nucleolin protein, the RRMs were less conserved in compared with the RRMs from various nucleolin protein. That means, for instance, the

conservation between RRM1, RRM2, RRM3 and RRM4 from human is less compared with the conservation between RRM1 from human and RRM1 from chicken (Ginisty *et al.*, 1999).

In animal, the central domain structure was analyzed precisely. The RRM consists of approximately 70-100 amino acid sequence with a topology $\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$. The fold consists of one four-stranded antiparallel β -sheets, which arranged from left to right in $\beta_4\beta_1\beta_3\beta_2$, and two α -helices packed against the β -sheets, which are involved in the interaction with single-stranded RNA (Bouvet *et al.*, 1997; Maris *et al.*, 2005; Clery *et al.*, 2008). The two highly conserved segments named Ribonucleoprotein 1 (RNP1) and Ribonucleoprotein 2 (RNP2) motifs are located in the central β -sheets, β_3 and β_1 , respectively.

In animal, RNP1 consists of eight amino acids in arrangements (R\K)G(F\Y)(G\A)(F\Y)VX(F\Y); while the RNP2 contains six amino acids in arrangements (L\I) (F\Y) (V\I) (G\K)(G\N)L. These motifs contain aromatic residues Y (Tyr); H (His), F (Phe); or Trp (W) at positions: 2 that is located in β_1 -sheet in RNP2 and 3 and 5 located in the β_3 -sheet in RNP1. The positions of aromatic residues are highly conserved, and they are participated in RNA-base-stacking interactions (Clery *et al.*, 2008). The positions of aromatic residues are highly conserved, and they are known to be involved in base-stacking interactions with RNA (Rao *et al.*, 2006). RNA binds to RRM by stacking of two specific bases of the 3' and the 5' nucleotides on an aromatic residue existent in in β_3

(RNP1-position 5) and β 1 (RNP2-position 2), respectively (Allain *et al.*, 2000; and Clery *et al.*, 2008). The third aromatic residue that is found in β 3 (RNP1-position 3) possesses hydrophobic interactions with the sugar rings between the dinucleotides which are stacked to the positions 2 and 5 (Clery *et al.*, 2008). In yeast each RRM has also two highly conserved RNP1 and RNP2 (Kondo and Inouye; 1992 and Gulli *et al.*, 1995).

Materials and Methods

Methods in sequence analysis

Plant nucleolin proteins (NUC-L1 and NUC-L2) sequences were obtained from (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and (<http://ppdb.tc.cornell.edu>) sites. Sequence alignments were carried out using *Multalin* program (Corpet, 1988). For study the secondary structure of RRMs from AtNUC-L1 and AtNUC-L2 proteins, we used *TMBpro* program at site (<http://www.ics.uci.edu/~baldig/tmb.html>) (Randall *et al.*, 2008).

Phylogenetic tree was generated with MEGA7 software (Kumar *et al.*, 2016), performing 1000 bootstrap tests were used in Neighbor Joining method.

Results

1. Study in nucleolin structure

In eukaryotes, Nucleolin is a highly conserved structure that composes of three domains in the same arrangements: acidic, RRMs and GAR domains. Plants and yeast nucleolin contain two RRMs in compare with four RRMs in animal nucleolin. To study the conservation of RRMs, we performed alignment for RRMs from nucleolin proteins from different species of plants, *Arabidopsis thaliana*, *Oryza sativa indica*, *Oryza sativa japonica*, *Zea mays*, *Populus trichocarpa*, *Sorghum bicolor*, *Medicago sativa*, *Nicotiana tabacum* and *Pisum sativum* as shown in Fig. 1. The alignment revealed the high conservation of all plant nucleolin RRMs. Moreover, the RRMs from different protein are more conserved than the same protein. For instance, the conservation among Nit_NUC1_RRM1 (*Nicotiana tabacum* nucleolin 1 RRM1) and Nit_NUC1_RRM2 is less compared with the conservation between RRM1 from *Nicotiana tabacum* and RRM1 from *Populus trichocarpa* (Pot_NUC1_RRM1). Thus, as shown in Fig.1 they are classified to two clusters, RRM1 cluster and RRM2 cluster.

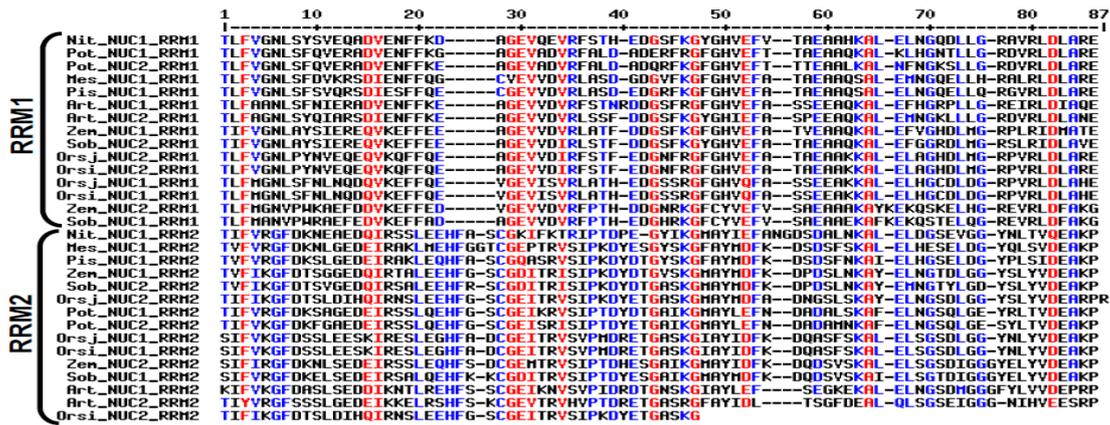


Figure 1: Alignment of different plant nucleolin RRM1 and RRM2 by using *Multalin* program. *Arabidopsis thaliana*_Q9FVQ1(ArtNUC1), Q1PEP5 (ArtNUC2), *Oryza sativa japonica*_Q6Z1C0 (OrsjNUC1), Q7XTT4 (OrsjNUC2), *Oryza sativa indica*_BGIOSIBCE026772 (OrsiUC1), BGIOSIBCE016635 (OrsiUC2), *Sorghum bicolor*_Sb07g005510 (SobNUC1), Sb01g019710 (SobNUC2), *Zea mays*_FGP025 (ZemNUC1), FGT019 (ZemNUC2), *Populus trichocarpa*_002310655 (PotNUC1), 002307174 (PotNUC2), *Medicago sativa*_T09648 (MesNUC1), *Nicotiana tabacum*_Q8LNZ4(NitNUC1) and *Pisum sativum*_T06458 (PsNUC-L1).

In another hand, the RRM domains alignment from different plant nucleolin proteins revealed that each RRM contains two highly conserved sequences, one of them: the highly conserved octamer sequences (RNP1) that is located in β_3 sheet and the other highly conserved hexamer sequences (RNP2) that is localized in β_1 sheet as shown in Fig. 3 and in Table 1. Excepting *Oryza sativa indica* nucleolin 2 (Ori_NUC-L2) that lacks RNP2 only from RRM2 (Table 1).

The presence of aromatic amino acids in RNPs is necessary for RRM binding to RNA (Clery *et al.*, 2008). For study the position of the aromatic acid we performed alignment of RRM1 and RRM2 from different plant nucleolin proteins. The positions of aromatic amino acids (Y (Tyr); H (His); F (Phe); or Trp (W)) are conserved and they are found at positions 2, in β_1 -sheet in RNP2 and the other are present at positions 3 and 5 in β_3 -sheet in RNP1 (Fig. 3 and Table 1).

Table 1. Alignment of the conserved RNP-1 and RNP-2 from two RRM motifs found in plants nucleolin proteins by using *Multalin* program.

	RRM1		RRM2	
	RNP2	RNP1	RNP2	RNP1
Nit_NUC-L1	LFVGNL	KGYGHVEF	IFVVRGF	KGMAYIEF
Mes_NUC-L1	LFVGNL	KGFGHVEF	VFVRGF	KGFAYMDF
Pis_NUC-L1	LFVGNL	KGFGHVEF	VFVRGF	KGFAYMDF
Zem_NUC-L1	IFVGNL	KGFGHVEF	VFIKGF	KGMAYMDF
Orsj_NUC-L1	LFMGNL	RGFGHVQF	IFVKGF	KGIAYIDF
Orsj_NUC-L2	LFVGNL	RGFGHVEF	IFIKGF	KGMAYMDF
Art_NUC-L1	LFAANL	RGFGHVEF	IFVKGF	KGIAYLEF
Art_NUC-L2	LFAGNL	KGYGHIEF	IYVRGF	RGFAYIDL
Zem_NUC-L2	LFMGNV	KGFCYVEF	IFIRGF	KGMAYIDF
Sob_NUC-L1	LFMANV	KGFCYVEF	IFVVRGF	KGMAYMDF
Orsi_NUC-L1	LFMGNL	RGFGHVQF	IFVKGF	KGIAYIDF
Sob_NUC-L2	IFVGNL	KGYGHVEF	VFIKGF	KGMAYMDF
Orsi_NUC-L2	LFVGNL	RGFGHVEF	IFIKGF
Pot_NUC-L1	LFVGNL	RGFGHVEF	IFVVRGF	KGMAYLEF
Pot_NUC-L2	LFVGNL	KGFGHVEF	IFVKGF	KGMAYLEF
Consensus	LFVGNL	KGFGHVEF	IFVKGF	KGMAYMDF

In topology study of RRM1, RRM2 and their separated region (linker), we observed that there are four β -sheets separated with two α -helices ($\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$) in each RRM as shown in Fig. 3. Moreover, the linker is longer in AtNUC-L1 (29bp) than AtNUC-L2 (21bp).

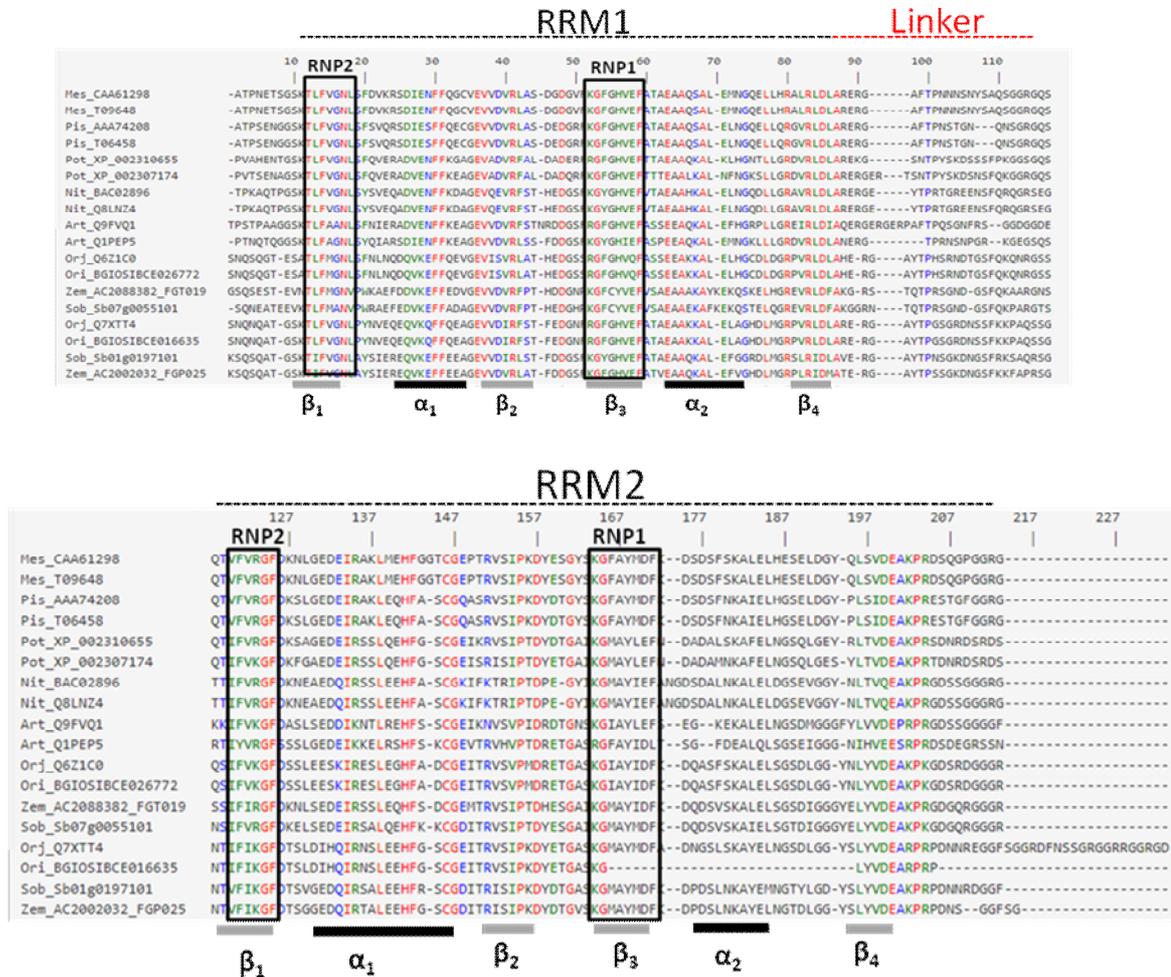


Figure 3. Amino acid sequences alignment (ClustalW) of nucleolin protein RRM1 and RRM2 from *Pisum sativum*, *Populus trichocarpa*, *Nicotiana tabacum*, *Arabidopsis thaliana* (*Art*), *Oryza sativa japonica*, *Medicago sativa*, *Zea mays* and *Sorghum bicolor*, *Oryza sativa indica*. Whereas, the black, red and gray dashed lines correspond to RRM1, linker and RRM2, respectively. Black boxes refer to RNP1s and RNP2s in the two RRM. RRM1 and RRM2 secondary structure elements are indicated below the sequences. Whereas black and gray lines represent α -helices and β -sheets

2. Evolutionary relationships of RRM2s and RRM1s from different types of nucleolin from plants

To study of evolutionary relationships among nine types (*Arabidopsis thaliana*, *Oryza sativa japonica*, *Zea mays*, *Oryza sativa indi-*

ca, *Populus trichocarpa*, *Medicago sativa*, *Sorghum bicolor*, *Nicotiana tabacum* and *Pisum sativum*) of plants depends on amino acid sequences of RRM1s and RRM2s from different types of nucleolin. The history of evolution was deduced using

the Neighbor-Joining method as shown in phylogenetic tree (Figure 4). By analysis of phylogenetic tree, the monocotyledon and dicotyledon sequences were arranged in two separated tight groups at the branch tips. In dicot. group, the two nuc-11 and nuc-12 are clustered on the same

branch but in monocot. nuc-11 from each *Oryza sativa japonica* and *Oryza sativa indica*; and *Sorghum bicolor* and *Zea mays* are on one branch, individually that is the same for nuc-12 from monocot. plants.

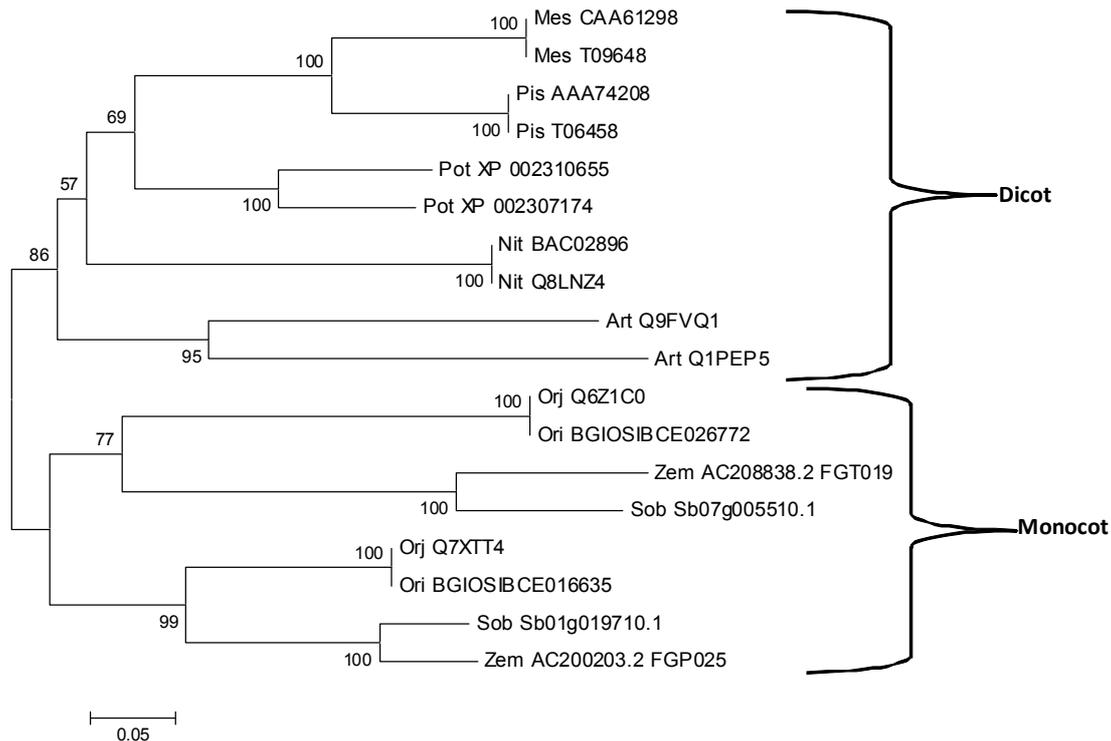


Fig 4. Phylogenetic tree of different plants depends on their RRM of nucleolin proteins using Neighbor Joining method. nucleolin proteins schematic from *Arabidopsis thaliana*, Q9FVQ1 (AtNUC-L1) and Q1PEP5 (AtNUC-L2), *Oryza sativa japonica*, Q6Z1C0 (OsjNUC-L1) and Q7XTT4 (OsjNUC-L2); *Oryza sativa indica*, BGIOSIBCE026772 (OsjNUC-L1) and BGIOSIBCE016635 (OsjNUC-L2), *Sorghum bicolor*, Sb07g005510 (SbNUC-L1), and Sb01g019710 (SbNUC-L2); *Zea mays* FGP025 (ZmNUC-L1) and FGT019 (ZmNUC-L2), *Populus trichocarpa*, 002310655 (PtNUC-L1) and 002307174 (PtNUC-L2), *Medicago sativa*, T09648 (MsNUC-L1), *Nicotiana tabacum* Q8LNZ4 (NtNUC-L1), *Pisum sativum* T06458 (PsNUC-L1).

Discussion

In plants and animal, RNA-recognition motif (RRM) structure is conserved during the evolution. Whereas RNP1 and RNP2 that are highly conserved in RRM from yeast and animal nucleolin, also they are

conserved in plants. The conservation is in their positions and sequences, where the aromatic amino acids are localized in RNP2 (position 2) and in RNP1 (positions 3 and 5). It is similar as in animal and yeast, the RNP1 and

RNP2 exist in β_3 -sheet and β_1 -sheet, respectively. Also, the arrangements of β and α sheets are quietly like that finding in animal nucleolin (Maris *et al.*, 2005 and Clery *et al.*, 2008). Binding of RNA to the RRM by stacking of two certain bases of the 3' and the 5' nucleotides on an aromatic residue found in β_3 (position 5 in RNP1) and in β_1 (position 2 in RNP2), respectively (Allain *et al.*, 2000 and Clery *et al.*, 2008). The third aromatic ring that is usually located in β_3 (position 3 of RNP1) has often hydrophobic interactions with the sugar rings between the dinucleotides which are stacked to the positions 2 and 5 (Clery *et al.*, 2008). It seems to be nucleolin use the same manner in the different species of plants and animals to achieve its function.

Plants show two genes encoding nucleolin proteins. Both proteins are structurally conserved and have the tripartite organization characteristic of nucleolin: i.e. the acidic, the RRM and the GAR domains. Protein sequence analysis revealed that the two RRM domains are highly conserved and suggests that the mechanisms and functions related to RNA are conserved in both plant nucleolin sequences. Study of evolutionary relationships among nine types revealed that monocots and dicots form two separated monophyletic group meaning that they composed of a collection of organisms, share a common evolutionary history and including the most recent common ancestor of all those organisms (Slobodian and Pastana, 2020).

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تحليل شامل لتتابعات منطقة الـ RRM في بروتينات النيوكليولين في النباتات

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الملخص

تحتوي النباتات، على عكس الخلايا الحيوانية والخميرة، على إثنين من الجينات التي تشفر للبروتينات النيوكليولين. وأوضح التحليلات الشاملة لتتابعات البروتين أن منطقة الـ RRM محافظة بشكل كبير جداً، مما يشير إلى أن الآليات والوظائف المتعلقة بالـ RNA المحتوي عليها أيضاً محافظة في كلاً من تتابعات البروتينات بالنوكليولين في النبات. أيضاً أوضحت تلك الدراسة أن تتابعات الـ RNP1 و RNP2 تكون متواجدة بصورة محافظة جداً في جميع الـ RRMs في بروتينات النيوكليولين المتواجدة في الخميرة والحيوان، أيضاً أظهرت النتائج أن تتابعات الـ RNP1 و RNP2 محافظة جداً في جميع النباتات تحت الدراسة. ومن الواضح أن بروتينات النيوكليولين في الأنواع النباتية المختلفة يمكن أن تستخدم نفس الوسيلة كما في الحيوان للقيام بوظائفها. وكشفت دراسة العلاقات التطورية بين تسعة أنواع نباتية مختلفة باستخدام تتابعات الـ RRMs أن نباتات أحادية الفلقة ونباتات ثنائية الفلقة تُشكل مجموعتين منفصلتين، مما يعني أنها تتكون من مجموعة من الكائنات الحية التي لها تاريخ تطوري مشترك وتشمل أحدث سلف مشترك لجميع هذه الكائنات الحية.