Determining Nutrient Deficiency Symptoms of Rice Seedling Using Modified Murashige-Skoog Solution Culture Technique

Ulysses A. Cagasan[*], Maria Jehan P. Libre[2], Joel A. Cantoneros[3], Carmenchita M. Tumaca[4], Diosdado R. Santiago[5], Aldwin D. Pablo[6], & George A. Hamora[7]

[*]*Department of Agronomy, Visayas State University, Baybay City, Philippines, orcid.org/0000-0003-1849-2261*

[2] *College of Agricultural Sciences, Visayas State University-Villaba Campus, Villaba Leyte, Philippines, orcid.org/0000-0002-2401-8526*

[3]*Regional Field Office VIII, Department of Agriculture, Tacloban City, Philippines, orcid.org/0000-0006-2366-8765*

[4]*Agricultural Department-College of Agriculture, Western Philippines University, Aborlan Palawan, Philippines, orcid.org/0000-0001-4798-9043*

[5]*College of Agriculture, Forestry and Sciences-Aklan State University, Banga, Aklan, Philippines, orcid.org/0000-0003-2718-7656*

[6]*Department of Plant Science, Nueva Vizcaya State University, Bayombong, Nueva Vizcaya, Philippines, orcid.org/0000-0002-5451-5435*

[7]*Agriculture Department-College of Agriculture, Western Philippines University, Aborlan, Palawan, Philippines, orcid.org/0000-0002-6726-3038*

Article Information

History:
Received:11NOV2020
Final Revision: 22JAN2021
Accepted: 01MAR2021

Keywords:
Chlorosis
Complete elements
Necrosis
Nutritional Disorder

Abstract: Nutrients play an essential role in plant growth. This study considered macronutrients such as nitrogen, phosphorus, potassium, magnesium, copper, iron, and zinc. The most limiting nutrient for rice in the field is nitrogen, followed by phosphorus and potassium. The study aimed to assess the effects of nutrient omission on the early seedling growth and identify the nutritional disorders in rice seedlings NSIC Rc160 variety using the Modified Murashige-Skoog technique. The omission of essential elements was established for the experiment and served as the treatments: without nitrogen (-N), without phosphorus (-P), without potassium (-K), without copper (-Cu), without iron (-Fe), and without zinc (-Zn). Rice plants receiving complete nutrient elements served as the control. The results revealed that rice plants without the macro-elements (N, P & K) severely exhibited yellowing of leaves (chlorosis), leading to necrosis. Likewise, rice plants without P, Ca and Fe exhibited stunted growth and yellowing of leaves. Moreover, rice plants without N had a very high root length (200%) compared to the shoot length, resulting in the root: shoot ratio's high value. This means that omitting the most limiting element (N) causes the plants to grow longer roots to find the plants' nutrients. This is a basic research that the findings are utilized for succeeding researches as basis for the treatment recommendations. Hence, the research results will contribute to the body of knowledge especially for the young researchers in developing their research studies.

I. INTRODUCTION

Rice is considered a primary staple food and commercial crop in the Philippines. It can grow in a wide range of environments, from humid tropics to frost-free temperate zones (Maucieri et al., 2017). In the Philippines, rice is commonly grown in lowland areas, especially in natural ecosystems. Due to continuous cropping of land for commercial rice production, soil nutrients are already depleted (FAO, 2016). This poses a problem for crop production and nutrient management. To address soil fertility deterioration, some agricultural scientists are looking at the possibility of a soilless crop cultivation system (Billen et al., 2018) such as hydroponics, which uses water and nutrient solutions (i.e., Murashige-Skoog solution) to grow crops.

One critical crop production problem that will affect rice yield is the deficiency of essential nutrient elements needed by the plant for its growth and development. The inadequate supply of one of these critical nutrients can cause metabolic disorders in plants. This will result in latent deficiency symptoms or the appearance of visual deficiency symptoms. This situation will lead to crop failure and reduced quality of yield (McCauley et al., 2011).

According to White and Brown (2010), the nutrients that are known to have practical importance to crops are the macronutrients needed by the crop in a more considerable amount and the micronutrients needed by the crop in a little amount. The macronutrients include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S), while the micronutrients include iron (Fe), zinc (Zn), molybdenum (Mo), and copper (Cu).

Identification of different nutrient deficiency symptoms exhibited by the plant at specific growth stages is critical in diagnosing the soil's capacity or the growth solution in hydroponics to sustain better crop growth. It is widely known that the productivity and quality of crops grown in hydroponic systems are markedly dependent on the extent of the plant nutrients acquisition from the growing medium (Hosseinzadeh et al., 2019).

Early diagnosis of nutrient deficiency symptoms is essential for the crop to recover once the disorder is detected and corrected. These metabolic disturbances to plants' growth and development result in yield losses and reduced quality and financial losses, particularly in intensive crop production. Visual and analytical diagnoses during plant growth and development provide two complementary methods for identifying plants' nutritional disorders. These complementary methods enable farmers to distinguish nutritional disorders from pathological damages (Jeyasubramanian complimentary). This study was conducted using the modified Murashige-Skoog solution culture technique to determine the effects of imbalanced nutrients and assess rice seedlings' deficiency symptoms (Nathan, 2016).

II. METHODOLOGY

Research Design

This study is under experimental research design with comparison analyses, treated by Statistical Tool for Agriculture Research (START) and Honestly Significance Difference (HSD) Test.

Research Samples

There were 24 containers (plant samples used in the study). The treatment samples were replicated three (3) times. Thirty (30) randomly selected sample seedlings were used in the data gathering.

Data Collection Method

The following are the research activities done to complete the research objectives.

Preparation of Materials

NSIC Rc160 rice seeds were soaked in water for 24 hours. Seeds were drained and wrapped in a moistened tissue paper. These were allowed to germinate until the root emerges. When the seeds produced primary root at about 24 hours, transplanting seedlings to the test containers was done by putting the seedlings into the holes with the roots submerged to the nutrient solution inside the container and the seeds top. Seeds with uniform size and length of roots were selected and used as the test plants. Sixteen plastic buckets were used as containers. These were washed and rinsed thoroughly with distilled water to control contamination. Holes were bored on each plastic cover using an electric driller. The containers were covered with black carbon paper to prevent the oxidation of culture solutions inside. The 16 containers were labeled as follows: complete, -N, -P, -K, -Cu, Fe, and - Zn. The treatments were replicated two times. The treatment plants were placed in the nursery for 16 days.

Preparation of Stock Solution

Table 1 was used as a guide in preparing the stock solution for the different salts. The weight of the solute was only half because we only prepared a 500 ml solution. Reagent bottles were always rinsed with distilled water. Each bottle was appropriately labeled, and solutions were stored in the laboratory. This stock solution was carefully prepared to minimize the contamination of different treatments.

Table 1

Composition of the Modified Murashige-Skoog Solution

Salts	Weight of solute (g) to prepare 1 L solution	The desired concentration of the solution	The volume of salt needed to prepare 1 L solution
NH_4NO_3 (Ammonium Nitrate)	174	0.007 M	3 ml
NaH_2PO_4 (Monosodium phosphate)	50	0.007 M	3 ml
K_2SO_4 (Potassium sulfate)	100	0.005 M	3 ml
$CaCl_2{\cdot}2H_2O$ (Calcium chloride dihydrate)	202	0.002 M	3 ml
$MgSo_4{\cdot}7H_2O$ (Magnesium sulfate heptahydrate)	199	0.004 M	3 ml
Ferric (Fe)-EDTA	15	0.0015%	1 ml
$ZnSo_4$ (Zinc sulfate)	25	0.0025%	1 ml
Traced Elements:			
$CuSO_4{\cdot}5H_2O$ (Cupric sulfate pentahydrate)	0.0025	-	1 ml
H_3BO_3 (Boric Acid)	0.062	-	1 ml
$MnCl_2$ [Manganese (II) Chloride]	0.223	-	1 ml
Na_2MoO_4 (Sodium Molybdate)	0.0025	-	1 ml

Reagent bottles were always rinsed with distilled water. Each bottle was appropriately labeled, and solutions were stored in the laboratory. This stock solution was carefully prepared to minimize the contamination of different treatments**.**

Preparation of Nutrition Solution

The required volume of salt was measured for the different treatments, as indicated in Table 1, and the solution was diluted to 1 L with distilled water. Five liters (5 li) of the solution was prepared for each salt solution, and the culture solutions were stored in the laboratory ready for the experiment. Only one pipette was used for the different salt/stock solutions. To avoid contamination, washing the pipette with distilled water was done before using it for every stock solution placed in the container. Approximately 1.5 liters of the solution or up to the test container's neck level was dispensed into each of the sixteen properly labeled plastic buckets (See Table 2).

Planting and Maintenance of Plants

Twenty-four (24) containers were used in the experiment. These were washed thoroughly and rinsed with distilled water to minimize contamination. Seedlings with uniform root lengths were selected and rinsed with distilled water. The roots of these seedlings were inserted slowly into the holes of the buckets' cover. It was sure that the plant roots were immersed in the nutrient solution. The solution level was checked daily, ensuring that it reaches up to the container's neck level to prevent dehydration of the test plants. The solution was added to each container whenever necessary.

After establishing the setup, these were placed under the shaded condition for two days to acclimatize the newly transplanted rice seedlings. Two days after, the containers were placed under the sun as the normal plant environment. However, the experimental plants were placed inside the mosquito net to control the rats' entry, birds, and other pests.

Table 2

Checklist of Salts Needed for Different Treatment Solutions

Treatment	NH_4NO_3	NaH_2PO_4	K_2SO_4	$CaCl_2$	$MgSo_4$	Fe	Zn	Trace Elements
Complete	X	X	X	X	X	X	X	X
Minus N		X	X	X	X	X	X	X
Minus P	X		X	X	X	X	X	X
Minus K	X	X		X	X	X	X	X
Minus Ca	X	X	X		X	X	X	X
Minus Mg	X	X	X	X		X	X	X
Minus Fe	X	X	X	X	X		X	X
Minus Zn	X	X	X	X	X	X		X

ISSN 2449-4577 (online) 2408-283X (print)

Every other day, the test plants were checked to maintain the solution's level in the container's neck to control the dehydration of the test plants. The pH of the solutions was also maintained to 6.0-7.0 (neutral) by adding potassium hydroxide if the solution becomes acidic and hydrochloric acid if the solution becomes alkaline. The 6.0-7.0 solution pH is recommended as the plants' neutral pH controls the culture solution's acidity and alkalinity (Chin et al., 2015). The observation of the test plants was done until the study was finished. The abnormalities which were likely caused by the omission of an essential element in the treatment plants were described and recorded.

Harvesting

Thirty (30) randomly selected sample seedlings were used in the data gathering. The harvested seedlings were placed in an oven and dried for 24 to 48 hours at 70C until a constant weight was attained and dry weights were taken. These were measured, counted, and weighed, and essential data was gathered to get the final data (see Table 3).

Data Analysis

The number of leaves, length of roots and shoots (cm), root to shoot ratio, fresh and dry weights of biomass per seedling were recorded at harvest at 20 days after planting using 30 seedlings as the sample plants. Deficiency symptoms of the minus elements were recorded and discussed. All data gathered were analyzed using the Statistical Tool for Agricultural Research (STAR) to analyze variance (ANOVA). The comparison of treatment means was made using the Honestly Significant Difference (HSD) Test.

Ethical Consideration

The authors declare no conflict of interest. They also ensure that in the conduct of the study all experimental protocols have been observed especially on safeguarding the environment.

III. RESULTS AND DISCUSSION

Micronutrient deficiencies are becoming more common. It is crucial to identify and correct them wherever they occur. The study was conducted to know the deficiency symptoms of rice seedlings if some essential elements are missing. Results revealed that, if elements are not available or imbalanced, rice plants significantly ($p<0.05$) exhibited deficiency symptoms such as stunted growth, yellowing of leaves (chlorosis), leading to necrosis. These manifestations suggested that the rice plants had inferior growth, abnormal root growth, and cannot complete its life cycle if one of the essential element is missing (Figure 1).

Figure 1

Photographs of Rice Seedlings Under Modified Murashige-Skoog Solution Culture Technique

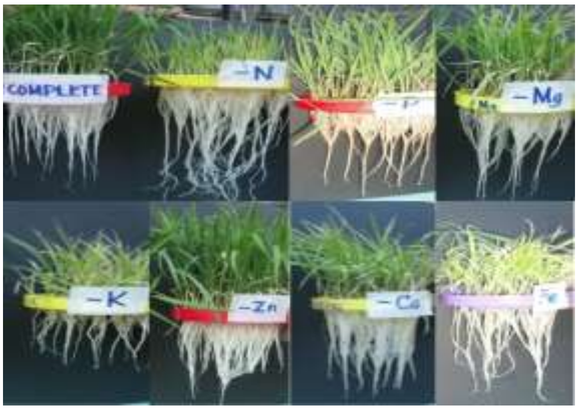


Table 3 presents the growth parameters such as shoot, root, fresh and dry weights, and percent dry matter of rice seedlings under modified Murashige-Skoog solution culture technique. The variance analysis showed that most of the growth parameters were significantly affected by the treatments except the number of leaves, percent dry matter, and percent moisture content of the rice seedlings. Results also revealed that the rice plants in all the treatments produced only two (2) leaves after a 16day period of the study regardless of the nutrients omitted. Most extended root length (cm) was produced when the rice seedlings were not applied with Nitrogen solution, or N was omitted. Roots of the plants in the -N treatment were longer but thinner. However, seedlings not applied with P, Ca, and Mg produced comparable shoot length (cm) with the seedlings applied with complete nutrient solution. The shortest shoot length (cm) was observed in the plants not applied with K, Fe, and Zn. This result can be attributed to the absence of N. Under this condition, the seedlings took a longer time to develop because it had to look for more nutrients needed for its nourishments, resulting in longer root length at the expense of shoot development. This result confirms Bown et al. (2010) findings that low N fertilization can enhance root length and diameter. In contrast, the application of higher N levels had been shown to reduce root growth but cause higher biomass production.

In terms of fresh weight (g), treatments with -P, -Mg, and -Zn elements had the highest fresh weight (g) comparable to the plants applied with complete nutrients, resulting in a higher percent dry matter yield the seedlings. This result further confirms Gruber et al. (2016) findings that plants exposed to -Zn and -Mg still managed to produce good plant herbage but did not give high marketable yield. Moreover, some parts of the plants whose growth can be sacrificed at the expense of the other plant parts (Song et al., 2011). The results of this study also conform to the research findings of Fageria et al. (2013) that macro elements are essential nutrients for ecosystem structure, processes, and function since their availability affects the production of plant biomass and growth.

For example, N and P's combined application increase root surface area, root length, and root-shoot mass. Different nutrient levels have been shown to influence both root length and branching plasticity in Arabidopsis plant species.

Visual Description of the Deficiency Symptoms of Rice Seedlings

Nitrogen (N) Deficiency

Nitrogen deficiency is the most commonly detected nutrient disorder in rice. Nitrogen is so vital because it is a significant component of chlorophyll. This compound enables plants to use sunlight to produce sugars from water and carbon dioxide (i.e., photosynthesis).

It is also a significant component of amino acids, the building blocks of proteins. Nitrogen deficient rice plants' general symptoms include yellowing of leaves and early stunted growth (Figure 2).

Figure 2

Nitrogen (N) Deficiency

The leaves of N deficient rice seedlings are tiny and thinner as compared to those plants receiving complete nutrients. Nitrogen deficiency symptoms usually affect both the older and younger leaves (Fitts et al., 2014). This is evident in the plant shoots, which showed stunted growth and generally smaller leaf sizes than the plants receiving complete nutrients. It has long roots

Table 3

Growth Parameters of Rice Under Modified Murashige-Skoog Solution Culture Technique

Treatment	No. of leaves	Mean Root Length (cm)	Mean Shoot Length (cm)	Root: Shoot Ratio	Fresh weight/ seedling (g)	Oven dry weight/ seedling (g)	Percent Dry Matter (%)	Percent of Moisture Content
Complete	2.0	10.32b	19.53a	0.53b	0.86a	0.11a	13.29	86.71
-N	2.0	19.71a	9.32b	2.11a	0.45b	0.06b	13.33	86.67
-P	2.0	13.30b	16.70a	0.70b	0.76a	0.09b	11.84	88.16
-K	2.0	7.47c	9.57b	0.79b	0.32b	0.04b	12.50	87.50
-Ca	2.0	12.45b	17.54a	0.70b	0.56b	0.07b	13.39	86.61
-Mg	2.0	12.73b	12.16a	0.45b	0.89a	0.17a	11.86	88.14
-Fe	2.0	9.90c	16.25a	0.61b	0.46b	0.05b	11.96	88.04
- Zn	2.0	8.52c	20.34a	0.42b	0.99a	0.12a	12.63	87.37
F-test	ns	*	*	*	*	*	ns	ns
% CV	0.0	2.54	4.78	7.54	10.54	3.54	4.34	6.43

The same element in a column is not significantly different at the 5% level, HSD test.

ISSN 2449-4577 (online) 2408-283X (print)

with less lateral secondary roots as compared to the plants receiving complete nutrients.

Phosphorus (P) Deficiency

Phosphorus (P) deficiency in soil is a major constrain for rice production. Phosphorus is one of the primary three nutrients most commonly found in fertilizers and is the "P" in the NPK balance listed on fertilizer recommendations. Phosphorus is essential in rice culture and helps promote rapid tillering, root development, and early flowering. However, if this element is deficient, rice plants often become stunted and appear very dark green to almost bluish.

Figure 3

Phosphorus (P) Deficiency

The general symptoms of phosphorus-deficient rice seedlings (Figure 3) include spotting of basal leaves, drying up of basal leaves, localized yellowing of older leaves with interveinal chlorosis. It showed an intense browning of the tips or edges of leaves with reduced leaf area compared to the treatment receiving the complete nutrients. It helps a plant convert other nutrients into usable building blocks with which to grow.

Potassium (K) Deficiency

The deficiency symptoms of potassium deficiency occur mainly on the older leaves (Figure 4). The general symptoms exhibited by rice plants include brown necrotic lesions or spots that develop within the leaves' chlorotic zones, severe yellowing of leaves (chlorosis) in the interveinal and marginal zones. In extreme cases, the leaves will turn whitish or completely lose their green coloration. The shoots of rice seedlings, which are deficient in potassium, generally exhibit stunted growth (Asadi et al., 2012).

Figure 4

Phosphorus (P) Deficiency

This has tremendous implications on rice roots development since potassium physiologically affects the root formation of rice. Other signs and symptoms of K deficiency include rusty brown spots on tips of older leaves that later spread over the whole leaf, causing it to turn brown and dry if K deficiency is severe. Stunted plants have smaller leaves and short and thin stems.

Zinc (Zn) Deficiency

Zinc (Zn) deficiency is one of the most important nutritional problems after micronutrient deficiency in crop production. It is now considered the most widespread nutrient disorder in lowland rice (Magahud et al. 2015). Zinc deficiency symptoms are more common on young or middle-aged leaves. Dusty brown spots appear on upper leaves of stunted plants, sometimes two to four weeks after transplanting, with uneven plant growth and patches of poorly established hills. Under severe deficiency, tillering decreases, and time to crop maturity may be increased. On the other hand, elevated concentrations of Zn can lead to toxicity symptoms and growth inhibition in rice (Song et al.,.2011).

The general symptoms of zinc deficiency are also chlorosis and necrosis of the younger leaves. As zinc stimulates auxin production, one symptom is that the plant becomes low and dense with small and dry old leaves. However, the expected symptoms did not manifest in our experiment (Figure 5). The overall features of the zinc-deficient plants were almost the same as those of the treatment receiving complete nutrients except for the very slight yellowing of the older leaves. This could be because the other nutrients available in the solution were able to help the plants to grow almost normally.

Figure 5

Zinc (Zn) Deficiency

Calcium (Ca) Deficiency

The primary symptoms of calcium deficiency in rice plants include the necrosis of young expanding leaves which may lead to failure of the leaf to develop and finally to the death of the apex. These symptoms were manifested in our experiment (Figure 6). In calcium deficiency, the necrosis spread from irregular patches especially along the lateral margins nearing the petiole and extends inward mainly in the interveinal tissues.

Figure 6

Calcium (Ca) Deficiency

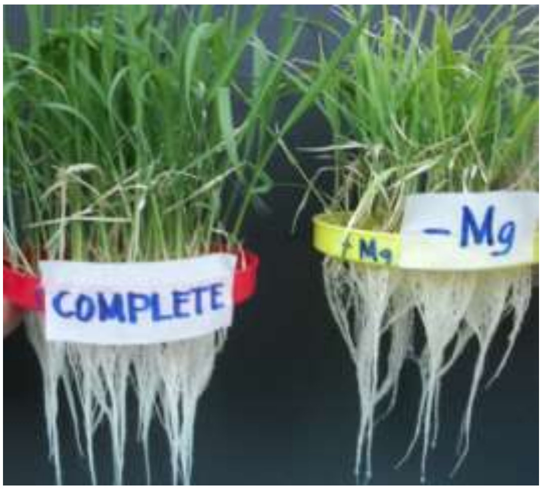
Generally, calcium deficient plant fail to extend its growth as shown by its inability to increase the shoot length due to shoot "die back". All the rice shoots we used failed to increase its length as shown in the pictures This is because the root tips of rice without calcium treatment becomes rotten/swollen and failed to grow making it impossible for the absorption of other nutrients to occur. In general plant growth is stunted. Because Ca is not re-translocated to new growth, deficiency symptoms usually appear first on young leaves. Roots are much shorter and look dark Brown. Calcium deficiency can result in impaired root function and might predispose the rice plant to iron deficiency (Haifa, 2020).

Magnesium (Mg) Deficiency

Magnesium (Mg) deficiency affects several enzyme activities in plants, including carbon dioxide (CO_2) assimilation and protein synthesis, and the cellular pH and the cation-anion balance activation. The general symptoms of magnesium deficiency in rice plants include interveinal chlorosis of the older leaves and the yellowing of the younger leaves. As the chlorosis intensifies, the dark green color of the leaves changed to yellow green, pale yellow to golden yellow (Figure 7).

Figure 7

Magnesium (Mg) Deficiency



The shoots are generally very thin with the younger leaves showing severe chlorosis aside from its stunted growth. In severe cases, chlorosis progresses to yellowing and finally necrosis in older leaves. Leaf number and leaf length are greater in Mg-deficient plants, and Mg-deficient leaves are wavy and droopy due to an expansion in the angle between the leaf sheath and leaf blade (Alghobar & Suresha, 2016). If deficiency becomes more severe, green coloring appears as a "string of beads" in which green and yellow stripes run parallel to the leaf.

Iron (Fe) deficiency

Iron (Fe) is an essential element for most living organisms. One major distinguishing feature of iron deficiency in rice as shown by our samples is the chlorosis of the leaves leading to

ISSN 2449-4577 (online) 2408-283X (print)

necrosis. In iron deficiency, the youngest leaves will show the greatest intensity of chlorosis. Severely affected leaf blades become necrotic, with the necrosis usually spreading from the tip and margin into interveinal zones (Figure 8). However, as the deficiency intensifies, the leaves will be severely necrotic and die with its color transforming to dark brown (Bashir et al., 2013).

Figure 8

Iron (Fe) Deficiency

The shoots are generally very thin with the younger leaves showing severe chlorosis aside from its stunted growth. In severe cases, chlorosis progresses to yellowing and finally necrosis in older leaves. Leaf number and leaf length are greater in Mg-deficient plants, and Mg-deficient leaves are wavy and droopy due to an expansion in the angle between the leaf sheath and leaf blade (Alghobar & Suresha, 2016). If deficiency becomes more severe, green coloring appears as a "string of beads" in which green and yellow stripes run parallel to the leaf.

Therefore, it can be concluded that macro elements are very important components for plant's structure, processes, and physiological function since their availability is necessary to complete its life cycle. The study is considered basic research that the findings are utilized for the succeeding researches as basis for the treatment recommendation. Hence, this study can contribute to the young researchers in developing their research studies.

IV. CONCLUSION

The results revealed that rice plants without the macro-elements (N, P & K) severely exhibited yellowing of leaves (chlorosis), leading to necrosis. Likewise, rice plants without P, Ca and Fe exhibited stunted growth and yellowing of leaves. Moreover, rice plants without N had a very high root length (200%) compared to the shoot length, resulting in the root: shoot ratio's high value. This means that omitting the most limiting element (N) causes the plants to grow longer roots to find the plants' nutrients. This results validates farther the essentiality of nutrient elements to the rice plants for its normal growth and development. The absence of the essential nutrients plants cannot complete its life cycle. This is a basic research that the findings can be utilized for succeeding researches as basis for the treatment recommendations.

ACKNOWLEDGEMENT

The researchers would like to thank the research committee for their efforts to check and enhance the output of this study.

REFERENCES

Alghobar, M. A., & Suresha, S. (2016). Effect of Wastewater Irrigation on Growth and Yield of Rice Crop and Uptake and Accumulation of Nutrient and Heavy Metals in Soil. *Applied Ecology and Environmental Sciences* , *4*(3), 53–60. https://doi.org/10.12691/aees-4-3-1

Asadi, M., Saadatmand, S., Khavari-Nejad, R. A., Ghasem-nejad, M., & Fotokian, M. H. (2012). Effect of Zinc (Zn) on Some Physiological Characteristics of Rice Seedling. *Biomedical & Pharmacology Journal*, *5*(2), 203–210. https://doi.org/10.13005/bpj/345

Bashir, K., Nozoye, T., Ishimaru, Y., Nakanishi, H., & Nishizawa, N. K. (2013). Exploiting new tools for iron bio-fortification of rice. *Biotechnology Advances*, *31*(8), 1624–1633. https://doi.org/10.1016/j.biotechadv.2013.08.012

Billen, G., Garnier, J., & Lassaletta, L. (2013). The nitrogen cascade from agricultural soils to the sea: modelling nitrogen transfers at regional watershed and global scales. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621), 20130123. https://doi.org/10.1098/rstb.2013.0123

Bown, H. E., Watt, M. S., Clinton, P. W., & Mason, E. G. (2010). Influence of ammonium and nitrate supply on growth, dry matter partitioning, N uptake and photosynthetic capacity of Pinus radiata seedlings. *Trees*, *24*(6), 1097–1107. https://doi.org/10.1007/s00468-010-0482-1

Chin, J. H., Gamuyao, R., Dalid, C., Bustamam, M., Prasetiyono, J., Moeljopawiro, S., … Heuer, S. (2011). Developing Rice with High Yield under Phosphorus Deficiency: Pup1 Sequence to Application. *Plant Physiology*, *156*(3), 1202–1216. https://doi.org/10.1104/pp.111.175471

Fageria, N. K., Moreira, A., & dos Santos, A. B. (2013). PHOSPHORUS UPTAKE AND USE EFFICIENCY IN FIELD CROPS. *Journal of Plant Nutrition*, *36*(13), 2013–2022. https://doi.org/10.1080/01904167.2013.816728

FAO. (2016). *Cereal Supply and Demand Brief World Cereal Production in 2014 to Surpass the Record in 2013*, Rome, 20.

Fitts, P. W., Walker, T. W., Krutz, L. J., Golden, B. R., Varco, J. J., Gore, J., … Slaton, N. A. (2014). Nitrification and Yield for Delayed-Flood Rice as Affected by a Nitrification Inhibitor and Coated Urea. *Agronomy Journal*, *106*(5), 1541–1548. https://doi.org/10.2134/agronj13.0586

Gruber, B. D., Giehl, R. F. H., Friedel, S., & von Wirén, N. (2013). Plasticity of the Arabidopsis Root System under Nutrient Deficiencies. *Plant Physiology*, *163*(1), 161–179. https://doi.org/10.1104/pp.113.218453

Haifa. (2020, June 28). *Crop Guide: Rice Nutrition*. Haifa Group. https://www.haifa-group.com/rice-0/crop-guide-rice-nutrition.

Hosseinzadeh, S., Liu, Z., De Graeve, J., BKheet, M., Libbrecht, W., De Clercq, J., & Van Hulle, S. (2019). Recirculating Water Treatment in Closed Hydroponic Systems: Assessment of Granular Activated Carbon and Soft Templated Mesoporous Carbon for Adsorptive Removal of Root Exudates. *Environmental Processes*, *6*(1), 1–23. https://doi.org/10.1007/s40710-019-00347-0

Magahud, J., Badayos, R., Sanchez, P., & Sta. Cruz, P. (2015). Levels and sources of potassium, calcium, sulfur, iron and manganese in major paddy soils of the Philippines. *International Journal of Philippine Science and Technology*, *8*(2), 1–8. https://doi.org/10.18191/2015-08-2-014

Maucieri, C., Barbera, A. C., Vymazal, J., & Borin, M. (2017). A review on the main affecting factors of greenhouse gases emission in constructed wetlands. *Agricultural and Forest Meteorology*, *236*, 175–193. https://doi.org/10.1016/j.agrformet.2017.01.006

McCauley , A., Jones, C., & Jacobsen , J. (2011, June). *Plant Nutrient Functions and Deficiency and Toxicity Symptoms* . Nutrient Management. https://landresources.montana.edu/nm/documents/NM9.pdf.

Nathan, M. (2016, July 15). *Diagnosing Nutrient Deficiencies* . Integrated Pest Management. https://ipm.missouri.edu/ipcm/2016/7/diagnosing_nutrient_deficiencies/.

Song, A., Li, P., Li, Z., Fan, F., Nikolic, M., & Liang, Y. (2011). The alleviation of zinc toxicity by silicon is related to zinc transport and antioxidative reactions in rice. *Plant and Soil*, *344*(1-2), 319–333. https://doi.org/10.1007/s11104-011-0749-3

White, P. J., & Brown, P. H. (2010). Plant nutrition for sustainable development and global health. *Annals of Botany*, *105*(7), 1073–1080. https://doi.org/10.1093/aob/mcq085

.

ISSN 2449-4577 (online) 2408-283X (print)