Computational prediction of microRNA genes and their targets in the Beluga genotype of Common Bean

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is one of the most agriculturally and economically important legumes known for its high protein and fiber contents. Beluga is a large seeded, well adapted full season white kidney bean variety, which blooms at 37 days after planting (DAP), with a maturation range of 100 - 108 DAP, and immune to diseases such as rust, anthracnose, and Bean Common Mosaic Virus (BCMV). This study was aimed at understanding the expression and roles of microRNAs (miRNAs) in Beluga at 50% and 100% maturity. The libraries for small RNAs isolated at 50% and 100% seed maturity in Beluga were prepared and sequenced on Illumina HiSeq 2500. The quality of small RNA reads was tested by using FastQC and further analyzed for miRNAs utilizing CLCBio Genomics Workbench and miRPlant. The gene targets for known and novel miRNA classes were identified by using psRNATargets. In total, ~100 million small RNA reads were sequenced but only less than ten percent of these were annotated after eliminating other classes of RNA. In this study, eight (8) most abundantly expressed miRNA families including miR166, miR319, miR399, miR482, miR519, miR1514, miR2118, and miR2119, and ~150 novel miRNAs were identified in Beluga at 50% maturity and 100% maturity. However, the level of known miRNAs was highest at 50% maturity than at 100% maturity, suggesting that these miRNAs play important role in early maturity. Further, the novel miRNA classes and targets predicted in Beluga may aid in determination of miRNAs involved in transition from vegetative to reproductive phase.

MATERIALS & METHODS

Plant material

- Beluga, a well adapted and large seeded white kidney bean variety was developed in 1998 jointly by Michigan Agricultural Experiment Station and USDA-ARS by crossing the Italian Borlotto bean with the Lassen white kidney bean cultivars.
- The attributes of Beluga include: upright growth habit, full-season maturity, good canning quality, resistant to lodging, immune to rust, resistant to race 65 and 73 of anthracnose, and Bean Common Mosaic Virus (BCMV).

microRNAs (miRNAs)

- **Biogenesis**: miRNA genes are transcribed by RNA polymerase II to produce primary-miRNA transcript (Pri-miRNA), which are processed in the nucleus by Drosha and DGCR8 to yield precursor-miRNA (pre-miRNA) and are exported into the cytoplasm by exportin 5. In the cytoplasm, the pre-miRNA is processed by Dicer enzyme to generate mature miRNA, which further targets genes (mRNA) by high sequence complementarity.
- miRNAs regulate several key physiological processes in plants.
- Recently, the roles of miRNAs have been reported in regulation of nutrient-stress and manganese toxicity in common bean.

Methods

- Small RNA extraction protocol
- Illumina HiSeq 2500
- Computational tools: FASTQC, CLCBio Genomics Workbench, miRPlant, and psRNATargets.

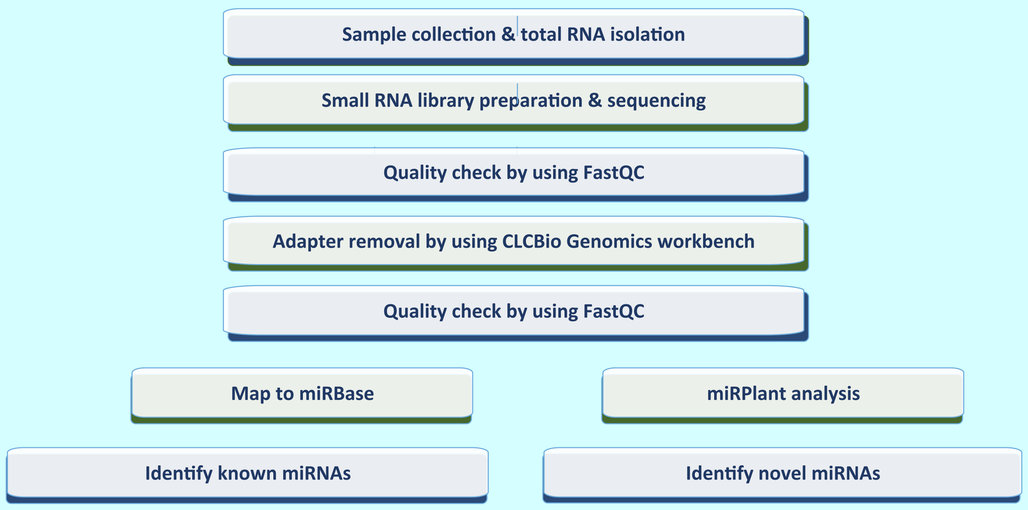

Figure 1. The schematic work flow of microRNA prediction in common bean

OBJECTIVES

1. To identify miRNAs at 50% maturity in Common Bean.
2. To identify miRNAs at 100% maturity in Common Bean.
3. To identify known and novel miRNAs and their targets.

RESULTS & DISCUSSION

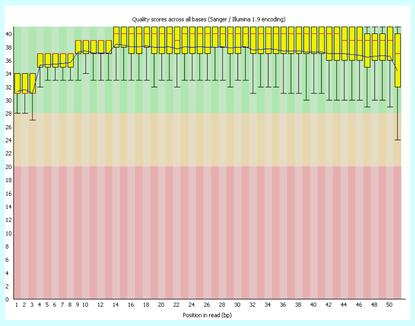

Figure 2a. QC before trimming

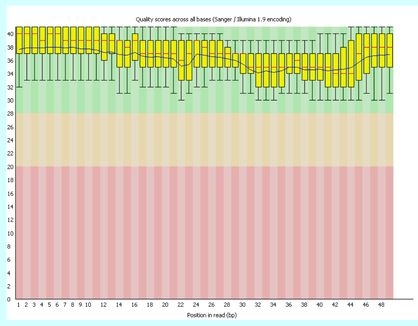

Figure 2b. QC after trimming

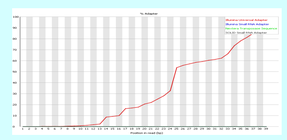

Figure 3a. Adapter contamination before trimming

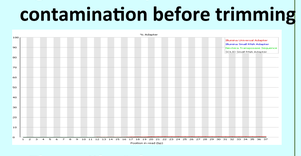

Figure 3b. Adapter content after trimming

Table 1. The most abundantly identified miRNAs in Beluga at 50% maturity

Name	Resource	Match type	Feature ID	Expression values	Exact mature	Mature	Unique exact mature	Unique mature
MIR159a	*Phaseolus vulgaris*	Mature 3'	TTTGGATTGAAGGGAGCTCTA	213115	46	213115	46	210625
MIR319c	*Phaseolus vulgaris*	Mature 3'	TTGGACTGAAGGGAGCTCCTT	136093	599	136093	599	133602
MIR166a	*Phaseolus vulgaris*	Mature 5'	TCGGACCAGGCTTCATTCCCC	41277	375	41277	375	41277
MIR2118	*Phaseolus vulgaris*	Mature 3'	TTGCCGATTCCACCCATTCCTA	15764	65	15764	65	15764
MIR482	*Phaseolus vulgaris*	Mature 5'	GGAATGGGCTGATTGGGAAGCA	8864	0	8864	0	8864
MIR1514a	*Phaseolus vulgaris*	Mature 5'	TTCATTTTGAAAATAGGCATTG	3313	23	3313	23	3313
MIR482	*Phaseolus vulgaris*	Mature 3'	TCTTCCCAATTCCGCCCATTCC	455	0	455	0	455
MIR399a	*Phaseolus vulgaris*	Mature 5'	TGCCAAAGGAGAGTTGCCCTG	328	1	328	1	328
MIR159a	*Phaseolus vulgaris*	Mature 5'	CTTCCATATCTGGGGAGCTTC	190	0	190	0	190
MIR2119	*Phaseolus vulgaris*	Mature 3'	TCAAAGGGAGTTGTAGGGGAA	66	0	66	0	66

Table 2. The most abundantly identified miRNAs in Beluga at 100% maturity

Name	Resource	Match type	Feature ID	Expression values	Exact mature	Mature	Unique exact mature	Unique mature
MIR159a	*Phaseolus vulgaris*	Mature 3'	TTTGGATTGAAGGGAGCTCTA	193264	71	193264	71	191190
MIR319c	*Phaseolus vulgaris*	Mature 3'	TTGGACTGAAGGGAGCTCCTT	136211	867	136211	867	134137
MIR166a	*Phaseolus vulgaris*	Mature 5'	TCGGACCAGGCTTCATTCCCC	24858	69	24858	69	24858
MIR482	*Phaseolus vulgaris*	Mature 5'	GGAATGGGCTGATTGGGAAGCA	18766	0	18766	0	18766
MIR2118	*Phaseolus vulgaris*	Mature 3'	TTGCCGATTCCACCCATTCCTA	8532	24	8532	24	8532
MIR1514a	*Phaseolus vulgaris*	Mature 5'	TTCATTTTGAAAATAGGCATTG	5696	38	5696	38	5696
MIR482	*Phaseolus vulgaris*	Mature 3'	TCTTCCCAATTCCGCCCATTCC	580	1	580	1	580
MIR159a	*Phaseolus vulgaris*	Mature 5'	CTTCCATATCTGGGGAGCTTC	416	0	416	0	416
MIR399a	*Phaseolus vulgaris*	Mature 5'	TGCCAAAGGAGAGTTGCCCTG	132	0	132	0	132
MIR2119	*Phaseolus vulgaris*	Mature 3'	TCAAAGGGAGTTGTAGGGGAA	6	0	6	0	6

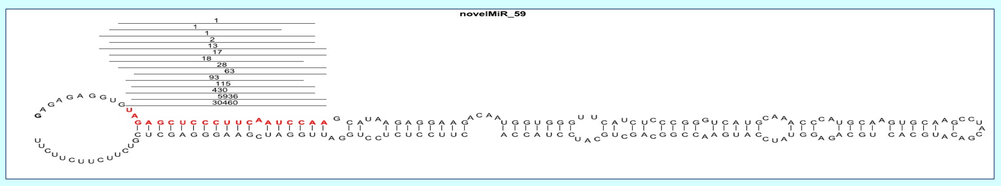

Figure 4. Representative hairpin structure of novel miRNA predicted in common bean

- Eight (8) highly expressed miRNA families including miR166, miR319, miR399, miR482, miR519, miR1514, miR2118, and miR2119 were identified.
- In total, we identified about 150 novel miRNAs in Beluga at 50% and 100% maturity.

Future direction of work

- The prediction of gene targets for novel miRNA classes in Beluga may aid in determination of miRNAs involved in transition from vegetative to reproductive phase.
- Identification of stress-responsive miRNAs in common bean.

CONCLUSION

- This study identified over 20 miRNA families and ~150 novel miRNAs in the Beluga variety of common bean using computational tools.

REFERENCES

- Kang, Wenjing, and Friedländer, Marc R. *Computational prediction of miRNA genes from small RNA sequencing data*. 1st ed. Stockholm: National Center for Biotechnology Information, 2015. Web. 30 June 2015.
- Kelly, J.D, and Copeland, L.O. *Beluga: A New Alubia Bean For Michigan*. 1st ed. Lansing: Michigan State University Extension, 1998. Web. 29 June 2015.

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