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Role Of MicroRNAs In Adaptive Responses Of *Lablab Purpureus* To Phosphate Deficiency

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Abstract

Nutrient stress, either low or excessive availability of essential nutrients is one of the crucial environmental stresses that encumber plant growth, development, and consequently reduce crop yield. Phosphorus, a vital macronutrient is one of the limiting factors for plant growth. Responses of hyacinth bean to phosphate deficiency were evaluated in terms of lateral root formation, secretion of acid phosphatase and microRNA (miRNA) expression profile which is known to account for maintenance of phosphate homeostasis. While lateral roots and secretion of acid phosphatase were increased, miRNA expression showed mixed response. Wherein, miRNA 164 and miRNA 398 were down-regulated and miRNA 399 was up-regulated. NAC1 and 1-Aminocyclopropane-1-carboxylic acid oxidase the targets of miRNA 164 and miRNA398 respectively showed up-regulation, while UBC 24 target of miRNA 399 was down-regulated.

Key words: Hyacinth bean, phosphate starvation, acid phosphatase, differential expression, miRNA targets.

Abbreviations: P **(**Phosphorous), Pi (orthophosphate), miRNA (microRNA), RT-qPCR (Reverse Transcription quantitative PCR), ACC oxidase (1-Aminocyclopropane-1-carboxylic acid oxidase).

Introduction

Plants require at least 17 essential elements for normal growth and fecundity, of these non-mineral nutrients such as C, H and O are absorbed from air and water, rest are mineral nutrients provided by the soil through plant roots. Phosphorus is a vital primary mineral macronutrient, forming an integral component of genetic, metabolic, structural and regulatory molecules. Phosphorus (P) is the 11th most abundant element in the earth's crust, and its concentration in soils generally lies between 100 and 3,000 mg kg^{-1} soil [1]. Despite the abundance of phosphorous in the soil, its availability in the form of orthophosphate (Pi) for uptake by plants [2], is usually present at a low level in the soil due to its precipitation with cations and conversion to organic matter [3], [4], [5]. This

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makes phosphorous a common limiting factor for plant growth and crop production in arable soils. Concentration of phosphorus ranges from 0.1 to 0.5% of dry weight. Applications of chemical P fertilizers to agricultural land have improved soil P fertility and crop production. However, modern agriculture is dependent on phosphorus derived from phosphate rock, which is a non-renewable resource and current global reserves are estimated to be depleted in 50–100 years [6]. Also, excess application of P fertilizer results in eutrophication and affects biodiversity. Thus, it is imperative to adopt sustainable practices by understanding the mechanisms by which plants respond and adapt to the P-deficient growth medium, and minimize the use of P fertilizers.

Plants can overcome P deficiency by exhibiting adaptive mechanisms such as; modification of root architecture to maximize P acquisition [7], [8], enhanced expression of root Pi transporters [9], [10], acidification of root rhizosphere by exudation of organic acids to solubilize P that is bound with cations and other mineral ligands in the soil [11], and increased secretion of acid phosphatases from roots to hydrolyze soil organic P [12], [13]. Numerous P starvation-responsive genes and transcription factors involved in transcriptional regulation of response to phosphate deficiency have been identified [14], [15]. In recent past, miRNAs are investigated as regulatory molecules in plant responses to nutrient stresses via mediation of post-transcriptional regulation of various effector genes [16].

Lablab purpureus, commonly known as hyacinth bean is widespread as a food crop throughout the tropics, especially in India, Africa, Bangladesh, and Indonesia. *Lablab* is cultivated as fodder crop, as well as, cover crop. Its use as green manure provides organic matter, minerals and fixes nitrogen, thereby improving crop yields in an economic and environmentally friendly manner. As phosphorous is an important nutrient that determines the plant productivity, it was felt worthwhile to investigate, how hyacinth bean which has proven ability to sustain under abiotic stress, would respond to Pi starvation, and identify any significant player, which can be exploited for crop improvement in Pi deficient soils. Further from the view point of gene regulation under Pi deficiency, explicating possible role of miRNAs and their targets are subject matter of this study.

Materials and methods

Plant materials and stress conditions

The seeds of Hyacinth bean (cv. HA-4) were obtained from National Seed Project, University of Agricultural Sciences, GKVK, Bengaluru, India. Seeds were surface sterilized with 0.1% (w/v) mercuric chloride for 1 min, rinsed immediately with large volume of distilled water, and soaked overnight in distilled water. The overnight soaked seeds were sown in trays containing vermiculite and acid-washed sand (1:1 w/w) and watered daily, once. The germination was carried out under natural greenhouse conditions; day/night temperature and relative humidity were 30/25°C, and 75/70%, respectively. The

average photoperiod was 12 h light/12 h dark. The seedlings were grown in green house conditions for 10 days, after which they were transplanted into one-half-strength modified Hoagland solution containing 0.2 µM KH_2PO_4 and 1 mM KH_2PO_4, that provides limited Pi concentrations and normal Pi concentrations, respectively. Root samples were drawn after 2, 4, 6, 8, 10 days for further analysis.

Estimation of orthophosphate

Total phosphate was extracted from about 100 mg oven dried (70ºC for 3 days) root samples of seedlings at different duration of growth by acid digestion. Phosphate was estimated according to the method of Fiske - Subbarow (1925) [17], using KH_2PO_4 as standard.

Assay of *in-situ* root Acid Phosphatase activity

The acid phosphatase activity of intact roots was assayed using the method of Mclachlan (1980) [18]. Plant roots were washed in distilled water, blotted dry and then immersed in 50 mM acetate buffer (pH 5.2) containing 50 mM *p*-nitrophenyl phosphate at 30° C for 1 h. After 1 h an aliquot was transferred to test tubes containing 0.1 M NaOH solution. Blanks contained all reagents except the root sample. The amount of *p*-nitrophenol liberated was measured by recording the absorbance at 405 nm. Enzyme activity is expressed as µmoles of *p*-nitrophenol liberated per gram root fresh weight per hour.

Total RNA isolation and cDNA synthesis for characterization of miRNA

Total RNA was extracted by homogenizing root tissue in liquid nitrogen and pre-chilled Tri-Reagent (Sigma–Aldrich) at room temperature. The quality of total RNA was evaluated by electrophoresis on 1.0% agarose gel. The purity was assessed by reading the A260/ A280 ratios and A260/A230 ratios using Biomate 3S UV-Visible spectrophotometer (Thermo Scientific). The miRNA primers and cDNA synthesis for stem-loop RT-qPCR experiment was designed according to Chen et al. (2005) [19] and Varkonyi-Gasic et al. (2007) [20]. Briefly, 1µg total RNA and 50 nM stem–loop primers were mixed with RNase-free water to a total volume of 12.5 µL and incubated at 65°C for 5 min followed by ice-cooling. Then, 4 µL RT-Buffer, 2µL of dNTP mix 10 mM each (Thermo Scientific), 0.5 µL Ribolock RNase inhibitor (Thermo Scientific) and 1 µL RevertAid Reverse Transcriptase 200U (Thermo Scientific) were added to a final volume of 20 µL. Reverse Transcription reaction was performed on Eppendorf mastercycler by incubating at 42°C for 60 min, followed by 70°C for 10 min to inactivate the enzyme. The cDNA samples were stored at - 80°C until required.

Quantitative Real-time RT-PCR

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qPCR reactions were carried out with Biorad iQ5 Multicolor Real Time PCR Detection System, using Biorad SYBR green Supermix in a 20 µL reaction volume containing 0.5µL cDNA, 10 µL 2X SYBR Green mix, 0.5 µL of 1µM forward and 0.5 µl of 1µM reverse primer. The reaction conditions were: an initial denaturation step of 95°C/4min, followed by 40 cycles of 95°C/30s for denaturation, 58°C/30s for annealing and 72°C/20s for extension. Each reaction was run in triplicate. A non-template control was also included in each run. The real time data was normalized by relative quantification method [21], using miRNA 156 as reference gene which was experimentally proved to be stable in *Lablab purpureus* [22]. Table 1 shows the primer sequences used for RT-qPCR amplification.

Table 1 Primer sequences used for RT-qPCR analysis of miRNAs

miRNAs	Primer Sequence 5' to 3'
miR164	SLP: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGCACG FP: TTCCTTGGAGAAGCAGGGCA
miR398	SLP: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAGGGG FP: CGGCCTGTGTTCTCAGGTCA
miR399	SLP: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAGGGC FP: CGGCCTGCCAAAGGAGATTT
miR156	SLP: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTGCTG FP: CGCGCCTGACAGAAGAGAGT
Universal Reverse primer: GTGCAGGGTCCGAGGT	

Target Prediction and validation of miRNA targets

Potential targets for miR164, miR398 and miR399 were predicted using online algorithm psRNA Target program available at http://plantgrn.noble.org/psRNATarget. The target genes predicted by psRNATarget follows the criteria proposed by Schwab et al. (2005) [23]; allowing one mismatch in the region complementary to nucleotide positions 2-12 of the miRNA, and three additional mismatches between positions 12-22, but with no more than two continuous mismatches.

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Predicted significant target genes were validated by performing RT-qPCR. cDNAs were synthesized from 1µg total RNA and 5µM oligo dT primers. RT- qPCR reactions were carried out as described for miRNAs using actin as reference gene. The primer sequences used for RT-qPCR are shown in table 2.

Table 2 Primer sequences used for RT-qPCR analysis of target mRNAs

Target Gene	GenBank Accession	Primer (5'–3')	Product Size
NAC1	AAY46121	**Forward** GGACTACCCAATAGCCCAAATCA **Reverse** GACCCAAGTAATCCATTTCCAAAAG	119
ACC oxidase	CV542243	**Forward** TGGCACCAAAGTTAGCAACTA **Reverse** ATTCTGGTGCCATCCGTTTGA	270
PHO2/ UBC24	NC_003071.7	**Forward** AGGTTTGAAGCTCCACCCTCA **Reverse** CCCAAGATGTGATTGGAGTTCC	70
Actin	NM_001252731.2	**Forward** CGGTGGTTCTATCTTGGCATC **Reverse** GTCTTTCCCTTCAATAACCCTA	142

Statistical analysis

The experiments were set up in a completely randomized design. All data are expressed as means of triplicate experiments. Comparisons of means were performed using Graph Pad prism 5.0 software. Data were subjected to a one-way analysis of variance (ANOVA), and the mean differences were compared by lowest standard deviations (LSD) test. Comparisons with $P \leq 0.05$ were considered significantly different.

Results and Discussion

Plants have evolved over several decades to circumvent potential detrimental effects of phosphorus deficiency in the soil by modification of their root architecture, with increased acid phosphatase secretion, and altered gene expression. Varying Pi concentrations produced a marked effect on the root architecture of hyacinth bean. While P-limiting conditions (0.2 µM) resulted in excessive lateral root formation, normal phosphorous conditions (1 mM) caused significantly lesser lateral root growth (Fig 1). Difference in lateral root formation was observed from 6th day and became more evident

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on 8th and 10th day under low phosphate levels. Modification of root growth and architecture is a well-documented response to Pi starvation in plants. Plant roots typically respond to phosphorous deficiency through allocation of more carbon to roots leading to increased root growth, enhanced lateral root formation and increased number of root hairs for greater exploration of the surface soil and for efficient absorption of Pi [24].

Fig1. Root development in hyacinth bean on 6th day, 8th day and 10th day after treatment with normal Pi concentration (A) and limited Pi concentration (B).

Secretion of acid phosphatase enzymes by roots in P-deficient conditions is an universal response in plants. Acid Phosphatases are synthesized prominently in plants, and their activities have traditionally been used as markers for P-deficiency [25], [26]. Acid phosphatase activity in the root secretions from Phosphate deficient roots of hyacinth bean exhibited gradual elevation with extended period of phosphate limiting condition. Greater elevation in acid phosphatase activity beyond 6 days indicated sensitive phosphate sensing mechanism in hyacinth bean. Depleting Pi signals increased the secretion of acid phosphatase which may be required to generate sufficient Pi from breakdown of organic phosphorous compounds in soil. On the other hand, no alteration in the level of acid phosphatase activity was observed in root secretions under normal phosphate levels (Fig 2).

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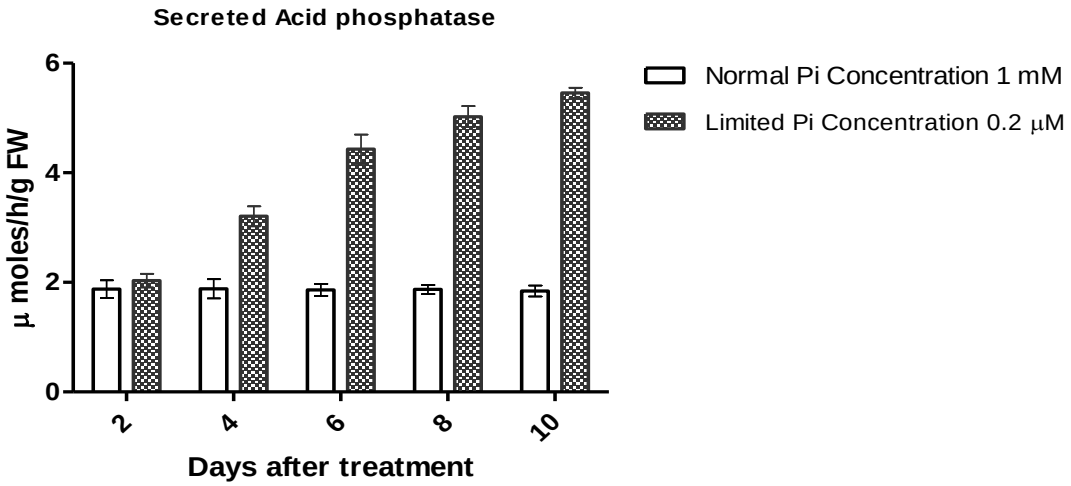

Fig 2. Secreted root acid phosphatase activity of Hyacinth bean exposed to normal (1 mM) and limited (0.2 µM) Pi concentration. Values are expressed as µmoles/h/g fresh weight.

Pi concentrations in normal control roots (1mM) were higher and remained almost constant due to sufficient availability of Pi in the growth medium. Pi concentrations in the plant roots treated with low phosphate (0.2 µM) was lower than normal control roots and was found to decrease slightly with extended starvation as shown in figure 3. Such alteration in Pi levels in response to Pi starvation have been reported in *Arabidopsis* [27].

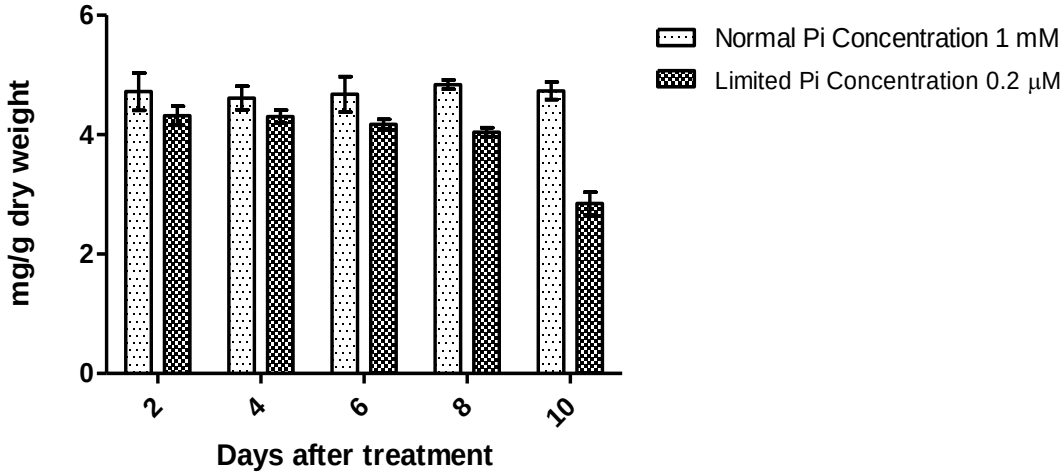

Fig 3. Inorganic phosphate (Pi) concentrations in roots of hyacinth bean exposed to normal (1 mM) and limited (0.2 µM) Pi concentration. Values are expressed as mg/g dry weight.

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In addition to modification of root morphology, and secretion of acid phosphatase enzyme, as an adaptive response to phosphate deficiency, a number of molecular events including altered gene expression and miRNA expression are found to contribute to conservation and acquisition of Pi [16]. Few Pi stress- specific miRNAs are found to be conserved among plant species as a part of Pi starvation signaling. However, there appears to be diversity in terms of their expression levels under Pi stress. RT-qPCR pattern of miRNAs in the root tissues of hyacinth bean exposed to phosphate limiting conditions for 8 days showed down-regulation of miR164, miR398 and up-regulation of miR399. Countervailing pattern of these miRNAs was reflected in the expression pattern of their target mRNAs (Fig 4). Wherein, the predicted targets of miR164 and miR398, NAC1 and ACC oxidase, respectively were up-regulated, the target of the up-regulated miR399, UBC 24 was down-regulated.

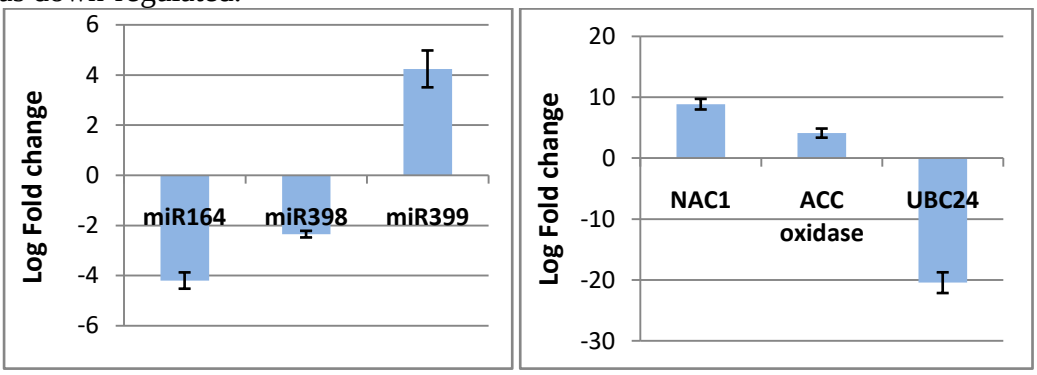

Fig 4. Differential expression pattern of miR 164, miR 398, miR 399 and their target genes NAC1, ACC oxidase and UBC24 respectively, in the roots of hyacinth bean under phosphate limiting conditions.

NAC1 transcription factor, a member of the largest families of plant-specific transcription factors mediate auxin signaling to promote lateral root development. It's over-expression as studied by RT-qPCR, coinciding with down-regulation of its regulator miRNA, miR164 and observed increase in lateral root under Pi stress in hyacinth bean substantiates its role in Pi homeostasis. Such correlation between NAC1 expression and enhanced lateral root growth have also been confirmed by transgenic *Arabidopsis* over-expressing NAC1, as well reduced lateral root growth under NAC1 antisense expression [28]. Predicted target of miR398, ACC oxidase is known to catalyze the oxidation of ACC to form ethylene. It's accumulation under Pi starvation, correlating to down-regulation of miR398, suggested its role in elevated production of ethylene, which has been found to promote auxin signaling for lateral root development.

An important phosphate deficiency responsive miRNA, miR399 controls inorganic phosphate (Pi) homeostasis by regulating the expression of UBC24 encoding a ubiquitin-conjugating E2 enzyme [29]. Over-expression of miR399 and down-regulation of its

predicted target UBC 24 or PHO 2, under phosphate starvation in *lablab* also suggested possible role miR399 in phosphate homeostasis (Fig 4). UBC 24/PHO 2 has been implicated in ubiquitin-proteosome pathway, wherein it could negatively regulate Pi transporters by facilitating their degradation via ubiquitination. This has been corroborated from studies of miR399 over-expressing *Arabidopsis thaliana* and Pi over accumulator mutant PHO 2 [30].

Conclusion

From the foregoing discussion, it is concluded that adaptation to Pi starvation in *lablab* involves conserved miRNAs, miR164, miR398 and miR399, along with their predicted targets as part of a phosphate homeostasis network at post-transcriptional regulatory level. This indicates the primary role of miRNAs in phosphate homeostasis and possibly provides an opportunity to engineer plants for survival under phosphate limiting conditions.

References

1. Mengel, K. "Agronomic measures for better utilization of soil and fertilizer phosphates," *Eur. J. Agron.* Vol 7, pp. 221– 233, 1997.
2. Smith, F., Mudge, S., Glassop, A.R.D. "Phosphate transport in plants," Plant Soil, Vol. 248, pp. 71-83, 2013.
3. Marschner, H. "Mineral Nutrition of Higher Plants," Ed 2. Academic Press, London. 1995.
4. Comerford, N. B. "Soil phosphorus bioavailability. In: Lynch JP, Deikman J, eds. *Phosphorus in plant biology: regulatory roles in molecular, cellular, organismic, and ecosystem processes,"* Rockville, MD, USA: American Society of *Plant Physiology*, 136 – 147. 1998.
5. Cordell, D., Drangert, J.O., White, S. "The story of phosphorus: Global food security and food for thought," *Global Environmental Change*, Vol. *19*(2), 292–305, 2009.
6. Steen, I. "Phosphorus availability in the 21st Century: Management of a non-renewable resource," *Phosphorus and Potassium*, Vol. 217, pp. 25-31, 1998.
7. Lynch, J.P., Brown, K.M. "Topsoil foraging–an architectural adaptation of plants to low phosphorus," *Plant and Soil, Vol.* 237, pp. 225 – 237, 2001.
8. Lynch J. "Root architecture and plant productivity," *Plant Physiol. Vol.* 109, pp. 7- 13, 1995.
9. Smith, F. W. "Sulphur and phosphorus transport systems in plants," *Plant Soil*, Vol. 232, pp. 109 – 118, 2001.
10. Vance, C. P., Uhde-Stone, C., Allan, D. L. "Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource," *New Phytologist*, Vol. *157*(3), pp. 423–447, 2003.

11. Ryan, P., Delhaize, E., Jones, D. "Function and mechanism of organic anion exudation from plant roots." *Annual Review of Plant Physiology and Plant Molecular Biology* 52(1): 527-560, 2001.
12. George, T.S., Richardson, A.E., Simpson, R.J. "Behaviour of plant-derived extracellular phytase upon addition to soil," *Soil Biol. Biochem*. Vol. 37, pp. 977–988, 2005.
13. Tran, H.T., Hurley, B.A., Plaxton, W.C. "Feeding hungry plants: the role of purple acid phosphatases in phosphate nutrition," *Plant Sci* Vol. 179, pp. 14–27, 2010.
14. Wu, P., Ma, L., Hou, X., Wang, M., Wu, Y., Liu, F., Deng, X.W. "Phosphate starvation triggers distinct alterations of genome expression in *Arabidopsis* roots and leaves," *Plant Physiology* 132: 1260–1271, 2003.
15. Hammond, J.P., Bennett, M.J., Bowen, H.C., Broadley, M.R., Eastwood, D.C., May, S.T., Rahn, C., Swarup, R., Woolaway, K.E., White, P.J. "Changes in gene expression in *Arabidopsis* shoots during phosphate starvation and the potential for developing smart plants," *Plant Physiology, Vol.* 132, pp. 578–596, 2003.
16. Kuo, H. F., Chiou, T. J. "The role of microRNAs in phosphorus deficiency signaling," *Plant Physiol*. Vol. 156, pp. 1016–1024, 2011.
17. Fiske, C. H., Subbarow, Y. "The Colorimetric Determination Of Phosphorus," *Journal of Biological Chemistry*, Vol. *66*(2), pp. 375–400, 1925.
18. Mclachlan, K. B. "Acid phosphatase activity of intact roots and phosphorus nutrition in plants. I. Assay condition and phosphatase activity," *Australian Journal of Agricultural Research* Vol. 31, pp. 429–40, 1980.
19. Chen, C., Ridzon, D.A., Broomer, A.J., Zhou, Z., Lee, D.H., Nguyen, J.T., Barbisin, M., Xu, N.L., Mahuvakar, V.R., Andersen, M.R., Lao, K.Q., Livak, K.J., Guegler, K.J. "Real-time quantification of microRNAs by stem-loop RT-PCR," *Nucleic Acids Res.* Vol. 33 (20), e179, 2005.
20. Varkonyi-Gasic, E., Wu, R., Wood, M., Walton, E. F., Hellens, R. P. "Protocol: A highly sensitive RT-PCR method for detection and quantification of microRNAs," *Plant Methods*, Vol. *3*(1), pp. 12, 2007.
21. Livak, K.J., Schmittgen, T.D. "Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method," *Methods*, Vol. 25, pp. 402–408. 2001.
22. Thilagavathy, A., Devaraj, V.R. "Evaluation of appropriate reference gene for normalization of microRNA expression by Real-time PCR in *Lablab purpureus* under abiotic stress conditions," Biologia, Vol. 71(6), pp. 660-668, 2016.
23. Schwab, R., Palatnik, J. F., Riester, M., Schommer, C., Schmid, M., Weigel, D. "Specific Effects of MicroRNAs on the Plant Transcriptome," *Developmental Cell*, Vol. 8, pp. 517–527, 2005.
24. Williamson, L.C., Ribrioux, S.P., Fitter, A.H., Leyser, H.M. "Phosphate availability regulates root system architecture in *Arabidopsis*," *Plant Physiol.* Vol. 126, pp. 875–882, 2001.

25. Duff, S.M.G, Sarath, G., and Plaxton, W.C. "The role of acid phosphatases in plant phosphorus metabolism," *Physiol Plant*. Vol. 90, pp. 791–800, 1994.
26. Baldwin, J.C., Karthikeyan, A.S., Raghothama, K.G. "*LEPS2*, a phosphorus starvation-induced novel acid phosphatase from tomato," *Plant Physiol.*, Vol. 125, pp. 728–737, 2001.
27. Li, W.F., Perry, P.J., Prafulla, N.N., Schmidt, W. "Ubiquitin-Specific Protease 14 (UBP14) Is Involved in Root Responses to Phosphate Deficiency in *Arabidopsis," Molecular Plant, Vol.* 3 (1), pp. 212–223, 2010.
28. Xie, Q., Frugis, G., Colgan, D., Chua, N.H. "*Arabidopsis* NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development" *Genes Dev*. Vol. 14(23), pp. 3024–3036, 2000.
29. Fujii, H., Chiou, T., Lin, S., Aung, K., Zhu, J. "A miRNA involved in phosphate-starvation response in *Arabidopsis*" *Current biology Vol. 15*(22), pp. 2038–2043, 2005.
30. Aung, K., Lin, S., Wu, C., Huang, Y., Su, C., Chiou, T. "*pho2*, a phosphate overaccumulator, is caused by a nonsense mutation in a microRNA399 target gene," *Plant Physiol.*, Vol. 141, pp. 1000–1011, 2006.