

Involvement of purple acid phosphatase gene into nitrogen uptake of oil palm (*Elaeis guineensis*)

SIGIT DWI MARYANTO^{1,*}, ZULFIKAR ACHMAD TANJUNG¹, ROBERDI¹,
WIDYARTINI MADE SUDANIA¹, PUJIANTO², HAIRINSYAH³, CONDRIO UTOMO¹, TONY LIWANG¹

¹Department of Biotechnology, Plant Production and Biotechnology Division, PT. SMART Tbk. Jl. Raya Cijayanti, Babakan Madang, Bogor 16810, West Java, Indonesia. Tel.: +62- 21-3925720. *email: biotechnology@sinarmas-agri.com

²Department of Agronomy, SMART Research Institute, PT. SMART Tbk. Jl. Raya Sam Sam, Kandis, Siak 28686, Riau, Indonesia

³PT. Dami Mas Sejahtera. Tapung Hilir, Kampar 28464, Riau, Indonesia

Manuscript received: 29 December 2020. Revision accepted: 19 February 2021.

Abstract. Maryanto SD, Tanjung ZA, Roberdi, Sudania WM, Pujiyanto, Hairinsyah, Utomo C, Liwang T. 2021. Involvement of purple acid phosphatase gene into nitrogen uptake of oil palm (*Elaeis guineensis*). *Biodiversitas* 22: 1385-1390. Nitrogen is the most important nutrient element in terms of plant growth. Plant purple acid phosphatases (PAPs) are known to participate in the phosphate (Pi) acquisition and utilization. Moreover, *PAP* gene plays an important role in nitrogen fixation. A single nucleotide polymorphism (SNP) was previously detected in the exon 7 of *EgPAP3*, based on SNP mining analysis of oil palm (*Elaeis guineensis* Jacq.) genome database. This study was aimed to obtain a Cleaved Amplified Polymorphic Sequences (CAPS) marker based on SNP within *EgPAP* associated with efficient nitrogen uptake oil palm progenies. Primer pairs were designed and used for PCR amplification of 3 oil palm progenies that showed low N-content, 3 progenies with moderate N-content, and 3 progenies with high N-content. The amplicon was purified prior to single-pass DNA sequencing analysis. Based on Pearson's chi-square and odds ratio statistical analysis, the SNP has strong positive correlation with the phenotype. The SNP is located at chromosome 13 with a distance of 17.7771 cM from start codon. The sequencing analysis revealed that three progenies with high N-content samples had GG allele motif, while moderate N-content progenies had GA allele and low N-content progenies had AA allele motifs respectively. In addition, a restriction site of *NlaIV* was found to be adjacent to the SNP location, thus the PCR products of all samples were digested with *NlaIV* restriction enzyme. *NlaIV* was able to distinguish between high, medium and low efficient DNA samples. Whole high N-content progenies with GG allele motifs were undigested indicating a single band size of 670 bp identical to the untreated PCR product (control). Moderate N-content progenies produced a 670 bp, 550 bp, and 120 bp bands because of digested by *NlaIV*. Low N-content progenies also resulted in double bands of 550 bp and 120 bp due to digested by *NlaIV*. Furthermore, *NlaIV* restriction enzyme was applied to digest other 54 oil palms DNA samples with unknown genotypes. Whole GG samples were consistently shown to have single band, GA and AA samples were also consistent in producing two bands with different lengths. Based on this result, CAPS marker based on SNP in *EgPAP3* was successfully developed to screen between high and low efficient N-uptake of oil palm progenies.

Keywords: CAPS marker, *EgPAP3*, N fixation, oil palm, polymorphism, SNPs

INTRODUCTION

Oil palm (*Elaeis guineensis*, Jacq.) is an important perennial crop with the highest yielding oil in the world (Corley and Tinker 2015). High yield can be achieved by supplying optimum mineral nutrition consisted of the supply, absorption, and utilization of the essential nutrients (Fageria et al. 2010). Nitrogen (N) is the most important nutrient in plant growth, its availability being a major limiting factor for crop productivity (Mohidin et al. 2015). Nitrogen plays many roles in growth and development of plants such as constituents of amino acids, proteins, protoplasm, and chlorophyll (Fita et al. 2011). The proportion of nitrogen absorbed and used by plants is called nitrogen use efficiency (NUE). An improvement in nitrogen use efficiency and fertilizer efficiency is vital to make agriculture more sustainable (Baligar and Fageria 2015).

Nitrogen in the form of nitrate (NO_3^-) and ammonium (NH_4^+) was absorbed from the soil by plants and

transported through xylem as nitrate, and dissolved ammonia, amino acids from the root to the shoot (Zhao et al. 2018). Several nitrate transporters were taken up of nitrate that uses a proton gradient to power the transport mechanism. Roots reduce only a small fraction of the absorbed nitrate to ammonia, while nitrate reduction was carried out in the shoots. Ammonia is incorporated into amino acids via the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway. A significant amount of ammonium ions were transported in the shoots, whereas whole ammonia is usually incorporated into amino acids in the root. Furthermore, biological nitrogen fixation converts N_2 into ammonia. Nitrogen fixation is a converted process of molecular nitrogen from air into ammonia (NH_3) or related nitrogenous compounds in soil. Plant purple acid phosphatases (*PAPs*) encoding gene is known to participate in nitrogen fixation. Ammonium ions from nitrogen fixation processes were absorbed by the plant through ammonia transporters (Feng et al. 2020).

The largest group of plant acid phosphatases (APases) (EC 3.1.3.2) is plant purple acid phosphatases. The *PAPs*

are called because of their purple color in water solution. This color results from the charge-transfer transition between ferric ion and the tyrosine residue. The presence of seven variant amino acid residues in five conserved motifs (DXG/GDXXY/GNH(D/E)/VXXH/GHXH) of PAP is essential for the formation of a binuclear metal center (Yin et al. 2019). Whole PAPs are capable of hydrolyzing phosphate esters such as ADP, ATP, and glycolipid (Wang 2011). They play multiple roles during plant development, including the accumulation of phosphate, modulation of ascorbate biosynthesis, flower development, cell wall biosynthesis, control of seed germination, and the generation of reactive oxygen species (Tian et al. 2012; Li et al. 2008). The PAP is associated with phosphate (Pi) utilization, and they considered to be induced by phosphate deprivation. Pi is the second most limiting nutrient in oil palm, playing most important role in plants energy transfer and storage. Pi is a structural component of nucleic acids, nucleotides, and coenzymes (Xie and Shang 2018). PAPs have biochemical function in regulating ADP levels and energy charge. The ratio of ATP or ADP and the energy charge in nodule cells of *Astragalus sinicus* was changed in silenced PAPs, leading to the damage of nitrogenase activity (Wang et al. 2015). Nitrogenase activity was regulated by the ratio of ATP or ADP, energy charge and ATP content in nodule cells (Ott et al. 2005).

The *PAP* gene also has an important role related to nitrogen fixation. The *PAP* genes play important role in nodule formation including nodule primordium formation, nodule early senescence, nitrogen fixation and induced hairy roots. This affected root nitrogen uptake and is essential to increase biological nitrogen fixation (Wang et al. 2020). Thirteen SNPs in soybean *PAP* gene are significantly associated with phosphate limitation tolerance (Ning et al. 2016).

Plant breeding is the manipulation processes of plant species to create desired phenotypes and genotypes for specific purposes (Daryono et al. 2012). Molecular markers can be used to identify useful genotypes for inclusion in plant breeding (Daryono and Maryanto 2018). The variation in a single position in DNA sequence among individuals of same species is called Single Nucleotide Polymorphism. SNP is a type of molecular marker, which can be used as a selection tool and crop genetic diversity analysis for plant breeding programs (Borlay et al. 2017). SNPs have important applications as molecular markers that reflect both natural genetic variability and a genetic drift created by breeders in plants (Shavrukov 2016).

SNP markers have been applied to select nitrogen use efficient plants in rice (*Oryza sativa*) (Duan and Zhang 2015). The polymorphism in *EgFUM1* was reported to be associated with N uptake in oil palm (Maryanto et al. 2020). SNPs in this gene could be used to select oil palm plants with high N-uptake efficiency. SNP could be detected in several methods including Sanger-sequencing, Single Nucleotide Amplified Polymorphism (SNAP), Cleaved Amplified Polymorphic Sequences (CAPS), High Resolution Melting (HRM), and Kompetitive Allele Specific PCR (KASP). CAPS markers also known as polymerase chain reaction-restriction fragment length

polymorphism (PCR-RFLP) (Konieczny and Ausubel 1993). CAPS are applicable for SNP detection in a wide range of populations (Liu et al. 2016). The advantages of CAPS markers are co-dominant, locus-specific, easy scored and interpreted, easy conducted in laboratory, rapid, and do not require radioactive isotopes. Previously, efforts have been initiated to convert SNPs to CAPS markers in many crops. CAPS marker was applied in the validation of SNP on rice (Lee et al. 2009), soybean (Shu et al. 2011), and *EgSHELL* for selection of Dura, Pisifera and Tenera in oil palm fruits (Babu et al. 2017). SNP-based CAPS markers were also conducted for molecular characterization of natural oil palm populations (Ong et al. 2015). However, there are no reports of the CAPS marker applied for SNP validation of the *EgPAP3* gene associated with nitrogen uptake in oil palm. So, the aim of this study is to develop CAPS marker for SNP validation in *EgPAP3*, an important gene for efficient N uptake of oil palm.

MATERIALS AND METHODS

Trials and planting materials

Tenera (DxP) seedlings used in this study were obtained from the crossing design of three Dura female parents and a Pisifera male parent. The Dura palms were selected based on their N-leaf content (high, moderate and low) ranged from 1.99% to 2.89% (Fadhila 2015; Maryanto et al. 2020). Thus, there were three progenies comprised of each high, moderate, and low N efficient uptake. Three plants from each progeny were used in Sanger's sequencing analysis to reveal any SNPs that may be associated with the respective phenotype. In addition, the number of samples enlarged up to 18 plants in each progeny for the CAPS marker development. N-leaf content was measured from 7-month-old tree leaves that were dried in an oven at 70–80° C for 12 hours using the Kjeldahl method with 3 repetitions (Gholizadeh et al. 2009; Rahmawati and Santoso 2017).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

Plant DNA was isolated using Nucleospin Plant II Kit (Macherey-Nagel, Duren, Germany) according to the manufacture instruction. Quantity and quality of DNA were measured using Nanodrop Spectrophotometer 2000C (Thermoscientific, MA, USA). DNA integrity was visualized using 1% agarose gel (Bioron, Ludwigshafen, Germany) electrophoresis in TAE 1x buffer (0.04 M Tris, 0.001 M EDTA-Na₂ 2H₂O, 0.02 M acetic acid pH 8.5). The gel was visualized using UV transilluminator Gel-DocTM XR (Biorad, USA).

The primer pair for PCR amplification targeting the SNP region at exon 7 of *EgPAPs* gene was designed using Primer3 software (<http://simgene.com/Primer3>). The primer consisted of forward 5'-TTCTACGATGTCTTCGGCCG-3' and reverse 5'-CAATCGAAATGAACTTCTTCCCC-3'. The primer was designed to produce a PCR amplicon of 670 base pairs (bp). Furthermore, PCR amplification was performed using

the KOD FX Neo 200U kit (Toyobo, Osaka, Japan) based on manufacturer instruction. The PCR reaction was comprised of 12.5 µL of 2x PCR buffer for KOD FX, 5 µL of 0.4 µM dNTP, 1.5 µL of 0.3 µM each primer forward and reverse, 1 µL of 1 u/50 µL of DNA polymerase, 1 µL of DNA template, and 4,5 µL of ddH₂O. The PCR reaction was performed initially by a denaturation cycle at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and DNA synthesis at 72°C for 1 minute. Finally, an extension cycles at 72°C for 7 min. Each PCR product was visualized using 1% agarose DNA gel electrophoresis.

Single-pass sequencing analysis based on Sanger's methods was performed to determine the allele variants in all DNA samples conducted at the Integrated DNA Technologies, Ltd, Malaysia. The recognition site of a restriction enzyme nearby the SNP position was done using Geneious version 9.0.5 software (Biomatters Ltd). The *Nla*IV (GGN|NCC) was designed to digest of amplicon of *EgPAP3* gene. The *Nla*IV digestion was conducted based on protocol from manufacturer's instruction. Total final solutions were 10 µL consisted of 1 µL of 10x NEbuffer (NEB Company, Ipswich, Massachusetts, United States), 0.2 µL of 2,000 U/ml restriction enzyme, 5 µL/40 ng template of amplicon, and 3.8 µL ddH₂O. The solution was incubated in water bath on 37° C for 3 hours.

Data analysis

N-leaf content was categorized based on Fairhurst (2015) approach with critical nutrient level of N is 2.50%. Sequence variants were analyzed using Geneious version 9.0.5 software (Biomatters Ltd). CAPS marker development was analyzed statistically using Pearson's chi-square and odds ratio with two-by-two table analysis with *Simple Interactive Statistical Analysis* (SISA) online software (<http://www.quantitativeskills.com>) (Maryanto et al. 2020).

RESULTS AND DISCUSSION

N-leaf content

The result showed that fifty-four palms were clustered into 2 groups, the N-leaf content in the high group (more than 2.50%) was ranged from 2.60% to 2.89%, while in low group (below 2.50%) it was ranged from 1.99 to 2.34 % (Figure 1).

Single nucleotide polymorphism

Initially, SNPs in the oil palm *EgPAP3* gene were identified based on a local database *in silico* analysis. The *EgPAP3* gene consisted of 3,232 bp with 7 exons and 6 introns. The total length of exon was 996 bp. Primer was designed in SNP flanking region. Nine palm samples (3 samples from each progeny) were amplified and sequenced using Sanger methods. One SNP motif was found among samples on 17.7771 cM position. The allele variants were GG, GA, and AA (Figure 2) for efficient, moderate, and non-efficient progenies, respectively.

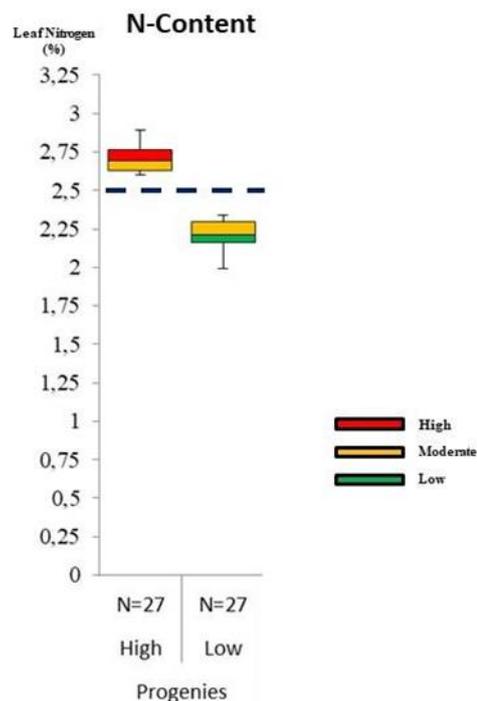


Figure 1. Nitrogen content in oil palm leaf

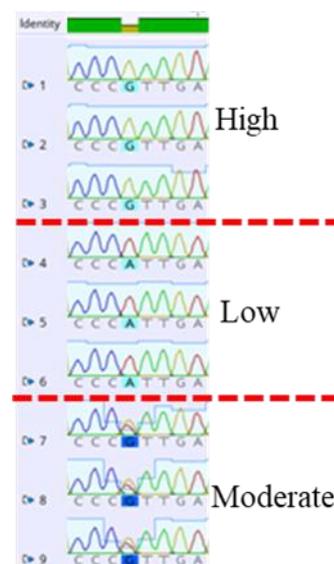


Figure 2. Electropherogram of *EgPAP3* gene

Development and validation of a CAPS marker

A recognition site of *Nla*IV was found nearby to the SNP based on analysis with Geneious version 9.0.5 software. CAPS marker has been attempted to develop for SNP that associated with high and low N efficient uptake progenies. Eighteen palms per progeny were used to verify the ability of *Nla*IV to distinguish among progenies. Sample with GG allele showed a band at 670 bp, while sample with AA allele showed double band with 550 bp

and 120 bp. Furthermore, GA allele showed triple band in 670 bp 550 bp, and 120 bp (Figure 3).

The result was validated with 54 oil palm samples consists of 27 high and 27 low N uptake efficiency palms. All of GG genotypes were consistent showed single band; AA genotypes were showed double band with different amplicon lengths, and GA was also consistent showed triple band (Figure 4).

Fifty-four palms were divided into 2 groups including the higher group consisted of eleven palms containing GG allele, ten palms had GA allele, and six palms had AA allele; while in lower group one palm had GG allele, nine palms had GA allele, and seventeen palms had AA allele (Table 1). Based on statistical analysis, SNP had strong positive correlation with N uptake based on N-content in the leaf parameters (Table 2). The GG genotype was related to high N uptake; GA genotype was related to moderate N uptake efficiency, while AA genotype was related to low N uptake.

Discussion

Nitrogen is essential nutrition for oil palm vegetative growth and fruit development (Wang et al. 2012). The *PAP* gene has important role related to nitrogen fixation and nodule formation. The *PAP* gene was reported to contribute to nitrogen fixation (Wang et al. 2015). Nitrogen fixation is a process by which nitrogen molecules in the air are converted into ammonia (NH₃) or related nitrogenous compounds in soil. Nitrogen fixation process occurs at root. The impact of increasing nitrogen fixation is plant absorbs more nitrogen. The *PAP* gene would induce nodule formation, consisted of nodule primordium formation and nodule early senescence in *A. Sinicus* (Wang et al. 2015).

Table 1. Allele variants of *EgPAP3* gene in 54 oil palm samples

Sample	Category of N-content	Allele	Sample	Category of N-content	Allele
N1	H	AA	N28	L	GA
N2	H	AA	N29	L	GA
N3	H	AA	N30	L	AA
N4	H	AA	N31	L	AA
N5	H	AA	N32	L	AA
N6	H	GA	N33	L	AA
N7	H	GA	N34	L	GA
N8	H	GG	N35	L	AA
N9	H	GA	N36	L	AA
N10	H	GA	N37	L	AA
N11	H	GG	N38	L	AA
N12	H	GA	N39	L	AA
N13	H	AA	N40	L	AA
N14	H	GG	N41	L	GA
N15	H	GG	N42	L	AA
N16	H	GG	N43	L	AA
N17	H	GA	N44	L	GA
N18	H	GG	N45	L	GA
N19	H	GA	N46	L	AA
N20	H	GA	N47	L	GA
N21	H	GG	N48	L	AA
N22	H	GG	N49	L	AA
N23	H	GG	N50	L	AA
N24	H	GA	N51	L	GA
N25	H	GG	N52	L	GA
N26	H	GG	N53	L	GG
N27	H	GA	N54	L	AA

*Note: N: sample code; H: high N-leaf content; L: low N-leaf content

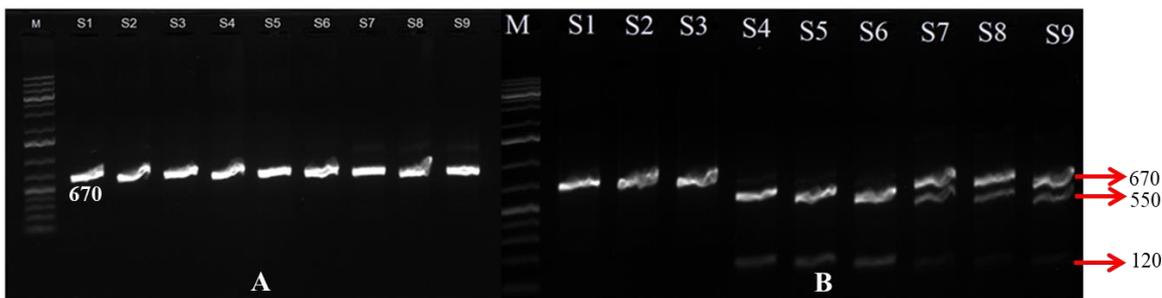


Figure 3. PCR profiles of oil palm samples A) Before digestion; B) After digested with *NlaIV*. Note: S1-S3: GG (single band); S4-S6: AA (double bands), and S7-S9: GA (triple bands).

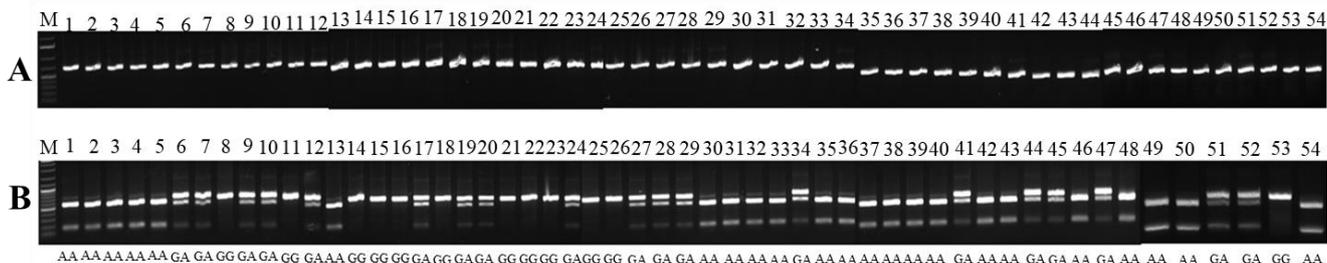


Figure 4. PCR profiles of oil palm samples with *EgPAP3* gene, A) Visualization before digestion; B) After digested with *NlaIV*

Table 2. Statistical analysis of SNPs *EgPAP3* gene

SNP (cM)	Flanking	Allele	Genotype	High	Low	P-value	Odds Ratio (OR)	95% Confidence Interval (CI)	Finding
17.7771	CC(G>A)TT	G, A	GG	11	1	10.71	17.88	2.10 >17.88 > 151.89	Significant, strong positive correlation
			Non GG	16	26		0.056	0.007 >0.056 > 0.475	

The *EgPAP3* gene was highly upregulated which may be presumed to stimulate hairy root at low nitrogen condition in oil palm. Increasing nitrogen fixation could be stimulated by hairy root growth. Increasing nitrogen fixation would influence root nitrogen uptake in the plant. Nitrogen fixation will increase the converted nitrogen in the air into ammonia. Ammonia will be absorbed by plants to the leaf through the ammonia transporter mechanism. They may affect the nitrogen activity and N-content in leaf according to the result in a previous study that under low N condition glutamine synthetase-glutamate synthase (GS) plays the important role in N uptake and utilization (Shah et al. 2017).

The polymorphism of *EgPAP3* gene was associated with nitrogen fixation in oil palm. One SNP was found among samples with different N-leaf content, located in chromosome 13 on 17.7771 cM positions. The allele variants were GG, GA, and AA. Furthermore, this SNP was converted to CAPS marker for validation. Single-band was indicated homozygote allele (GG), while triple band with 670 bp, 550 bp, and 120 bp were indicated GA and double band with 550 bp and 120 bp were indicated AA alleles. Statistical analysis of SNPs shown that there was strong positive correlation with the phenotype. The GG allele was correlated with efficient N-uptake which is indicated from majority of palm which GG allele has highest N-content in leaf. The GA allele was correlated with moderate N-uptake which is indicated from palm which GA allele has middle N-content in leaf. Furthermore, the AA allele was correlated with not efficient N-uptake which is indicated from palm which AA allele has low N-content in leaf. The *EgPAP3* gene which GG allele might induce hairy root at low nitrogen condition in oil palm, so that nitrogen fixation (convert from N₂ into ammonia) would be faster. Increasing nitrogen fixation would induce a lot of nitrogenous compounds in root. Ammonia transporter mechanism will be absorbed of ammonia into leaf. The amount of N-content in the leaf will be high, which means that N-uptake would be efficient. Furthermore, The *EgPAP3* gene which AA allele might not induce hair roots at low nitrogen conditions in oil palm, so that nitrogen fixation (convert from N₂ into ammonia) would be slow. An ammonia supply would be affected into leaf, when N-uptake would be not efficient.

In conclusion, the *EgPAP3* gene from oil palm encodes a purple acid phosphatase which participates in nitrogen fixation. One SNP was found in 17.7771 cM in *EgPAP3* gene. CAPS marker based on SNP was developed and could be used for the molecular assisted selection of oil

palm. It was found a strong positive correlation between polymorphism and N-leaf content phenotype.

ACKNOWLEDGEMENTS

We thank the management of PT Sinar Mas Agro Resources & Technology (SMART) Tbk. for their support in completing this study. Our appreciation to Reno Tryono, Marcelinus Rocky Hatorangan, and Victor Aprilyanto for proofreading the manuscript and Wulan Artutiningsih, Olivia S. Purba, Tengku Imam Saputra, Ranny Hanifah, and Ainun Najib for their support and technical assistance in laboratory and greenhouse activities.

REFERENCES

- Babu BK, Mathur RK, Kumar PN, Ramajayam D, Ravichandran G, Venu MVB, Babu SS. 2017. Development, identification and validation of CAPS marker for SHELL trait which Governs Dura, Psifera and Tenera fruit forms in oil palm (*Elaeis guineensis* Jacq.). PLOS ONE 12 (2): 1-16. DOI: 10.1371/journal.pone.0171933
- Baligar VC, Fageria NK. 2015. Nutrient use efficiency in plants: An overview. Commun Soil Sci Plant Anal 32:7-8 (2001): 921-950. doi:10.1007/978-81-322-2169-21
- Borlay AJ, Roberdi R, Suharsono S, Liwang T. 2017. Development of Single Nucleotide Polymorphism (SNPs) marker for oleic acid content in oil palm (*Elaeis guineensis* Jacq.). Pak J Biotechnol 14 (1): 55-62.
- Corley RHV, Tinker PB. 2015. The Oil Palm. Blackwell Publishing, Oxford.
- Daryono BS, Maryanto SD. 2018. Keanekaragaman dan Potensi Sumber Daya Genetik Melon. Books, Gadjah Mada Universty Press. Yogyakarta. [Indonesian]
- Daryono BS, Hayunintyas SD, Maryanto SD. 2012. Perakitan melon (*Cucumis melo* L.) kultivar melodi gama 3 dalam rangka penguatan industri pertanian nasional. Proceeding. UNNES, 245-256. [Indonesian]
- Duan D, Zhang H. 2015. A single SNP in NRT1.1B has a major impact on nitrogen use efficiency in rice. Life Sci 58 (8): 827-828. doi: 10.1007/s11427-015-4907-3
- Fadhila M. 2015. Effect of Progeny and Doses of Nitrogen Fertilizer on The Growth of Oil Palm in The Main Nursery [Thesis]. Agricultural Faculty, Stiper Agricultural Institute, Yogyakarta. [Indonesian]
- Fageria NK, Baligar VC, Jones CA. 2010. Growth and Mineral Nutrition of Field Crops. 3rd ed. CRC Press, New York. <http://dx.doi.org/10.1201/b10160>.
- Fairhurst T. 2015. Minimization of Error in Leaf Analysis Samplings and Analysis. Tropical Crop Consultants Limited.
- Fita A, Nuez F, Picó B. 2011. Diversity in root architecture and response to P deficiency in seedlings of *Cucumis melo* L. Euphytica 181 (3): 323-339. <http://dx.doi.org/10.1007/s10681-011-0432-z>.
- Feng H, Fan X, Miller AJ, Xu G. 2020. Plant nitrogen uptake and assimilation: Regulation of cellular pH homeostasis. J Exp Bot 71 (15): 4380-4392. DOI: 10.1093/jxb/eraa150
- Gholizadeh A, Amin MSM, Anuar AR, Aimrun W. 2009. Evaluation of leaf total nitrogen content for nitrogen management in a Malaysian

- paddy field by using soil plant analysis development chlorophyll meter. *Am J Agric Biol Sci* 4 (4): 278-282.
- Konieczny A, Ausubel FMA. 1993. Procedure for mapping arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. *Plant J* 4 (2): 403-410. DOI: 10.1046/j.1365-313X.1993.04020403.x
- Lee GA, Koh HJ, Chung HK, et al. 2009. Development of SNP-based CAPS and dCAPS markers in eight different genes involved in starch biosynthesis in rice. *Mol Breeding* 24: 93-101. DOI: 10.1007/s11032-009-9278-7.
- Liu S, Gao P, Zhu Q, Luan F, Davis AR, Wang X. 2016. Development of cleaved amplified polymorphic sequence markers and a CAPS based genetic linkage map in watermelon (*Citrullus lanatus* [Thunb.] Matsum. and Nakai) Constructed using whole-genome re-sequencing data. *Breeding Sci* 66 (2): 244-259. DOI: 10.1270/jsbbs.66.244
- Li WY, Shao G, Lam HM. 2008. Ectopic expression of *GmPAP3* alleviates oxidative damage caused by salinity and osmotic stresses. *New Phytol J* 178 (1): 80-91. DOI: 10.1111/j.1469-8137.2007.02356.x
- Maryanto SD, Tanjung ZA, Sudania WM, Kusnandar AS, Roberdi, Pujianto, Utomo C, Liwang T. 2020. Polymorphism of Fumarate Hydratase 1 (*FUM1*) gene associated with Nitrogen uptake in oil palm (*Elaeis guineensis*). *Biodiversitas* 21 (6): 2462-2466. DOI: 10.13057/biodiv/d210618
- Mohidin H, Hanafi MM, Rafii YM, Abdullah SNA, Idris AS, Man S, Idris J, Sahebi M. 2015. Determination of optimum levels of nitrogen, phosphorus and potassium of oil palm seedlings in solution culture. *Bragantia* 74 (3): 247-254. DOI: 10.1590/1678-4499.0408
- Ning L, Kan G, Du W, Guo S, Wang Q, Zhang G, Hao CH, Yu D. 2016. Association analysis for detecting significant single nucleotide polymorphisms for phosphorus- deficiency tolerance at the seedling stage in soybean *Glycine max* (L) Merr. *Breed Sci* 66 (2): 191-203. DOI: 10.1270/jsbbs.66.191
- Ong PW, Maizura I, Abdullah NAP, Rafii MY, Ooi LCL, Low ETL, Singh R. 2015. Development of SNP markers and their application for genetic diversity analysis in the oil palm (*Elaeis guineensis*). *Genetics Mol Res* 14 (4): 12205-12216. DOI <http://dx.doi.org/10.4238/2015>
- Ott T, van Dongen JT, Gunther C, Krusell L, Desbrosses G, Vigeolas H, Bock V, Czechowski T, Geigenberger P, Udvardi MK. 2005. Symbiotic leghemoglobins are crucial for nitrogen fixation in legume root nodules but not for general plant growth and development. *Curr Biol* 15 (6): 531-535. DOI: 10.1016/j.cub.2005.01.042
- Rahmawati L, Santoso EP. 2017. Application of the LSU (Leaf Sampling Unit) method for analysis of essential elements in the samples of the palm leaf (*Elaeis guineensis* Jacq.). *Agrisains J* 3 (1): 14-17. [Indonesian]
- Shah JM, Bukhari SAH, Zeng J, Quan X, Ali E, Muhammad N, Zhang G. 2017. Nitrogen (N) metabolism-related enzyme activities, cell ultrastructure and nutrient contents as affected by N level and barley genotype. *J Integr Agric* 16 (1): 190-198. DOI: 10.1016/S2095-3119(15)61308-9
- Shavrukov Y. 2016. Comparison of SNP and CAPS markers application in genetic research in wheat and barley. *BMC Plant Biol* 16 (1): 47-51. DOI 10.1186/s12870-015-0689-9
- Shu Y, Li Y, Zhu Z, Bai X, Cai H, Ji W, Guo D, Zhu Y. 2011. SNPs discovery and CAPS marker conversion in soybean. *Mol Biol Rep* 38 (3): 1841-1846.
- Tian J, Wang C, Zhang Q, He X, Whelan J, Shou H. 2012. Overexpression of *OsPAP10a*, a root-associated acid phosphatase, increased extracellular organic phosphorus utilization in rice increased extracellular organic phosphorus utilization in rice. *J Integr Plant Biol* 54 (9): 631-639. DOI: 10.1111/j.1744-7909.2012.01143.x
- Wang Y, Yang Z, Kong Y, Li X, Li W, Du H, Zhang C. 2020. *GmPAP12* is required for nodule development and nitrogen fixation under phosphorus starvation in soybean. *Front Plant Sci* 11, 450-462. DOI: 10.3389/fpls.2020.00450
- Wang J, Si Z, Li F, Xiong X, Lei L, Xie F, Chen D, Li Y, Li Y. 2015. A purple acid phosphatase plays a role in nodule formation and nitrogen fixation in *Astragalus sinicus*. *Plant Mol Biol J* 88 (6): 515-529. <http://10.1007/s11103-015-0323-0>
- Wang YY, Hsu PK, Tsay YF. 2012. Uptake, allocation and signalling of nitrate. *Trends Plant Sci* 17 (4): 458-467. DOI: 10.1016/j.tplants.2012.04.006
- Wang L, Li Z, Qian W, Guo W, Gao X, Huang L, Wang H, Zhu H, Wu JW, Wang D, Liu D. 2011. The arabidopsis purple acid phosphatase AtPAP10 is predominantly associated with the root surface and plays an important role in plant tolerance to phosphate limitation. *Plant Physiol* 157 (3): 1283-1299. DOI: DOI: 10.1104/pp.111.183723
- Xie L, Shang Q. 2018. Genome-wide analysis of purple acid phosphatase structure and expression in ten vegetable species. *BMC Genomics J* 19 (1): 1-12. DOI: 10.1186/s12864-018-5022-1
- Yin C, Wang F, Fan H, Fang Y, Li W. 2019. Identification of tea plant purple acid phosphatase genes and their expression responses to excess iron. *Intl J Mol Sci* 20 (8), 1954.
- Zhao L, Liu F, Crawford NM, Wang Y. 2018. Molecular regulation of nitrate responses in plants. *Intl J Mol Sci* 19 (7): 2039. Doi:10.3390/ijms19072039