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Phylogenetic analysis of target of rapamycin (TOR) kinase gene of some selected plants species

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ABSTRACT

TOR kinase has been reported to regulate number of biological processes, including central dogma, which collectively contributes to cell growth in all the organisms. The major role of the target of rapamycin (TOR) kinase is to encourage cell growth in response to favorable conditions. Up to some extent,nucleotide and amino acidsequencesof TOR kinase were found to be similar in all the organismswherein it has been identified or characterized.In order to assess the phylogenetic relationship and conservative nature of TOR gene among 32 different plants species TOR gene sequences and protein sequence retrieve from public repository database NCBI.Sequence length and GC% of each sequence were determined. Maximum GC% was found in TOR kinase gene of *Brachypodium distachyon* (46.18%). All the 32 TOR gene sequences contained more than 43% of GC. Phylogenetic tree constructed using Neighbor-joining method separated TOR kinase into two distinct groups *i.e.* monocots and dicots. Sequences of TOR kinase from similar family of plants were grouped together signifyingits conserved nature within the family. The phylogenetic tree of TOR gene at both nucleotides and proteins level from different species perfectly reflects phylogenetic relationships of the species. This strong conservation of *tor* genes among all the species including in this investigation advocate the general significance of this kinase and, consequently, the entire TOR pathway.

KEY WORDS: CELL GROWTH; NEIGHBOR-JOINING; PHYLOGENETIC TREE; *TOR* KINASE

INTRODUCTION

The modulation of growth rate in a particular environmental condition such as nutrient availabilityis necessary for continued existence. Plant growth is largely dependent on surrounding environmental information. It includes cell growth coupled with cell proliferation and cell expansion depends onexogenous factors such as stresses and nutrient availability. Unlike animals, in plants, postembryonic growth is directly influenced

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by availability of nutrients and energy source that are present outwardly or generated by various cellular processes (Nanjareddy et al. 2016). However, so far, very few secrets have been revealed on mechanism that how this information is perceived and transduce into coherent growth and developmental decisions.One of the most important pathways that are found in all eukaryotes is the one related to the target of rapamycin (TOR) protein kinase.

The target of rapamycin (TOR), a Ser/Thr protein kinase, has emerged as a key player of nutrient, energy, and stress signaling networks (Dobrenel et al. 2013; Yuan et al. 2013). It is a large protein that belongs to the phosphoinositide 3-kinase-related kinase family and is highly conserved among all eukaryotes (Robaglia et al. 2012). Numerous components of the TOR signalingmachinery have beenidentified in model plant *Arabidopsis*. Various members of the TOR complex such as the RAPTOR1/RAPTOR2, LST8-1/LST8-2, S6K1/S6K2, ribosome protein small subunit6 (RPS6A/B), type 2A-phosphatase-associated protein 46 kD (TAP46), and ErbB-3 epidermal growth factor receptor binding protein have been reported in photosynthetic eukaryotes through sequence homology searches from *C. reinhardtii* to Arabidopsis plants (Creff et al. 2010; Ahn et al. 2011; Moreau et al. 2012; Ren et al. 2012; Xiong and Sheen, 2012).

In this era of high throughput gene and genome sequencing, prediction of function of a gene is a key step. Various reverse genetics techniques like site directed mutagenesis and RNAi are effective to solve this purpose. Another easier way to predict function of a particular gene and phylogenetic relation between different species iswith the use of bioinformatics. Nowadays, increasing sequenced genomesof diverse plants are providing new opportunities to study gene families in an evolutionary context. Based on these facts, present investigation was conducted to evaluate the phylogenetic relationship and sequence similarity of TOR kinase genes from different plants species and make an efforts to know the conserve nature of TOR gene at both nucleotide and protein sequence level. Phylogenetic tree analysis on basis of conserved nature and similarity of known sequence helps us to predict the function of a gene and also exhibit the phylogenetic relationships of the species (John et al. 2011). However, for a deeper understanding of the gene function, it is helpful to go beyond cataloguing of similarities and differences and to understand how and even why these similarities and differences arise.

MATERIAL AND METHODS

Nucleotide sequence of TOR kinase gene of *Zea mays* was retrieved from NCBI database for its further use as bait sequence to isolate other sequences of different plant species using BLASTn (http://www.ncbi.nlm.nih.gov/BLAST/). TOR gene nucleotide sequences of different plants were selected on the basis of E-value (within 0 to 1e – 50). DNA sequences were aligned with ClustalW (Thompson et al. 1994) and alignments were subsequently adjusted manually using BioEdit (Hall, 1999). Sequence length and GC% of each sequencewas calculated by MEGA6 (Tamura et al. 2011). Protein sequences were also deduced from all retrieved sequences of TOR kinase genes and were aligned using ClustalW and go for construction of phylogenetic tree.

To evaluate the genetic relationship between retrieved nucleotide sequences of TOR kinase gene, a phylogenetic tree was constructed using Neighbor-joining (NJ) method. The output data was processed using MEGA 6 to draw the phylogenetic tree. The bootstrap consensus tree (Felsenstein, 1985) inferred from 1000 replicates was selected to represent the evolutionary history of the 32 TOR kinase genes under study. Phylogenetic tree was also constructed for amino acid sequences of TOR kinase genes two know the sequence homology at protein level.

RESULTS AND DISCUSSION

In this study, TOR kinase gene sequences of 32 plants species belongs to 11 familieswere selected and retrieved from GenBank for phylogenetic analysis. The sequence lengths of all the TOR genes with GC% are given in Table 1. Maximum GC% was found in TOR kinase gene of *Brachypodium distachyon* (46.18%). All the 32 TOR gene sequences containedmore than 43% of GC.

The evolutionary relationships between the plants were evaluated by phylogenetic analysis of the aligned nucleotides and amino acids sequence of their TOR kinase gene. TOR kinase gene sequences currently available in the database are either full length mRNA sequences or predicted sequences obtained from annotation of genome. The phylogenetic tree obtained by Neighbor-joining method showed two distinctphylo-groups of TOR kinase genes from monocots and dicots (Fig 1). Group I containing only monocots included *Zea mays, Setaria italica, Sorghum bicolor, Brachypodium distachyon, Oryza sativa, Oryza brachyantha, Phoenix dactylifera, Elaeis guineensis* and *Musa acuminate*. Cluster of monocots further divided in two subgroups, wherein subgroup I-acontained three gene sequences, two from family Arecaceae and one from family Musaceae. The plant species *Elaesis guineensis* and *Phoenixdactylifera* belongs to same family Arecaceae and also exhibited high degree of similarity than plant species *Musaacuminata* belongs to family Musaceae in subgroup I-a.

This result also revealed that the relatedness of these two families were higher than the other family included in the present studies. While, subgroup I-b comprised 7

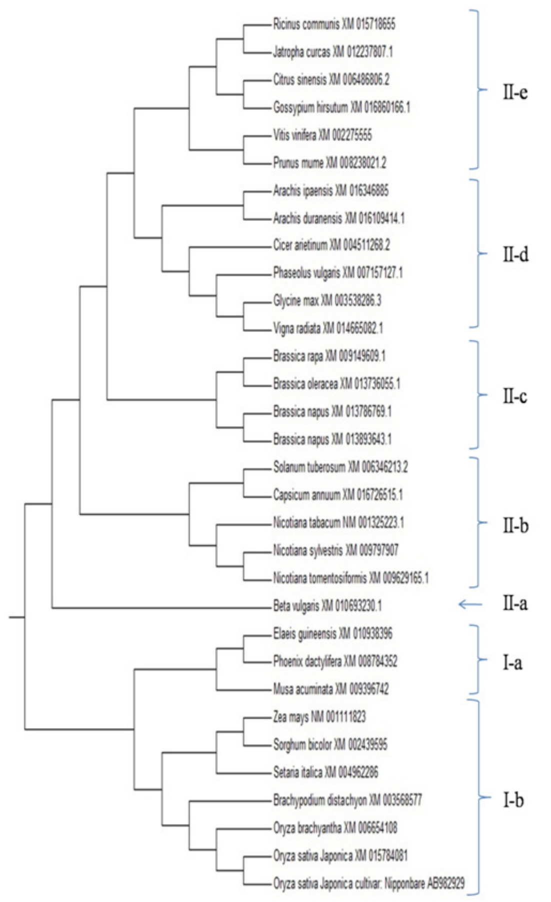

FIGURE 1. Phylogenetic tree showing the relationships between TOR nucleotide sequences from different plants.

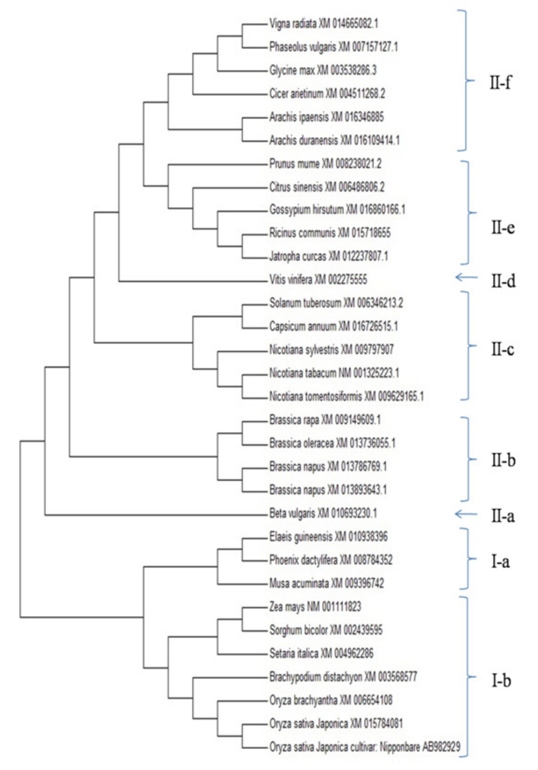

FIGURE 2. Phylogenetic tree showing the relationships between TOR protein sequences from different plants

sequences,all belongs to Poaceae family. In this group the plant species of genus *Oryza* is closely related with *Bracypodiumdistachyon* while *Zeamay* sexhibited higher similarity with plant species *Sorghum bicolor* and *Setaria italica*. In group II, all the sequences of TOR kinase gene of dicot plants clustered into 5 subgroups. TOR gene sequence of *Beta vulgaris* was lone species in subgroup II-a whereas, remaining 4 subgroups clustered according to their family such as subgroup II-b consist of 5 species of Solanaceae, Subgroup II-cwas occupied bythe 4 species of Brasicaceae while 6 species of Fabaceae family gathered in subgroup II-d. On the other hand, five families namely Euphorbiacea, Malvaceae, Rootaceae, Rosaceae and Vitaceae grouped into subgroupII-e.

Inphylogenetic tree of amino acid sequences of TOR kinase divided into two groups of monocots and dicots (Fig 2) with a similar clustering pattern to nucleotide sequenc eexcept *Vitis vinifera* which formed a separate group II-d in phylogenetic tree of protein sequences. In group I of monocots, two subgroups of 7 and 3 plants were formed. In group II of dicots, six subgroups were formed. *Vitis vinifera* and *Beta vulgaris* fell into two separate groups whereas, other groups contained 4,5 and 6 species of Brasicaceae, Solanaceae and Fabaceae respectively whereas, 5 genera clustered inII-e. Above mentioned results showed the highly conserved nature of TOR gene among different plant species at family level. The results obtained are in accordance with previous research finding reported by John et al. (2011) as they observed similar pattern of clustering during phylogenetic analysis of TOR proteins of different species included animal kingdom, fungi, algae and higher plants. In higher plants all the studied plants species are separated into two cluster i.e. monocots and dicots. The plants species *Oryzasativa*, *Sorghum bicolor and Zea mays* clustered together exhibited the similarity of results obtained during present studies. The amino acid sequences were found to be conserved among a wide variety of plants including all the cereals andin many dicots. Our results are also supported by another investigation carried out by Dobrenel et al. (2011) in which protein sequences of TOR kinase from plants, animals, yeasts, algae and moss were aligned and monocots and dicotsgrouped in separate clusters. The plants species

Table 1. List of source plants of *tor* genes utilized in investigation and their features

Plant	Accession number	Family	Size (bp)	GC%
Zea mays	NM_001111823	Poaceae	7691	44.80
Nicotiana sylvestris	XM_009797907	Solanaceae	6552	43.86
Setaria italica	XM_004962286	Poaceae	7899	45.80
Sorghum bicolor	XM_002439595	Poaceae	7544	44.55
Brachypodium distachyon	XM_003568577	Poaceae	8263	46.18
Oryza sativa	XM_015784081	Poaceae	7659	45.05
Oryza brachyantha	XM_006654108	Poaceae	7708	44.75
Oryza sativa	AB982929	Poaceae	7398	44.59
Elaeisguineensis	XM_010938396	Arecaceae	7722	44.28
Musa acuminata	XM_009396742	Musaceae	7722	44.51
Vitisvinifera	XM_002275555	Vitaceae	7791	44.15
Phoenix dactylifera	XM_008784352	Arecaceae	3530	43.63
Ricinuscommunis	XM_015718655	Euphorbiaceae	7993	43.69
Arachisipaensis	XM_016346885	Fabaceae	8105	44.45
Nicotiana tabacum	NM_001325223.1	Solanaceae	7488	44.78
Solanum tuberosum	XM_006346213.2	Solanaceae	7929	43.93
Capsicum annuum	XM_016726515.1	Solanaceae	7581	44.00
Citrus sinensis	XM_006486806.2	Rutaceae	7929	43.65
Nicotiana tomentosiformis	XM_009629165.1	Solanaceae	6982	43.81
Jatropha curcas	XM_012237807.1	Euphorbiaceae	6910	43.14
Gossypiumhirsutum	XM_016860166.1	Malvaceae	7904	44.00
Arachisduranensis	XM_016109414.1	Fabaceae	8144	44.51
Prunusmume	XM_008238021.2	Rosaceae	7991	44.50
Glycine max	XM_003538286.3	Fabaceae	8287	42.97
Vigna radiata	XM_014665082.1	Fabaceae	8372	43.11
Cicer arietinum	XM_004511268.2	Fabaceae	8411	42.42
Beta vulgaris	XM_010693230.1	Amaranthaceae	8031	43.71
Brassica rapa	XM_009149609.1	Brassicaceae	7800	44.44
Brassica napus	XM_013786769.1	Brassicaceae	7581	44.60
Brassica napus	XM_013893643.1	Brassicaceae	7880	44.37
Brassica oleracea	XM_013736055.1	Brassicaceae	7630	44.39
Phaseolus vulgaris	XM_007157127.1	Fabaceae	4186	43.17

Oryza sativa subsp. Japonica, *Oryza sativa* subsp. Indica, *Bracypodiumdistachyon*, *Sorghum bicolor and Zea Maysare* grouped together and formed the separate cluster of monocot species which showed similarity with results obtained during present investigation.

During phylogenetic analysis and sequence similarity search of wheat TOR gene with other plant species Sapre et al. (2016) observed the same clustering pattern of plant species as obtained in this investigation. The results of this investigation are not contradicted the results obtained by Nanjareddy et al. (2016) during the phylogenetic analysis of bean TOR gene which confirmed that this gene belongs to the legume group and is closely related to the *G. max* and *M. truncatula* TOR genes which showed the highly conserved nature of TOR gene at family level. In our studies all the plants species including in present investigation are clustered together according to their family showed the similar nature of gene as mentioned by Nanjareddy et al. (2016).

CONCLUSION

During present investigation phylogenetic analysis was carried out separately among TOR kinase gene sequences and amino acid sequences of 32 plants retrieve from public repository database NCBI. The phylogenetic tree of

TOR gene and its homolog's from several plants including both monocots and dicots revealed a close relationship between plant species.All sequences were grouped separately in two groups i.e. monocots and dicots. Further, sequences from similar family grouped together perfectly reflect the conserve nature of TOR kinase gene at nucleotide and protein sequence level.This strong conservation of TOR geneamong all the studied plant speciesadvocates the general significance of this kinase and, consequently,the entire TOR pathway.

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