Australian Journal of Crop Science AJCS
AJCS 9(3):215-222 (2015) ISSN:1835-2707

Genome-wide identification of miRNAs in pigeonpea (*Cajanus cajan* L.)

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Abstract

MicroRNAs (miRNA) are endogenous small RNAs that play essential roles in plant growth, development and response to biotic and abiotic stress. With the availability of draft genome sequence of pigeonpea, understanding miRNA repertoire of several crops has begun to be facilitated. In the present study, we have attempted to find miRNA sequences in pigeonpea using genome-wide computational approaches. Further whole genome sequence (WGS) based comparative studies using homology and secondary structure analysis was done. A total of 142 potential conserved miRNAs belonging to 48 families were identified and considered for this study. The size of these 48 miRNA families ranged from one to ten members while the length of miRNAs ranged from 19 nt to 24 nt. Furthermore pigeonpea pre-miRNA sequences were identified which varied from 62 to 203 nt while these sequences were found to have high negative minimal folding free energy (MFE), adjusted MFE (AMFE) and MFE index (MFEI) which is in agreement with the published data from crop sequences. Furthermore, this criterion distinguishes miRNAs from other coding and non-coding RNAs. Among the miRNAs, Uracil was found to be dominant nucleotide base in the first position at the 5' end of the mature miRNAs. A total of 423 potential miRNA targets were identified for newly identified pigeonpea miRNAs using psRNATarget tool. These target genes include a number of transcription factors that control plant growth and development, linked to metabolic enzymes involved in stress response. We believe these identified miRNA target genes would help us to know more about the important roles of miRNAs, suggesting that genome-wide computational analysis is a good alternative strategy for identifying new miRNAs and their targets.

Keywords: miRNAs, target validation, stress related genes, plant growth.

Introduction

Pigeonpea (*Cajanus cajan* L.) is one of economically important edible legume crops belonging to Fabaceae family and has immense agricultural and medicinal value. About 90 % of the world production of pigeonpea is grown in India. Because of its high value protein content, it plays a role in the vegetarian diet and nutrition of major population of India and Eastern Africa. The presence of flavonoids and isoflavonoids makes pigeonpea one of the important medicinal plants reported to be beneficial in treatment of various ailments. A member of leguminaceae, pigeonpea enriches soil through symbiotic nitrogen fixation. Furthermore, it is very drought resistant making it to be grown in areas with less annual rainfall. In spite of considerable progress achieved in terms of increasing the productivity in the crop through conventional breeding approach, application of biotechnological and genomics tools are still infancy in pigeonpea. With the recent draft genome sequence available, it is important to exploit this information for understanding metabolic processes in pigeonpea (Varshney et al., 2011; Singh et al., 2012). A specific class of non-coding RNAs (ncRNAs), *viz.* microRNAs (miRNAs) are abundant in all eukaryotes known to regulate majority of biological processes. The miRNA genes loci represent about 1-2% of eukaryotic genome and constitute an important class of fine-tuning regulators that are involved in several physiological and cellular processes. MiRNAs (miRNAs) are small about 21 nt in length and usually transcribed from RNA polymerase II with the polymerase often binding to a promoter. The biogenesis of miRNAs and conserved pathways has been well discussed in plants (Millar and Waterhouse 2005; Jones Rhoades et al., 2006; Voinnet 2009; Meng et al., 2011; Jones Rhoades 2012). With an approximate 60% of the human genes known to be regulated by miRNAs, research on miRNAs have caught a tremendous interest in other organisms (Friedman et al., 2009). A total of 24521 miRNAs have been identified from 206 species so far including plant, animal, virus and fungi. There is still a dearth of perceptible factor for miRNAs considering the large numbers of available miRNAs. From miRBase (release 19.0; August 2012; *Mirbase:* http://www.mirbase.org/index.shtml), a set of 6009 plant miRNAs were reported from species with fully sequenced genomes or the plants having large number of ESTs and GSSs including *Arabidopsis thaliana* (Wang et al., 2004), *Arabidopsis lyrata* (Ma et al., 2010; Fahlgren et al., 2010) *Brassica napus* (Wang et al., 2007; Xu et al., 2012), *Brassica oleracea*, *Brassica rapa*, *Brachypodium distachyon* (Unver and Budak et al., 2009), *Citrus aestivum* (Song et al., 2009), *Cucumis melo* (Gonzalez-lbeas et al., 2011), *Chlamydomonus reinhardtii*, *Glycine max* (Subramanian et al., 2008; Zhang et al., 2008), *Gossypium herbecium*, *Gossypium hirsutum* (Ruan et al., 2009), *Malus domestica* (Varkonyi-Gasic et al., 2010), *Medicago truncatula*, *Nicotiana tabacum* (Frazier et al., 2010), *Oryza sativa* (Sanan-Mishra et al., 2009; Jian et al., 2010; Li et al., 2010), *Pinus taeda*, *Populus tricocarpha* (Barakat et al., 2007), *Polpulus euphratica* (Li et al., 2011) *Phaseolus vulgaris* (Arenas-Huertero et al., 2009) *Solanum lycopersicum*, *Sorghum bicolor*, *Triticum aestivm* (Han et al., 2009; Xin et al., 2010), *Vitis vinifera* (Pantaleo et al., 2010), *Vitis amurensis Rupr*. (Wang et al., 2012) and Z*ea mays* (Zhang et

al., 2009). The miRNAs are also known to play diverse and complex roles in plant development, *viz.* developmental regulation (Kidner and Martienssen 2005; Glazinska et al., 2009; Arenas-Huertero et al., 2009; Li et al., 2010), flowering time (Glazinska et al., 2009), nodulation (Subramanian et al., 2008), epigenetic modifications (Vaucheret 2006), mRNA splicing, biotic and abiotic stress responses (Sunkar et al., 2012), protein synthesis, seed germination (Wang et al., 2011), continuous cropping (Yang et al., 2011) and chromatin modifications (Verdel et al. 2009). While miRNAs are evolutionarily conserved from mosses to higher plants, a significant number of them have also been recognized as species-specific non-conserved miRNAs (Jones-Rhodes et al., 2006; Sanan-Mishra et al., 2009; Xin et al., 2010; Wang et al., 2011). Some of the non-conserved miRNAs are integrated into plant-specific regulatory networks implying that there is a regulation of tissue-specific pathways. This strengthens the hypothesis that the conserved nature of miRNA sequences has the basis for homologous identification of miRNAs in other plants species. Many target genes of miRNAs encode transcription factors, each of which further regulates a set of downstream genes (Bartel 2004; Zhang et al., 2006). The majority of plant miRNAs studied till date negatively regulates the target gene expression at the post-transcriptional level. While miRNAs are known to interfere in expression patterns, they manifest in altered agronomic characters allowing the candidate genes carrying these traits towards development of useful transgenic plants (Perez-Quintero et al., 2010; Liu and Chen 2010; Sablok et al., 2011; Zhou and Luo 2013). Computational strategies developed based on this principle provide a valuable and efficient approach to predict miRNA genes and their targets. This approach has been preferred over experimental approaches such as direct cloning and deep sequencing, because of the reason that it is low cost and the basic requirement being the sequence data is available in public databases that are utilized to mine the putative miRNA sequences. However, this method is limited by the number of sequences available in the database. Identification of miRNAs using bioinformatics tools in the recent past has proved to be highly efficient, fast and comprehensive method. Finding homologous sequences of known miRNAs both within a single genome and across genomes of related organisms has steadfastly grown. While the major advantage of the method is the ability to identify miRNAs independent of their abundance or spatial and temporal expression pattern, there is a firm belief that identification of new miRNAs and their targets in pigeonpea will help in identifying key players in the regulation of plant developmental pathways. In this work, we report genome-wide analysis of pigeonpea draft genome to identify conserved miRNAs and their targets.

Results

Identification of pigeonpea miRNAs

For the computational identification of conserved miRNAs in pigeonpea, a reference set of 2437 non-redundant plant miRNAs was searched against whole genome sequence of pigeonpea (*See reference*). Sequences with less than four mismatches to the miRNAs were subjected to Mfold 3.2 for screening based on secondary structure. A set of 142 potentially conserved miRNAs were identified (Supplementary Table 1). Although mature miRNA sequences are highly conserved among plants, we found that a majority of these miRNAs having 1 to 4 mismatches were compared to miRNAs in other plant species. Of the 142 pigeonpea miRNAs identified, 68 (~48%) were located in 5' arm of the stem-loop hairpin structure while 74 (~52%) were located in 3' arm, suggesting that pigeonpea miRNAs are located in both the arms of the structure void any preference (Supplementary Table 1). This property of miRNAs is in agreement with plant species in which mature miRNAs are typically confined to the stem-loop hairpin structure (Zhang et al., 2008; Sanan-Mishra et al., 2009; Han et al., 2009; Frazier et al., 2010; Wang et al., 2012). On the basis of similarity in mature miRNA sequences, the miRNAs were grouped into families with members frequently variable in 1-2 nucleotides. The identified 142 miRNAs belonged to 48 families with the number of miRNAs in each family ranging from one to ten whereas from our annotation, the miRNAs were found to be distributed randomly (Fig 2). Again this type of distribution pattern is in consensus with confined miRNAs in other plants (Zhang et al., 2008; Zhang et al., 2009; Wang et al., 2012). Major characteristics of the potentially conserved miRNAs are varied amongst families with the size evenly distributed (Fig 2 and 3). Additionally we observed that among the miRNAs U (uracil) was the more dominant nucleotide base in the first position at the 5' end of the mature miRNAs (Fig 4). Pigeonpea pre-miRNAs sequences showed great variability in their length from 62 to 203 nt (Supplementary Table 2 and Supplementary Table 3) with an average length of 109 $\pm$ 34 nt. Nevertheless, the majority of the pre-miRNA sequences were only between 80 -100 nt in length and accounted for 43.6% of the total pigeonpea pre-miRNAs (Fig 5). The length distribution of pre-miRNAs in pigeonpea is similar to those reported for other plant species such as soybean, cotton, maize, tobacco, wheat and *Porphyra yezoensis* (Zhang et al., 2008; Zhang et al., 2009; Han et al., 2009; Liang et al., 2010; Frazier et al. 2010; Wang et al., 2012). Cca-MIR4416a exhibited the shortest precursor length of 62 nt, whereas Cca-MIR159b exhibited the longest precursor length of 203 nt. The percentage compositions of the four nucleotides (A, C, G and U) in pigeonpea pre-miRNAs were not equal. Uracil was dominant and comprised 29.8 $\pm$ 4.6% of the total nucleotide composition while adenine constituted 24.9 $\pm$ 4.7% followed by guanine at 23.9 $\pm$ 4.9% and cytosine at 21.3 $\pm$ 4.2% (Supplementary Table 3). The nucleotide composition of the identified potential pigeonpea pre-miRNA precursor sequences had (A+U) content ranged from 27.37 to 72.09 with an average of 54.8 $\pm$ 7.25 and (G+C) content ranging from 27.9 to 72.6 with an average of 45.25 $\pm$ 7.25 (Supplementary Table 3). The average A/U ratio of the potential pigeonpea pre-miRNA precursor sequences was 0.85 $\pm$ 0.20 (Supplementary Table 3). The identified pigeonpea pre-miRNA sequences can be found in supplementary table 6.

Prediction of potential pigeonpea miRNA targets suggests that the stem-loop structure is not unique to miRNAs alone

We observed that pigeonpea pre-miRNAs have negative MFEs which ranged from 14.20 to 96.5 kcal/mol. (Supplementary Table 3 and Fig 6). However, MFEs are strongly and positively correlated with their pre-miRNA sequence length. Longer the pre-miRNA sequences, more the degree of freedom (and lower the MFEs) with which the sequences have to form stable secondary structures. To normalize the potential bias caused by the pre-miRNA sequence length on MFE, adjusted MFE (AMFE) was calculated (Zhang et al., 2006). While AMFE of pigeonpea

Table 1. Six selected *Cajanus* miRNAs with their corresponding predicted target genes. Complementary base pairing is indicated by *solid lines* whereas G:U wobble base pairing is indicated by *dotted lines*

Cca-MIR156a (3'-5') squamosapromoter-binding-like protein (5'-3') (gi|352914255|gb|AFSP01036324.1)	miRNA 21 ACACGAGUGAGAGAAGACAGU 1 ||||||| ||||||||||| Target 385 UGUGCUCUCUCUCUUCUGUCA 405
Cca-MIR159a (3'-5') auxinresponse factor (5'-3') (gi|352948424|gb|AFSP01002448.1)	miRNA 21 GCCUCGAGGGAAGUGAGGUUA 1 | ||||||||||| :|||| Target 385 CUGAGCUCCCUUCACGUCAAU 405
Cca-MIR164c (3'-5') NAC domain protein (5'-3') (gi|352949819|gb|AFSP01001053.1)	miRNA 20 CGUGCACGGGACGAAGAGGU 1 ||| |||||||||||| Target 386 GCAAGUGCCCUGCUUCUCCA 405
Cca-MIR171a (3'-5') SCARECROW-like protein (5'-3') (gi|352937037|gb|AFSP01013542.1)	miRNA 21 CUAUAACCGUGCCGAGUUAGU 1 ||||||||:|||||||||| Target 386 GAUAUUGGCGCGGCUCAAUCA 406
Cca-MIR172a (3'-5') floralhomeotic protein apetala 2-like (5'-3') (gi|352913152|gb|AFSP01037427.1)	miRNA 22 ACGUCGUAGUAGUUCUAAGAGU 1 ||||||||||||:||||| | Target 389 UGCAGCAUCAUCAGGAUUCCCU 410
Cca-MIR162a (3'-5') ABC transporter a family member 2-like (5'-3') (gi|352940346|gb|AFSP01010526.1)	miRNA 20 ACCUACGUCUCCAAAUAGCU 1 |||| ||||| ||||||| Target 408 UGGAAGCAGAUCUUUAUCGA 427

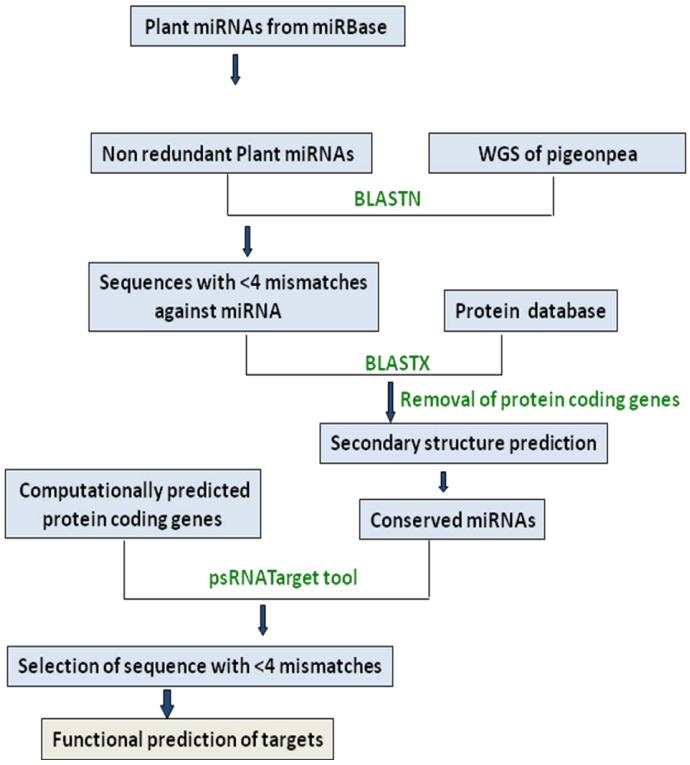

Fig 1. Flow chart for Genome wide identification of microRNAs from pigeonpea (*Cajanus cajan* L.).

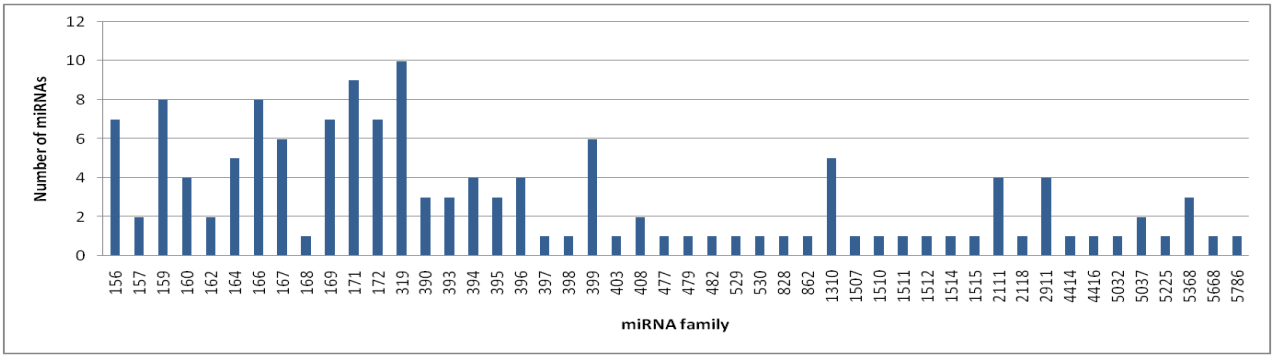

Fig 2. Size of miRNA families in *Cajanus.*

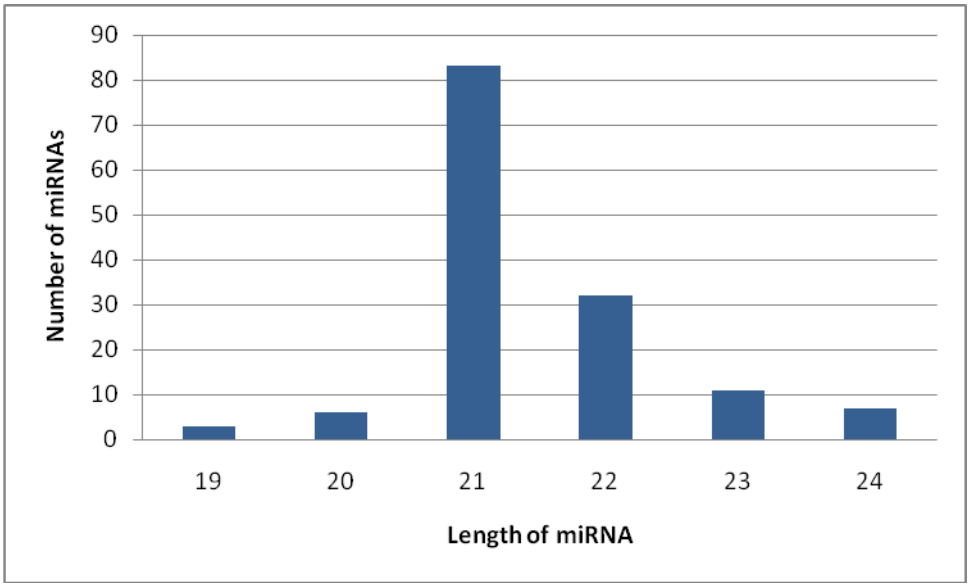

Fig 3. Size distribution of miRNAs in *Cajanus.*

pre-miRNAs ranged from 16.54 to 62.32 kcal/mol with an average of 45 ± 8.18 kcal/mol (Supplementary Table 3 and Fig 7), we found that pre-miRNAs have high negative MFEs and AMFEs suggesting that there is no significant difference between MFEs and AMFEs of pre-miRNAs and other RNAs (Zhang et al., 2006). Furthermore, to better distinguish miRNAs from other RNAs, MFE index (MFEI) was calculated which combines the three important parameters of RNAs: MFE, length of pre-miRNA and G+C %. All previous studies have reported that MFEI of pre-miRNA precursors was significantly higher than that of other types of RNAs; candidate RNA sequence is more likely to be an miRNA when the MFEI is greater than 0.85. MFEI of pigeonpea pre-miRNAs ranged from 0.49 to 1.46 kcal/mol with an average of 1.01 ± 0.19 kcal/mol with a 81% (115) of the identified pigeonpea miRNAs having an MFEI value higher than 0.85 (Supplementary Table 3 and Fig 8). In the present study, we observed high conserved nature of 169 family pre-miRNAs among different plants (Fig 9). All the predicted secondary structures can be found in supplementary fig 1.Mature miRNAs control the gene expression at the post transcriptional level by binding mostly to mRNAs within coding sequence or sometimes to UTRs which are located in the beginning or at the end of coding sequences leading to target mRNA cleavage or translational repression respectively. A total of 423 potential miRNA targets were identified in pigeonpea using psRNATarget server with at least one target mRNA identified for most of the pigeonpea miRNA families (Table 1). These targets belonged to a variety of gene families with diverse biological and physiological functions. Majority of the identified target mRNAs were found to be transcriptional factors, while other were involved in metabolism and development and response to biotic and abiotic stresses (Supplementary Tables 4 and 5). Again, these results were in agreement with previously reported plant species such as maize, tobacco, wheat, soybean, Arabidopsis, *Euphorbiaceae* plants and citrus (Wang et al., 2004; Zhang et al., 2008; Arenas-Huertero et al., 2009; Zeng et al., 2010; Song et al., 2009; Zhang et al., 2009; Han et al., 2009; Frazier et al., 2010). Prominent transcriptional factors targeted by miRNAs include MYB, ARF, LRR protein and WRKY that are known to regulate plant development. Other conserved miRNA targets include Squamosa promoter-binding (miR156), Auxin response factor (miR159), ABC transporter (miR160), NAC domain protein (miR162 and 408), SCAREROW-like protein (miR169 and 477), APETALA2 (miR172). These transcription factors are known to play role in the control of expression of the genes involved in regulation of metabolic processes.

Discussion

Profitable cultivation of the crop is plagued by the problems of insects-pests and diseases. Many germplasm lines have been identified as potential source of resistance against biotic and abiotic stresses. It has been shown that majority of the plant metabolic processes and responses to environment are

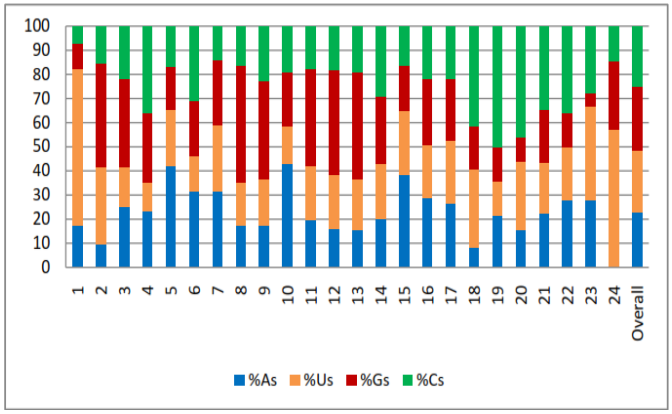

Fig 4. Nucleotide frequency of pigeonpea mature miRNAs

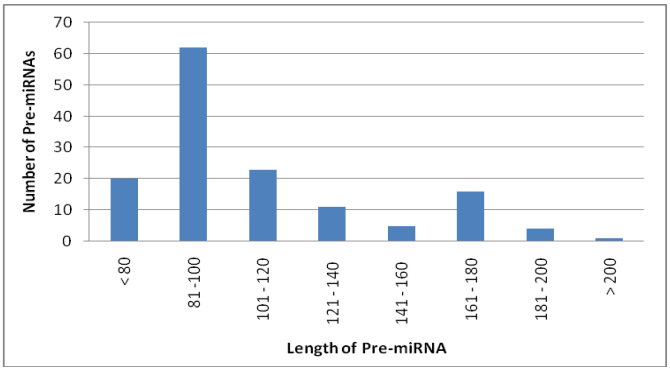

Fig 5. Size distribution of pre-miRNAs in *Cajanus*

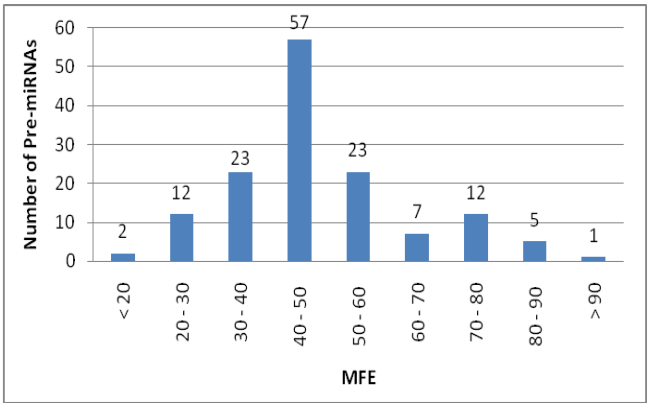

Fig 6. MFE distribution pattern in *Cajanus*

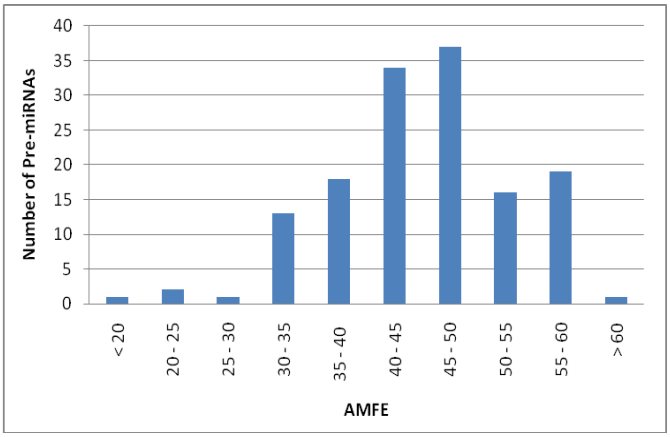

Fig 7. AMFE distribution pattern in *Cajanus.*

controlled by miRNAs (Khraiwesh et al., 2012). Identification of miRNAs and their targets in pigeonpea will help not only in understanding the mechanism of control of cellular processes but also help in controlling the traits. In this report, we have used pigeonpea genome sequence to predict miRNAs and their targets and found 142 potential miRNAs belonging to 48 families. Newly identified pigeonpea miRNAs have similar characteristics to the miRNAs identified in other plant species. Our report is in consensus with the fact that U being a predominant nucleotide at the 5' end of mature miRNA sequences may play an important role in biogenesis through recognition of targeted miRNAs by the RNA-induced silencing complex (Zhang et al. 2006; Zhang et al., 2008; Wang et al., 2012). Furthermore, the distribution of miRNAs among various miRNA families in pigeonpea is similar to other plant species, such as soybean, cotton, maize and tobacco (Zhang et al., 2008; Zhang et al., 2009; Frazier et al., 2010; Wang et al., 2012). This uneven distribution of miRNAs may have different evolutionary history and play a major role in pigeonpea plant growth and development. Several studies as discussed earlier have proved that pre-miRNAs have negative MFE values while we identified pre-miRNAs with MFEI values greater than 0.85 suggesting that majority of pre-miRNAs were likely to produce mature miRNAs. To dodge the regulation by miRNAs *via* structural genes, we searched candidate targets of pigeonpea miRNAs using psRNATarget tool with 142 identified miRNAs against computationally predicted protein coding genes. A total of 423 potential miRNA targets were identified and most miRNA genes were found to have multiple target sites suggesting that these conserved miRNAs may be functionally divergent. Most of these identified potential miRNAs play an important role by regulating genes which are mostly involved in plant developmental pathways and response to biotic and abiotic stress responses. Analysis and annotation of the predicted target genes showed that they were with diverse functions, ranging from genes encoding enzymes involved in metabolism, genes regulating transport, genes encoding various kinases, and genes encoding isomerase and helicase. Further confirmation and validation of the identified targets is needed and would help us gain insight into the roles these newly identified miRNAs play during pigeonpea development. These results demonstrated that miRNAs identified in pigeonpea are conserved in nature. From an evolutionary view, it is plausible that the conserved miRNAs are keen to play role in cellular and developmental pathways. Transcription factors are important in controlling gene expression even as they bind to enhancer or promoter regions of DNA adjacent to the genes that they regulate. Based on the functional studies conducted in model crops, it was deduced that a number of miRNAs identified in pigeonpea target transcriptional factors that regulate plant development and are conserved (Zeng et al., 2010). In this study, we found that miR-172 targets the APETALA2 gene in pigeonpea suggesting that the function of a miR-172 is highly conserved among plants. Our analysis revealed that most of the predicted targets in pigeonpea have conserved function with miRNA targets in Arabidopsis and a wide variety of plant species. Consistent with previous reports, most of these targets in pigeonpea were plant specific transcription factors. Furthermore pigeonpea miRNA targets have a miRNA-complementary site located in their coding region. The expression of miRNAs and their target gene pattern might provide clues about miRNA functions in plant development. Comprehensive characterization of all the identified pigeonpea miRNAs and their targets genes in different tissues

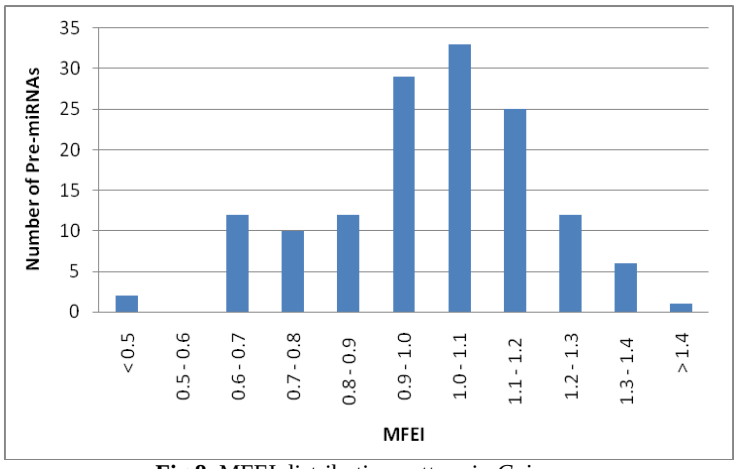

Fig 8. MFEI distribution pattern in *Cajanus.*

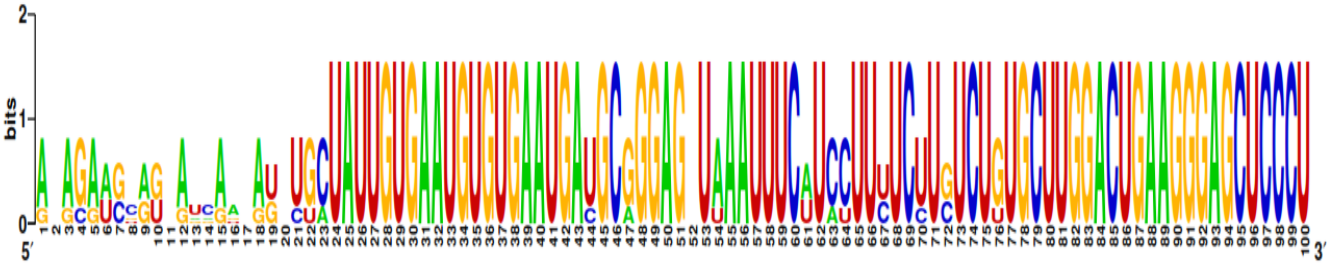

Fig 9. Highly conserved nature of pre-miRNA 169 in different plants.

would be helpful to understand the tissue specific expression of all the miRNAs. The observation that experiments may not divulge in identification of new targets has been subtly discussed earlier (Schwab R et al. 2005). However, *in silico* based prediction of other types of targets specifically involved at the phase of translation or post translation and which may not be identified experimentally might prove to be useful. Thanks to numerous *in silico* approaches where we are able to identify extensive complementary regions to the miRNAs with *bona fide* predictions with the computational methods that can be applied to plants.

Materials and Methods

Plant species and reference datasets

Pigeop Pea (Cajanus cajan L)

A total of 6009 miRNAs from different plant species deposited in miRBase (release 19.0, August, 2012 *Mirbase:* http://www.mirbase.org/index.shtml) were downloaded. Among them 2437 non-redundant plant mature miRNA sequences including 483 from *Arabidopsis* species, 67 from *Brachypodium distachyon*, 268 from *Glycine max*, 308 from *Medicago truncatula*, 428 from *Oryza sativa*, 187 from *Psychometrilla patens*, 75 from *Populus* species, 208 from *Saccharum* species, 55 from *Selaginella moellendorffii*, 81 from *Vitis vinifera*, 147 from *Zea mays* and the remainder from other plant species like *Brassica species, Citrus species, Sorghum bicolour, Solanum lycopersicum*, and *Triticum aestivum* were used as a reference set to identify their peers in pigeonpea. A complete set of 72,922 pigeonpea whole genome shotgun assembly sequences were used in the present investigation.

Whole Genome Sequence-based comparative genomic resources

Homology based tools, *viz.* BLASTN and BLASTX from NCBI (*see reference*) were used for comparing mature miRNAs against pigeonpea whole genome sequence and eliminating protein coding sequences respectively. Sequence surrounding the miRNA hit regions in all WGS was extracted using in-house written Perl script. The prediction of secondary structures of miRNA precursors were performed using the web-based Mfold server (*Zuker M 2003;* http://mfold.rna.albany.edu). The mature non-redundant miRNA sequences were subjected to a BLASTN search against all available pigeonpea WGS assemblies. To improve the BLASTN search, the parameters settings were adjusted to e-values set at 1,000 to increase the number of potential hits; the default word-match size between the query and database sequences was set at seven; and the number of descriptions and alignments were increased to 1,000. If the BLASTN search revealed partial sequence similarity to any miRNA sequence, the non-aligned regions were manually checked and compared to determine their potential for coding miRNAs. The precursor sequences of approximately 800-nt were extracted (390 nt upstream and 390 nt downstream to the BLAST hit region) and checked for the formation of stable hairpin secondary structure prediction. All the sequences with no more than 4 mismatches were selected for further investigation. An 800-nt sequence surrounding the miRNA hit region in all WGS was extracted using in house written perl script. The extracted sequences were searched for comparing against each other to remove repeated sequences so as to retain only non-protein coding sequences. Selected non-protein coding sequences were subjected to prediction of secondary structure as well as minimal folding energy (MFE) using web-based Mfold tool (Zuker, 2003). The sequence was considered as potential miRNA candidate if the predicted

mature miRNA has not more than 4 mismatches compared with a known mature miRNA, the selected sequence could fold into an appropriate stem-loop hairpin secondary structure, the mature miRNA localized in any one arm of the stem-loop structure, no loop or break in the miRNA or miRNA* sequences, no more than 6 mismatches between the predicted mature miRNA sequence and its opposite miRNA* sequence in the secondary structure and the predicted secondary structure has high negative MFE and Minimal Folding Energy Index (MFEI) values. All the Mfold output of the sequences that satisfied the aforementioned criteria were exported into an excel file, (the length of mature miRNA, length of pre-miRNA, number of each nucleotide (A, G, C and U), (A+U) %, (G+C) %, and minimal folding energy (MFE)). To avoid the potential effect of nucleotide length on MFEs, adjusted MFE (AMFE) values, adjusted minimal folding energy (AMFE) and minimal folding energy index (MFEI) were calculated according to Zhang et al., 2009 using this formulae AMFE = (MFE/ length of pre-miRNA)*100 and MFEI = AMFE/ (G+C) percentage. Previous reports indicated that precursor miRNA sequences have considerably higher MFEI compared to coding or non-coding sequences and the candidate miRNA sequence are more likely to be miRNAs when the MFEI was greater than 0.85 (Frazier et al., 2010; Zhang et al., 2008; Song et al., 2009). We followed the same in identifying the putative pre-miRNA sequence. The Plant Small RNA Target Analysis Server (psRNATarget) was used to predict miRNA targets in the pigeonpea against computationally predicted protein-coding gene database (Dai and Zhao, 2011). Due to limited number of protein-coding sequences available for pigeonpea, target search against protein database of other model plant species was used to identify potential target genes (Fig 1*)*.

Conclusions

We have mined miRNA target genes of pigeonpea which include a number of transcription factors that control plant growth and development. This study is attempted to know more about the important roles of miRNAs, suggesting that genome-wide computational analysis is a good alternative strategy for identifying new miRNAs and their targets. Our results may apparently are *in silico* mined but we believe this identification of conserved miRNAs has resulted in significant enrichment of the repertoire of pigeonpea miRNAs further providing rich insights into miRNAs regulation of genes expressed in pigeonpea.

Acknowledgements

The authors gratefully acknowledge Velu Mani Selvaraj, Directorate of Oilseeds Research, Hyderabad towards pictorial representation of figures and analysis.

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