EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH OF PERENNIAL RICE UNDER PHOSPHORUS TREATMENT

Khin Yuzana[1], Fang Liu[2], Yujiao Zhang[3], Fengyi Hu[4]

Abstract

This experiment was explored the effect of arbuscular mycorrhizal fungi (AMF) on perennial rice 23 growth allocation under phosphorus conditions. We carried out a pot experiment of inoculating *Rhizaphagus irregularis* (AM) fungus with perennial rice 23, and set up three different phosphorus (P) levels (40 µM, 80 µM and 160 µM KH_2PO_4). AM fungus inoculation had significant effect on perennial rice physiological and associated gene expression compared to the control group. Plants phosphate transporter (PT) genes (*OsPT11* and *OsPT13*) were significantly up-regulated with AMF colonization under different P conditions. The inoculation with AMF showed improved plant height, plant biomass of perennial rice 23 (PR 23). In addition, phosphorus treatment also significantly increased the AMF colonization in roots of perennial rice. AMF plays a vital role in diverse functions such as soil health improvement, plant health improvement, and protection of plants against pathogen. Therefore, the molecular and functions understanding of AMF in perennial rice is very important to select an efficient species to reap the full beneficial effects from these fungi.

Keywords: Perennial rice, AMF, Phosphorus

Introduction

Perennial crops can provide effective yields and through maintaining the environment (Glover *and Regamol.* 2010). The main characteristic of perennial crops is no need for replanting or tillage as it can regrow after harvest. Consequently, the production of perennial rice would decrease labor costs and field management tasks (Zhang *et al.* 2018a, 2018b). Perennial crops can preserve soil biodiversity and aid the development of natural soil systems with improved soil health, high drought resilience, and long-term stability. Moreover, the perennial cropping gives a greater above-ground biomass production. Perennial rice efficiently utilizes water and nutritional resources from the subsoil. It is therefore a solution to the current climate change and depletion of nutrients and water due to intensive agriculture (Burmeister, 2021; Sanford *et al.* 2021). Lack of tillage and use of repeated pesticides may allow the evolution of pathogenic fungi, pests and weeds in host plants (Shim, 2012). In terms of soil fertility, no-tillage and low organic matter in soil along with the developed root system of plants can accumulate high carbon concentrations to the extent that nitrogen and phosphorus are imbalanced. In cases where perennial crops require nutritional support, these inputs would be optimally utilized and leveraging the capability of their root systems to capture nutrients (Qaswar *et al.* 2019). In order to prevent soil erosion, pathogens presence, and nutrient scarcity such as phosphorus, perennial rice has to be grown with inoculated microbes as biofertilizers.

[1] Department of Botany, University of Mandalay
[2] School of Agricultural, Yunna University (YNU) Research Center for Perennial Rice Engineering and Technology in Yunnan
[3] School of Agricultural, Yunna University (YNU) Research Center for Perennial Rice Engineering and Technology in Yunnan
[4] School of Agricultural, Yunnan University (YNU) Research Center of Perennial Rice Engineering and Technology in Yunnan

Microbial communities include a pivotal role in the functioning of plants by influencing their physiology and development (Bjorn *et al*. 2016). Among these microorganisms, arbuscular mycorrhizal fungi (AMF, Phylum Glomeromycota) are important components of soil microbial communities. AMF can exchange the nutrient via rice roots in flooded fields and AMF colonization of rice roots under wetland conditions has even been the subject of debate (Vallino *et al*. 2009; Lumini *et al*. 2011; Watanarojanaporn *et al*. 2013).

AMF is considered as an important weapon in a soil with low endogenous P (Panigrahy *et al*. 2009). Plants have developed sophisticated mechanisms to attract and interact with the fungi (Gutjahr and Parniske 2013) and P transporters have evolved and specifically expressed in infected cells (Paszkowski *et al*. 2002; Javot *et al*. 2011; Yang *et al*. 2012). Under high P conditions, AM infection is generally low, which is partly due to the P- and N-dependent synthesis of strigolactone, the plant hormone essential for the establishment of the symbiosis (Yoneyama *et al*. 2012) but also on the nitrogen status of the plant and root inherent processes that impair colonization (Javot *et al*. 2011). The rice genome contains thirteen P transporter genes in the Pht1 family (Paszkowski *et al*. 2002). Of these, ORYsa; *PHT1*;*1* (*PT1*), *PT2*, *PT6*, and *PT8* have been implicated in direct Pi uptake from the soil (Ai *et al*. 2009a). PT2 has been classified as a low affinity and *PT6* as a high-affinity transporter (Ai *et al*. 2009b). The transporter genes *PT11* and *PT13* are specifically expressed in AM-colonized roots and both are required for the establishment of AM symbiosis (Paszkowski *et al*. 2002; Gutjahr *and Parmiske* 2008). However, it was recently shown that *PT11* alone is required and sufficient for symbiotic P uptake (Yang *et al*. 2012).

The aim and objectives of the present study is to determine the interaction between perennial rice and AMF under phosphorus condition and to support for improving perennial rice productivity and sustainable agricultural development by the inoculation of AMF.

Materials and Methods

Plant and AM fungal materials

The perennial rice 23 was used in this experiment. Seeds of perennial rice were kindly provided by the breeding and foundation seed program at the School of Agriculture, Yunnan University, Kunming, China.

A commercial inoculum of AM fungi *Glomus intraradices* (*Glomus intraradices* Schenck & Smith DAOM 197198 (recently reassigned to *G. irregulare* and the *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schubler comb. nov.; Stockinger *et al.*, 2009) were selected to establish and promote symbiosis with perennial rice in greenhouse.

Experimental Design

The pot experiment was conducted in a greenhouse (24.50°N, 102.51°E) at the Institute of Resource Botany, Yunnan University. First, the preparation of PR 23 seedlings and the potting experiment were designed. Rice seeds were sterilized with 70 % (v/v) sodium ethanol solution for 15 min, and before sowing, the seeds were soaked in moist filter paper sterilized at about 30°C for 48 h until the emergence of radicle. Germinated seeds were planted in pots containing 800 ml of substrate (seedling height 12 cm ,volume 1 L, 3 plants per pot) with or without AMF (spore addition of 1000 Rhizophagus irregularis per pot). The substrate consisted of 75% soil and

25% sand. The field soil and sand were sterilized three times (121°C for 20 min each time). The potted plants were maintained in a greenhouse at a temperature of 26-30°C day/night and 60% relative humidity until harvest. Each treatment was replicated four times for a total of 27 pots.

Three treatments: unsterilized soil, sterilized soil without AMF inoculation (control), and sterilized soil with AMF, AMF and phosphate levels (40 μM: P1, 80 μM: P2, 160 μM: P3). After 6 weeks of planting, the fresh and dry weights of the ground and lower part of the rice were determined for the different treatment groups. The mycorrhizal symbiosis rate was calculated by staining the rice root system. Total RNA was extracted from rice roots for transcription and sequencing to isolate phosphate transporter genes (*OsPT11* and *OsPT13*).

Mycorrhizal symbiosis rate statistics

The rice root system was washed, dried, cut into 1 cm long root segments, and fixed with FAA fixative for more than 4 h. The root segments were then dried and cut into 1 cm long root segments. The root segments were removed and sequentially washed by 10% KOH solution at 90°C, and heated in water bath for 1 h; poured into KOH. Afetr rinseing it several times in running water, it was stained by 2% hydrochloric acid for 5 min. Then, 0.05% triphenylmethyl blue staining solution was added at 90°C water bath heating for 30 min. After pouring out the staining solution, lactic acid glycerol was added. The decolorizing solution was applied several times until most of the excess dye was removed from the root segments, and the sections were placed under a microscope for observation. The AMF infiltration rate of the root segments was graded using the method.

The infection intensity M% was calculated as:

$$M\% = \frac{95 * n5 + 70 * n4 + 30 * n3 + 5 * n2 + n1}{sample\ total\ root\ segments}$$

RNA Isolation, cDNA Synthesis, and qRT-PCR Assay

Frozen root and leaf samples of rice were ground in liquid nitrogen for nucleic acid extraction. The isolated RNA from the tissue powder was extracted by using Trizol RNA isolation kit (Sangon, Shanghai, China) according to the manufacturer's protocol. Subsequently, the RNA was pretreated with DNAse I, followed by verification of sufficient removal of genomic DNA contamination by PCR using primers exclusively targeting genomic DNA. Quality of the extracted RNA was quantified using a Nano Drop 2000. RNA purity was estimated by calculating the A260/A280 ratio. One microgram of RNA for each sample was reverse-transcribed to synthesis cDNA with PrimeScriptTM RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China) (Gutjahr *et al*. 2008; Pimprikar *et al*. 2016). Quantitative real-time qRT-PCR was carried out using SYBR® Green qRT-PCR assay. The 20-μL PCR reaction contained: 0.5 μL of each primer, 1 μL of cDNA template, 8 μL of double-distilled H_2O, and 10 μL of SYBR Premix Ex Taq II (TaKaRa). The PCR procedure was as follows: denaturation at 94°C for 4 min, then 20 cycles at 94°C for 45 s, 58 or 60°C for 25 s, and 72°C for 10 s, and finally extension at 72°C for 10 min. For each gene, the experiment was repeated three times and each biological replicate was a mean of three technical replicates. Normalized expression analysis was conducted following the $2^{-\Delta\Delta CT}$ method as described by Livak and Schmittgen (2001), and the transciriptional levels for each gene were normalized using the reference gene. The housekeeping genes were used for the normalization of gene expression values in the respective species.

Table 1 Gene-specific primers for qRT-PCR analysis

	Forward Primer (5'-3')	**Reverse Primer (5'-3')**
OsPT11	CATATCCCAGATGAGCGTATCATG	GAGAAGTTCCCTGCTTCAAGCA
OsPT13	CTTCTCCATCTCCCTTGTCG	TTTTGTCGCCTAGCCAGCC
Ubiquitin	CATGGAGCTGCTGCTGTTCTAG	CAGACAACCATAGCTCCATTGG

Results

Analysis of plant biomass

In the present study, the biomass of perennial rice under interactive treatments of three phosphorus (40 µM, 80 µM and 160 µM) concentration and AMF was performed. As Figure 1 showed, phosphorus condition showed no significant influence on shoot dry weight of perennial rice neither in non-sterilized or in sterilized soil. Whereas, under the same phosphorus condition, planting in non-sterilized soil showed higher plant shoot biomass compared to sterilized soil. Inoculation with AMF in sterilized soil has increased the shoot biomass to the highest among different treatments ($p<0.001$). When AMF inoculation with sterilized soil, the shoot biomass reached the maximum under three phosphorus treatment, which was 87%, 93% and 95% compared to non-sterilized soil without AMF inoculation and followed by sterilized soil with non-inoculated about 25%, 57% higher than the control in non inoculated AMF with non-sterilized soil. The total biomass of root difference significantly under AMF inoculation with phosphorus addition 88% under 40 µM, 92% in 80 µM and 96% for 160 µM compared to non-inoculation AMF with non-sterilized soil and sterilized soil, respectively. Analysis of the shoot and root data revealed a significant increase with AM fungi inoculation in three phosphorus than that of the non-mycorrhizal plant in non-sterilized soil and sterilized soil. A significant increase in shoot and root with AMF inoculation at three phosphorus level was observed in this experiment. To the overall, the highest shoot and root biomass increase was observed in the highest phosphorus. But under the same phosphorus levels, there was no difference in the shoot and root dry weight of perennial rice plants between non-sterile soil with and without AMF inoculation. Furthermore, mycorrhizal inoculation was resulted in significantly higher than non-mycorrhizal inoculation in sterile soil (Figure 1 A and B).

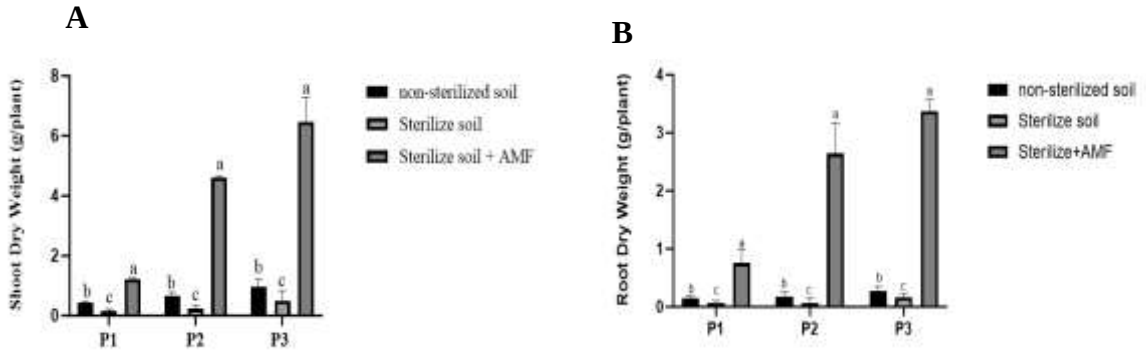

Figure 1. Biomass of shoot (A) and root (B) under different levels of phosphorus treatment

Note: Biomass of shoot phosphate content of rice: B. Biomass of root phosphate content of rice. The different letters designate the parameters ($p<0.05$)

Analysis of the plant height

One of the growth indices, plant height was investigated from the perennial rice at 42 days after inoculation with AMF (Figure 2). The plant height of AMF-inoculated rice was significantly improved as compared to that of sterilized soil without AMF inoculation under three phosphorus levels. Inoculation of AMF and P supplementation resulted in improved plant height at increase of 24%, 42% and 57%, respectively than that planted in sterilized soil with P supplement. The inoculated AMF with P supplement can reduce non-sterilized soil without AMF with P supplement about 19%, 36% and 49%. Non inoculated with sterilized soil exhibited reduced plant height 39%, 96% and 100% respectively by AMF at three phosphorus (40 µM, 80 µM and 160 µM).

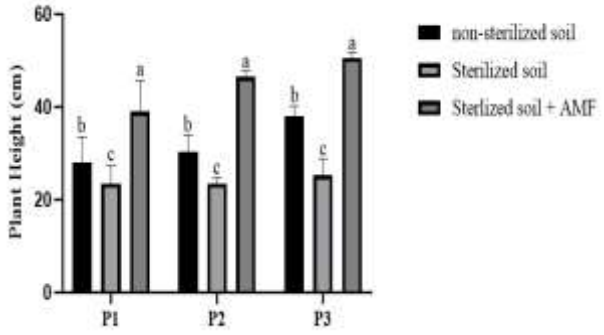

Figure 2. Comparison of plant height of rice plants under phosphorus treatment

Note: Effect of *Rhizophagus irregularis* on the plant height of PR 23 under different phosphorus level of non-inoculated and inoculated AMF. The values of each parameter labeled by different letters indicated significant differences (p<0.05)

Analysis of AM fungal rate in rice

AMF was inoculated with different soil types and phosphorus levels and grown in a greenhouse. Under different P treatments, perennial rice inoculated with AMF showed highest colonization percentage (87%, 90%, and 93%). As showed in Figures 3-5, the sterilized soil inoculated with AMF with the highest phosphorus treatment level was significantly higher than the low and medium phosphorus treatments. Under the three phosphorous levels, sterilized soil with AMF showed the highest colonization rate. In all the three phosphorus level treatments, colonization rate in the non AMF inoculated and non-sterilized soil was significantly lower than and non inoculated sterilized (Figure 3).

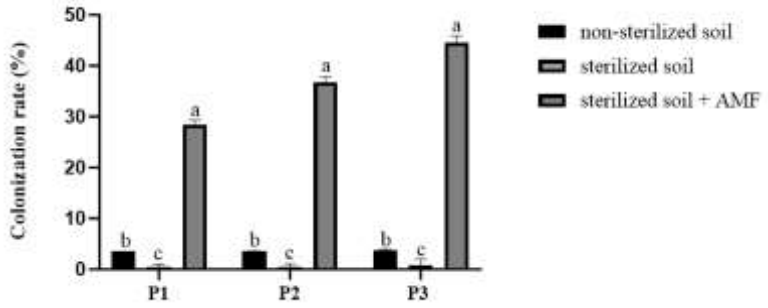

Figure 3. Comparison of root colonization rate of arbuscular mycorrhizal fungi under phosphorus treatment

Note: Effect of Rhizophagus irregularis on the rice root colonization rate of PR 23 under different phosphorus concentration of non-inoculated and inoculated AMF. The values of each parameter labeled by different letters indicate significant differences assessed ($p<0.05$)

AM Fungal colonization characteristics in roots

Roots of all the perennial rice plants inoculated/non inoculated with AMF under different phosphorus conditions were examined and AMF colonization was characterized by intraradical colonization (Figure 4, A), intercellular hyphae and vesicles (Figure 4, B), intracellular hyphae with arbuscules (Figure 4, C) and Arbuscule and intercellular hyphae (Figure 4, D).

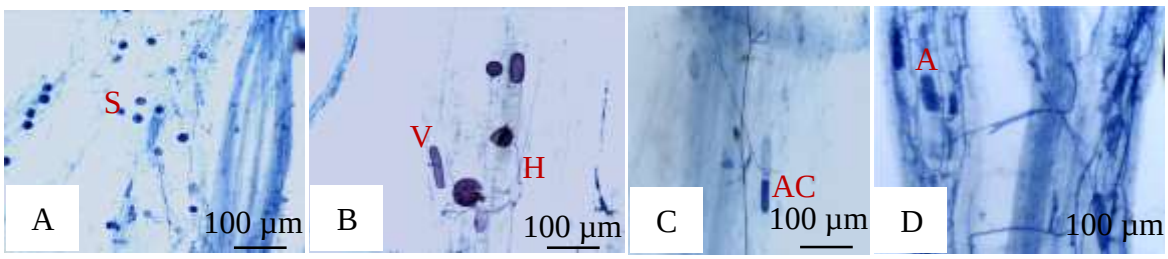

Figure 4. Aruscular mycorrhizal (AM) fungal colonization in the rice root

Note: A- intraradical colonization; B- intercellular hyphae and vesicles; C- intracellular hyphae with arbuscules; D- Arbuscule and interacellular hyphae. Scale bars 100µm

Analysis of phosphate transporter

Expression of two arbuscular mycorrhizal fungi (AM)-mediated P uptake (*OsPT11* and *OsPT13*) were analyzed in root samples of perennial rice genotypes at 6 weeks after sowing by quantitative polymerase chain reaction. Data was generated by taking average values of perennial rice in three different soil types. *OsPT11* and *OsPT13* cDNA were isolated from the total RNA of mycorrhizal rice roots, followed by PCR amplification using primers derived from the gene sequence. The same primers were used for amplification of the structural gene. Expression of P-transporter genes was quantified in root samples. PT11 and PT13 were specifically expressed in AM-colonized roots. This study has therefore showed the relative upregulation of *OsPT11* gene by 1.16-fold, 1.30-fold and 2.2-fold in mycorrhizal groups than with the non-mycorrhizal groups under different phosphorus concentration and soil feature. Likewise, OsPT13 has also revealed a significant regulation by 0.84-fold ($p<0.01$), 1.03-fold ($p<0.05$) and 2.12-fold ($p<0.05$) under different level of phosphorus treatments (Figure 5, A and B).

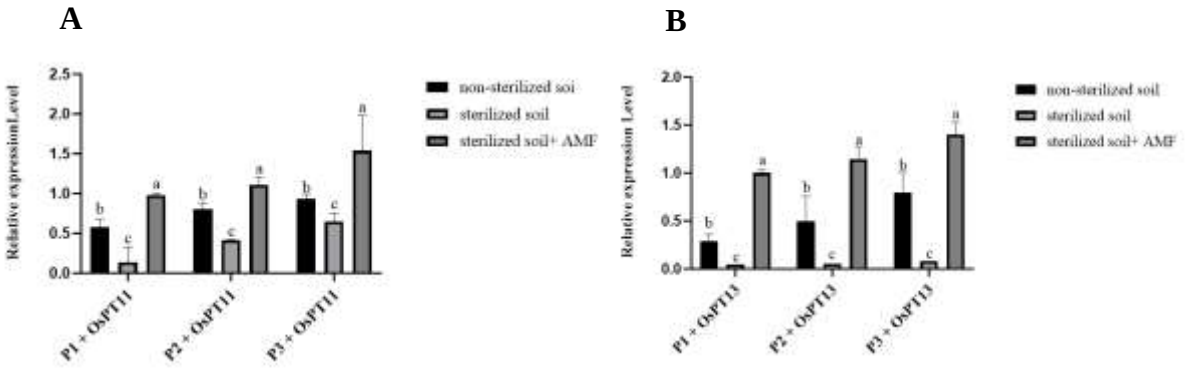

Figure 5. Relative gene expression of *OsPT11* and *OsPT13* from the root of perennial rice grown in different phosphorus levels

Discussion

Phosphorous (P) is a key essential, non-replaceable element for plant growth and development. Phospholipids mainly composed of P are components of the cell membrane. Notably, P is used in energy transfer, photosynthesis, metabolism, intracellular signaling and gene replication and expression. The low availability of P is the main constraint in much of the low input agriculture worldwide. There is a huge importance of P in several crops such as rice, barley, maize, sugar beet, and common bean. Hence growth and productivity of major crops world wide are limited by P availability.

AMF inoculation improves phosphorus acquisition, especially when the availability of soil P is low. The extending hyphae of AMF can greatly increase the absorption area and can help to solubilize P in the soil. For a species with poor root development, AMF association could enhance P uptake and promot plant growth and yield. This study is therefore demostrating the alleviation role of AMF under phosphorus treatment in particular to an increased rice plant growth, colonization rate, shoot and root growth and upregulation of responsive genes.

In the different concentration of phosphorus, above ground and underground biomass of rice in the AMF inoculate group was significantly enhanced compared with the AMF non-inoculated group. AMF had little effect on both aboveground and underground rice biomass with phosphorus in the AMF non-inoculated with non-sterilized soil and sterilized soil. With the increase in phosphorus concentration, the effect of AMF on rice biomass gradually appeared and became more and more obvious.

In non-sterilized soil, due to the potential indigenous mycorrhiza propagules, there was significant difference than sterilized soil with non-inoculated AMF under different phosphorus stress. However, inoculated sterilized soil was significantly different from both non-sterilized and sterilized soils under three phosphorus levels.

The present study showed that the AMF-inoculated plants had higher P acquisitions, lower P uptake, increased expressions of *OsPT11* and *OsPT13* compared with non-mycorrhizal plants in non-sterilized soil and sterilized soil. The increased expressions of *OsPT11* and *OsPT13* were also in line with previous studies conducted by Paszkowski *et al.* (2002) and Glassop *et al.* (2007), which showed that the expressions of *OsPT11* were significantly up-regulated in rice associated with AMF. In addition to the higher concentration of P detected in mycorrhizal plant

tissue, the appearance of *OsPT11* in rootissue can be considered as one of the indications of symbiotic success (Paszkowski *et al.* 2002).

The *OsPTs* (*OsPT1-13*) are known to contribute to the accumulation of phosphate (Goff *et al.* 2002). The AMF symbiosis increased *OsPT11*, which was supposed to be localized in the arbuscule-containing cell (Rausch and Bucher 2001; Paszkowski *et al.* 2002). The changes of *OsPT11* and *OsPT13* only play parts of the program of taking up phosphate by the rice plant when colonized by AMF as other *OsPTs* are also responsible for the P acquisition.

Conclusion

In the present study, inoculation of perennial rice (PR 23) with arbuscular mycorrhizal species of *Rhizophagus irregularis* showed a higher performance than the non-sterilized and sterilized soils without AMF inoculation under different phosphorus stress. The importance of AMF was evident by the significant correlation between mycorrhizal colonization, physiological, and relative expression (*OsPT11* and *OsPT13*). The higher content of P in mycorrhizal plants can led to the higher physiological, and relative expression. *Rhizophagus irregularis* could increase the phosphate transporter of rice in mycorrhizal plants and decreased in non-sterilized soil and sterilized without AMF inoculation. The increased expression of *OsPT11* and *OsPT13* were the most important factors that led to the significantly higher P concentration in plant tissues.

Acknowledgments

I would like to express my sincere thanks to the following individuals and organizations who played an important role in completing this study:

I am deeply thankful to my supervisor, Fengyi Hu, (Research Center of Perennial Rice Engineering and Technology in Yunnan, School of Agriculture, Yunnan University) for his unwavering support, guidance, and expertise throughout the research process, his guidance has been invaluable, and I am fortunate to have had the opportunity to work under his supervision. Special thanks to Dr. Fang Liu, associate Prof. unjian Xu, and associate Prof. Yujiao Zhang for their advices and support for this research. Their insights, feedback, and help shape the direction of this research.

I would like to express my heartful thanks to Dr Kalaya Lu, Professor and Head, Department of Botany, University of Mandalay, for her permission to report this research paper in the Myanmar Art and Science.

References

Ai, P. H., S. B Sun, J. N Zhao, X. R Fan, W. J. Xin, Q. Guo, L. Yu, Q. R. Shen, P. Wu and A. J. Miller, (2009a) "Two rice phosphate transporters, OsPht1;2 and Os Pht1;6, have different functions and kinetic properties in uptake and translocation" Plant. Journal, 57: 798-809.

Ai, P., S. Sun, J. Zhao and G. Xu, (2009b) "Regulation and function of Pht1 family phosphate transporters in rice. Proceedings of the International Plant Nutrition Colloquium XVI. UC Davis" Retrieved from: http://escholarship. org/uc/ item/3657w1q3

Bjorn, B., J. Pump and M. G. Dumont, (2016) "Microbial Community Structure in the Rhizosphere of Rice Plants" Frontiers in Microbiology, Vol. 6. doi:10.3389/fmicb.2015.01537

Burmeister, J., (2021) "Promotion of ground beetles by integrating perennial energy crops into existing agricultural landscapes" Biomass Bioenergy 146. https://doi.org/10.1016/j.biombioe.2021.105973

Glassop, D., R. M. Godwin, S. E. Smith and F. W. Smith, (2007) "Rice phosphate transporters associated with phosphate uptake in rice colonized with arbuscular mycorrhizal fungi" Canadian Journal of Botany, Vol. 85(7): 644-651

Glover, J. D., and J. P. Reganold, (2010) "Perennial grains food security for the future" Issues in Science Technology, Vol. XXVI, No.2. Doi:10.1007/0-306-48155-3-1

Goff S. A., D. Ricke, T. H Lan, G. Presting, R. Wang, M. Dunn and J. Glazebrook, (2002) "A draft sequence of the rice genome (*Oryza sativa* L. ssp. japonica)" Science 296: 92 100

Gutjahr, C., and M. Parniske, (2013) "Cell and developmental biology of arbuscular mycorrhiza symbiosis" Annu Rev Cell Dev Biol 29: 593617

Javot, H., R. V. Penmetsa, F. Breuillin, K. K. Bhattarai, R. D. Noar, S. K. Gomez, Q. Zhang, D. R. Cook and M. J. Harrison, (2011) "Medicago truncatula mtpt4 mutants reveal a role for nitrogen in the regulation of arbuscule degeneration in arbuscular mycorrhizal symbiosis" Plant Journal 68: 954-965

Lumini, E., M.Vallino, M. M. Alguacil, M. Romani and V. Bianciotto, (2011) "Different farming and water regimes in Italian rice fields affect arbuscular mycorrhizal fungal soil communities" Ecological Applications 21(5), pp.1696-1707.

Panigrahy M., D. N. Rao and N. Sarla, (2009) "Molecular mechanisms in response to phosphate starvation in rice" Biotech Advance, Vol. 27(4), 389-397

Paszkowski, U., N. Requena, S. A. Rensing, C. Roux, I. R. Sanders, Y. Shachar-Hill, G. Tuskan, J. P. W. Young, V. Gianinazzi-Pearson and F. Martin, (2012) "The transcriptome of the arbuscular mycorrhizal fungus Glomus intraradices (DAOM 197198) reveals functional trade offs in an obligate symbiont" New Phytologist, 193:755-769

Paszkowski, U., S. Kroken, C. Roux and S. P. Briggs, (2002) "Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis" Proceedings of the National Academy of Sciences of the United States of America 99(20): 13324-13329

Qaswar, M., W. Ahmed, H. Jing, F. Hongzhu, S. Xiaojun, J. Xianjun, L. Kailou, X. Yongmei, H. Zhongqun, W. Asghar, A. Shah and H. Zhang, (2019) "Soil carbon (C), nitrogen (N) and phosphorus (P) stoichiometry drives phosphorus lability in paddy soil under long-term fertilization: a fractionation and path analysis study" PLoS One 14, 0218195. https://doi.org/10.1371/journal.pone.0218195

Rausch, C., and M. Bucher, (2002) "Molecular mechanisms of phosphate transport in plants" Planta Vol. 216, No.1, 23-37

Sanford, G. R., R. D Jackson, E. G. Booth, J. L. Hedtcke and V. Picasso, (2021) "Perenniality and diversity drive output stability and resilience in a 26-year cropping systems experiment" Field Crops Research, 263, 108071. https://doi.org/10.1016/j.fcr.108071

Shim J. H., (2012) "Perennial Rice; improve rice productivity for a sustainable upland ecosystem" SABRAO Journal of Breeding and Genetics 44(2)191-201

Stockinger, H., C. Walker and A. Schussle, (2009) "*Glomus intraradices* DAOM197198, a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*" New Phytologist. 183:1176-1187

Vallino, M., D. Greppi., M. Novero., P. Bonfante and E. Luppotto, (2009) "Rice root colonization by mycorrhizal and endophytic fungi in aerobic soil" Annals of Applied Biology, 154(2), 195-204. ISSN 003-4746

Watanarojanaporn, N., N. Boonkerd, P. Tittabutr, A. Longtonglang, J .P. W. Young and N. Teaumroong, (2013) "Effect of rice cultivation systems on indigenous arbuscular mycorrhizal fungal community structure" Microbes and Environments. Vol. 28, No. 3, 316-324

Yang, S. Y., M. Gronlund, I. Jakobsen, M. S. Grotemeyer, D. Rentsch, A. Miyao, H. Hirochika, C. S. Kumar, V. Sundaresan, N. Salamin, S. Catausan, N. Mattes, S. Heuer and U. Paszkowski, (2012) "Nonredundant regulation of rice arbuscular mycorrhizal symbiosis by two members of the phosphate transporter1 gene family" Plant Cell 24:4236-4251. doi: 10.1105/tpc.112.104901

Yoneyama K., X. N. Xie, H. I. Kim, T. Kisugi, T. Nomura, H. Sekimoto, T. Yokota and K. Yoneyama, (2012) "How do nitrogen and phosphorus deficiencies affect strigolactone production and exudation?" Planta 235: 11971207. https://doi.org/10.1007/s00425-011-1568-8

Zhang, J., H. Zhang, J. R. Botella and J. K. Zhu, (2018a) "Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the *Waxy* gene in elite rice varieties" Journal of Integrative Plant Biology 60, 369-375. https://doi.org/10.1111/jipb.12620

Zhang, S., L. Huang, G. Huang, J. Zhang, Y. Zhang and F. Hu, (2018b) "Perennial rice: sustainable rice producction system. In: Scaling up Agroecology to Achieve the Sustainable Development Goals" Proceedings of the Second FAO International Symposium. pp. 216-217