# Rice-Based Biosystems Journal

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Philippine Rice Research Institute
Central Experiment Station
Maligaya, Science City of Muñoz, 3119 Nueva Ecija


# ABOUT THE COVER

Achieving abundant harvest in the Philippines is limited by traditional farming practices such as manual transplanting. Thus, appropriate rice-based technologies and researches are conducted to improve farming practices and increase the competitiveness of the Filipino rice farmers. Research results on proper nutrient application, increased seed viability, and profitable rice-based products are published in this issue as the scientific community's way of helping uplift the lives of rice growers.

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# EDITORIAL TEAM

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# FIELD APPLICATION OF POTASSIUM FERTILIZER MITIGATES IMPACT OF REPRODUCTIVE STAGE DROUGHT STRESS ON RICE YIELD

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## Abstract

Success in increasing rice grain yield in drought-prone rainfed lowlands has yet to be fully achieved. Hence, it is important that agronomic and genetic management strategies focus on use of available soil moisture for growth and yield. Field experiment in 2017 and 2018 dry seasons assessed the morphological, growth, and grain yield responses of three rice varieties on increasing K fertilizer application and its effect on stress mitigation following reproductive stage drought stress. The K levels were: 0-0-0, 120-40-60, and 120-40-120 kg NPK ha$^{-1}$ applied before imposing the 20-day drought stress at reproductive stage (60-80 DAT or DAT). In response to drought stress, average soil water content decreased to 9.4% while soil strength increased to 2.3 MPa. Higher K levels showed mitigating effects on the impact of reproductive drought stress; however, it is genotype independent. Comparing with NSIC Rc 222 and NSIC Rc 418, NSIC Rc 282 had better response to application of 120-40-120 kg NPK ha$^{-1}$ in mitigating reproductive drought stress with slower progression of leaf rolling, higher panicle exsertion rate, lower spikelet sterility, higher spikelet number m$^{-2}$, and ultimately resulting in higher grain yield and smaller percent yield reduction based on the well-watered control. Thus, NSIC Rc 282 with application of 120-40-120 kg NPK ha$^{-1}$ is recommended for use in drought-prone rainfed lowlands in the Philippines specifically for reproductive stage drought.

**Keywords:** *Grain Yield, Growth, Leaf Rolling, Potassium Fertilizer, Reproductive Drought Stress, Rice Variety, Soil Moisture Status.*

## Introduction

Abiotic stresses such as drought, salinity, submergence, and nutrient deficiencies limit rice production in rainfed environments (Lafitte at al., 2014). Reports have shown that damage due to biotic and abiotic stresses resulted in more than 50% crop damage worldwide (Ansari et al., 2015). Average grain yield of rice in rainfed lowland ecosystem is about 2 t ha$^{-1}$, but may vary depending on the intensity and duration of drought stress (Fischer et al., 2004), sensitivity of growth stage, and crop management.

With long period of El Niño in the Philippines, which is associated with persistent warmer than average sea surface temperatures and consistent changes in wind and rainfall patterns, drought has become more damaging to rice production, especially in drought-prone areas. The average grain yield in these areas is 2.3 t ha$^{-1}$ (DA, 2010).

Drought has numerous meanings in relation to crop production (Passioura, 2006; Serraj et al., 2011). To a meteorologist, drought has the lowest rank of annual rainfall. To an agronomist, yield is limited by too little water. To a farmer, drought is a risk management issue, e.g., how to match cultivar and agronomic operations such as land preparation, transplanting, and fertilizer application to the developing season; and how to minimize possible severe damage to a critical process such as floral fertility induced by severe water deficits during flowering. These terms and issues are relevant in improving crop production under limited water. Agronomists and breeders can help improve seasonal water balance in the field by matching crop development to its environment. Physiologists, biochemists, and molecular biologists can help by identifying ways of improving the competence of particular organs especially at a most sensitive stage to water deficit, i.e., reduction division stage to heading or panicle exsertion followed by flowering, in which yield is reduced significantly due to high spikelet sterility.

Drought stress affects the physiological, morphological, and biochemical properties of plants (Wang et al., 2016). In addition to the complexity of drought itself, plant responses to water deficits are complex due to unpredictable factors in the environment and interaction with other abiotic and biotic factors (Nevo and Chen, 2010). To alleviate the adverse effects of drought to rice crop, use of drought-tolerant varieties is more practical than supplying more irrigation to drought-prone areas considering inadequate water. Following exposure of the rice crop to drought stress at vegetative stage, Bunnag and Pongthai (2013) categorized three levels of tolerance based on more distinguishing features such as leaf rolling, leaf death, and leaf water status. CT9993 was considered highly drought-tolerant; KDML 105, IR58821, IR57514, IR52561, and BT were moderately drought-tolerant; while IR62266 was sensitive to drought.

In drought tolerance studies, application of potassium (K) fertilizer has been shown to mitigate the adverse effects of drought on the growth of barley (Andersen et al., 1992) and rice (Tiwari et al., 1998). The higher K requirement of plants during abiotic stresses appears to be related to the inhibitory role of potassium against oxidative stress (Cakmak, 2005). Zain et al. (2014) assessed the effects of different periodical water stress combined with K fertilization regimes on growth, yield, leaf gas exchanges, and biochemical changes in rice cultivar MR220 grown in pots and compared the regimes with standard local grower practices. The pot study included common application of 120 kg N ha$^{-1}$ and 70 kg $P_2O_5$ ha$^{-1}$ [i.e., 30% at 3-leaf stage or 3 days after seeding and 70% at booting stage or 50-55 days after seeding]. Although the yield of 8.2 ± 0.4 t ha$^{-1}$ with drought stress for 25 days at vegetative stage and 120 kg $K_2O$ ha$^{-1}$ applied in two phases was higher than the yield of 7.3 ± 0.3 t ha$^{-1}$ with periodical drought stress for 15 days at vegetative stage and 120 kg $K_2O$ ha$^{-1}$ applied in three phases (i.e., 30% at three-leaf stage or 3 DAS, 30% at booting or 50-55 DAS, and 40% during the stress cycle at 80-90 days after seeding), the latter increased drought tolerance based on increased water use efficiency, peroxidase, catalase and proline levels, maximum efficiency of photosystem II, and lower minimal fluorescence.

In field studies, it is critical that agronomic and genetic management strategies focus on the efficient use of available soil moisture for crop establishment, growth, and yield to develop an overall strategy for rice drought tolerance. Hence, this study assessed the soil moisture status, leaf rolling as a morphological response, growth, and grain yield of transplanted rice varieties when K fertilizer application is increased. Its effect on stress mitigation following drought stress at reproductive or flowering stage was also assessed.

## Materials and Methods

### *Location and Crop Establishment*

The field experiment was conducted at PhilRice Central Experiment Station in Science City of Muñoz, Nueva Ecija, Philippines in the dry seasons (DS) of 2017 and 2018. Soil type was Maligaya clay (Ustic Epiaquert). NSIC Rc 418, Rc 282, and Rc 222 with growth durations of 112, 115, and 109 days, respectively, were tested. Seed rate was 40 kg ha$^{-1}$. After growing the plants in the seedbed for 21 days, seedlings were carefully pulled out from the seedbed and transplanted in the experimental field at 20 cm x 20 cm between hills and density of 2-3 seedlings per hill. Plot size was 10.6 m$^2$ per treatment and replicated three times.

### *Nutrient Management*

Three fertilizer (F) treatments were used: (a) F1: 0-0-0 kg NPK ha$^{-1}$, (b) F2: 120-40-60 kg NPK ha$^{-1}$, and (c) F3: 120-40-120 kg NPK ha$^{-1}$. All of $P_2O_5$ at 40 kg ha$^{-1}$ and $ZnSO_4$ at 25 kg ha$^{-1}$ were applied at 14 days after transplanting (DAT). In each of F2 and F3 treatments, 1/3 of N and 1/3 of $K_2O$ were applied at 14 DAT, and the remaining 2/3 N and 2/3 $K_2O$ were applied at 40 DAT.

### *Drought Stress Imposition*

Drought stress was imposed by withholding irrigation water 60-80 DAT coinciding with the reproductive stage, i.e., from heading (panicle exsertion) or early flowering to complete flowering. After reproductive drought stress treatment, the field was re-irrigated with 2-3 cm floodwater depth until 2 weeks before crop maturity. In the well-watered control, 2-3 cm floodwater depth was maintained from vegetative stage until 2 weeks before crop maturity.

### *Soil Water Content, Bulk Density, and Soil Strength*

During the treatment period, replicated soil samples were obtained 0.05-0.15 m below the soil surface (i.e., representing the mid 0.10 m soil layer) for the assessment of soil water content ($\theta_g$) by gravimetric method and expressed in g g$^{-1}$ or %.

At representative sites in the test area, a standard pre-weighed metal core (5 cm diameter and 5 cm height) was gently pushed into the 0.05-0.15 m below the soil surface. The extracted soil samples were oven-dried at 100$^0$C. Bulk density (BD) was obtained by dividing the oven dry weight of the soil by the volume of the soil core with a unit g cm$^{-3}$ or Mg m$^{-3}$. The gravimetric soil water content ($\theta_g$) was multiplied by BD to obtain the volumetric soil moisture content ($\theta_v$) with a unit $cm^3$ water $cm^{-3}$ soil.

Soil strength was measured in the field up to 0.10 m below the plow pan using a cone penetrometer. The surface of the plow pan was usually observed at 0.20 m depth from the soil surface.

### *Morphological Response, Growth, Grain Yield, and Yield Components*

Morphological or visible plant response to drought was assessed using the IRRI Standard Evaluation System (IRRI, 2013) with modification. Leaf tip drying was scored between 1-5 wherein 1- slight tip drying, 2- tip drying extended to ¼ length in most leaves, 3- drying extended to ½ length of all leaves, 4- drying extended to more than 2/3 length of all leaves, and 5- whole plant dried/dead. Leaf rolling scores were quantified using the method of O'Toole and Cruz (1980) with modification in which 1- leaves fully open, 2- for slight leaf rolling, 3- C-shaped leaf rolling, 4- start of inward leaf rolling, and 5- tightly rolled leaves or closed cylinders.

Newly emerging panicles from the main tiller were selected and marked for measurement of exsertion rate. Panicle exsertion rates of four plants from each replicate were measured daily with a metric ruler. At physiological maturity, panicles from a 5 $m^2$ area in the center of each plot and were harvested, threshed, and sun- or oven-dried before weighing. Grain yield was expressed in t $ha^{-1}$ adjusted to 14% grain moisture content. Whole plant samples for grain yield components (i.e., panicle number $m^{-2}$, spikelet number $panicle^{-1}$, % filled spikelets or spikelet sterility, and 1000-grain weight in g) were obtained from three 4-hill samples located outside the three corners of the 5 $m^2$ grain yield sampling area.

### *Statistical Analysis*

Treatments were laid out in a Randomized Complete Block Design with Split-Split-Plot. Soil moisture treatment, fertilizer level, and rice variety were assigned as main plot, sub-plot, and sub-sub-plot, respectively. The analysis of variance and mean separation (Tukey's Test) were performed using the Statistical Tool for Agricultural Research (STAR). The standard error (SE) of the mean was calculated by dividing the standard deviation by square root of the number of measurements.

### *Weather Condition*

Measurements of air temperatures (maximum and minimum), rainfall, and irradiance were obtained from the Automatic Weather Station (Davis Vantage) in the experiment station.

## Results

### *Weather Condition and Crop Phenology*

During the 20-day reproductive or flowering stage treatment period from 60-80 DAT when irrigation was withheld, maximum air temperatures ranged 27.9-36.6 $^0$C, minimum air temperatures ranged 20.3-23.9 $^0$C, and irradiance ranged 19.3-23.9 MJ $m^{-2}$ in 2017 DS (Figure 1A). Similar trends were observed in 2018 DS when maximum air temperatures ranged 28.3-36.8 $^0$C, minimum air temperatures ranged 19.0-23.8 $^0$C, and irradiance ranged 16.7-23.7 MJ $m^{-2}$ (Figure 1B). In both years, there was no rainfall during the 20-day reproductive stage drought treatment period.

### *Changes in Soil Water Content and Soil Strength*

During the 20-day treatment period from 60 to 80 DAT in 2017 DS, average gravimetric soil water contents in the well-watered control in the mid 0.10 m soil layer below the soil surface ranged 58.0-67.5% in plots with 0-0-0 kg NPK $ha^{-1}$ (Figure 2A), 63.9-68.0% in plots with 120-40-60 kg NPK $ha^{-1}$ (Figure 2B), and 45-71.4% in plots with 120-40-120 kg NPK $ha^{-1}$ (Figure 2C) across rice varieties. During progressive drought treatment, average soil water content in the mid 0.10 m soil layer below the soil surface 60-80 DAT decreased from 70.4 to 9.5% in plots with 0-0-0 kg NPK $ha^{-1}$ (Figure 2A), 74.7-10.1% in plots with 120-40-60 kg NPK $ha^{-1}$ (Figure 2B), and 75.1-8.2% in plots with 120-40-120 kg NPK $ha^{-1}$ (Figure 2C) across rice varieties. Across rice varieties, soil water contents at 80 DAT or end of the 20-day drought treatment period were fairly similar and ranged 9.3-10.0% with 0-0-0 kg NPK $ha^{-1}$, 9.7-10.6% with 120-40-60 kg NPK $ha^{-1}$, and 7.2-9.0% with 120-40-120 kg NPK $ha^{-1}$.

After multiplying the above gravimetric soil water contents ($\theta_g$) by the average soil BD of 1.08 ± 0.06 g $cm^{-3}$ for Maligaya clay soil, volumetric soil water contents ($\theta_v$) in the control in 2017 DS ranged 0.49-0.77 $cm^3$ water $cm^{-3}$ soil at 60 DAT, 0.49-0.72 cm3 $cm^{-3}$ at 70 DAT, and 0.50-0.77 $cm^3$ $cm^{-3}$ at 80 DAT across rice varieties and fertilizer levels. In the drought treatment, $\theta_v$ ranged 0.71-0.90 cm3 $cm^{-3}$ at 60 DAT, 0.16-0.18 $cm^3$ $cm^{-3}$ at 70 DAT, and 0.08-0.11 cm3 $cm^{-3}$ at 80 DAT across rice varieties and fertilizer levels.

In 2018 DS, similar trends were observed and average gravimetric soil water contents across rice varieties ranged 79.4-81.4% with 0-0-0 kg NPK $ha^{-1}$, 79.2-80.7% with 120-40-60 kg NPK $ha^{-1}$, and 78.0-84.7% with 120-40-120 kg NPK $ha^{-1}$ in the well-watered control during the 20-day treatment period (Figures 3A to 3C). After irrigation was withheld

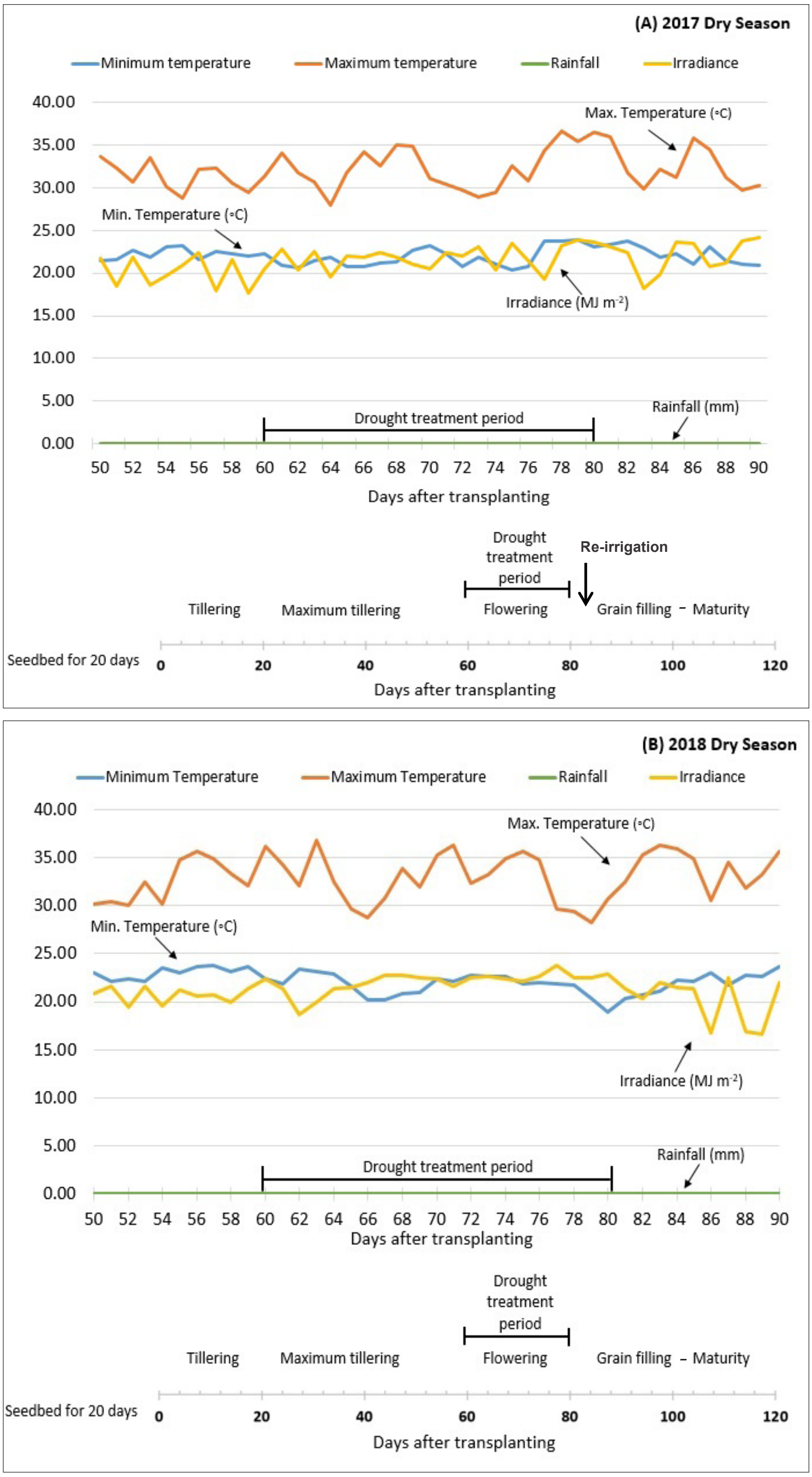



**Figure 1.** Air temperature (minimum and maximum), rainfall, and irradiance from 10 days before and 10 days after the 20-day reproductive or flowering stage drought treatment 60-80 DAT in (A) 2017 DS and (B) 2018 DS when irrigation was withheld and re-irrigated after the 20-day drought treatment period. The control was irrigated with 2-3 cm floodwater throughout the growth stages. PhilRice Central Experiment Station, Muñoz, Nueva Ecija, Philippines.

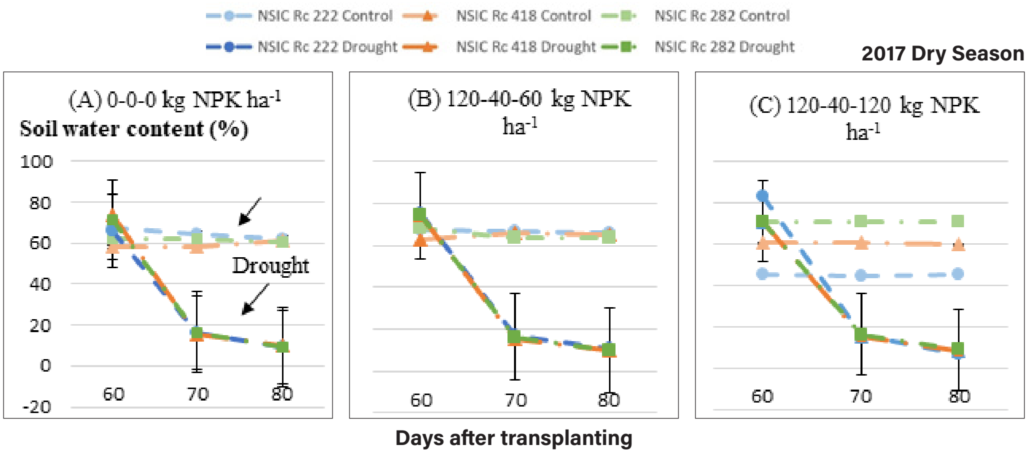



**Figure 2.** Percent soil water content in the mid 0.10 m soil layer below the soil surface in the control (well-watered) and drought treatment plots grown to NSIC Rc 222, Rc 418, and Rc 282 plots with fertilizer treatments (A) 0-0-0, (B) 120-40-60, and (C) 120-40-120 kg NPK ha$^{-1}$ in 2017 DS. Drought was imposed by withholding irrigation 60-80 DAT at reproductive or flowering stage. The control was irrigated with 2-3 cm floodwater throughout the growth stages. Vertical bar is the standard error of the mean (± SE).

in the drought treatment 60-80 DAT, average soil water contents decreased from 75.8 to 9.4% with 0-0-0 kg NPK ha$^{-1}$ (Figure 3A), from 76.4 to 9.7% with 120-40-60 kg NPK ha$^{-1}$ (Figure 3B), and from 78.9 to 9.4% with 120-40-120 kg NPK ha$^{-1}$ (Figure 3C) across rice varieties. At 80 DAT or end of the 20-day drought treatment period, average soil water contents ranged 9.2- 9.6% with 0-0-0 kg NPK ha$^{-1}$, 9.6-9.8% with 120-40-60 kg NPK ha$^{-1}$, and 9.0-9.6% with 120-40-120 kg NPK ha$^{-1}$.

In the control in 2018 DS, $\Theta_v$ ranged from 0.77 to 0.96 cm$^3$ water cm$^{-3}$ soil at 60 DAT, 0.80-0.90 cm$^3$ cm$^{-3}$ at 70 DAT, and from 0.84 to 0.89 cm$^3$ cm$^{-3}$ at 80 DAT across rice varieties and fertilizer levels. In the drought treatment, $\Theta_v$ ranged 0.38-0.40 cm3 cm$^{-3}$ at 60 DAT, and 0.10-0.11 cm3 cm$^{-3}$ at 80 DAT across rice varieties and fertilizer levels.

Soil water content, measured at a single point (i.e., mid 0.10 m soil layer below the soil surface) during the 20-day treatment period from 60-80 DAT, was above the field capacity soil water potential ($\Psi_{soil}$ > -0.033 MPa) in the well-watered control across rice varieties and fertilizer levels. This was inferred from the reported soil water retention curve for clay soil (Wopereis et al., 1996). In the drought treatment from 60 to 80 DAT, soil water content decreased

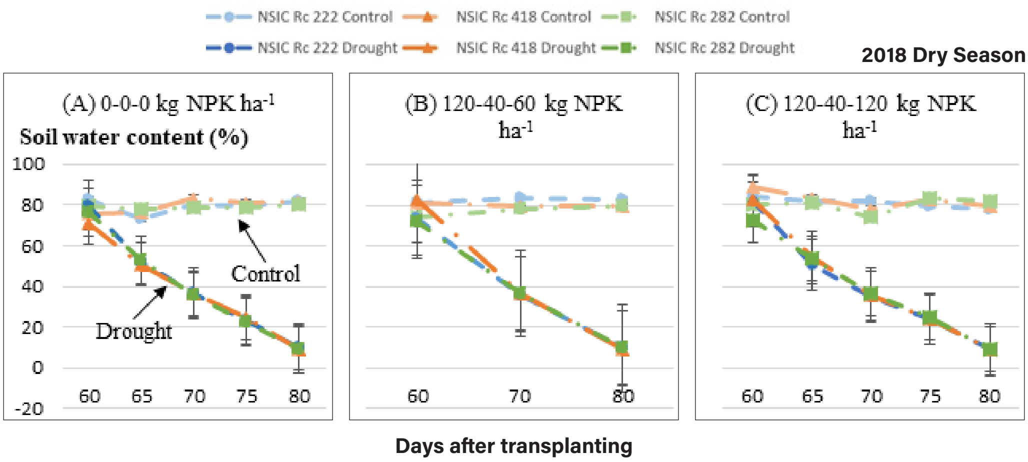



**Figure 3.** Percent soil water content in the mid 0.10 m soil layer below the soil surface in the control (well-watered) and drought treatment plots grown to NSIC Rc 222, Rc 418, and Rc 282 plots with fertilizer treatments (A) 0-0-0, (B) 120-40-60, and (C) 120-40-120 kg NPK ha$^{-1}$ 2018 DS. Drought was imposed by withholding irrigation 60-80 DAT at reproductive or flowering stage. The control was irrigated with 2-3 cm floodwater throughout the growth stages. Vertical bar is the standard error of the mean (± SE).

progressively to a level much below the field capacity but did not appear to be influenced by fertilizer levels in 2017 DS (Figure 2) and 2018 DS (Figure 3) across rice varieties.

Soil strength in the plow pan layer in the well-watered control in 2017 DS was close to 0.5 MPa across rice varieties in the different fertilizer treatments during the 20-day treatment period (Figures 4A, 4B, and 4C). As soil water content decreased during the 20-day drought treatment period, the Maligaya clay soil became more cohesive and soil strength increased. Average soil strength increased from 0.5 to 2.4 MPa in plots with 0-0-0 kg NPK ha$^{-1}$, from 0.5 to 2.3 MPa in plots with 120-40-60 kg NPK ha$^{-1}$, and from 0.5 to 2.2 MPa in plots with 120-40-120 kg NPK ha$^{-1}$ across rice varieties. Across varieties, soil strengths at the end of the drought treatment were fairly similar ranging 2.2-2.5 MPa with 0-0-0 kg NPK ha$^{-1}$, 2.3 MPa with 120-40-60 kg NPK ha$^{-1}$, and 2.1-2.3 MPa with 120-40-120 kg NPK ha$^{-1}$.

In 2018 DS, similar trends in soil strength were observed. In the well-watered control, average soil strength was 0.5 MPa across rice varieties in the different fertilizer treatments during the 20-day treatment period. In response to drought, average soil strength increased from 0.5 to 2.3 MPa across fertilizer treatments and rice varieties during the 20-day treatment period (Figures 5A, 5B, and 5C).

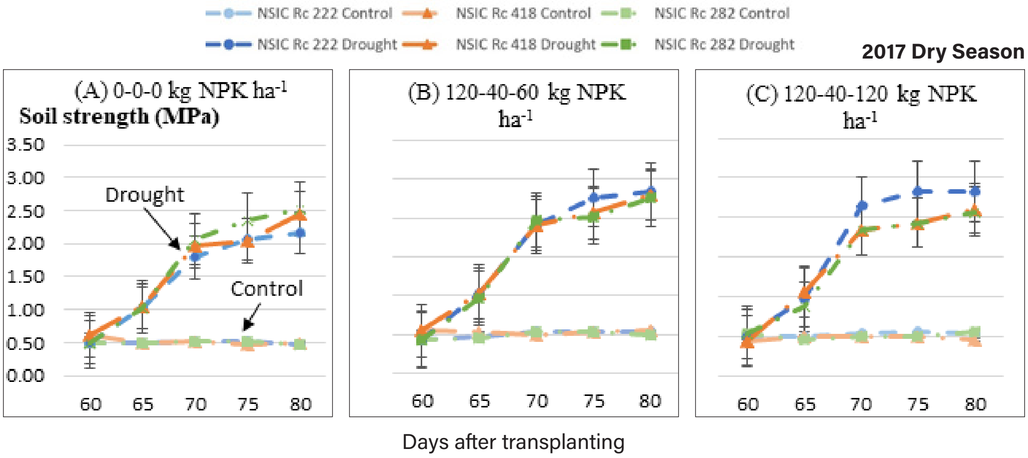



**Figure 4.** Soil strength up to 0.10 m below the plow pan (usually at 0.20 m below the soil surface) in the control (well-watered) and drought treatment plots grown to NSIC Rc 222, Rc 418, and Rc 282 with fertilizer treatments (A) 0-0-0, (B) 120-40-60, and (C) 120-40-120 kg NPK ha$^{-1}$ in 2017 dry season. Drought was imposed by withholding irrigation 60-80 DAT at reproductive or flowering stage. The control was irrigated with 2-3 cm floodwater throughout the growth stages. Vertical bar is the standard error of the mean (± SE).

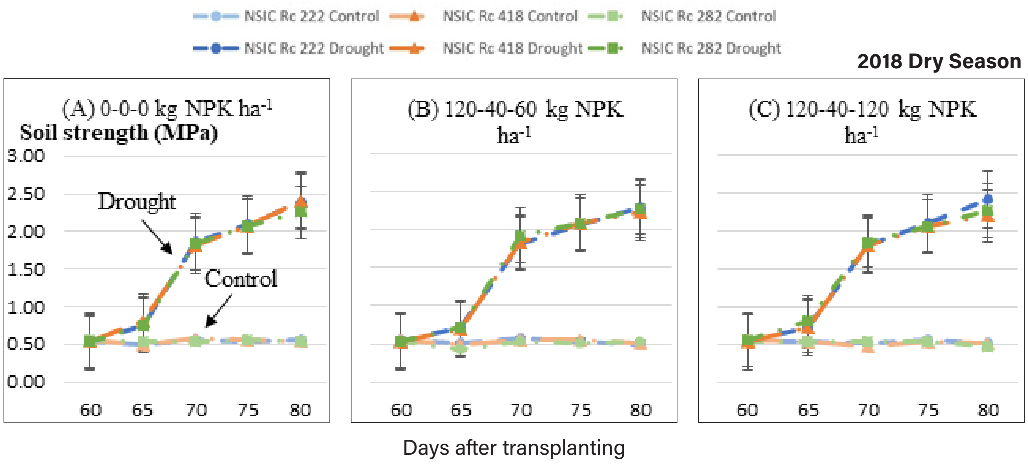
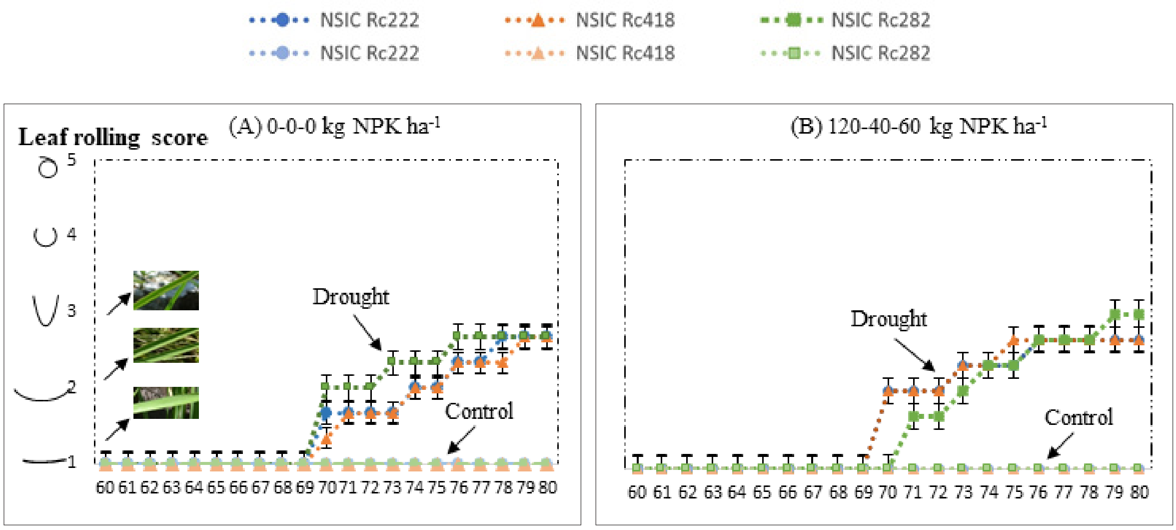
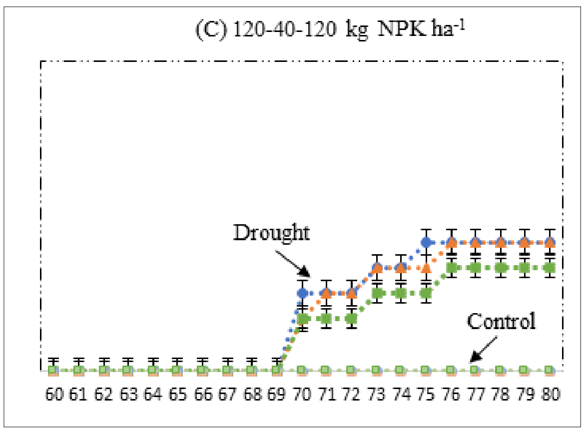



**Figure 5.** Soil strength up to 0.10 m below the plow pan (usually at 0.20 m below the soil surface) in the control (well-watered) and drought treatment plots grown to NSIC Rc 222, Rc 418, and Rc 282 with fertilizer treatments (A) 0-0-0, (B) 120-40-60, and (C) 120-40-120 kg NPK ha$^{-1}$ in 2018 dry season. Drought was imposed by withholding irrigation 60-80 DAT at reproductive or flowering stage. The control was irrigated with 2-3 cm floodwater throughout the growth stages. Vertical bar is the standard error of the mean (± SE).

In the well-watered control in 2017 DS, leaf rolling score was 1 across rice varieties and fertilizer levels during the 20-day treatment period (Figures 6A, 6B, and 6C). In response to drought stress at 70 DAT or 10 days after withholding irrigation, average leaf rolling scores increased to 1.7 with 0-0-0 kg NPK ha$^{-1}$ (Figure 6A), 1.7 with 120-40-60 kg NPK ha$^{-1}$ (Figure 6B), and 1.8 with 120-40-120 kg NPK ha$^{-1}$ (Figure 6C) across rice varieties. At 80 DAT or 20 days after withholding irrigation, average leaf rolling scores increased to 2.7 with 0-0-0 kg NPK ha$^{-1}$, 2.8 with 120-40-60 kg NPK ha$^{-1}$, and 2.6 with 120-40-120 kg NPK ha$^{-1}$ across rice varieties. The decrease in average leaf rolling score to 2.6 with increased K level (i.e., 120-40-120 kg NPK ha$^{-1}$) was attributed to a lower leaf rolling score of 2.3 for NSIC Rc 282 at the end of the drought period (Figure 6C). Leaf rolling score was 2.7 for NSIC Rc 222 and Rc 418.

In 2018 DS, similar trends in leaf rolling scores were observed for the well-watered control and drought stress across rice varieties and fertilizer levels during the 20-day treatment period (Figures 7A to 7C). With increased pre-drought stress potassium level (i.e., 120-40-120 kg NPK ha$^{-1}$) and drought, NSIC Rc 282 had lower leaf rolling scores of 1.3 at 70 DAT or 10 days after withholding irrigation and 2.3 at 80 DAT (Figure 7C). Leaf rolling scores for NSIC Rc 222 and Rc 418 were 2.0 at 70 DAT and 3.0 at 80 DAT.




**2017 Dry Season**

**Days after transplanting**

**Figure 6.** Leaf rolling score of NSIC Rc 222, Rc 418, and Rc 282 in the drought and control (well-watered) plots with fertilizer treatments (A) 0-0-0, (B) 120-40-60, and (C) 120-40-120 kg NPK ha$^{-1}$ fertilizer during the 20-day reproductive stage treatment 60-80 DAT in 2017 dry season. A leaf rolling score of 1 referred to leaves that were fully open while a score of 5 referred to leaves that were tightly-rolled. Vertical bar is the standard error of the mean (± SE).

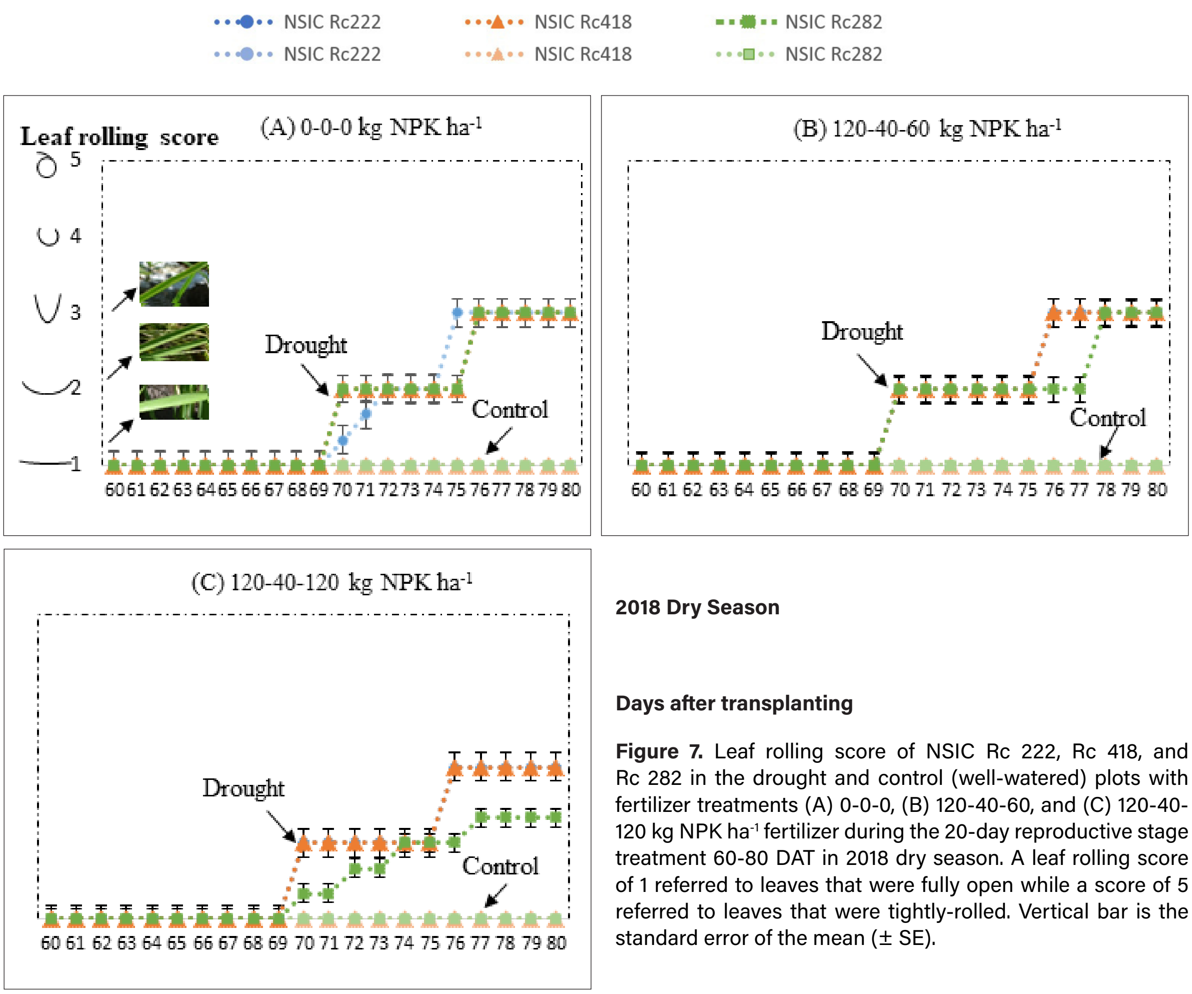



**Figure 7.** Leaf rolling score of NSIC Rc 222, Rc 418, and Rc 282 in the drought and control (well-watered) plots with fertilizer treatments (A) 0-0-0, (B) 120-40-60, and (C) 120-40-120 kg NPK ha$^{-1}$ fertilizer during the 20-day reproductive stage treatment 60-80 DAT in 2018 dry season. A leaf rolling score of 1 referred to leaves that were fully open while a score of 5 referred to leaves that were tightly-rolled. Vertical bar is the standard error of the mean (± SE).

In both well-watered control and drought stress treatments, leaf tip drying score was 1 (no tip drying to slight tip drying) across rice varieties and fertilizer levels during the 20-day treatment period in the dry season of 2017 and 2018.

***Panicle Growth, Spikelet Sterility, Other Yield Components, and Grain Yield in Response to Potassium Level and Reproductive Stage Drought Stress***

In 2017 and 2018 DS across rice varieties in the well-watered control, panicle growth rates or panicle exsertion rates ranged 0.97-1.65 mm day$^{-1}$ with 0-0-0 kg NPK ha$^{-1}$ 1.76-2.22 mm day$^{-1}$ with pre-treatment application of 120-40-60 kg NPK ha$^{-1}$, and further increased from 2.08 to 3.35 mm day$^{-1}$ with pre-treatment application of 120-40-60 kg NPK ha$^{-1}$ (Figures 8A to 8C). Across fertilizer treatments and rice varieties, NSIC Rc 282 with 120-40-120 kg NPK ha$^{-1}$ had significantly higher panicle exsertion rate. In response to 20-day drought stress at reproductive stage, panicle exsertion rates were significantly lower than the control but likewise increased with higher in pre-drought stress K fertilizer application. With reproductive stage drought stress, the average panicle exsertion rates ranged 0.46-1.00 mm day$^{-1}$ with 0-0-0 kg NPK ha$^{-1}$, 0.54-1.19 mm day$^{-1}$ with 120-40-60 kg NPK ha$^{-1}$, and 0.73-1.27 mm day$^{-1}$ with 120-40-120 kg NPK ha$^{-1}$. Following the same trend in the control, NSIC Rc 282 with 120-40-120 kg NPK ha$^{-1}$ had significantly higher panicle exsertion rate across rice varieties and fertilizer treatments. There was no significant interaction among water treatments, fertilizer treatments, and rice varieties for panicle exsertion rate.

With the increase in panicle exsertion rates across rice varieties, well-watered and drought treatments, and elevated levels of K fertilizer application prior to drought stress (Figures 9A to 9C), percent spikelet sterility (i.e., based on the ratio of number of unfilled spikelets to total number of spikelets) decreased. For well-watered and drought treatments, NSIC Rc 282 with 120-40-120 kg NPK ha$^{-1}$ were significantly higher across fertilizer treatments and rice varieties. Spikelet sterilities across rice varieties in the well-watered control were significantly lower than in drought stress treatment. Combined analysis of 2017 and 2018 DS results showed that across rice varieties in the well-watered control, spikelet sterility ranged 29.63-33.30% with 0-0-0 kg NPK ha$^{-1}$ (Figure 9A), 25.45-29.00% with 120-40-60 kg NPK ha$^{-1}$ (Figure 9B), and 16.45-21.05% with 120-40-120 kg NPK ha$^{-1}$ (Figure 9C). In response to reproductive stage drought stress across rice varieties, spikelet sterility ranged 42.47-49.12% with 0-0-0 kg NPK ha$^{-1}$, 30-.27-35.95% with pre-drought application of 120-40-60 kg NPK ha$^{-1}$, 23.86-28.95% with 120-40-120 kg NPK ha$^{-1}$. There was no significant interaction among water treatments, fertilizer treatments, and rice varieties for spikelet sterility.

In 2017 and 2018 DS across rice varieties in the well-watered control, the spikelet number m$^{-2}$ significantly increased with the level of K fertilizer application and significantly higher than in the drought stress treatment. In the well-watered control across rice varieties, spikelet number m$^{-2}$ ranged 31.34-36.55 x 10$^{3}$ with 0-0-0 kg NPK ha$^{-1}$ (Figure

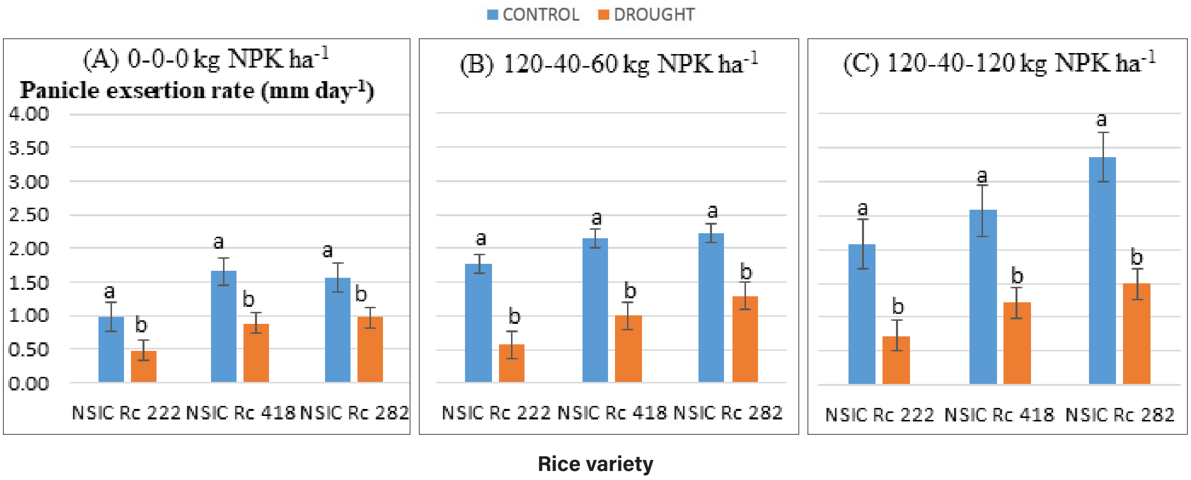



**Figure 8.** Mean panicle exsertion rate (mm day$^{-1}$) for NSIC Rc 222, Rc 418, and Rc 282 in the control (well-watered) and drought treatments with (A) 0-0-0, (B) 120-40-60, and (C) 120-40-120 kg NPK ha$^{-1}$ during the 20-day reproductive stage treatment period 60-80 DAT in 2017 and 2018 DS. Combined analysis of variance of the 2017 and 2018 DS spikelet sterility did not differ significantly at 5% level. Vertical bar is the standard error of the mean (± SE). Means followed by a common letter are not significantly different at 5% level by Tukey's Test.

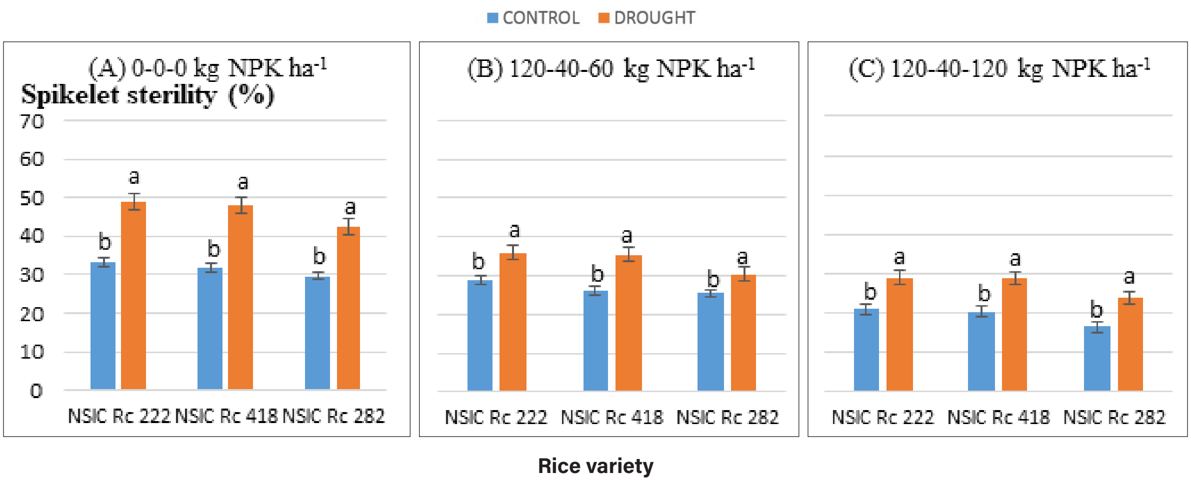



**Figure 9.** Mean percent spikelet sterility of NSIC Rc 222, Rc 418, and Rc 282 in the control (well-watered) and drought treatments with (A) 0-0-0, 120-40-60, (B) 120-40-60, and (C) 120-40-120 kg NPK ha-1 fertilizer in 2017 and 2018 DS. Combined analysis of variance of the 2017 and 2018 DS spikelet sterility did not differ significantly at 5% level. Vertical bar is the standard error of the mean (± SE). Means followed by a common letter are not significantly different at 5% level by Tukey's Test.

10A), 46.11-56.22 x $10^3$ with 120-40-60 kg NPK $ha^{-1}$ (Figure 10B), and 66.81-75.44 x $10^3$ with 120-40-120 kg NPK $ha^{-1}$ (Figure 10C). In response to reproductive stage drought stress across rice varieties, spikelet number $m^{-2}$ ranged 16.15-23.64 x $10^3$ with 0-0-0 kg NPK $ha^{-1}$, 23.60-30.95 x $10^3$ with 120-40-60 kg NPK $ha^{-1}$, and 36.52-46.14 x $10^3$ with 120-40-120 kg NPK $ha^{-1}$. Fertilizer treatment of 120-40-120 kg NPK $ha^{-1}$ had significantly increased spikelet number $m^{-2}$ compared with other fertilizer rates across rice varieties for both well-watered and drought treatments. NSIC Rc 282 had significantly higher spikelet number $m^{-2}$ with 120-40-120 kg NPK $ha^{-1}$ in the well-watered control and drought treatments. There was no significant interaction among water treatments, fertilizer treatments, and rice varieties for spikelet number $m^{-2}$.

The increasing trends in panicle exsertion rates, decreasing trends in the yield component percent spikelet sterility, and increasing trends in the yield component spikelet number $m^{-2}$ in response to increasing level of K fertilizer application in the well-watered control and drought stress treatments across rice varieties were not as evident in the yield component 1000-grain weight. There was no significant difference among fertilizer treatments and rice varieties. In 2017 and 2018 DS across rice varieties in the well-controlled, 1000-grain weight ranged 21.88-23.54 g with 0-0-0 kg NPK $ha^{-1}$ (Figure 11A), 24.07-24.66 with pre-treatment application of 120-40-60 kg NPK $ha^{-1}$ (Figure 11B), and 24.91-25.58 g with pre-treatment application of 120-40-120 kg NPK $ha^{-1}$ (Figure 11C). In response to reproductive stage drought stress across rice varieties, 1000-grain weight

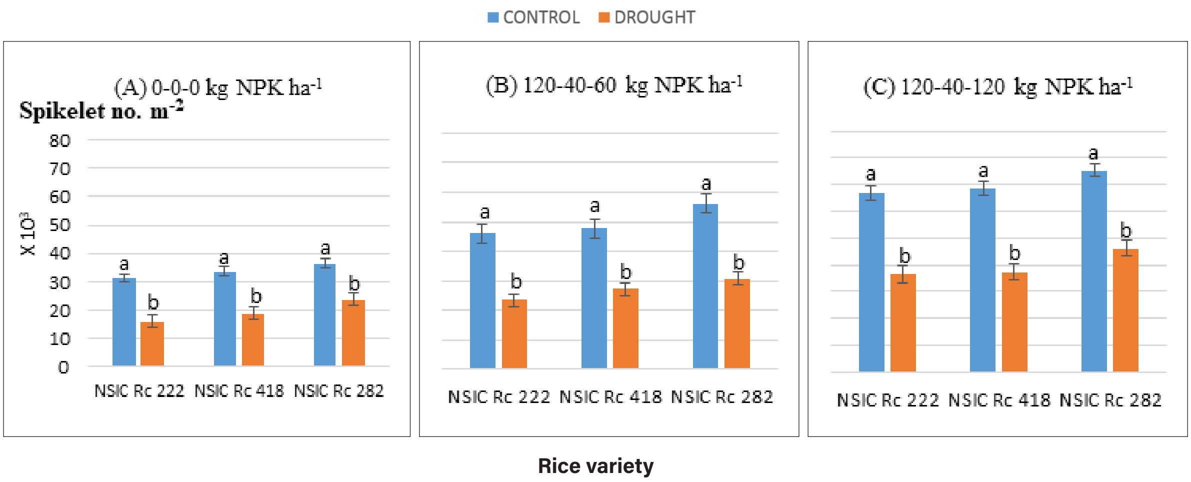



**Figure 10.** Average number of spikelets $m^{-2}$ of NSIC Rc 222, Rc 418, and Rc 282 in the drought and control (well-watered) treatments with 0-0-0, 120-40-60, and 120-40-120 kg NPK $ha^{-1}$ fertilizer treatment in 2017 and 2018 DS. Combined analysis of variance of the 2017 and 2018 DS spikelet sterility did not differ significantly at 5% level. Vertical bar is the standard error of the mean (± SE). Means followed by a common letter are not significantly different at 5% level by Tukey's Test.

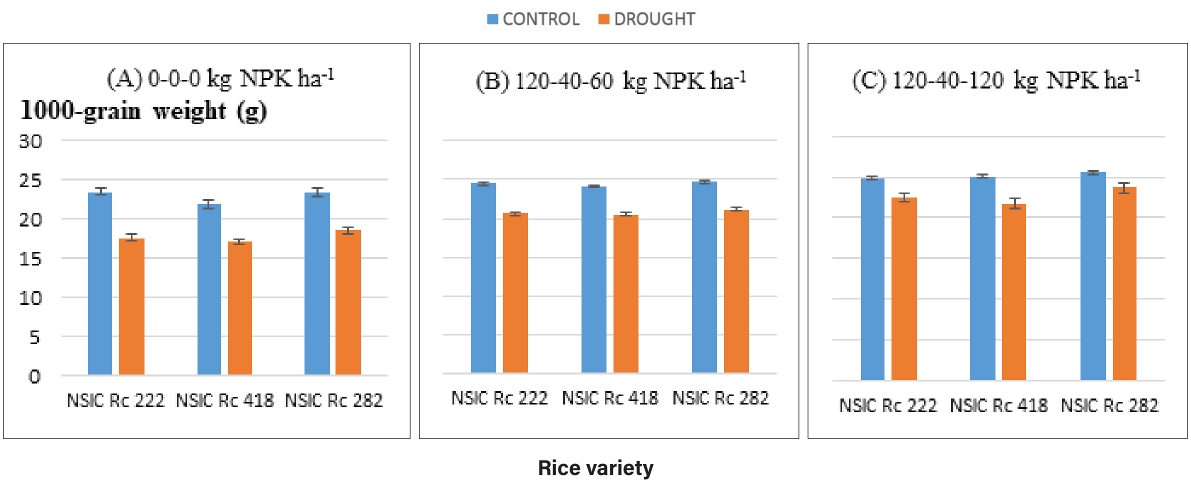



**Figure 11.** 1000-grain weight (g) of NSIC Rc 222, Rc 418, and Rc 282 in the drought and control (well-watered) treatments with (A) 0-0-0, (B) 120-40-60, and (C) 120-40-120 kg NPK $ha^{-1}$ fertilizer treatment from 60 to 80 DAT in 2017 and 2018 DS. Vertical bar is the standard error of the mean (± SE).

was lower ranging 17.12-18.55 g with 0-0-0 kg NPK $ha^{-1}$, 20.49-21.15 g with pre-drought application of 120-40-60 kg NPK $ha^{-1}$, and 21.76-23.71 g with pre-drought application of 120-40-120 kg NPK $ha^{-1}$. In response to reproductive stage drought stress, NSIC Rc 282 had relatively higher 1000-grain weight with pre-drought application of 120-40-120 kg NPK $ha^{-1}$.

In 2017 and 2018 DS across rice varieties in the well-watered control, grain yields of across rice varieties were significantly higher in increased K levels (i.e., 120-40-60 and 120-40-120 kg NPK $ha^{1}$) than 0-0-0 kg NPK $ha^{-1}$ (Table 1). In response to reproductive stage drought stress across rice varieties, grain yields were significantly lower than the well-watered treatments with 120-40-60 kg NPK $ha^{-1}$ and 120-40-120 kg NPK $ha^{-1}$ except for NSIC Rc 282 with pre-drought application of 120-40-120 kg NPK $ha^{-1}$ (4.3 t $ha^{-1}$). Percent reduction (enclosed in parentheses) in grain yield due to drought was calculated based on the well-watered control and respective fertilizer treatment (Table 1). Percent reduction in grain yields across varieties generally were decreased with increased levels of K fertilizer with 120-40-120 kg NPK $ha^{-1}$ having the lowest average percent reduction of 18.6% (Table 1). NSIC Rc 82 had the lower percent reduction in grain yield of 9.7% with 120-40-120 kg NPK $ha^{-1}$ than NSIC Rc 222 and NSIC Rc 418.

**Table 1.** Average grain yields of NSIC Rc 222, Rc 418, and Rc 282 in the well-watered control and reproductive stage drought stress treatments with application of different potassium levels, i.e., (F1) 0-0-0, (F2) 120-40-60, and (F3) 120-40-120 kg NPK $ha^{-1}$ and stage of application before imposing reproductive stage drought stress 60-80 DAT in 2017 and 2018 DS. In a column, means followed by a common letter are not significantly different at 5% level by Tukey's Test.

| Water Treatment | NPK (kg $ha^{-1}$): 1/3 each of N & K & all P applied at 14 DAT, & remaining 2/3 each of N & K at 40 DAT or maximum tillering/ early panicle initiation | Grain Yield (t $ha^{-1}$) | | | Average Grain Yield Reduction Based on the Control (%) |
|---|---|---|---|---|---|
| | | NSIC Rc 222 | NSIC Rc 418 | NSIC Rc 282 | |
| Control (well-watered) | 0-0-0 | 4.1 b | 3.8 b | 4.0 b | - |
| | 120-40-60 | 4.8 a | 4.9 a | 5.1 a | - |
| | 120-40-120 | 5.1 a | 4.8 a | 4.7 a | - |
| Reproductive stage drought stress | 0-0-0 | 2.4 c (42.2%) | 2.5 c (36.2%) | 2.6 c (35.7%) | 38.0 |
| | 120-40-60 | 3.5 b (27.9%) | 3.8 b (22.4%) | 3.7 b (27.6%) | 26.0 |
| | 120-40-120 | 4.0 b (22.0%) | 3.6 b (24.2%) | 4.3 a (9.7%) | 18.6 |

**Note:** Combined analysis of variance of the 2017 and 2018 DS grain yields did not differ significantly at 5% level. Number in parenthesis is the percent reduction in grain yield based on the well-watered control for each fertilizer level.

Among yield components, spikelet sterility ultimately influenced grain yield responses. Correlation and regression analysis of spikelet sterility and grain yield showed that 82.64% ($R^2$ or coefficient of determination) of variation of the grain yield can be affected by the spikelet sterility (Figure 12). With R or coefficient of correlation value of -0.9091, the relationship of spikelet sterility and grain yield was perfectly negative (i.e., if spikelet sterility decreases, grain yield increases).

## Discussion

### *Influence of Soil and Weather Conditions on the Rice Crop*

In this study, the 20-day reproductive stage drought treatment was successfully carried out due to the absence of rainfall. Hence, the rice crop relied on stored soil moisture to meet the evapotranspiration demand. Changes in soil moisture content and soil strength were similar for the test rice varieties (NSIC Rc 222, Rc 418, and Rc 282), and did not appear to be influenced by fertilizer management. During the 20-day drought treatment period, average soil moisture decreased from 75.1 to 9.4%. Soil strength increased from 0.5-2.3 MPa. Root growth has been reported to decrease at soil strength of 2.1 MPa (Duiker, 2002). Soil strength was measured in the field up to 0.10 m below the plow pan using a cone penetrometer. Although soil samples were obtained from a single point (i.e., 0.05-0.15 m soil layer below the soil surface to represent the mid 0.10 m soil layer) rather than from the different soil layers and relate changes in soil moisture to root system distribution (Mambani and Lal, 1983; Kamoshita et al., 2000), the whole plant (shoot and root) can be considered as an integrator in the soil-plant-atmosphere continuum. Hence, the influence of soil moisture, soil water pressure potential, other soil properties, and weather condition (e.g., air temperature, rainfall, irradiance, wind, and humidity) during the drought period can be integrated by the rice crop through its morphological, physiological, growth, and yield responses and the degree to which it can withstand mild to severe drought stresses through mechanisms at the cellular level (Bordner et al., 2015). Quantification of physiological and morphological responses of rice to drought stress is essential to predict the impact of soil and weather conditions on rice production using process-based crop simulation models (Woperies et al., 1996). Drought may delay the phenological development of the rice crop (Inthapan and Fukai, 1988) and affect physiological processes such as transpiration, photosynthesis, respiration, and translocation of assimilates to the grain (Turner, 1986). Leaf area development may be hampered due to reduced leaf area expansion, leaf rolling and early senescence, and tillering and panicle development may be reduced (O'Toole and Cruz, 1980; O'Toole and Baldia, 1982). Leaf rolling and leaf tip drying are visible plant responses to drought. These symptoms are used in practical visual scoring to evaluate drought tolerance of rice genotypes. Leaf rolling can also be associated with drought avoidance mechanism by reducing leaf surface area exposure to sunlight (Neealam et al., 2017).

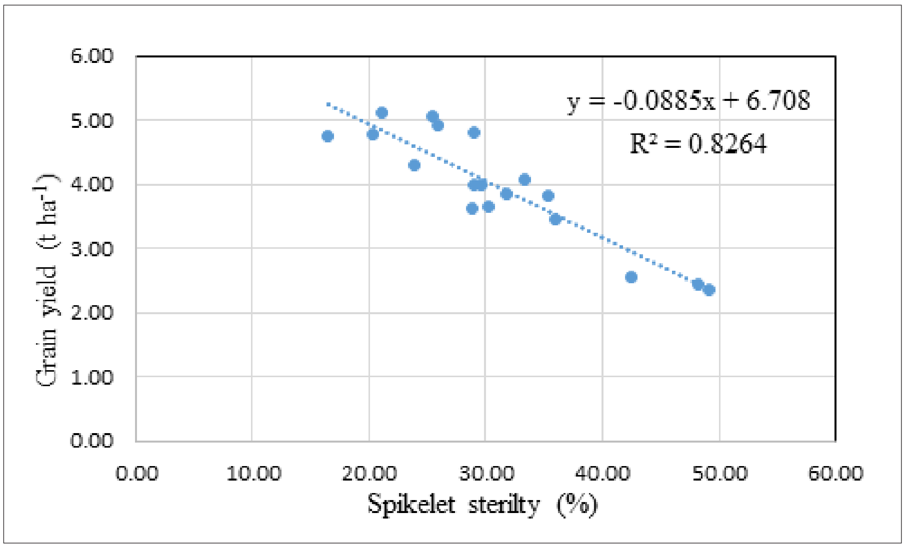



**Figure 12.** Simple linear regression of spikelet sterility (independent variable) and grain yields (dependent variable) of NSIC Rc 222, Rc 418, and Rc 282 in the drought and control (well-watered) treatments with (A) 0-0-0, (B) 120-40-60, and (C) 120-40-120 kg NPK $ha^{-1}$ fertilizer treatment 60-80 DAT in 2017 and 2018 DS.

### *Impact of Pre-Drought K Fertilization on Crop Morphology, Growth, and Yield*

Results of this study showed that as drought stress progressed, leaf rolling scores increased from 1 to 3 as average soil moisture content decreased to 9.4% during the 20-day reproductive drought stress. Based on the soil moisture retention curve of Wopereis et al. (1996) for clay soil, the soil moisture content of 9.4% is estimated to be much lower than the soil water pressure potential -200 kPa. The study of O'Toole and Moya (1978) demonstrated that leaf rolling and leaf water potential were highly correlated with decreasing soil moisture. Woperies et al. (1996) showed that the first observed effect in a drought period in the vegetative phase was a decline in rice leaf expansion rate. Leaf expansion stopped completely with root zone soil water pressure potential $h$ in the range of -50 to -250 kPa, depending on crop age and growing season. Leaf rolling and leaf senescence started at $h <$ -200 kPa or lower and were linearly related with log $(|h|)$.

Genotypes maintaining relatively high leaf water potential during drought stress utilize an "avoidance" mechanism while adjustment to lower leaf water potential is "tolerance" mechanism (Levitt, 1972). Osmotic adjustment involves the accumulation of ions and soluble sugars such as proline that maintains tissue viability; hence, delaying onset leaf rolling and leaf drying (Kadioglu and Terzi, 2007). In the present field study, tolerance mechanism could be associated with increasing potassium levels. Increased K levels may have enhanced osmotic adjustment at lower leaf water potential, maintaining positive pressure potential of plant tissues, and slowing the progression of leaf rolling. It is possible that NSIC Rc 282 exhibited greater leaf osmotic adjustment than NSIC Rc 222 and Rc 418 in response to pre-drought application of higher K level (i.e., 120-40-60 kg NPK $ha^{-1}$ vs. 120-40-120 kg NPK $ha^{-1}$) and 20-day reproductive drought stress. However, differences in osmotic adjustment across rice cultivars can be established after carefully assessing the development drought stress for each rice cultivar taking into consideration the degree of osmotic adjustment in relation to cumulative stress days above a threshold cumulative leaf water potential (Turner et al., 1986).

In the screenhouse pot experiment, leaf tip drying had a score of 5 when most leaves had a leaf rolling score of 4-5 at 0.5 % soil moisture content (Faustino et al., unpublished data). In the field experiment, the highest leaf rolling score was 3 and leaf tip drying score was 1 until the end of the 20-day reproductive drought stress period when soil moisture content decreased to 8.9%. Hence, due to smaller soil volume in pots in the screenhouse than in the field, soil moisture depletion was faster resulting in lower soil moisture content and higher degree of leaf rolling and leaf tip drying.

The rapid rolling of the leaves can be associated with the reduced leaf area and photosynthesis (Ji et al., 2012). Leaf rolling decreases the effective leaf area for light interception resulting in increased diffusive resistance to $CO_2$; thereby, reducing photosynthesis (Hsiao et al., 1984). In this study, NSIC Rc 282 had slower progression of leaf rolling with pre-drought application of 120-40-120 kg NPK $ha^{-1}$ than with pre-drought application of 120-40-60 kg NPK $ha^{-1}$, and presumably had relatively higher photosynthetic rate. Although there were studies on genes controlling leaf rolling such as *Abaxially Curled Leaf 1* (ACL1), ACL2, *Rice Outermost Cell-specific* gene (Roc5), and *Rolled and Erect Leaf 1* (REL1) (Zou et al., 2011; Chen et al., 2015), the extent of leaf rolling regulation (e.g., moderate leaf rolling) in relation to leaf photosynthesis and transpiration needs further studies in the screenhouse or controlled environment and field.

The reproductive or flowering stage of rice is very sensitive to drought stress (Fageria, 2007). In this study, drought stress was imposed at 60-80 DAT or flowering stage, in which anthesis occurs and seeds or spikelets are established via fertilization of receptive stigma by viable pollen (Stone, 2001). Garrity and O'Toole (1994) reported that anthesis and fertilization were among the most drought-sensitive processes during reproductive stage, together with panicle exsertion and anther dehiscence. The ability of the rice genotype or variety to recover from drought is also crucial. There are cases that even after re-watering, a rice variety that underwent reproductive drought stress may not flower at all or exhibit high spikelet sterility (Jain et al., 2013).

Drought stress reduces panicle exsertion rate; thereby, leaving some portion of the panicle unexserted in the leaf sheath. All spikelets in the unexserted portion of the panicle were completely sterile (Cruz and O'Toole, 1984). Panicle exsertion is primarily influenced by peduncle elongation (Rang et al., 2011). In the present study, panicle exsertion rates across varieties and fertilizer levels in the well-watered control and drought stress treatments decreased during the 20-day treatment period. When compared with well-watered control, the significantly lower panicle exsertion rates in the drought-stressed plants were associated with lower spikelet sterility.

In response to reproductive stage drought stress, panicle exsertion rate generally increases with higher level of K fertilizer. Compared with NSIC Rc 222 and Rc 418, Rc 282 had significantly higher panicle exsertion rate, lower spikelet sterility, higher number of spikelets $m^{-2}$ and 1000-grain weight with pre-drought application of 120-40-120 kg NPK $ha^{-1}$ across fertilizer treatments. Our results agree with the study of O'Toole and Namuco (1983), which showed that 25-30% of spikelet sterility was associated with the final length of the panicles that did not completely exsert. A full dose of K helps produce more grains through increased photosynthesis during the grain filling stage (Zou et al., 2007; Hasanuzzaman 2018). The study of Islam and Muttaleb (2016) showed that applying 100 kg K $ha^{-1}$ reduced spikelet sterility by 22.6%. Plants without K application registered spikelet sterility of 30.3%. Thus, increasing the rate of potassium may play a role in improving the rate of panicle exsertion during reproductive drought stress. Spikelet sterility was shown to have high negative correlation and coefficient of determination values with grain yield (i.e., R = -0.9091 and $R^2$ = 0.8264). This may imply that spikelet sterility greatly affected the grain yields.

The same trends were observed for well-watered and reproductive drought treatments in terms of increasing fertilizer rate application to the crop morphology, growth, and grain yield parameters; hence, there was no water treatment x fertilizer treatment x rice variety interactions. Genotypic variations in response to higher K levels, on the other hand, were eminent. NSIC Rc 282 with application of 120-40-120 kg NPK $ha^{-1}$ exhibited better reproductive drought stress mitigation than NSIC Rc 222 and Rc 418, especially manifested in the percent grain yield reduction based on the well-watered control (e.g., 9.7% vs. 24.2%). The drought stress mitigation due to increased levels of K will be genotype independent; hence, genotypic variations were observed.

Then et al. (2011) described quantitative trait loci (QTLs) of backcross-introgression lines of IR64 identified in chromosomes 1 and 2 (thick root morphology) had increased panicle exsertion rate and lower spikelet sterility compared with the backcross parent IR64 in response to reproductive drought stress. Panicle exsertion rate was negatively correlated with percent spikelet sterility. Spikelet sterility was due to inhibited peduncle elongation. Florets kept under the leaf sheath or after anthesis were all sterile. Thick root morphology is required to support panicle exsertion, high filled-grain percentage, and grain yield in response to reproductive drought. Rice varieties with high and efficient K absorption have thick root system; hence, have more root surface area and higher root to shoot ratio (Yang et al., 2003; Quampah et al., 2011).

Physiological, growth, and grain yield processes can be improved by increasing the rate of potassium application. Adequate application of K can enhance leaf area and dry mass accumulation of crop plants exposed to drought stress. Potassium regulates the stomatal closure and photosynthetic rates (Egilla et al., 2001; Marschner, 2012). It also plays a key role in the translocation of photoassimilates to roots, resulting in an increase in root surface area for higher uptake of available water from the soil (Romheld and Kirkby, 2010). Potassium also enhances the cell membrane stability during drought stress (Wang and Huang, 2004). Overall, K not only increases leaf area and yield, but also enhances the plant tissue water retention through regulated osmotic adjustment and improved cell membrane stability under drought stress conditions. These parameters serve as a good platform for studying or developing drought tolerance in rice.

Wang et al. (2013) reviewed the role of K in the drought resistance of plants in relation to reactive oxygen species (ROS) production. Plants exposed to drought stress can form ROS, damage the leaf tissue damage, and reduce photosynthetic efficiency. The impairment of the photosynthetic $CO_2$ fixation activates the molecular $O_2$, which leads to extensive generation of ROS (Cakmak, 2005). Maintaining adequate K nutrition enhances photosynthetic $CO_2$ fixation and transport of photosynthates to sink organs. It also inhibits transfer of photosynthetic electrons to $O_2$; thereby, reducing ROS production (Cakmak, 2005).

Another important role of K in mitigating the ROS due to drought stress is the enhancement of production of antioxidant enzymes such as catalases and peroxidases that scavenge ROS. Zain et al. (2014), in their screenhouse study, reported that augmented application of 120 kg K $ha^{-1}$ under drought stress condition increased the activity of catalase and peroxidase as defense mechanism. Catalase primarily scavenges $H_2O_2$ (ROS) and converts the $O_2^-$ and $H_2O_2$ to water and molecular $O_2$ with the combined action of peroxidase (Alexanderson et al., 2005).

In Table 2, yield and yield reduction of NSIC Rc 282 based on the well-watered control following reproductive stage drought stress were compared to published results of other researchers for MR220, IR64, and IR72. NSIC Rc 282 with pre-drought application of 120-40-120 kg NPK $ha^{-1}$ had the lowest average percent yield reduction of 9.7% based on the well-watered control. Following reproductive stage drought stress, percent reductions in grain yield were 30% for MR220 (Zain et al., 2014), 20.0% for IR64 and 14.0% with IR72 (Venuprasad et al., 2007). However, for MR220 that was exposed to vegetative stage drought stress, percent reduction in grain yield was only 5.0% (Zain et al., 2014). Hence, results of our two dry season studies in field under rainfed lowland system give the confidence to assess the reproductive stage drought tolerance of rice genotypes and minimize reduction in grain yield with agronomic

**Table 2.** Reported drought stress imposition (duration and timing), nutrient management, and grain yield for various rice varieties. DAS = days after sowing.

| Rice Variety | Duration of Drought Stress and Growth Stage | Nutrient Applied (kg NPK $ha^{-1}$) | Grain Yield (t $ha^{-1}$) | Yield Reduction Based on the Control (%) | Reference |
|---|---|---|---|---|---|
| NSIC Rc 282 | 20 days at flowering stage (60-80 DAT) in the field with Maligaya clay soil | 120-40-120<br>1/3 each of N and K and all P applied at 14 DAT, & remaining 2/3 each of N and K at 40 DAT or maximum tillering/early panicle initiation | 4.3 | 9.7 | Present study: average of 2017 and 2018 DS |
| MR220 | 25 days at vegetative stage in Bakau series soil in screenhouse tank | 120-70-120 | 8.2 | 5.0 | Zain et al. (2014) |
| | 15 days at reproductive stage in Bakau series soil in screenhouse tank | N and K were applied at 15 DAS; K was applied in 2 splits: 30% at 15 DAS and 70% at booting stage (50-55 DAS) | 6.1 | 30.0 | |
| IR 64 | Drought stress around flowering by reduction of irrigation once every 10-12 days from 7 weeks after sowing until harvest in the field with Maahas clay loam | 100-40-40<br>P and K were applied at sowing; N was applied in 3 equal splits: at sowing, 30 DAS and 60 DAS | 0.4 | 20.0 | Venuprasad et al. (2007) |
| IR 72 | | | 0.4 | 14.0 | |

management such as application of higher level of K and optimum levels of N and P (e.g., 120-40-120 kg NPK $ha^{-1}$) prior to reproductive stage drought stress.

## Conclusions

The experiment conducted in the dry season of 2017 and 2018 assessed the morphological, growth, and yield responses of rice varieties NSIC Rc 222, Rc 418, and Rc 282 to K fertilizer application and its impact on drought stress mitigation at a very sensitive reproductive growth stage or flowering from 60-80 DAT under a rainfed lowland system. There was no rainfall during the treatment period; thus, the development of reproductive drought stress was unperturbed and the rice crop relied on the available soil moisture to meet the evapotranspiration demand.

Mitigation of the impact of reproductive drought by increased K fertilizer application will depend on the relative response of the rice variety. It is important in the future studies to evaluate more rice genotypes to the reproductive drought stress mitigation of higher K level application (i.e., 120 kg K $ha^{-1}$). Compared with NSIC Rc 222 and Rc 418, Rc 282 had better response to pre-drought application of 120-40-120 kg NPK $ha^{-1}$ in mitigating reproductive drought stress with slower progression of leaf rolling and had higher panicle exsertion rate, lower spikelet sterility, and higher spikelet number $m^{-2}$, which will ultimately result in higher grain yield and smaller percent reduction in grain yield based on the well-watered control.

Combining agronomic and genetic management strategies, it is recommended that NSIC Rc 282 be applied with 120-40-120 kg NPK $ha^{-1}$ in drought-prone rainfed lowlands in the Philippines specifically for reproductive stage drought.

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# SPORE PRODUCTION AND GROWTH RATE OF TEN *AZOLLA* HYBRIDS

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## Abstract

Several studies had supported the effectiveness of *Azolla* as alternative or supplementary fertilizer in flooded rice cropping system. The vegetative means of multiplying *Azolla*, which was the main basis for selection of *Azolla* poses a great problem to farmers whenever they need to re-inoculate. The hydrological condition in the paddies may change abruptly that it may threaten the survival of *Azolla* as hydrophyte. For *Azolla* sp. to coincide with the growth and development of irrigated lowland rice in two cropping cycles with dry fallow period between the two cropping cycles, its spores must produce for at least 50% of the time within one year with sporulation index (SI) $\geq$ 50% during maximum spore production. When the SI value is below 50%, the megaspore (MGS) and microspore (MCS) count can be considered. The desired peak of sporulation is at least a month before the withdrawal of floodwater in rice paddies, i.e., two months before harvest date. Among the 10 *Azolla* UPLB hybrids evaluated, *Azolla microphylla* 4099, *Azolla microphylla* 4113, *Azolla microphylla* 4098, *Azolla mexicana* 2033, and *Azolla mexicana* 2030 are the varieties recommended with these parameters.

**Keywords:** *Azolla, Biofertilizer, Rice-Azolla, Azolla Spore, Azolla Spore Technology.*

## Introduction

*Azolla* is an aquatic fern with a symbiotic association with a nitrogen (N) - fixing blue green algae, *Anabaena azolla*. The *Azolla* - *Anabaena* symbiosis had the ability to fix N at high rates. It can fix about 1.2 kg N ha$^{-1}$ daily or about 864 kg N ha$^{-1}$ annually (Lumpkin and Plucknett, 1980). A full bloom of *Azolla* in one-hectare paddy field weighs around 20 t and gives 30 kg of N (NAAP-UPLB-DA, 1989). The rice crop yield increases proportionately to the amount of *Azolla* added as green manure. As an aquatic fern, it can be grown with rice in irrigated fields to serve as an organic N source. The same rice yield will be obtained when at least 50% of the inorganic N requirement are replaced with *Azolla*. An abundant growth of the fern can help increase rice yields by 50-100% more than rice not fertilized at all (NAAP, 1985). *Azolla* as green manure has major benefits. When cultured with rice, *Azolla* increases the yield of the rice crop by providing it with nutrients, especially nitrogen. It also increases humus content, which ultimately improves the physical, chemical, and biological soil conditions. *Azolla* cover reduced the rate of nitrogen losses (Villegas and San Valentin, 1989). Furthermore, *Azolla* inter-cropped with rice significantly also reduced weed population (Satapathy and Singh, 1985).

Field observations indicate the persistence of certain *Azolla* species in some areas alternately flooded and drained during the year-round cropping cycle (PhilRice, 2014). The persistence of *Azolla* appears to be associated with the production of spores and favorable environmental condition to induce sporulation (Mondejar and San Valentin, 2015). *Azolla* multiplies sexually by spores and vegetatively by fragmentation. The vegetative multiplication of *Azolla* is an extremely efficient process because it can double its mass in two to three days under favorable condition. Vegetative propagation of *Azolla* has limitations as the fern dies when it dries. The spore-producing ability of *Azolla* was recently discovered as the main explanation of the presence of fern in some rice paddies in the Philippines (Mondejar and San Valentin, 2017). This trait with other desired biological characters of *Azolla* will solve the sustainability problems in the use of *Azolla* as biofertilizer in rice culture. The potential uses of *Azolla* in agricultural production were already recognized by the government in the 1980s with the establishment of National Azolla Action Program (NAAP). However, sporulation of *Azolla* was not yet considered important in the adaptability tests of the plant. NAAP Biology Team started the investigation of the reproductive biology of *Azolla* because clonal propagation may suffer from genetic stagnation and does not allow for selection to improve the gene pool of sporulation of

*Azolla*. Previous procedure in determining sporulation of *Azolla* included only taking 100 plants randomly from the propagation trays monthly (Payawal and De Macale, 1994). Intensity of sporulation was measured as the relative percentage of spore-bearing plants. This is termed as the sporulation index. Setting parameters in the selection of desirable traits (with focus on sporulation) include taking account of the following: (a) the *Azolla* should produce spores ample enough as sources of inoculum for the next cropping season; (b) the sporulation should synchronize with the rice cropping, the *Azolla* already sporulated before the field is drained with water in preparation for harvesting; and (c) the *Azolla* should be fast enough to grow and cover the entire paddy maximize the incorporation of *Azolla* in the soil and be used as biofertilizer.

In this study, previous evaluation and selection parameters for *Azolla* were reviewed. These included weekly monitoring of the sporulation for the whole year, categorizing the sporocarp into megasporocarp and microsporocarp, and measurements of growth rates during linear phase of *Azolla* growth under normal and high floodwater temperature.

## Materials and Methods

### *Plant Materials and Cultivation*

The study was conducted in the plastic roofed facility at PhilRice Los Baños, College, Laguna from January 13, 2015 to February 16, 2017. The plant material used were the ten *Azolla* hybrid lines developed using the *in-vitro* method of hybridization in 1988 and reported by Payawal and de Macale (1994) as prolific spore producers in Los Baños. The ten hybrids were recovered from the *Azolla* collection of the International Rice Research Institute (IRRI). The parents of the hybrids are presented in Table 1 along with the identification of materials.

Plants were grown in 33 x 28 x 10 - cm (l x w x h) plastic trays filled with paddy soil and maintained with 2-cm height of water. The paddy soil used in the plant cultivation and all the experiments in this study were collected from the UPLB Central Experiment Station. Daily mean, maximum, and minimum air temperatures during the whole experimental period were 25.2°C, 31.1°C, and 21.7°C, respectively. Meanwhile, the mean, maximum, and minimum floodwater temperatures were 24.8°C, 29.5°C, and 21.3°C, respectively. The ferns were grown in 50% of full sunlight at 49.03-64.9-klux for growing *Azolla* at normal condition.

### *Measurement of Spore Production*

Spore production of *Azolla* was induced by allowing the plants to fully cover the 33 x 28 x 10-cm plastic trays making the plant population unable to undergo vegetative reproduction and reach maturity. The *Azolla* hybrids were grown under full sunlight from 96.9 to 126.46-klux to induce sporulation. Sporulation was monitored weekly from February 18, 2016 to February 16, 2017. Spore production was monitored by taking 100 sample fronds at random from the population. Sporulation index (SI) was computed using the formula by Payawal and de Macale (1994):

$$\text{Sporulation index or SI (\%)} = \frac{\text{total number of spore bearing plants}}{\text{total number of samples collected}} \times 100$$

Highest SI value, peak of spore production, megasporocarp (MGS) counts, and microsporocarp (MCS) count were also recorded.

### *Measurements of Growth Rates*

Growth rates of *Azolla* hybrids were determined from January 13 to February 28, 2015. Mean, maximum, and minimum temperatures for both ambient and floodwater during the growth periods were 23.5°C, 29.5°C, and 20.0°C, respectively. Three

**Table 1.** Plant identification of 10 *Azolla* hybrids used in the experiment.

| UPLB Hybrids | | UPLB Code | **Parents (female x male)** |
|---|---|---|---|
| *Azolla mexicana* | 2024 | UPLB 1 | *A. microphylla* 4018 x *A. mexicana* 2001 |
| *Azolla microphylla* | 4123 | UPLB $7_8$ | *A. microphylla* 4018 x UPLB 1 (line 8) |
| *Azolla microphylla* | 4119 | UPLB $7_4$ | *A. microphylla* 4018 x UPLB 1 (line 4) |
| *Azolla microphylla* | 4124 | UPLB $7_9$ | *A. microphylla* 4018 x UPLB 1 (line 9) |
| *Azolla mexicana* | 2030 | UPLB $2_3$ | *A. mexicana* 2002 x UPLB 1 (line 3) |
| *Azolla microphylla* | 4114 | UPLB $6_3$ | *A. microphylla* 4003 x *A. caroliniana* 3004 (line 3) |
| *Azolla mexicana* | 2033 | UPLB $2_6$ | *A. mexicana* 2002 x UPLB 1 (line 6) |
| *Azolla microphylla* | 4113 | UPLB $6_2$ | *A. microphylla* 4003 x *A. caroliniana* 3004 (line 2) |
| *Azolla microphylla* | 4099 | UPLB $3_2$ | *A. microphylla* 4018 x UPLB 1 (line 2) |
| *Azolla microphylla* | 4098 | UPLB $3_1$ | *A. microphylla* 4018 x UPLB 1 (line 1) |

fronds for each *Azolla* hybrids were selected and grown in plastic containers with the dimension of 11 x 8 x 5-cm containing 250 g of soil. The collected soil was dried, pulverized, and sieved using 0.5 mm opening mesh to ensure uniformity of particle sizes. Three newly grown fronds were selected, weighed, and inoculated. *Azolla* growth was determined by weighing using analytical balance every after eighth days starting from the initial inoculation (day 0) for 48 days. The experiment was replicated four times in a complete randomized design (CRD).

### *Evaluation under High Floodwater Temperature*

*Azolla* hybrids were put in a designed culture baths (Figure 1) for ten days, where floodwater temperatures were elevated to an average of 36°C with ± 3.5°C fluctuation. The temperatures fluctuated to 3.5°C when ambient temperature was at the lowest, 5 am (25 to 27°C) and at the highest, 1 pm (35 to 41°C). Mean, maximum, and minimum ambient temperature were recorded at 31°C, 42°C, and 25°C, respectively. A total of 2 g each plant was placed in a plastic container with 9 cm diameter and filled with soil. Floodwater was maintained at 2 cm level. The floodwater temperature was sustained by immersing the plastic containers to a bigger container filled with hot water heated by sensor submersible heaters. Ambient and floodwater temperature inside the plastic containers were monitored using a precision hygrometer every 15 minutes. Aerator underwater was used to level the temperature. The plants were weighed every five days. Due to the limitation of space, the experiment was time replicated for three times. First incubation at elevated high floodwater temperatures was conducted from April 14 to 25, 2016, then April 25-May 5, 2016, and May 16-27, 2016 for the 2nd and 3rd incubations, respectively.

### *Statistical Analysis*

Statistical analyses for growth rates were performed by using general linear model. Analysis of variance (ANOVA) was performed to test the line and SI, MGS, and MCS counts. The period of sampling and replication were considered as random effects. Significant treatment effects were explored using Tukey's least significant difference (LSD) test.

## Results

### *Spore Production*

*Azolla microphylla* 4123, 4124, and *Azolla mexicana* 2024 had similar sporulation mechanism (Table 2). Recorded sporulation of these three UPLB *Azolla* hybrids was less than 25 sampling times and started during the 3rd week of February, with less than 50% highest SI value during the 4th week of February. *Azolla mexicana* 2030, 2033, and *Azolla microphylla* 4114 had higher number of weeks of observed sporulation than the previous three hybrids. *Azolla mexicana* 2030 sporulated starting February until September. *Azolla mexicana* 2033 continued producing spores from January to May and in other months except December. *Azolla microphylla* 4114 had distinct sporulation cycle with alternate sporulation for 11 weeks and did not produce spores for four weeks in June, September, and December. *Azolla microphylla* 4098, 4099, and 4113 had almost the same sporulation mechanism. These three hybrids produced spore almost all year round

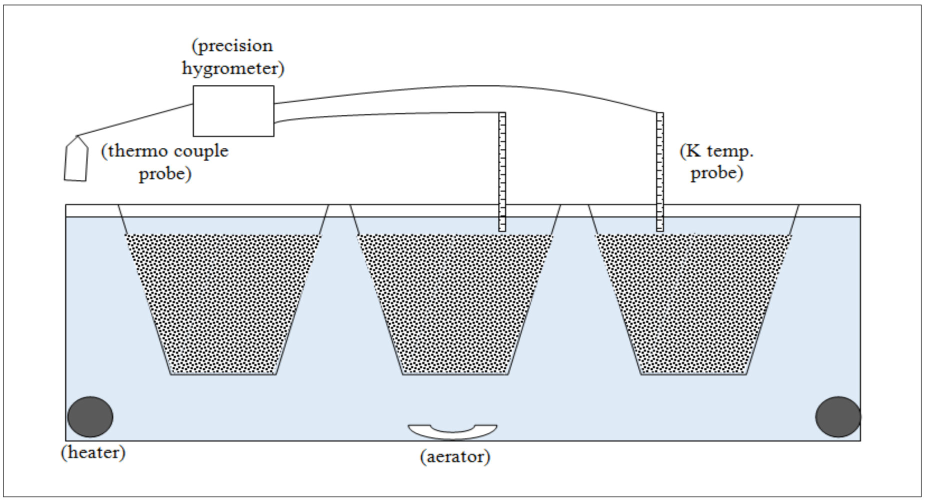


**Figure 1.** Designed culture baths used to elevate high floodwater temperatures.

with an SI value of more than 70%. High variability on the SI value, MGS, and MCS counts among UPLB *Azolla* hybrids was observed. Mean difference of the SI value, MGS, and MCS counts of hybrids were highly significant ($P$ < 0.0001). *Azolla microphylla* 4099 had the highest SI value, followed by *Azolla microphylla* 4113 and 4098 (Table 3). These three hybrids were the only entries with significant mean difference to *Azolla mexicana* 2024, which obtained the lowest SI value. Similarly, these three hybrids had the highest MGS and MCS counts and the only entries with significant mean difference to *Azolla mexicana* 2024, which registered the lowest MGS and MCS counts (Table 4). MCS of hybrids were recorded 39 to 65% higher than MGS.

### *Growth Rates*

Growth rates expressed in g/day calculated as the slope of the line differed significantly among hybrids ($P$ < 0.0001). *Azolla mexicana* 2033 was the fastest in

**Table 2.** Recorded sporulation and highest sporulation index (SI) of 10 UPLB *Azolla* hybrids during weekly monitoring of spore production from February 18, 2016 to February 16, 2017 in Los Baños, Laguna.

| **UPLB Hybrids** | **Recorded Sporulation** | | **Highest SI recorded** | |
|---|---|---|---|---|
| | **no. of weeks** | **periods of spore production** | **%** | **peak period** |
| *Azolla microphylla* 4123 | 14 | from 3rd week of February to 4th week of April | 47 | 4th week of February |
| *Azolla microphylla* 4124 | 21 | from 3rd week of February to 1st week of July | 47 | 4th week of February |
| *Azolla mexicana* 2024 | 18 | from 3rd week of February to 2nd week of May | 37 | 4th week of February |
| *Azolla mexicana* 2030 | 27 | 3rd week of February to 2nd week of September | 51 | 3rd week of February |
| *Azolla mexicana* 2033 | 34 | starting at 1st week of January until 4th week of May; observed sporulation in other months except December | 60 | 3rd week of February |
| *Azolla microphylla* 4114 | 38 | observed sporulation cycle; alternate sporulation for 11 weeks and no spore production for 4 weeks during June, September and December | 44 | 3rd week of July |
| *Azolla microphylla* 4119 | 27 | starting 3rd week of January until 1st week of July; then from 2nd week of October to 1st week of December | 41 | 3rd week of March |
| *Azolla microphylla* 4098 | 44 | observed sporulation from January to November except October | 84 | 3rd week of February |
| *Azolla microphylla* 4099 | 50 | spore production all year round | 72 | 2nd week of February |
| *Azolla microphylla* 4113 | 50 | spore production all year round | 73 | 4th week of March |

**Table 3.** Mean sporulation index (SI) value, megasporocarp (MGS), and microsporocarp (MCS) of 10 UPLB *Azolla* hybrids during weekly monitoring of spore production from February 18, 2016 to February 16, 2017 in Los Baños, Laguna.

| UPLB Hybrids | **SI value* (%)** | **MGS** | **MCS** |
|---|---|---|---|
| *Azolla microphylla* 4099 | 26.77 *a* | 1.25 | 1.89 |
| *Azolla microphylla* 4113 | 22.89 *ab* | 1.22 | 1.99 |
| *Azolla microphylla* 4098 | 19.11 *bc* | 1.32 | 2.30 |
| *Azolla microphylla* 4114 | 12.83 *cd* | 1.12 | 1.86 |
| *Azolla mexicana* 2033 | 11.77 *cd* | 1.35 | 2.37 |
| *Azolla microphylla* 4119 | 6.49 *d* | 1.00 | 2.26 |
| *Azolla mexicana* 2030 | 6.30 *d* | 1.01 | 1.62 |
| *Azolla microphylla* 4124 | 5.66 *d* | 0.86 | 2.19 |
| *Azolla microphylla* 4123 | 4.43 *d* | 1.32 | 3.04 |
| *Azolla mexicana* 2024 | 3.25 *d* | 0.97 | 1.98 |

*Highly significant at 99% level of confidence (*P<0.0001*).

**Means with the same letter are not significantly different.

***Sporulation index or SI (%) = total no. of spores bearing plants / total no. of samples collected x 100.

reproducing asexually among the hybrids (Table 5). *Azolla microphylla* 4099 and 4113 were the slowest and only hybrids with significantly mean difference with *Azolla mexicana* 2033.

### *Response to High Floodwater Temperatures*

There were no significant differences in the growth rates among *Azolla* hybrids that were exposed to high floodwater temperature (36°C). *Azolla microphylla* 4114 and *Azolla microphylla* 4119 underwent senescence at eighth day while other hybrids remained healthy after exposing to elevated floodwater temperatures. *Azolla* hybrids registered the fastest growth rates at ambient temperature, and the slowest growth rates at elevated floodwater temperatures.

## Discussion

No standard breeding procedure for *Azolla* were available in the 1980s and it posed a problem during the NAAP operation (NAAP, 1986). Doubling time (DT) was typically used as a measure of vegetative growth and adaptability of the *Azolla* to the agro climatic environment (NAAP-UPLB-DA, 1988). It is computed using the formula: $DT = 0.693\ t / \ln (A_f / A_0)$, where t is the number of days required to fill up a specified area, $A_0$ and $A_f$ are the initial and final biomass, and ln as natural logarithm. The DT of *Azolla* should be $\leq$ 7 days to be recommended for deployment in different parts of the country. However, DT as a mode of measurement is not always applicable such as in the

**Table 4.** Mean megasporocap (MGS) & microsporocarp (MCS) of 10 UPLB *Azolla* hybrids during weekly monitoring of spore production from February 18, 2016 to February 16, 2017 in Los Baños, Laguna.

| UPLB Hybrids | MGS | MCS |
|---|---|---|
| *Azolla microphylla* 4099 | 33.49 *a* | 50.64 *a* |
| *Azolla microphylla* 4113 | 28.00 *ab* | 45.62 *a* |
| *Azolla microphylla* 4098 | 25.21 *ab* | 43.91 *a* |
| *Azolla mexicana* 2033 | 15.89 *bc* | 27.94 *ab* |
| *Azolla microphylla* 4114 | 14.38 *bc* | 23.92 *ab* |
| *Azolla microphylla* 4119 | 6.49 *c* | 14.64 *b* |
| *Azolla mexicana* 2030 | 6.34 *c* | 10.19 *b* |
| *Azolla microphylla* 4123 | 5.85 *c* | 13.45 *b* |
| *Azolla microphylla* 4124 | 4.85 *c* | 12.42 *b* |
| *Azolla mexicana* 2024 | 3.15 *c* | 6.45 *b* |

*Highly significant at 99% level of confidence (*P<0.0001*).

**Means with the same letter are not significantly different.

**Table 5.** Growth rates (g/day) of 10 UPLB *Azolla* hybrids in ambient and elevated floodwater temperatures (36°C).

| UPLB Hybrids | Growth Rate (g/day) | |
|---|---|---|
| | Ambient Temperature | Elevated Floodwater Temperature |
| *Azolla mexicana* 2033 | 1.90 *a* | 0.03 *a* |
| *Azolla mexicana* 2024 | 1.86 *a* | 0.04 *a* |
| *Azolla microphylla* 4124 | 1.83 *a* | 0.13 *a* |
| *Azolla mexicana* 2030 | 1.76 *a* | 0.14 *a* |
| *Azolla microphylla* 4098 | 1.59 *a* | 0.15 *a* |
| *Azolla microphylla* 4119 | 1.48 *a* | 0.15 *a* |
| *Azolla microphylla* 4114 | 1.34 *a* | 0.17 *a* |
| *Azolla microphylla* 4123 | 1.14 *ab* | 0.17 *a* |
| *Azolla microphylla* 4099 | 0.64 *b* | 0.18 *a* |
| *Azolla microphylla* 4113 | 0.53 *b* | 0.22 *a* |

*Highly significant at 99% level of confidence (*P<0.0001*).

**Means with the same letter are not significantly different.

***Growth rates were calculated as the slope of the line.

field experiments or during the linear or diminishing growth phases of the plant when growth will be slow. DT is usually used in unicellular organisms and during exponential growth only. Vegetative reproduction of *Azolla* as a lone parameter for selection to the agro climatic environment will not solve the problem on the use of *Azolla*. Constraints on using *Azolla* in lowland rice production include the limiting conditions in rice paddies such as water unavailability during the dry fallow period between the two cropping cycles, which results in the desiccation of *Azolla* (Mondejar and San Valentin, 2017). Mondejar and San Valentin (2017) reported the permanence of *Azolla* in irrigated rice lowland ecosystem maximizing the utilization of the plant as green manure in rice culture. They observed that both asexual and sexual reproduction should be the inherent growth traits for the *Azolla* varieties to survive the varying condition during rice cropping.

To produce genetically-improved *Azolla* varieties, 46 *Azolla* hybrid lines were developed using the *in-vitro* method of hybridization by NAAP Biology and Culture Team in 1988. These 46 hybrid lines were evaluated by Payawal and his colleagues under highland (Benguet) and lowland (Los Baños) conditions from 1989 to 1992. They monitored the sporulation index monthly. SI using the NAAP formula was used in this study.

*Azolla* does not always produce spores. Under Philippine condition, a year-round sporulation was reportedly only in cooler places, and sporulation occurs only in cooler months starting from November until February (NAAP-UPLB-DA, 1988). Previous evaluation and selection parameters for *Azolla* were reviewed, which included weekly monitoring of the sporulation for a year to determine the differences in the pattern of spore production. The ten prolific spore producers in Los Baños, Laguna reported by Payawal and De Macale (1994) were evaluated on its vegetative growth and spore production. Spore production was induced by making the plant population unable to undergo vegetative reproduction and reach maturity. Mature *Azolla* plant produces several pairs of sporocarps along the branches underneath the leaves. *Azolla* hybrids grown in plastic house facility under full sunlight from 96.9 to 126.46-klux sporulated consistently. Thus, hybrids used in this experiment were exposed to this condition to guarantee sporulation.

Maximum sporulation was recorded in February under lowland and highland condition by Payawal and De Macale (1994). Similarly, the majority of the hybrids attained maximum sporulation in February except for some hybrid, which produced more spores in March and July. *Azolla microphylla* 4119, 4099, 4098, and *Azolla mexicana* 2030 and 2033 were reported as the most prolific spore producers throughout the year with an average sporulation index greater than 50%. In this study, *Azolla microphylla* 4099 and 4098 were consistent as the most prolific spore producers with 84% and 73%, respectively, followed by *Azolla microphylla* with 72% SI value. These three hybrids also produced spore all year-round. *Azolla microphylla* 4099, 4113, including *Azolla mexicana* 2033 were observed to produce spores in Nueva Ecija, which has warmer environmental condition than in Los Baños in October and November 2016. *Azolla mexicana* 2024 was the first hybrid to sporulate all year round in 2015 reaching the highest SI value of 95%. However, it only attained an SI value of 37% and sporulated 35% in 2016. *Azolla mexicana* 2024 registered 95% SI value twice under partial sunlight in 2015, and may be the reason for the decline of SI value of *Azolla mexicana* 2024 under full sunlight in 2016. This can be explained by the cross-talk between environmental stresses and plant metabolism for reproductive success and continuity of the species through time similar with the sporulation of some species or varieties in highland but not in lowland or vice versa (Payawal and De Macale, 1994; Mondejar and San Valentin, 2017); indicating that the induction of the sporocarp formation used in this study may not be applicable to other varieties. To validate this observation, sporulation of *Azolla mexicana* 2024 can be evaluated under partial and full sunlight. MGS and MCS for each hybrid differed significantly and sometimes did not correspond to the SI value. MGS and MCS can be considered in the selection especially when SI value do not reach 50%. Among the 10 *Azolla* hybrids, the varieties fit in the criteria are *Azolla microphylla* 4099, *Azolla microphylla* 4113, *Azolla microphylla* 4098, *Azolla mexicana* 2033 and *Azolla mexicana* 2030.

Mondejar and San Valentin (2017) estimated that a thick mat of 8 kg $m^{-2}$ fresh biomass with SI value of 100% can produce 380,000 microsporocarps and 85,000 megasporocarps $m^{-2}$. *Azolla* hybrids sporulated with at least 50% SI value is safe enough to produce ample inoculum for the next growth cycle. The maximum sporulation of *Azolla* in Majayjay, Laguna attained an SI value of 65%, which produced 25,000 progenies per square meter (Mondejar and San Valentin, 2015). The sporulation of *Azolla* observed by Mondejar and San Valentin (2017) in Majayjay had obtained sporulation index of > 50, which happened twice in each cropping season, i.e., before maximum tillering and after flowering. Based on data obtained from this study, *Azolla* hybrids sporulated with at least 50% SI value in February, which is a month before withdrawal of floodwater in rice paddies in Los Baños. During this period, the rice crop is at an early reproductive stage. This implies that the sporulation of *Azolla* hybrids evaluated in Los Baños coincide with the rice cropping cycle in the area. The viability of dry spores reached up to one year with an increasing germination percentage in the first three months and decline in the germination percentage for the succeeding months with longer days to germination. *Azolla* survival was ensured with this viability span of spores and the synchronized high production of spores in the rice cropping cycle, which can result in the stability of *Azolla* in the rice cropping system.

The vegetative growth of *Azolla* should be fast enough to grow and cover the entire paddy. If growth of *Azolla* is rapid, more fronds will be available to produce spores as inoculum in the paddy soil. Fast growth rates are needed maximize *Azolla* as biofertilizer and provide the most nitrogen to the rice crop. When the fern is fast enough, *Azolla* can be incorporated into the soil up to three times. The application of *Azolla* can be synchronized with the method of growing rice (NAAP-UPLB-DA, 1988), for example during land preparation, and first and second weeding.

In this study, the linear growth model was used, in which the slope of the line (biomass accumulation per day) is the growth rate. Genetic differences were also observed using this method. *Azolla microphylla* 4099 and *Azolla microphylla* 4113 had the slowest growth rates. These varieties were observed to have the fastest growth rate at elevated floodwater temperature but not significantly different with other hybrid varieties. It can be assumed that the growth rates of *Azolla* hybrids under ambient temperature can be inversely proportional to its growth rates under elevated floodwater temperature. However, the two experiments were conducted independently making it not valid for comparison analysis. To validate this relationship, growth rates of these hybrids can be evaluated in normal and elevated floodwater temperature under similar agro climatic conditions and crop management.

Selected *Azolla* varieties should be adapted to the ecological conditions prevailing in the lowland rice fields. Intense solar radiation and high air temperature were identified as major constraints in *Azolla* cultivation. (NAAP, 1985). Hybrids were exposed to full sunlight (96.9-126.46-klux) as part of the method to induce sporulation. All hybrids produced spores. Some of the hybrids turned red or maroon but continued to occupy the available space and even produce spores. The air temperature threshold in rice is at maximum temperature of > 35°C for 10 days (IRRI, 2012). Reduction of rice yields is ~10% for every 1°C increase in minimum temperature (Peng *et* al., 2004). Presence of *Azolla* cover in rice paddies reduces the floodwater temperature to 1-2 °C (Villegas and San Valentin, 1989). However, the *Azolla* should survive first in those areas where the temperature threshold happened, before it can contribute to the reduction of temperature. Floodwater was considered in this study, and aside from it is easier to control than ambient temperature, the *Anabaena azollae* (symbiont) lives in the leaf cavities within the dorsal leaf lobe of *Azolla* (Peters, 1977; Lumpkin and Plucknett, 1980) located near the water surface. Increasing the floodwater temperature may influence the nitrogen (N) fixation capability of the symbiont; thereby, affect the biomass accumulation of the fern because of the strong interaction between photosynthesis and N fixation of *Azolla* (Tyagi *et* al., 1981). The majority of the hybrids survived incubation under high floodwater temperature except for *Azolla microphylla* 4119 and 4114, which had senesced at day eight. Minimal biomass accumulation was observed in all hybrid lines.

## Conclusion

*Azolla* varieties differ on spore production with highly variable sporulation pattern. Year-round sporulation of *Azolla* does not only occur in cooler places or highland condition. Maximum sporulation does not necessary happen during cooler months. *Azolla* to be deployed to farmers should be selected based on its pattern of spore production in a given environmental condition including rice cropping pattern. Selection parameters for recommended *Azolla* varieties should include their biological characteristics that sustains their presence in the rice ecosystem. Further evaluation should focus on describing the sporulation mechanism of *Azolla*.

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FULL PAPER

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# EXPERTS' PERSPECTIVE, CONSUMER PERCEPTION ON RICE-BASED PRODUCTS, AND MARKET TREND ON CONSUMER GOODS FOR RICE-BASED PRODUCT IDEA GENERATION

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## Abstract

Development and marketing of rice-based products provide additional income in rice-producing communities, which ultimately enhances their quality of life. This study explored experts' perception on health and wellness through focus group discussions (FGD, n=24) and determined consumer trends on products and acceptability of rice-based products through a market survey (n=339) in Central Luzon, Philippines. Survey results showed that the commonly purchased consumer goods featured convenience and health such as packed ready-to-eat bakery products for snacks; fast food and canned goods for meals; ready-to-drink beverages and instant coffee for beverages; body care and hair care for personal care products; and vitamins and minerals and pain relievers for pharmaceutical or nutraceutical products. Rice-based bakery product ideas were also highly preferred. Thus, product developers should create and innovate convenient, nutrient-rich, healthy, and functional rice-based products.

**Keywords:** *Consumer Trend, Convenience, Health, Market-Driven, Rice-Based Product.*

## Introduction

Development of high-value products from rice is a scientific and entrepreneurial activity. It serves as another milestone to advance and make the country's rice industry competitive. Rice value-adding and diversified rice-based farming products and by-products also enhance the nutritional and economic status of rural farming communities. Producing and marketing value-added products with better quality and nutrition becomes additional sources of income (PhilRice, 2016a).

At present, value-added products from rice such as rice bran oil, rice-based alcoholic beverage, rice milk, rice syrup, and rice starch are already available. Most of them are produced in Japan and Taiwan (Ernst & Woods, 2011) while extruded fresh rice noodle is popular in Thailand. There are also rice cake varieties in Asian countries like *"nenkau"* in China, *"mochi"* in Japan, and *"bibingka"* in the Philippines. Japan also produces vinegar from rice (FAO, 2015). Rice-based products with improved quality and nutritional value such as rice beverages, snacks, meals, antioxidant-rich supplements, and rice by-products are available in the Philippines (PhilRice, 2016b).

Despite successful value-adding activities, developers have difficulty making most of these products readily available and accessible to consumers and target clients. Few marketing initiatives, technology transfer, and commercialization scheme are among the major causes. It was noted that developing new products or venturing into innovation is a promising activity for growth but is associated with high risk for market failure. Two out of three new products no longer be available in the market within three years (Lucas, 2013). Possible reasons for product failure in the Philippines are the influences of being loyal to a particular brand, advertising, value for money, and consumer attitudes (Castillo, 2018). Filipino consumers are open to new concepts, however, they are highly loyal to certain product brands. A survey showed that around 80% of Filipinos are willing to buy new products from brands they are more familiar with rather than from a new brand (Lucas, 2013). Aside from that, most Filipinos only buy a new product if it has proven its quality and effectivity to its first buyers (Lucas, 2013). Filipino consumers are highly influenced by advertising; 78% has increased their brand preference due to commercials. Filipinos choose the product that offers the best value for their money and a great experience (Valentin, 2017).

To minimize the risk and make a new product popular in the market, the challenging factors must be addressed. New products must meet consumer needs, which other or previous products fail to do; be communicated clearly; possess a distinct innovation; and be marketed strategically (Lucas, 2013). This can be done through a consumer-driven approach in new product development.

A consumer-driven approach means that a product is developed based both on technical knowledge and market information (Costa & Jongen, 2006). Given the dynamic lifestyle and behavior of consumers, product development should focus more on consumers (Jreissat et al. 2017). According to Costa & Jongen (2006), the main pillars of consumer-driven product development are: 1) consumers as the starting point in product development, 2) fulfillment of consumer needs and understanding consumer value as the main target of the product development, and 3) success of the new product development is due to customer satisfaction and meeting the needs of the consumers. Knowing the market and understanding the consumers will help product developers create products that suit and satisfy consumer needs, wants, and expectations; thus, making the product competitive and marketable.

This study presents a consumer-driven approach to product development. It primarily explored and gathered market information that guide product developers in generating new products from rice. The study specifically collected rice-based product ideas from experts in food and health sectors and to determine consumers' awareness about rice-based products available in the market, consumer consumption trends on food product categories, and perception toward the collected rice-based product ideas.

## Materials and Methods

This exploratory market study was divided into two parts: a focus group discussion (FGD) and a market survey. In the first part, three FGDs with 24 participants from the fields of food product development and manufacturing, marketing, nutrition and health services, whose products and services featured health and wellness, were conducted. The purpose of the experts' FGD was to describe participants' perception of health and wellness and to gather rice-based food product ideas to be developed.

The second part of the study was a market survey conducted to determine the *market* potential for rice-based products and describe the demographics and attitudes of the products' target buyers.

### *Study Site*

The study was conducted in Central Luzon (Region III) located below North Luzon and above the National Capital Region, around 126 km away from Manila, the country's capital. It has seven provinces: Aurora, Bataan, Bulacan, Nueva Ecija, Pampanga, Tarlac, and Zambales. Aurora, Nueva Ecija, and Zambales provinces are mostly rural areas while Bataan, Bulacan, Pampanga, and Tarlac are highly-urbanized provinces.

Central Luzon is the largest rice-producing region contributing to one-third of the country's rice production (PSA, 2016). In terms of consumption, the diet of rice-based farming households in this region is mainly composed of rice and rice products. Rice contributed a significant amount to their total per capita calorie (49.1%), protein (11.7%), and iron (31.7%) intakes (Abilgos-Ramos & Ballesteros, 2018). It is usually served as food and snacks in the country, particularly the native rice snacks such as *biko, bibingkang suman, tamalis, bibingkang galapong, espasol, muryekos, puto, cutchinta,* and *pinipig* (PhilRice, 2013).

The FGDs were conducted at PhilRice, Maligaya, Science City of Muñoz, Nueva Ecija. PhilRice is a government corporate entity under the Department of Agriculture. It is mandated to develop high-yielding and cost-reducing technologies that will enable farmers to produce enough rice for the country. PhilRice also develops products and other technologies from rice and its environment to advance and make our country's rice industry competitive.

### *Sampling and Study Participants*

Out of 30 target individuals who were invited for the FGD, only 24 gave consent and participated. Participants were chosen based on their specialization, current affiliation, and years of work experience. These were considered to get a wide array of product and market ideas from individuals who specialized in either product development, marketing, or nutrition and health, from the public and private sectors. They had an average of 13 years of work experience. There were 17 female and seven male participants (Table 1).

Convenience and purposive-quota sampling techniques were employed in this study. In convenience sampling, geographical location and ease of collecting a number of respondents were considered. The survey was conducted in malls, supermarkets, and public markets in all seven provinces of Central Luzon that had given consent for the activity. In purposive sampling, the following characteristics of the target market were considered: 1) individuals who belong

**Table 1.** Specializations of the participants of the three FGDs, 2016.

| Batch | No. of Participants | Specialization/Profession |
|---|---|---|
| 1 | 8 | Health product developer, food scientist and developers, nutritionist, and entrepreneurs on nutrition and health products |
| 2 | 12 | Chefs and food developers, food scientists, nutritionists, entrepreneurs on nutrition and health products or health supplements, and managers of food distribution companies |
| 3 | 5 | Food developers, nutritionists, entrepreneur of nutritious food product, and manager of a food manufacturing company |

**Table 2.** Socio-demographic characteristics of the survey participants, Central Luzon, 2016.

| | | No. | Percentage % |
|---|---|---|---|
| Province | | | |
| Aurora | | 48 | 14.2 |
| Bataan | | 46 | 13.6 |
| Bulacan | | 50 | 14.7 |
| Nueva Ecija | | 48 | 14.2 |
| Pampanga | | 50 | 14.7 |
| Tarlac | | 49 | 14.5 |
| Zambales | | 48 | 14.2 |
| Average Age | 41.41± 15.7 | | |
| Gender | | | |
| Female | | 213 | 62.8 |
| Male | | 126 | 37.2 |
| Civil Status | | | |
| Married | | 192 | 56.6 |
| Separated | | 2 | 0.6 |
| Single | | 119 | 35.1 |
| Widow/Widower | | 26 | 7.7 |
| Educational Attainment | | | |
| Elementary | | 33 | 9.7 |
| High school | | 125 | 36.9 |
| College | | 164 | 48.4 |
| Vocational | | 7 | 5.0 |
| Average Income | PhP11,085.68 | | |

n=339

in the age group 15-64 years old, and 2) played a major part in buying groceries for the household. There were 339 respondents who participated in the survey, 63% were females and 37% were males with an average age of 41 years. More than half of them (56.6%) were married. Almost half of the respondents (48.4%) reached college, 36.9% graduated from high school, 9.7% had an elementary education, and 5% had a post-secondary course (vocational course). The average per capita monthly income was PhP11,085.68 (Table 2).

### *Data Collection*

In the FGD, perception of participants about health and wellness was obtained by asking them individually to write down three thoughts that would readily come to their minds when they hear the phrase "health and wellness." The participants were asked to share, explain, and discuss the reasons and experience regarding their thoughts written on the papers. Participants were also asked to think of different product ideas based on the market trends that can be developed from rice. Participants were requested to rank their top five rice-based product ideas and wrote these down in the colored papers. FGD participants then discussed and agreed among themselves about these chosen product ideas and their categories.

In the market survey, a pre-tested questionnaire was used by five trained enumerators to collect the following data: 1) socio-demographic characteristics of consumers, 2) consumers' level of awareness and understanding towards rice-based products, 3) usage and purchase behavior of consumers towards different products gathered from the FGDs (e.g., brands usually bought, purchase frequency, purchase quantity), 4) consumers' perception (e.g., product attributes that are most important for the consumers, perceived value of the product concepts, and the likelihood of purchase, and 5) rice-based product ideas with high consumer appeal. These rice-based product ideas were generated by the participants during the FGD. A qualifier question was also included on whether the prospect respondents were among the decision-makers in purchasing items for household use. If they answered yes, they were encouraged to participate in the survey. Otherwise, they were not included in the survey.

The importance of different product attributes was measured using a 5-point scale (5-very important to 1-very unimportant). Likewise, the degree of liking toward the different rice-based product ideas presented during the survey was measured using a 5-point scale (5-very like to 1-very dislike). To determine the likelihood of purchase for the rice-based product ideas, the respondents were asked to answer with yes or no to the question asking them if they will be willing to buy the product if it would be available.

### *Data Analysis*

All completed questionnaires were encoded in MS Excel 2013 and were analyzed in IBM SPSS version 20 software. Data collected in the survey were analyzed using descriptive statistics (e.g., means, frequencies, and percentage distribution). Multiple response analysis was also conducted for questions requiring more than one answer.

## Results

### *Experts' Perception of Health and Wellness*

Figure 1 shows the main thoughts of the FGD expert-participants whenever they would hear "health and wellness." Experts stated that health and wellness were about nutritious products and healthy diet, balanced quality of life, healthy lifestyle, exercise/ physical activity, clean environment, freedom from diseases, beauty and hygiene, and health care services (Figure 1).


**Figure 1.** FGD participants' primary ideas about health and wellness.

A food product development expert stated that health and wellness are about nutritious food, balanced diet, and relaxation. Claire said,

> "I have three [thoughts]. First, **nutritious** [food] and [second] **balanced diet.** If it's health and wellness, what came into my mind instantly was the famous phrase 'food is thy medicine.' So, I think it covers **nutritious** and **healthy food**, and **a balanced diet.** The last would be **relaxation** and a fit body. It includes going to a spa and exercise. Health and wellness mean the totality or the well-being of the person."

An expert from a well-known convenience store chain in the country mentioned that it is about one's diet. According to Lucas,

> "The first thing that comes to my mind about health and wellness is **diet**. When I went to a doctor for a check-up, I said to him that I want to know everything about my health condition. He asked me back about my expectations. I replied that I didn't expect anything good. He further said that I was right. He said that I should have a good lifestyle by eating the **right food** and to exercise."

Another expert on food research stated that health and wellness are about the food that is good for the body; nutritious, no preservatives, and fortified with necessary nutrients. Sam said,

> "What I think most about it is **food**. Maybe, because I have been so exposed to food research. So, the food that we make should be good for the body. If it's **good for the body**, then it should be **nutritious.** And as much as possible there are **no harmful preservatives**. Many young people are now health conscious and they are looking for products with no preservatives. There are also **food products that are fortified**, for example, vitamin A-fortified food."

An expert from a health service provider company associated health and wellness with healthy living for their patients. Grace shared that they recommended their patients to undergo diet counseling, consume food that have no hazardous chemical ingredients, and use organic products. According to Grace,

> "The first thing that comes to my mind is healthy living for the patients. When we talk about health and wellness, we have conducted **diet counseling** not just to patients but also to their families. Number two would be **no hazardous chemicals to be used as food ingredients**. Now, it has come to our knowledge the term '**Organic**', which is believed to promote life longevity in patients."

Aside from nutritious products and healthy diet, participants also associated other things with "health and wellness."

A nutritionist mentioned that health and wellness refer to a balanced way of life. For Natalie,

> "What it means to me is a **balance of the mental, psychological, spiritual, and physical** [states] of the body."

A chef teaching in a college institution mentioned that it is about beauty and healthy lifestyle. According to Mark,

> "The first one is beautification. For wellness, of course, we need **to be beautiful**, right? We need things that will make us beautiful, right? Because, when you are beautiful it will tell what kind of lifestyle you have. Second, if you have an unhealthy lifestyle, you need **detoxification**."

A drug and food regulator stated that it refers to exercise, diet, and nutrition. Hanah said,

> "The three core terms are **exercise, diet, and nutrition**. In general, when we look at the holistic aspect, we should consider not just the food and things that enter our body."

Another expert from a health service provider stated that health and wellness refer to absence of illnesses and the state of being healthy and fit. Emma said,

> "For me, because I am from a hospital, it is a **condition of being safe from diseases**, when **we don't have illnesses**. [It refers to] the **good overall condition** of someone's body and mind and the **state of being healthy and fit**."

A social entrepreneur associated it with being free from toxins, cancer, and pollutants. According to Lucy,

> "It's about **being free from toxins, cancer, and pollutant**. That kind of viewpoint came because my mother had cancer so I only become aware of health and wellness recently. I had grown up thinking that I am young and that I could always eat in a fast food. This went through until my Mom had cancer. So, I think health and wellness should be a priority not just for the upper class but for all. The government should also make efforts on promoting health and wellness.

***Rice-Based Product Ideas***

Experts were requested to list down product ideas that they would like to develop from rice and present them to the group. They were asked to discuss among themselves, which product ideas could be improved in terms of nutrition and health and would have a high market potential.

Experts from FGD Batch 1 generated, discussed, shortlisted, and categorized the following rice-based product ideas: 1) convenient rice products such as microwaveable rice meal packs or instant rice meals; 2) rice drinks such as probiotic drink, rice bran tea, brown rice tea with *malunggay* and *bignay*; 3) brown rice-based products such as high calorie energy bar and brown rice *am* for young children; 4) rice flour-based products such as packed cake mixes, nuggets, muffins, cakes, cookies, chips, and biscuits; and 5) rice-based personal care products such as rice-based cosmetics, lotion, scar/burnt ointment, and rash ointment.

These ideas came out based on the discussion that consumers now are more outdoor-engaged. Consumers do a lot of things outside their homes. Ronel, a food manufacturing business owner, shared,

> "*Outdoor Consumers*. This means consumers eat and play outside their homes. They do a lot of things outside their homes. Homes now are just for sleeping."

Claire agreed with Ronel,

> "From snacking to other activities, they do most things outside. And these products should be fast, convenient, and instant."

Experts from FGD Batch 2 created and selected the following product ideas based on their perception of the current market trend: 1) rice meals such as flavored rice, rice pasta flavored with *malunggay*, sushi-like rice products with filling, and instant *champorado* using different rice varieties; 2) rice snack/confectionery such as rice pie, pop rice filled with chocolate, rice praline, rice candy, rice ice cream, and chocolate bar filled with rice wine; 3) rice as functional ingredients such as natural food colorant (red mold rice), and other functional isolate compounds from other parts of rice and rice as food supplement; 4) rice drinks or beverages such as ready-to-eat breakfast cereal, flavored rice drink, rice juice, rice iced-drink, and chocolate drink; and 5) rice-based hygiene and beauty products such as red rice bran facial scrub, rice bran additive for beauty products, and milk soap with pigmented rice.

Gordon, an employee of a leading convenience chain store, shared that rice meals, pasta, and flavored water products had growing sales. He said,

> "Rice meals, pasta, pizza, and flavored water are offered for people on-the-go. These are our best-sellers."

Dimas added,

> "Fast food meals are also trendy nowadays, especially to children. Even if we wanted to eat at other restaurants, our children wanted to go in those famous fast food chains."

For snacks and drinks, Rolly mentioned,

> "During warm or hot days, the first thing you that want is a cold drink or an ice-drink, then ice cream or sundae."

For hygiene and beauty products, Fernand said,

> "The youth, even my mother, buy facial wash, facial scrub, whitening soap and lotion to beautify themselves."

Experts from Batch 3 FGD identified the following product ideas: 1) convenient rice-based products such as frozen-style and microwave-ready rice meal and brown rice instant meal; 2) rice snacks/confectionery such as chewy candies, ice cream, bread with added vegetable ingredients, and enriched/fortified rice biscuits or energy bar; and 3) hygiene and beauty products such as rice foot and facial scrub, and lotion.

Cora said,

> "I have chosen products that are convenient and easy to use because almost all people now are always in a hurry. That's why instant noodles and eating in fast food chains are popular."

Table 3 shows the summary of categories of all rice-based product ideas generated by the participants from three cohorts of FGD. Rice flour-based products (32.8%) ranked first. This category includes rice bread, chips, crackers, and cakes. Rice drinks/beverages (18.8%) such as ready-to-drink rice juices, energy drinks, and herbal drinks came second. Participants also liked the ideas of instant rice meals (15.6%) and rice-based personal care/hygiene products (15.6%). Other product ideas were rice as functional ingredient (6.3%), brown rice-based products (4.7%), fermented rice products (3.1%), and rice confectionery (3.1%).

**Table 3.** Rice-based products idea categories of FGD Participants, 2016.

| Product Category | No. | Percentage (%) |
|---|---|---|
| Rice flour-based products | 21 | 32.8 |
| Rice drinks/beverages | 12 | 18.8 |
| Instant rice meals | 10 | 15.6 |
| Rice-based personal care/ hygiene products | 10 | 15.6 |
| Functional ingredient | 4 | 6.3 |
| Brown rice-based products | 3 | 4.7 |
| Fermented rice products | 2 | 3.1 |
| Confectionery | 2 | 3.1 |

n=24; r=total number of product idea responses, 64

Out of these rice-based product ideas generated in the FGD, the top 21 product ideas were selected and were validated through the market survey by measuring consumer interest, preferences, and willingness-to-buy.

***Awareness Level Toward Rice-Based Products***

Survey respondents were asked if they were aware of any rice-based food and non-food products. Majority (98.8%) stated that they were aware; however, most consumers referred rice-based products to native rice-based products or rice cakes (*kakanin)* (88.7%) only rather than manufactured products (Table 4).

**Table 4.** Consumers' awareness of rice-based products, 2016.

| | No. | Percentage (%) |
|---|---|---|
| Aware of any rice-based products (food/non-food)? | | |
| Yes | 335 | 98.8 |
| No | 4 | 1.1 |
| Product | | |
| Native Rice-based Food Products | 298 | 88.7 |
| Manufactured Rice-based Product | 38 | 11.3 |

n=339

***Commonly Consumed Consumer Goods***

Survey respondents were asked to identify snack items usually included in their grocery list or items (Figure 2). Out of 976 snack items identified by the respondents, pre-packaged bakery food products (71%) were the most commonly purchased. Majority of the respondents (89.7%) bought two kinds of these products every time they went shopping or outdoor. This category included pre-packaged biscuits, bread, chips, cookies, crackers, doughnuts, and cakes. Noodles (16.7%) came second as indicated by 48.1% of the respondents. Yogurt and oats were also among the items commonly included in the grocery.

Figure 3 shows the usual drinks/beverages purchased by consumers when grocery shopping. Pre-packaged ready-to-drink beverages were the most purchased (44%). Most consumers (93.5%) habitually bought two types of ready-to-drink drinks/beverages at a time. Other common items mentioned by the majority of the respondents (50.3-64.79%) were instant coffee (14.7%), powdered juice (14.1%), and powdered milk (11.4%).

Figure 4 shows the personal care products that respondents usually purchased Of the 1,541 personal care items mentioned by the respondents, 37.1% were body care products. Consumers (58.2%) usually had two types of body care products in their list during grocery shopping. Hair care (26%) products were included among the usual items purchased as indicated by all the respondents. Most of them (89.1%) also included dental/oral care products (19.60%) in their shopping list.

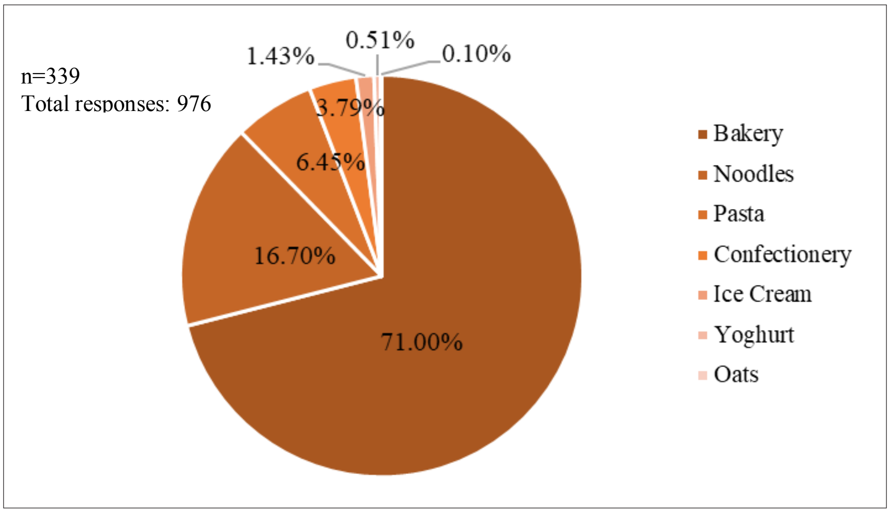


**Figure 2.** Snack items usually included in the grocery list or bought by the consumers, 2016.

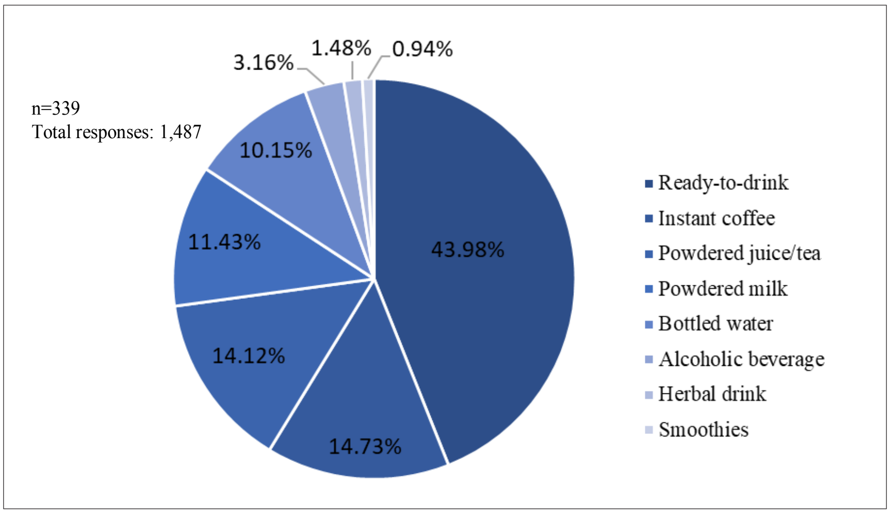


**Figure 3.** Drink/beverage items usually included in the grocery list or bought by the consumers, 2016.

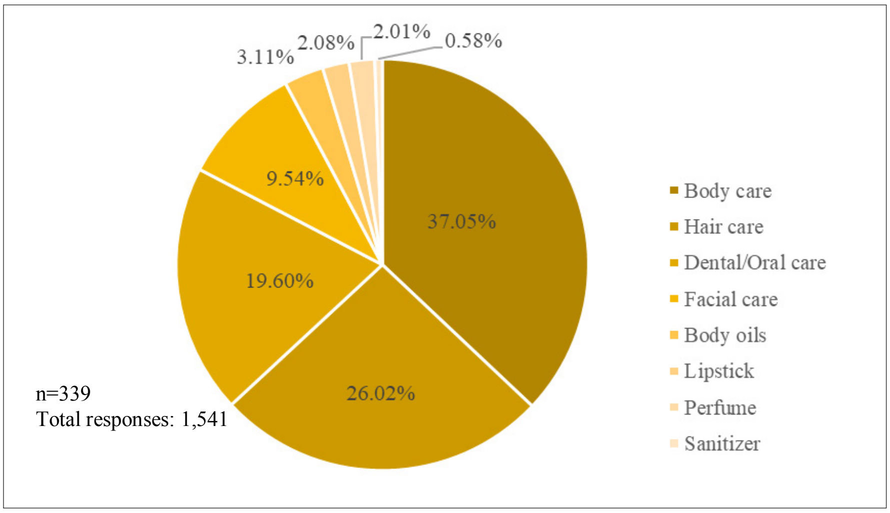


**Figure 4.** Personal care products usually included in the grocery list or bought by the consumers, 2016.

The respondents were also asked if they usually buy some of the pre-identified meal items stated in the questionnaire during grocery shopping or when going outdoor (Figure 5). Consumers indicated that they would buy fast food meals (29.3%), canned goods (28.3%), instant noodles (18.6%), *carinderia* (local eatery) meals (15.9%), restaurant meals (7.7%), and microwavable meals (0.2%).

Figure 6 shows the pharmaceutical or nutraceutical products that consumers would include in the grocery shopping list. Majority of the respondents mentioned pain relievers (internally taken; 45.3%) and vitamins and minerals (35.26%).

***Consumers' Perception of Food Product Attributes***

Table 5 shows how consumers perceived the different food product attributes in terms of their importance when deciding to buy. Results showed that consumers perceived high importance for all product attributes as indicated by high importance rating given to intrinsic and extrinsic attributes (3.8-4.7). Among intrinsic attributes, taste ranked first (4.7± 0.7), followed by nutritional and health benefits (4.6 ± 0.8). On the other hand, the availability of the product at any time ranked first (4.6±0.8) among all extrinsic attributes. This was followed by price (4.6±0.9).

**Table 5.** Consumers' importance rating (1 to 5) to different food product attributes, 2016.

| Attribute | Mean |
|---|---|
| *Intrinsic* | |
| Taste | 4.7 ± 0.7 |
| Nutritional and health benefits | 4.6 ± 0.8 |
| Aroma | 4.4 ± 0.9 |
| Texture | 4.2 ± 1.0 |
| Color | 4.0 ± 1.1 |
| *Extrinsic* | |
| Availability | 4.6 ± 0.8 |
| Price | 4.6 ± 0.9 |
| Accessibility | 4.6 ± 0.8 |
| Packaging | 4.2 ± 1.1 |
| Advertisement | 4.1 ± 1.1 |
| Online Presence | 3.8 ± 1.3 |

n=339

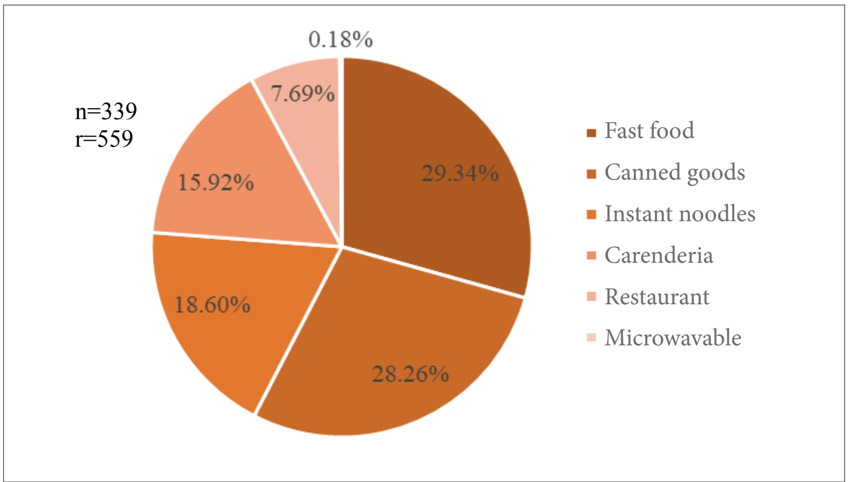


**Figure 5.** Meal items bought by the consumers, 2016.

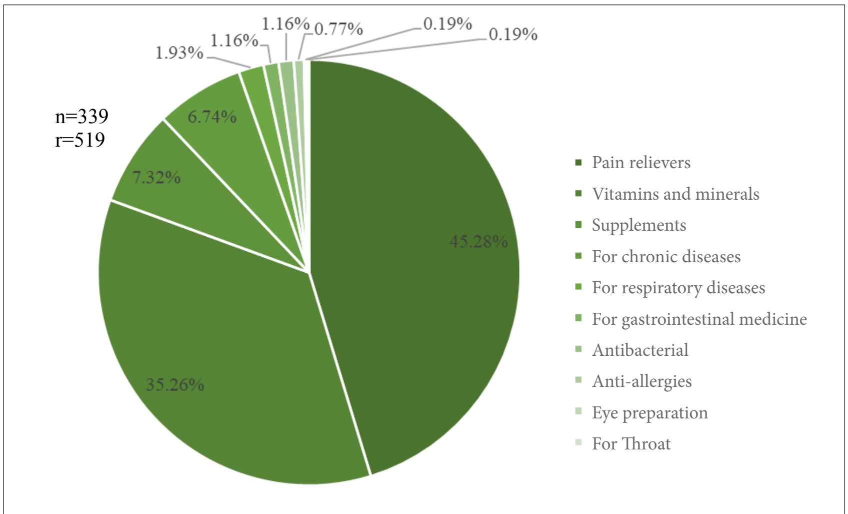


**Figure 6.** Pharmaceutical/ nutraceutical items usually included in the consumers' grocery list, 2016.

### *Consumers' Degree of Liking and Likelihood of Purchase Toward Different Rice-Based Product Ideas*

Table 6 shows the respondents' degree of liking and likelihood of purchase toward the different rice-based product ideas presented during the survey. All rice-based bakery food product ideas such as biscuit (4.6±0.8), cookie (4.5±0.9), cake (4.4±1.0), bread (4.31±1.11), cracker (4.23±1.1), and muffin (4.0±1.2) had high ratings. Some (51-85%) of the consumers expressed willingness to purchase all product ideas. Specifically, rice biscuit ranked highest among all rice-based product ideas in terms of the degree of liking. Most of the respondents (84.70%) also indicated that they would likely buy rice biscuit if it would be made available in the market. Rice- or rice-based health supplement (4.5±1.0) came second and many of the respondents (72.3%) would like to buy it if available.

**Table 6.** Consumers' degree of liking (1-5) and the likelihood of purchase (yes-no) toward the different rice-based product ideas, 2016.

| Rice-based Product Idea | Degree of Liking | Yes (%) | No (%) |
|---|---|---|---|
| Biscuit | 4.6 ± 0.8 | 84.7 | 15.3 |
| Health Supplement | 4.5 ± 0.9 | 72.3 | 27.7 |
| Cookies | 4.5 ± 0.9 | 75.2 | 24.8 |
| Cake | 4.4 ± 0.9 | 72.9 | 27.1 |
| Bread | 4.3 ± 1.1 | 72.9 | 27.1 |
| Cracker | 4.2 ± 1.1 | 69.3 | 30.7 |
| Chocolate Drink | 4.2 ± 1.2 | 67.6 | 32.4 |
| Soap | 4.1 ± 1.3 | 65.5 | 34.5 |
| Nugget | 4.1 ± 1.1 | 61.4 | 38.6 |
| Muffin | 4.0 ± 1.2 | 61.4 | 38.6 |
| Lotion | 4.0 ± 1.2 | 57.5 | 42.5 |
| Instant Rice | 4.0 ± 1.4 | 65.2 | 34.8 |
| Tea | 4.0 ± 1.3 | 67.6 | 32.4 |
| Facial Scrub | 4.0 ± 1.3 | 57.5 | 42.5 |
| Juice | 3.9 ± 1.3 | 65.8 | 34.2 |
| Ice Cream | 3.9 ± 1.3 | 61.1 | 38.9 |
| Facial Powder | 3.9 ± 1.3 | 56.9 | 43.1 |
| Microwavable food | 3.9 ± 1.3 | 62.8 | 37.2 |
| Facial Cream | 3.8± 1.4 | 56.0 | 44.0 |
| Wine | 3.1 ± 1.6 | 51.0 | 49.0 |
| Beer | 2.8 ± 1.6 | 44.5 | 55.5 |

n=339

## Discussion

### *The Health and Wellness Trend: An Opportunity*

A consumer-driven approach of product development entails looking at the current consumer trends to derive new product ideas. One of the biggest trends in today's market is health and wellness. FGD results showed that experts primarily associated health and wellness with the concepts of food, diet, and nutrition. This is supported by a report stating that good health is now a trend around the world, including the Filipinos who are willing to pay more for food products with added health and nutritional properties (Nielsen, 2015). It is believed that good health is best achieved through balanced and diversed diet including fruits, vegetables, and whole grains (Cornish & Moraes, 2015).

Additionally, a study by Ong et. al. (2014) showed that consumers perceived health status as the major determinant of quality of life. Consumers further stated that they could achieve good health with medicine intake, balanced diet, regular exercises; and by getting away from vices. Likewise, FGD experts in this market study associated health and wellness to quality diet, healthy lifestyle, clean environment, freedom from diseases, beauty and hygiene, and health care service. This suggests that there is a huge market opportunity for new rice-based products that will offer a wide range of health and nutritional benefits.

FGD participants further stated that convenient, healthy, and beauty products have high market potential. They suggested rice-based product ideas that were ready-to-eat (packed), easy-to-prepare, and ready-to-drink. Beauty and personal care/hygiene products, rice as a functional ingredient, and fortified or enriched rice-based products were also suggested.

### *Availability of Rice-Based Products*

The survey respondents showed low awareness of manufactured rice-based products. Based on a survey conducted by PhilRice (2013), manufactured rice-based products were already available in supermarkets and wet markets. However, the most common form of rice-based food products served as meals and snacks were native rice-based food products or rice cakes such as *biko*, *bibingkang suman*, *tamalis*, *bibingkang galapong*, *espasol*, *puto*, *cutchinta*, and *pinipig*. Manufactured rice-based products (e.g., packaged mixes such as *adobo* mix, *caldereta* mix, *kare-kare* mix, rice mate, baby food ingredient, seasonings, and cereal drink) comprised lower percentages (0.05-8.24%) in the rice-based products available in the market (PhilRice, 2013). These findings tell us that consumers have a limited understanding of rice-based products, and they were unaware that some manufactured food products contain rice as ingredient.

Development of products from rice flour is not new, and there are still possibilities for innovation. Rice flour's attributes such as bland taste, white color, ease of digestion, and hypoallergenic properties have made it a suitable ingredient to produce bakery food snacks (Arendt & Zannini, 2013). Rice could also be used as an aid to hypertension due to its low sodium and fat content and zero cholesterol. It is also widely used as an ingredient in baby food due to its hypoallergenic properties. It is also useful in oral rehydration of infants with diarrhea (Arendt & Zannini, 2013).

***Trends on Consumer Goods***

Survey respondents commonly purchased consumer goods that were convenient such as packed ready-to-eat bakery products for snacks, fast food and canned goods for meals, ready-to-drink beverages and instant coffee for beverages, body care and hair care for personal care products, as well as vitamins, minerals, and pain relievers for pharmaceutical or nutraceutical products.

Bakery food product group was a major food segment in the Philippine market that had steady high sales value (Agriculture and Agri-Food Canada, 2014). In fact, bread and biscuits were among the top 20 foods consumed in Filipino households based on the 2013 Food Consumption Survey conducted by the Department of Science and Technology - Food and Nutrition Research Institute (DOST-FNRI). Results of this market study were consistent with the reports about the Philippine market. Noodles, yogurt, and oat products were also included in the top list by the respondents. Noodles had consistent sales growth in the Philippine market with a Compound Annual Growth Rate (CAGR) of 6.9% in 2014. Additionally, pasta was among the top commonly consumed snack item. In fact, it was one of the products in the packaged food industry in the Philippines with the best growth having a CAGR of 10.2% (Agriculture and Agri-Food Canada, 2014). According to Kantar Worldpanel's data, yogurt and cereal products like oats had a remarkable growth of 11% and 12% in sales value from January 2015 to December 2016, respectively (Miraflor, 2017).

Ready-to-drink beverage category included pre-packaged ready-to-drink coffee, juices, teas, milk, cereal drinks, fermented drinks, and other flavored drinks. Most Filipinos now have an *on-the-go* lifestyle implying a need for convenient products that do not require a lot of preparations. Based on a survey, Filipinos were encouraged to spend more, from PhP288 in 2015 to PhP320 in 2016, for ready-to-drink juices that offer cholesterol management effect (Francia, 2017). Convenience is considered as one of the big four consumer trends in the Philippine market nowadays (PANA, 2013). Instant coffee was the second item usually bought. Instant coffee was also among the top 20 foods consumed by Filipino households (DOST-FNRI, 2015).

Hygiene and beauty are also big consumer trends in the Philippines (PANA, 2013). In the recent years, Filipino consumers are becoming more conscious in terms of beauty and cleanliness. Increase in the use of hygiene and beauty products has been observed lately. In this study, body care product category included items such as soap, body wash, lotion, and deodorant. This category had the highest percentage of all personal care products. Hair care product category followed, which is dominantly comprised by shampoo and conditioner (PANA, 2013).

Food service establishments have been growing both in number and in sales due to the rising popularity of eating outside the home. This was driven by increased purchasing power, busy lifestyle, desire for convenience, and entry of international brands in the industry (Claridades, 2016). Based on the study of Rufino (2015), the incidence of consuming "food away from home" among Filipino households have been increasing over the years with the highest incidence of 89.61% occurred in 2012. Rufino (2015) showed that the per capita consumption on "food away from home" (9.91% annual increase) was increasing at a higher rate than of food consumed at home (4.41% annual increase). Additionally, based on the survey conducted by Nielsen (2014), a decline of 13% in the monthly grocery spending of its Filipino respondents was noted in 2014 due to increased "dining out" instances. Consumers have gone to fast food chains and restaurants for family bonding or hanging out with friends over the weekends, after work, or after grocery shopping (Claridades, 2016). In the canned goods category, increased consumption of a healthier choice such as tuna or luncheon meat with less sodium was observed. Luncheon meat variants were purchased by 1.1 million Filipino households, and it garnered up to 27% sales increase in the market (Francia, 2017).

The increase in prevalence of health problems in a developing country is brought about by the changes in lifestyle, eating habits, and unstable rural habitations (Hussain et al, 2015). As a result, consumer demand shifted to food products offering desired health benefits as a means of prevention against non-communicable diseases (Hussain et al, 2015). Supported by Nielsen (2015), beneficial ingredients in foods such as vitamins, calcium, minerals, and micronutrients were found to be important among Filipinos.

Furthermore, multivitamins and minerals, B-complex vitamins and vitamin C were the most commonly consumed supplements by the Filipinos based on surveys (DOST-FNRI, 2014; Tanchoco & Cruz, 2000). Tanchoco & Cruz (2000) figured out that majority of the consumers were using nutraceuticals or food supplements because they perceived that these products could make them healthy. They also consume these products due to someone's recommendation. A Philippine study on knowledge, attitudes, and practices of patients towards the use of herbal dietary supplements found that patients generally have a positive attitude toward these products for health benefits and safety (Ong, 2014). However, some were cautious about using dietary supplements due to the lack of information about these products. In addition, consumers are always being warned about the usage of such supplements without medical prescription due to side effects like the antagonistic interaction that could happen between nutrients (DOST-FNRI, 2014). These products are highly regulated by the Food and Drug Administration (FDA) in the Philippines. FDA has constantly reminded the Filipino consumers to be careful in taking food/dietary supplements because these products have no curative and therapeutic effects despite the vigorous company advertisements and testimonials of people that these products have somehow helped or cured their diseases (DOH-FDA, 2012; 2013). Despite all of these, the local vitamins and supplements industry in the Philippines still poses a very promising market opportunity with a potential annual sale of Php150 billion and a compounded annual growth of 3% (PhilStar Global, 2011).

***Influence of Product Attributes on Consumers' Choice***

Survey results indicated that all product attributes were important to the consumers, especially taste, nutrition and health benefits, as well as the availability of the product. Product attributes, both intrinsic and extrinsic, are the major determinants of a consumer's buying decision (Hanis et al., 2012). Attributes are used as basis for evaluating product's quality. Consumers use attributes to characterize a product if it has the desired benefits from one aspect to another, as well as in comparing a product to its competitors (Oghojafor et. al., 2012). These entail that consumer purchases or repeat-purchases happen if food products have the perceived qualities and if these expected qualities are met after being consumed (Hanis et al., 2012). As such, product's attributes are important consideration in product development and marketing strategy formulation (Oghojafor et. al., 2012; Hanis et. al., 2012).

Taste is the most important among attributes based on functional foods study. It was also found that skepticism, bad taste, and costs were the major reasons why consumers did not buy these foods (Marina, et al., 2014). These implied that in generating rice-based food products, developers should seriously take into consideration that good taste must come along with being healthy and nutritious.

***Consumers' Preferences on Rice-Based Products***

Among the 21 rice-based products presented to the respondents during the survey, rice-based bakery product ideas were highly preferred. A Filipino household spent an average of 35% of the household budget for food products, which was the highest among the household expenditures (Agriculture and Agri-Food Canada, 2016). It is reported that Filipinos eat five times a day and mostly snacks (New Zealand Trade and Enterprise, 2013). Based on another report, the highly demanded products in the Philippine market included snacks/crunchers (e.g., biscuit, chips), individually-packed pastries (e.g., cakes, cookies, and cupcakes), soups, juices, sauces, cheese, canned goods, ready-to-eat packages, and nutraceutical products (e.g., food supplements and slimming products) (Agriculture and Agri-Food Canada, 2016).

The results implied that rice-based products with added value on health and convenience would likely become appealing to Filipino consumers. Healthy and nutritious rice-based bakery products are definite consumer demand in the Philippine local market in the future. This denotes that product developers should take special consideration on this when generating rice-based food products.

## Conclusions and Recommendation

Food, nutrition, and business experts associated "health and wellness" trend with healthy and nutritious food products. Convenience is also a strong market trend that goes along with it. Based on these major trends, experts suggested product ideas such as rice-based bakery products, rice beverages, instant rice meals, rice-based functional products, and rice-based personal care products.

Based on the survey, consumers are only aware of rice-based products as native food products or "*kakanin*" and not as manufactured rice-based products. Moreover, consumers rarely know about non-food rice-based products. Consumer trends for product categories were ready-to-eat/drink, beauty and personal care, and supplements. Consumers highly consider taste, price, nutritional content, aroma, packaging, texture, availability, and accessibility when they decide on household purchases. Among all rice-based product ideas, consumers in Central Luzon mostly prefer to buy bakery products if available in the market. From this study, it can be deduced that for the next 5 to 10 years, consumer preferences include products that are convenient, healthy, and for beauty and hygiene purposes. Product development should focus on instant, nutrient-rich with added health benefits rice-based food products (e.g., probiotic rice drink, instant rice meal, rice-bran soap and body scrub, rice as a functional ingredient in food supplements).

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# COMPARATIVE EFFECT OF CHICKEN MANURE AND EM-BASED *BOKASHI* AS INOCULANT TO RICE STRAW DECOMPOSITION

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## Abstract

Rice straw (RS) is a common farm waste in the rice-rice cropping pattern. While reincorporating this waste to the soil has benefits, its high C:N ratio would lead to slower mineralization and temporary immobilization of nutrients, particularly nitrogen. As such, hastening its decomposition in the paddy field becomes an interest. This study assessed the effect of direct incorporation of rice straw on the release of nutrients in paddy soil and compare the effectiveness of chicken manure and Effective Microorganism-Based Inoculant (EMBI) with rice straw. The treatments were the following: (1) control (untreated), (2) rice straw (RS), (3) rice straw with chicken manure as inoculant (RS+CM), and (4) rice straw with EM-based inoculant (RS+EMBI). The soil $NH_4$-N released is significantly higher in rice straw with chicken manure as inoculant compared with rice straw and rice straw with EM-based inoculant. However, rice straw with or without inoculant, negatively affected the availability of $NH_4$-N concentration in the soil up to 60 days as the $NH_4$-N is much lower than the control. Available soil Phosphorous (P) and exchangeable soil Potassium (K) were significantly higher in soil applied with RS (with or without inoculant) while the P and K released in rice straw with chicken manure as inoculant were higher than rice straw with EM-based inoculant. Rice straw, incorporated alone or in combination with chicken manure or EM-based inoculant, has significantly higher soil Iron (Fe), Zinc (Zn), and Manganese (Mn) than the control. Available soil Fe was highest in rice straw with EM-based inoculant and lowest in rice straw with chicken manure as inoculant. Available soil Zn was found highest in rice straw while available soil Mn was highest in rice straw with chicken manure as inoculant. Results showed that rice straw has a positive impact in increasing the availability of nutrients in paddy soil except for Nitrogen (N). Choosing chicken manure as an alternative inoculant over EM-based inoculant is a more practical option especially in increasing available soil P and soil K. It is also readily available within farming systems that include fowl or chicken raising.

**Keywords:** *Bokashi, Chicken Manure, Immobilization, Microbial Inoculant, Mineralization, Paddy Rice Soils, Rice Straw.*

## Introduction

Rice straw management is considered an important aspect in sustaining long-term fertility of soil in rice cropping systems, especially in lowland paddies because it is the major organic material easily available. In the Philippines, rice straw is usually removed from the field or burned in situ, incorporated in the soil, or used as mulch for the following crop. While burning is a cost-effective method of straw disposal that may reduce pest and disease populations, it causes almost complete N loss and losses in P (about 25%), K (about 20%), and S (5-60%). These nutrient losses (Dobermann and Fairhurst, 2002) and the potential air pollution are the reasons why burning rice straw is discouraged (Eagle et al. 2001).

Incorporating rice straw in the soil is an alternative to burning. It returns most of the nutrients back to the soil and helps conserve its nutrient reserves in the long-term. Although its short-term effects on grain yield are often small (compared with straw removal or burning), its long-term benefits are significant. Straw incorporation has resulted in increased N mineralization potential in non-rice and rice systems in the long term.

But while rice straw incorporation in the rice field increases the number of decomposers over time, its mineralization rate is usually lower and slower than composted materials (Cooperband et al., 2002; Hadas and Portnoy, 1997) owing to its high C:N ratio (Tyson and Cabrera, 1993; Kirchmann, 1989) cellulose content. Mineralization initially immobilizes nutrients including N. Nutrients are required to produce microbial biomass and stable soil Organic Matter (OM) and may not be able to meet the plant demand of N and all other nutrient elements necessarily for rice growth and grain production. The available N supply in the soil tends to increase after an initial equilibration period of up to three years following rice straw incorporation (Verma and Bhagat, 1992; Bacon, 1990). It is imperative, therefore, to hasten rice straw decomposition in situ by using enhancing materials such as high N fertilizers, inoculant like mixture of microorganisms, and use of manures with lower C:N ratio.

Effective Microorganism (EM) is a mixed culture of naturally occurring, beneficial microorganisms (predominantly lactic acid bacteria, yeast, actinomycetes, photosynthetic bacteria, and certain fungi), that has been used by Japanese farmers as traditional soil amendments to increase the microbial diversity of soil and supply nutrients to crop. In the Philippines, EM-Based Inoculant (EMBI) or Bokashi, a Japanese term for "fermented organic matter," has been adopted to facilitate composting of organic materials. The EMBI is usually made by fermenting organic matter (rice bran is used in this study) with EM. EMBI applied with fresh rice straw in situ had reduced the days of mineralization to 14 days after their incorporation (Javier and Tabien 2003).

Chicken manure (CM), on the other hand, has been used effectively as fertilizer due to its high N-P-K and micronutrient content (Magat and Recel, 1999). It is also considered the most economically efficient types of manure due to its high pH, low organic C, high inorganic N, and low C: N ratio compared with other types of manure. A large portion of N in poultry manure is in organic fractions. Under laboratory conditions, approximately 50% of the organic N in poultry manure was mineralized within 90 days. Based on a 330-day greenhouse study, an approximately 60% of the organic N in poultry manure was made available (Bijay-Singh, et al 1996). In Malaysia, 20-30% of chicken manure is usually combined with empty fruit bunches (by-product of oil palm making) to accelerate the composting process. In Korea, chicken manure is mixed with sludge, which was left after processing potato starch before composting.

Unlike Bokashi or EMBI, chicken manure is cheaper and readily available in the Philippines. As farmers encouraged to naturally decompose rice straw in-situ, this study was conducted to compare the effect of chicken manure and Bokashi as inoculant to rice straw decomposition and determine the rate of release of N, P, K, Zn, Fe, and Mn from different treatments.

## Materials and Methods

A greenhouse pot experiment was conducted from August to October 2006 at the Philippine Rice Research Institute (PhilRice) using *Maligaya clay soil, Ustic Epiaquerts*. A total of 50 kg of dry soils was placed in each of the plastic pail representing one experimental unit. The experiment was laid out in complete randomized design (CRD) with three replications. The treatments were: (1) control (untreated), (2) rice straw alone at the rate of 10 t/ha (RS), (3) rice straw with chicken manure as inoculant (RS+CM), and (4) rice straw with EM-based *Bokashi* as inoculant (RS+EMB). The volume of rice straw was computed based an equivalent of 60 kg nitrogen/ hectare in an oven-dry weight basis (ODW) for fair experimental treatments. Hence, the rate was not necessarily based on the actual harvested rice straw in the field.

Rice straw were chopped into small pieces, homogenized, and weighed accordingly. Then each EMB and air-dried and ground CM were weighed and mixed separately with the rice straw at the rate of 10:1 and 3:1 ratio, respectively. The mixture was incorporated manually into the paddy soil, after which the soils were submerged with water and maintained at 3-5 cm depth. An initial soil sample equivalent to 20 g each pot was taken and tagged as samples of 0 days after incorporation (DAI). The sampling was done every three days until 65 DAI. No rice plants were planted into the pot as the study focused on the availability of soil nutrient resulting from soil amendments.

The soil was sampled and extracted as wet soil for the analyses of N, P, and K while part of the same samples was air dried, pulverized, and extracted for the analysis of Zn, Mn, and Fe content. In saturated or flooded soil, Soil ammonium N ($NH_4$-N) was the dominant available form of N.

NH4-N was determined thru colorimetric method (at absorbance of 636 nM wavelength); available P by Olsen determination using sodium bicarbonate extraction and by colorimetric method (at absorbance of 720 nM); and available K thru flame photometer method (transmittance at 768 nM) using ammonium acetate extraction. For the micronutrients available Fe, Mn, and Zn were analyzed thru atomic absorption spectrophotometer (AAS) method using 0.05M HCl extraction. Data gathered were statistically analyzed using STAR Nebula® and presented as data means per treatment per sampling date.

## Results and Discussion

**On Macronutrient:**

***Nitrogen***

$NH_4$-N, the dominant form of nitrogen under submerged soil condition, had decreased as early as 3 DAI for treatments applied with rice straw with or without additives (Figure 1). The trend was constantly low from the soils applied with RS alone and RS+EMBI 3-34 DAI. It implies that the low soil N availability was very low when rice plants would have been actively growing and in need of N during tillering and leaf formation stages. The increasing trend, however,

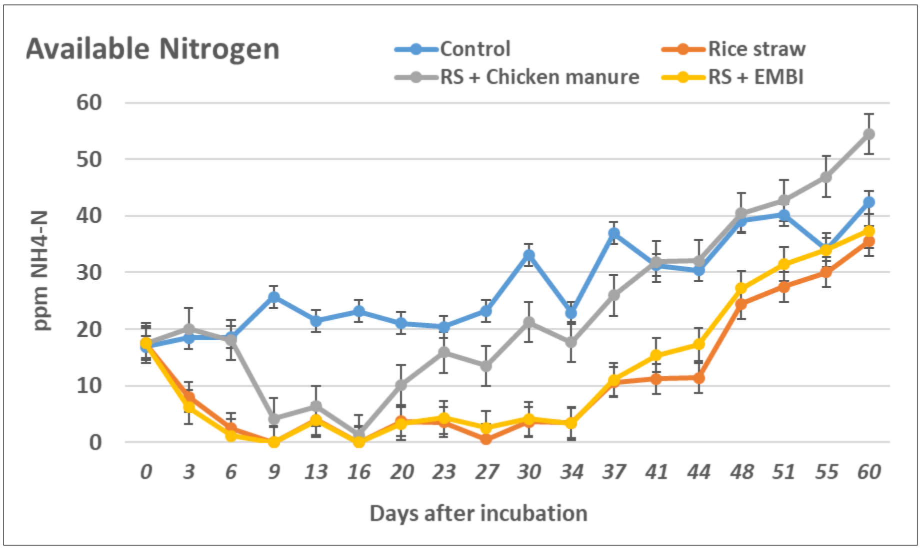


**Figure 1.** Available Nitrogen released in paddy soil treated with rice straw in combination with chicken manure and with *Bokashi* as inoculant.

at 37-60 DAT might be assumed to supply N during early panicle initiation until early flowering stage. For soils applied with RS+CM, the soil $NH_4$-N, which started to decrease at 6 DAI, showed increasing trend at 16 DAT up to 60 DAI and higher than those soils applied with RS and RS+EMBI. Apparently, chicken manure as inoculant showed possibly higher efficiency than the uninoculated rice straw and the rice straw inoculated with EMBI.

In cases when incorporation of rice straw in the soil with or without inoculants depressed $NH_4$-N concentration in the paddy soil, and when soil $NH_4$-N had decreased at a very low level, it contributed to temporary N immobilization due to microbial activities during the decomposition process (Cabrera, 1994; Eagle et al., 2001; Javier and Tabien 2003). Soil microorganisms usually utilize rice straw as their food and energy source. As these microorganisms decompose the material, they compete for the limited supply of available N as the residue does not provide adequate N to form proteins. This process is contrarily observed in the unapplied paddy soils where $NH_4$-N increased during sampling. Temporary N immobilization was not observed in the absence of organic matter or RS. Results showed that the soil total available $NH_4$-N in the control has the highest average N released (28 ppm) followed by RS+CM (23 ppm). RS+EMB and RS treatments yielded 12 and 11 ppm $NH_4$-N, respectively. The higher N in the control may be due to the undisturbed natural mineralization in the soil, while the lower N in the RS applied soils may be due to the temporary immobilization of N in the soil and in the RS during which microorganisms have utilized N in the decomposition process.

With reincorporation of rice straw into the soil, N released in the RS+CM set up may be due mainly to the N released from chicken manure, and/or to the combined effect of RS+CM on the released of soil N. Cabrera et al. (1994) confirmed this rapid mineralization was due to chicken manure; estimated that 35-50% of organic N could be mineralized within 14 days after incorporating chicken manure into the soil.

No significant difference was observed between RS and RS+EMB, which means that the added EM-based inoculant has less or no significant effect in releasing $NH_4$-N.

### *Phosphorous*

Available P was consistently increasing in the paddy soils when applied with RS+CM, and in higher content than other treatments (Figure 2). This is probably due to high P content of the chicken manure (Javier et al., 2004). It was observed that soil applied with RS and RS+EMBI has higher P content than soil with RS+CM treatment. However, P in unapplied paddy soils was higher than in soil treated with RS+CM. In clay soil with high indigenous P, the available P analyzed in all the treatments did not show deficiency level (5ppm). Instead, a very high available P was observed in the Maligaya clay soil series especially when flooded.

Results indicated that the highest available P was in RS+CM-treated soils with an average value of 33 ppm P, followed by RS and RS+EMB with 28 and 29 ppm P, respectively (*Table 1)*. The lowest P released was observed in the control with an average value of 26 ppm. The critical available P level for lowland rice based on Olsen P analyses should be more than 5-10 ppm to be sufficient (Dobermann and Fairhurst, 2000). The level of soil P, treated or not with rice straw, was more than 10 ppm.

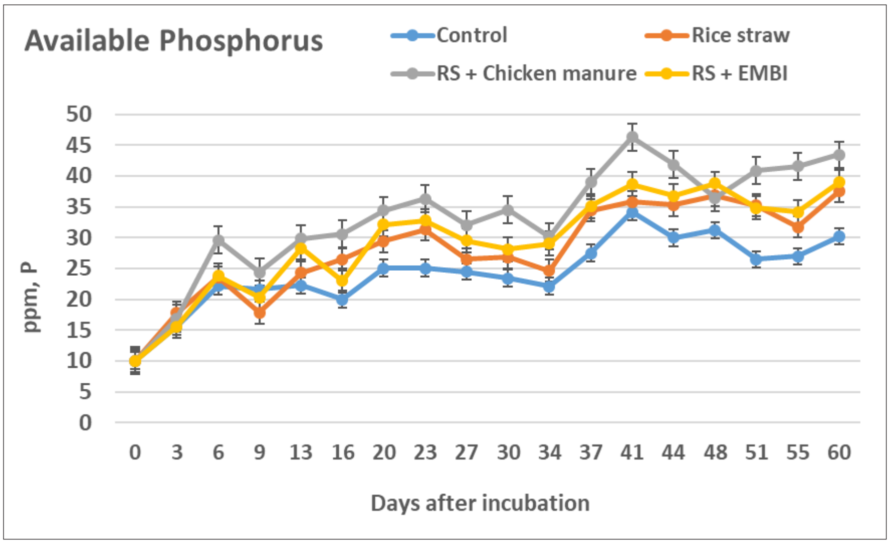


**Figure 2.** Available Phosphorus released in paddy soil treated with rice straw in combination with chicken manure and with *Bokashi* as inoculant.

**Table 1.** Average soil nutrient content 1-65 DAI as affected by the application of rice straw inoculated with chicken manure and EM-based inoculant.

| Treatment | NH4-N (ppm) | P (ppm) | K (m.e./100g soils) | Zinc (ppm) | Fe (ppm) | Mn (ppm) |
|---|---|---|---|---|---|---|
| Methods | Kjedahl | Olsen P | NH4OAc | DTPA | | |
| Control | 27.74 | 24.35 | 0.332 | 4.03 | 3,283.31 | 282.85 |
| Rice straw | 10.99 | 28.10 | 0.670 | 5.27 | 3,539.09 | 330.44 |
| RS + Chicken manure | 23.36 | 33.25 | 0.740 | 5.66 | 3,526.79 | 334.27 |
| RS + EMBI | 12.25 | 29.45 | 0.674 | 5.43 | 3,388.43 | 337.00 |
| Mean | 18.59 | 28.79 | 0.603 | 5.10 | 3,434.40 | 321.14 |
| CV (%) | 15.61 | 8.29 | 6.84 | 6.58 | 8.21 | 3.19 |

***Potassium***

The increase of soil K was observed at 3 DAI and consistently high until 65 DAI (*Figure 3*). On contrary, the exchangeable K observed from the control was consistently lower than those with rice straw, treated or not treated; predicting that the indigenous potassium from the irrigated lowland paddy soils remained constant. The higher exchangeable K in the paddy soils applied with RS may be due to its inherent high K content of the straw (Javier, et al, 2004), which can be compared with non-RS-treated soils.

After 60 days of experiment, the exchangeable K in the control has an average of 0.33 meq/100g soil and significantly lower than RS treatments (Table 1). For lowland rice, the available K is already below sufficiency level (Dobermann and Fairhurst, 2000). Meanwhile, the application of rice straw alone and inoculated RS yielded significant increase in exchangeable K. The highest available soil K was observed in the RS+CM (average 0.74 meq/100g soil) while the uninoculated rice straw and the RS+EMB has an average value of 0.67 meq/100g soil. Soil applied with RS, however, showed sufficient amount of K (>0.45 meq/100g soil). This implies that re-incorporation of RS had contributed high amounts of soil K, which can be assumed to be available and can be sufficient for rice plant K nutrition throughout its growing period.

## On Micronutrients

Several researches had shown high amount of micronutrients in crop residues and other organic fertilizers and their availability is a function of soil organic matter. They are released through decomposition of organic matter and chelating compounds. But while Fe, Cu, and Mn are more available and with greater content under waterlogged than aerated soil condition, their higher level in paddy soils may render imbalances and unavailability of some other nutrient elements important for good growth and higher yield.

***Iron***

Available Fe in the control (3,283 ppm Fe) and in treated soils (average 3485 ppm Fe) showed high concentration. These values are higher than the critical

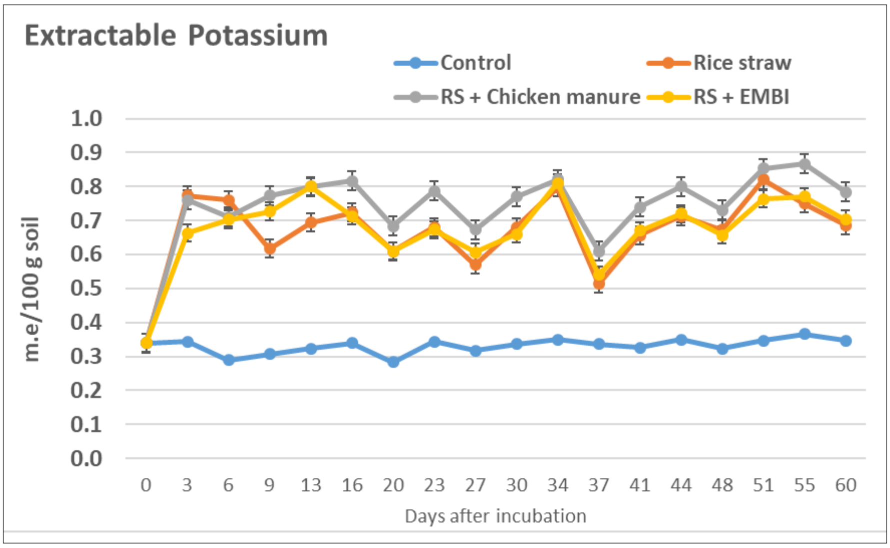


**Figure 3.** Exchangeable Potassium released in paddy soil treated with rice straw in combination with chicken manure and with *Bokashi* as inoculant.

concentration (>300 ppm Fe) where Fe toxicity is more likely to occur (Dobermann and Fairhurst, 2000). Inherent in the flooded Maligaya clay soils, Fe is apparently high, with or without any applied supplement. The soil Fe, due to the incorporation of rice straw alone, gave the highest input into the soil with 7.23% higher than the control, while RS+CM gave an increase of 6.90%. RS+EMBI only contributed 3.10% increase in the soil Fe (Table 1). With the results, Fe toxicity can be avoided by incorporating chicken manure into the rice straw or by adding EMBI to hasten decomposition. The peak of available Fe was at 30 and 60 DAI (Figure 4). High amount of Fe in the soil may lead to either P or Zn deficiency or unavailability for plant use due to its chelating capability as induced by some soil microorganism (Washington State University). This can also be true based on the Mulder's chart of antagonism and synergism of elements (Goldy, 2016).

***Zinc***

The peak of available soil zinc in this study was observed at 23 DAI then at 51 DAI (Figure 5). It was also observed that application of RS gave 26% higher soil Zn than the control. Among the RS treatments, RS+CM made soil Zn more soluble (average of 5.6 ppm Zn) than when RS+EMB was used (average of 5.23 ppm Zn) (Table 1). The soil Zn, even without amendments, was still higher than the deficiency level (1ppm Zn) (Dobermann and Fairhurst, 2000). This concentration may help in mitigating factors that may lead to zinc unavailability for plant uptake.

***Manganese***

Manganese in the soil, with no added intervention, showed the lowest content (average of 282.5 ppm) throughout the duration of the study (Figure 6). It was observed that the peak of its availability is at 27, 55, and 58 DAI (Figure 6). RS incorporation, regardless of combined CM and EMBI, showed the same peak of its release and consistently similar from the start of incorporation until 65 DAI. On average after the 65 DAI, the RS-applied soils (333 ppm or 15% higher than the control) had significantly higher soil Mn than the control (283 ppm) (Table 1). This level is already beyond the soil minimum requirement of 40 ppm Mn (0.1 M HCl extractable Mn) (Dobermann and Fairhurst, 2000).

## Conclusion

Rice straw had positive impact on the immediate availability of nutrients in the soil except for N. Incorporation of CM rather than EM-based *Bokashi* as inoculant to RS is a better option especially in increasing P and K and in chelating Fe-rich soil. Incorporating RS to paddy soil in time with transplanting is critical as rice straw can depress available N, which is necessary for early growth and development of the rice plants.

Incorporation of rice straw had increased micronutrients (soil Fe, Cu, and Mn) content in the soil, higher than the set critical level for paddy soils by Dobermann and Fairhurst (2000); rendering imbalances and unavailability of some other nutrients important for good growth and high yield.

It is recommended that chicken manure may be used as an inoculant to rice straw incorporation *in situ* when EM1 is unavailable or cannot be afforded by the farmers. This will enhance RS decomposition in the paddy field instead of RS burning or off-site decomposition.

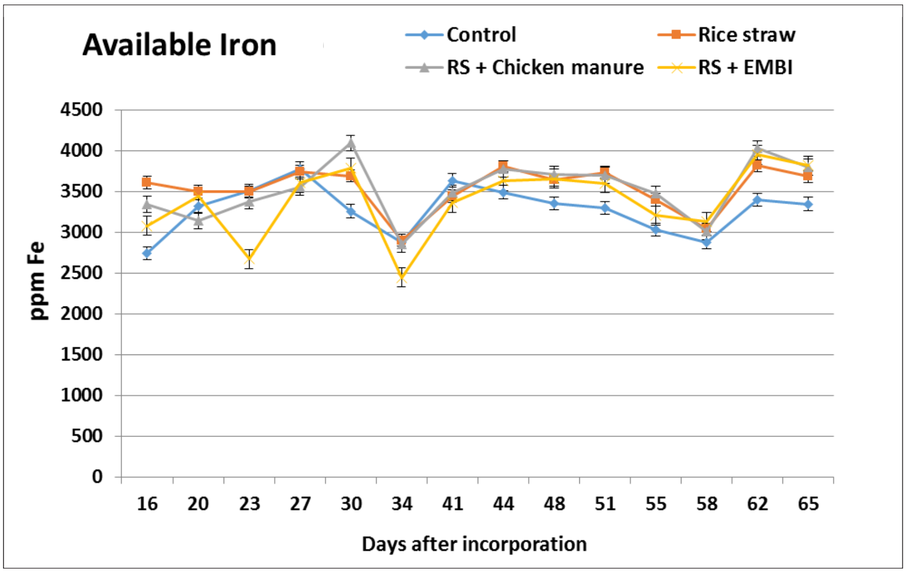


**Figure 4.** Available Iron released in paddy soil treated with rice straw in combination with chicken manure and with *Bokashi* as inoculant.

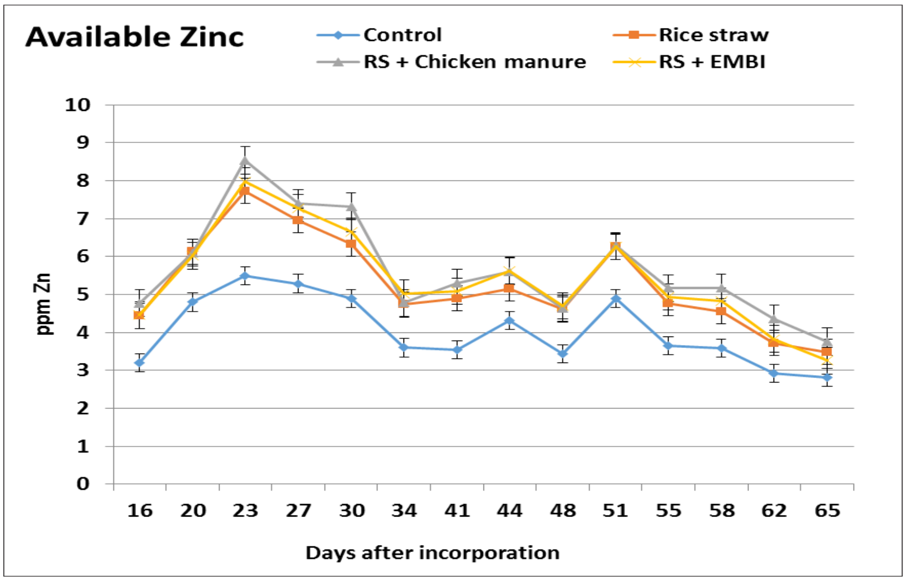


**Figure 5.** Available Zinc released in paddy soil treated with rice straw in combination with chicken manure and with *Bokashi* as inoculant.

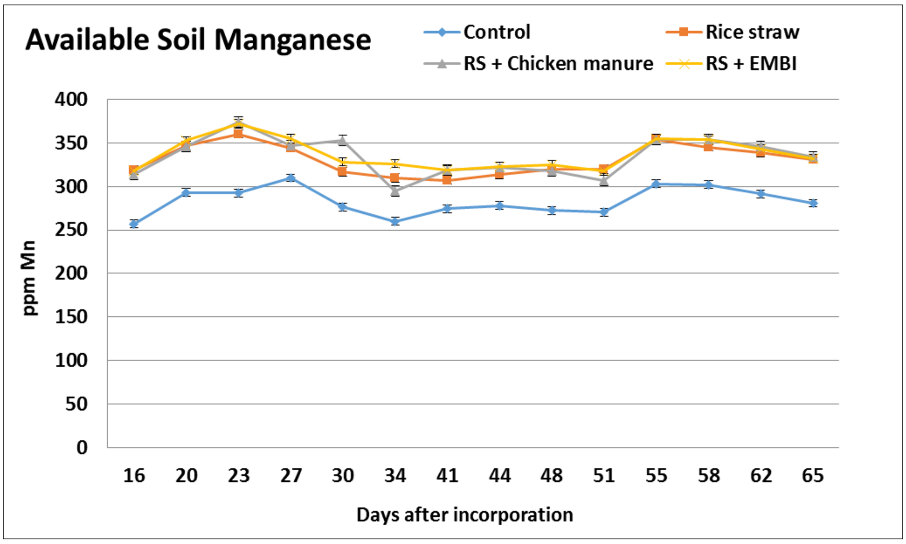


**Figure 6.** Available Manganese released in paddy soil treated with rice straw in combination with chicken manure and with *Bokashi* as inoculant.

With the soil's redox potential, further studies can still be done to determine the effect of rice straw incorporation on continuously flooded and in alternate submerged-and-saturated soil conditions when organic amendments are applied as alternate nutrient sources.

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RESEARCH NOTE

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# ENHANCED VIABILITY AND VIGOR IN RICE SEEDS THROUGH TREATMENTS WITH WATER HYACINTH (*EICHORNIA CRASSIPES*) DECOCTION

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## Abstract

Rice farmers who do not have access to quality seeds use old stock seeds, which usually have low germination rate. Farmers preferring traditional varieties also have similar challenges in seed viability. The water hyacinth decoction was evaluated for its possible effect in enhancing rice seed germination. Seeds of Red Rice, Black Rice, NSIC Rc 222, and NSIC Rc 226 were soaked for one week in varying concentrations: 25:75 (decoctions: distilled water, v/v), 50:50, 75:25, and distilled water only for control. Seed treated with decoctions had significantly improved germination rate. The radicle length of traditional rice varieties, Red Rice and Black Rice, were significantly increased. The plumule length had significantly increased in almost all genotypes except for NSIC Rc 222 in 25:75 treatment. Data analysis showed positive correlation between decoction concentrations and germination rate. The used of 75% water hyacinth decoctions: 25% distilled water (v/v) will significantly improve seed viability of poor-quality seeds and traditional varieties.

**Keywords:** *Decoction, Water Hyacinth, Seed Treatment, Seed Viability.*

## Introduction

Rice farmers who do not have access to quality seeds and storage facilities usually recycle seeds from previous season harvest. However, old stock seeds have low germination rate. Use of low-quality seeds entails higher seeding rate to compensate the low germination rate, which adds more cost for the farmers. Seed viability is also a concern on traditional rice varieties. Thus, seed germination on low-quality seeds and traditional rice varieties must be enhanced to help the farmers increase their harvest and reduce their production cost.

Mechanical, chemical, and microbial processes have been successfully used in this area of study (Kloepper *et* al. 1989). Some methods used microorganisms *Azospirillium* (Arsac *et* al. 1990), *Pseudomonas* (Hofte *et* al. 1991), *Trichoderma* (Doni *et* al. 2014), and an aqueous diffusate of *Striga densifolia* (Zuberi *et* al. 1991).

Water hyacinth is regarded a troublesome aquatic plant as it causes serious environmental hazard such as clogging irrigation, rivers, canals, and water supply ways, which results in flooding (Vidya and Girish, 2014). Currently, water hyacinth is explored for agriculture uses such as green manure (Vidya and Girish, 2014) and bioherbicides (Chai *et* al. 2013). Water hyacinth contains 64% methane suitable for biogas generation and water purification (Gopal, 1987). A large quantity of inorganic nitrogen and phosphorus accumulates in the roots of water hyacinth, which makes it suitable as a compost or fertilizer. The nutrients and microorganisms harboring on water hyacinth makes it a possible source for improving seed viability especially on rice.

This study evaluated the potential of water hyacinth extracts or decoctions in improving germination rate and rice seed vigor. The ideal concentrations of water hyacinth decoction to enhance the seed viability were also determined.

## Materials and Methods

### *Rice Genotypes*

Two inbred (NSIC Rc 222 and NSIC Rc 226) and two traditional (Red Rice and Black Rice) rice varieties were used in the study. The Bureau of Plant Industry-National Seed Quality Control Services (BPI-NSQCS) prescribed 85% as an acceptable normal germination rate for rice NSIC Rc 222 with germination rate of 85% were obtained from PhilRice Negros warehouse. NSIC Rc 226 was collected from the local market with 78% initial germination rate. Black and Red rices were purchased from the local market with germination rate of 81% and 79%, respectively.

### *Preparation of Water Hyacinth Decoction*

Water hyacinth was collected from the local fishpond in Victorias City, Negros Occidental, Philippines. The collected samples were washed thoroughly by distilled water to remove the impurities. The freshwater weed was cut into smaller pieces and crushed with the use of mortar and pestle. The finer pieces were weighed to 300 g and placed in a stainless-steel container with 1.5 li of distilled water then boiled in medium heat.

### *Seed Treatment and Germination Test*

Seeds were soaked for 24 hours on four different concentrations of water hyacinth treatment: 25 % distilled water: 75 % water hyacinth (25:75 v/v), 50:50, 75:25, and control (distilled water). After soaking the seeds for 24 hours, germination test was performed using modified ragdoll method. One hundred fully filled and uniform seeds were placed in a two-ply paper. The paper was moist with the water hyacinth treatment used. Each treatment was replicated three times arranged in a complete randomized design (CRD).

### *Data Collection and Analysis*

After a week, sheets were opened and germinated seeds were counted. Plumule and radicle length were also measured using micro caliper. Germination percentage was calculated using the following formula: percent germination = no. of seeds germinated / total no. of seeds x 100. Mean percentage germination was obtained in three replications. Seed viability index (SVI) was determined at seven days after observation and it was calculated using the formula suggested by Kharb *et* al. (1994). Seedling vigor index (SVI) of each genotypes and concentration was calculated using this formula: SVI = % germination rate x seedling length in mm x 100. Analysis of variance (ANOVA) for each trait and mean difference at 5% level of confidence were obtained using Statistical Tool for Agricultural Research (STAR).

## Results

### *Germination Rate*

Germination rate in response to varying concentrations of water hyacinth decoctions was highly significant. Analysis of variance showed highly significant in treatment ($p < 0.01$) and variety ($p < 0.01$). Treatment by variety variance was not significant implying no interaction between the two factors. Across varieties, treatment with 75% water hyacinth decoctions was significantly higher than other treatments with germination rate of 91.4% (Table 1). This was followed by treatment with 50% decoction but not significantly different with the treatment containing 25% decoction. All the seeds treated with water hyacinth decoction were at acceptable germination percentage (≥85%) (Figure 1). The control or distilled water registered the lowest germination rate at 80.58%.

**Table 1.** Germination rate of rice seeds treated with varying levels of water hyacinth concentrations across varieties.

| Levels of Water Hyacinth Decoctions in the Solution (%) | Germination Rate * (%) |
|---|---|
| 0 | 80.56 a |
| 25 | 85.42 b |
| 50 | 87.08 b |
| 75 | 91.42 c |

*Means with the same letter are not significantly different
Highly significant at 1% level of probability

Treatment with 75% decoction concentration gave the highest increase in germination rate across varieties. Black Rice got the highest improved average germination rate followed by NSIC Rc 222, Rc 226, and Red Rice. The increase in germination rate is attributed to the application of decoctions ranging 7-16%. Germination rate of old stock seeds of NSIC Rc 226 increased significantly from 76.7% (control) to 83.3% at 75% decoction concentration (Figure 2). Similarly, germination rate of Red Rice seeds significantly increased when soaked to 75% decoction concentration from 79.3% (control) to 91.7%. Germination rate of NSIC Rc 222 seeds was improved when treated with 75% water hyacinth decoction from 84.5% (control) to 93.7% (75% decoction concentration). Meanwhile, Black Rice seeds treated with 25%, 50%, and 75% had significantly increased on germination committed with control, which showed no significant difference among concentration treatments.

### *Radicle Length*

Analysis of variance resulted in significant response ($p<0.01$) and variety by treatment interaction ($p<0.05$). However, treatment variance was not statistically significant. The different rice genotypes/varieties showed variable response to varying treatment concentrations as expected because varieties used have diverse characteristics. Treatments with 50% and 75% decoction showed significant difference over the control but not at 25% (Figure 3). Radicle length of NSIC Rc 226 and NSIC Rc 222 seeds treated with decoction had no significant difference with the control. In Black Rice seeds, treatments with water hyacinth decoctions have significant difference with

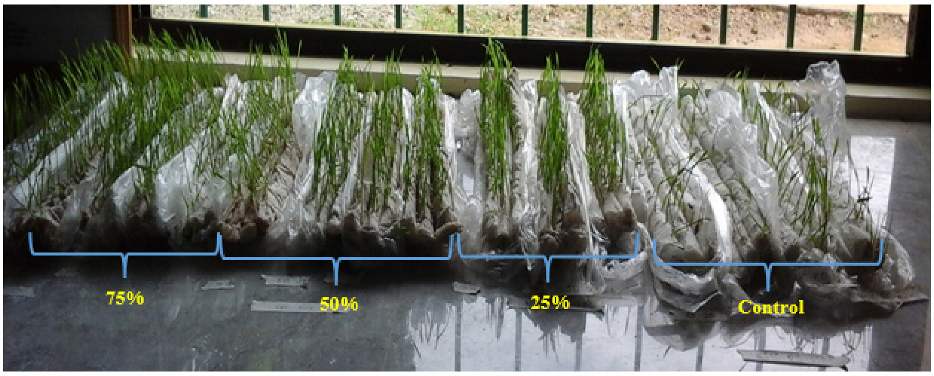


**Figure 1.** Germination and seedling vigor of various rice varieties soaked in varying concentrations of water hyacinth decoctions.

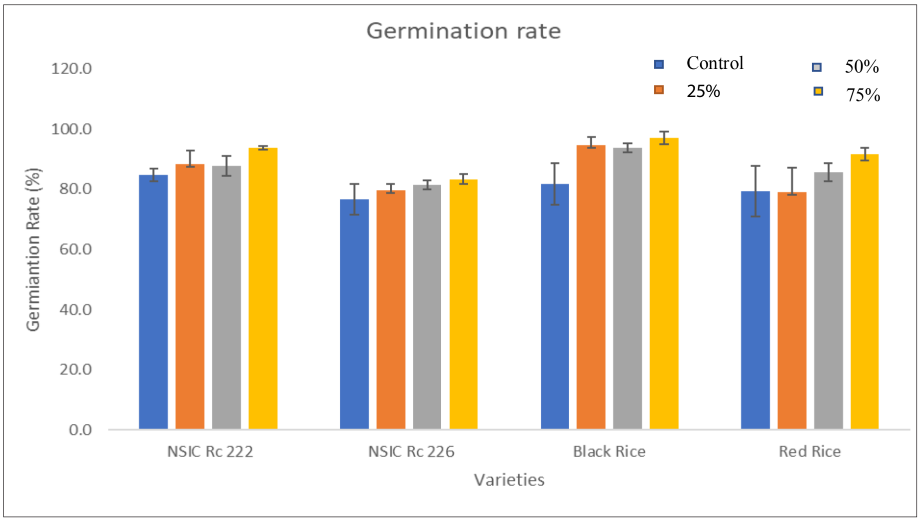


**Figure 2.** Germination rate of different rice seed varieties treated with varying levels of water hyacinth concentrations.

the control but no significant difference with each other. No significant difference for the treatments in Red Rice was observed but an increasing radicle length was noted when concentration levels were increased.

### *Plumule Length*

Analysis of variance showed highly significant differences on variety, treatment, and variety by treatment ($p<0.01$). A significant increase in plumule length was observed in varieties applied with water hyacinth decoctions except for NSIC Rc 222 with 25% concentration, which did not differ significantly over the control (Figure 4).

### *Seed Vigor Index*

Analysis of variance showed that seed vigor index is highly variable among genotypes and highly variable responses on various concentrations. Significant genotype by treatment interaction was also observed implying that seed vigor response of genotypes is highly affected by the varying concentrations of decoctions. Increase in seed vigor index among genotypes was observed on increased water hyacinth decoctions except for NSIC Rc 226, in which 75% concentration is significantly lower than 25% and 50% (Figure 5). Black Rice had the highest seed vigor index across concentrations. Across genotypes, seed treatment of 75% water hyacinth decoction concentration resulted in higher seed vigor index than other treatments except for NSIC Rc 226.

### *Correlation of Traits*

Result showed positive correlation between percent decoction concentration and germination rate ($p< 0.05$).

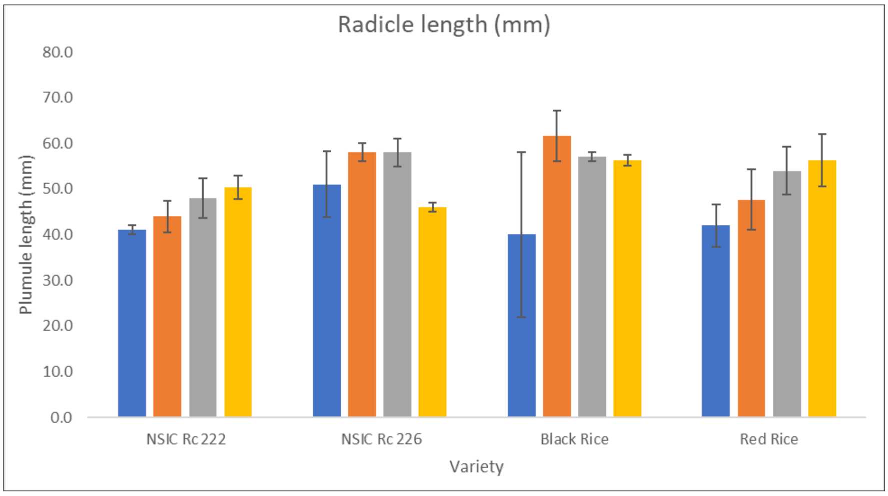


**Figure 3.** Radicle length (mm) of different varieties treated with varying levels of water hyacinth concentrations.

222 226

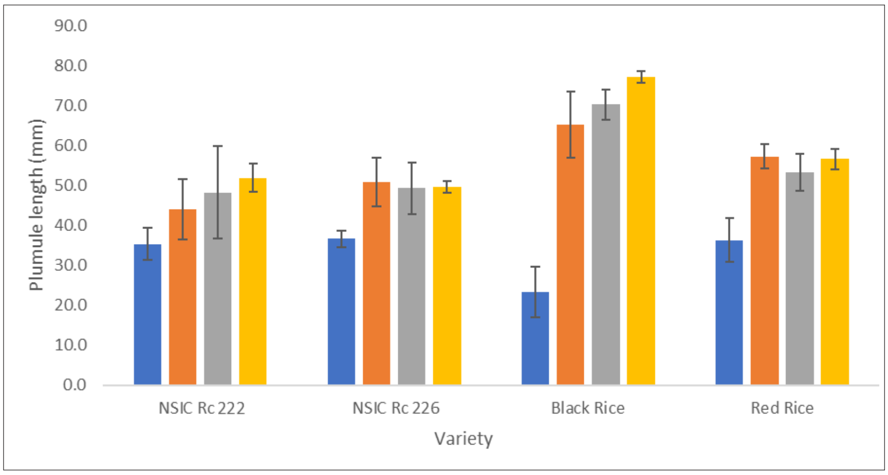


**Figure 4.** Plumule length (mm) of different varieties treated with varying levels of water hyacinth concentrations.

## Discussion

Farmers still have limited access to high-quality seeds despite its availability while some farmers prefer using their own seeds as its cost less than high-quality seeds. This research could address the farmers' problems on low seed germination rate of old and recycled seeds and traditional rice varieties. Effect of water hyacinth application on germination conforms to the study of Vidya and Girish (2014), which showed that application of water hyacinth manure had significant effect on the germination rate of wheat seeds. The possible presence of naturally-occurring microorganisms and biological products in water hyacinth enhance the germination and early growth of the seeds (Zuberi *et* al. 1991). Increasing the concentration of water hyacinth leads to increase germination rate of rice seeds. The 75% concentration of water hyacinth is the most effective rate to increase germination of low-quality seeds. Result gathered from Black Rice seed samples implies that different genotypes have different response to varying decoction concentration. A 25% water hyacinth decoction concentration can already have significant effect on Black Rice seeds.

Radicle emergence in the seeds indicates a germinating seed. The root system responsible for anchorage and nutrient uptake for growing embryo emerges from the radicle. A well-developed radicle is essential for the proper growth and development of the emerging seedling. Water hyacinth concentration is only effective in increasing radicle length of traditional varieties such as Black and Red Rice, but not as effective on NSIC Rc 222 and NSIC Rc 226, which are modern rice varieties. Vigorous radicle is important in the development of adaptable root system, an essential adapted feature of traditional rice varieties to environmental stresses such as drought or moisture stress. The plumule gives rise to the shoot system of the germinating seeds, which captures sunlight needed for photosynthesis. Enhancing plumule growth by applying water hyacinth decoctions will give a good head start to the growing seedlings.

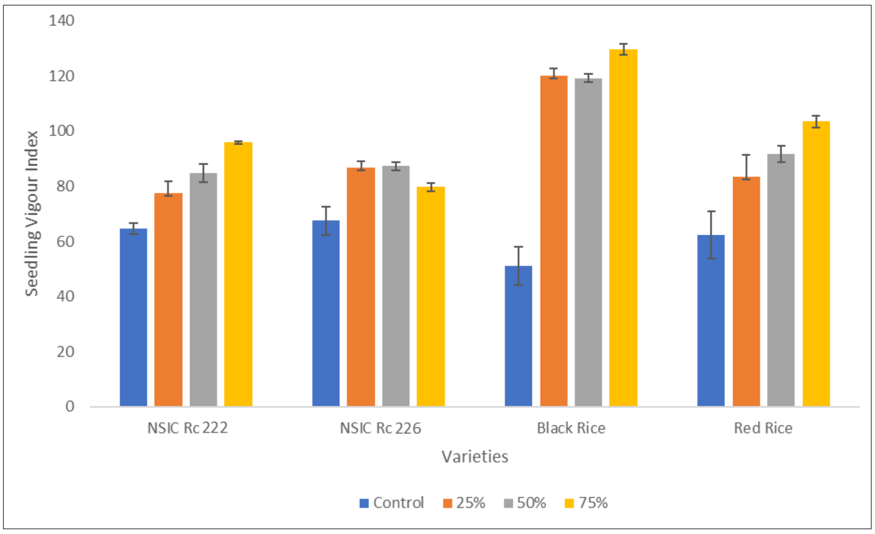


**Figure 5.** Seed vigor index of different varieties treated with varying levels of water hyacinth concentrations.

Traditional varieties, which are generally taller than modern varieties, have longer plumule length in all treatments than the modern varieties under study. Correlation analysis showed that only germination rate had positive correlation with increasing concentration of water hyacinth decoction. Seed treatment of water hyacinth decoction will lead to increase germination rate of rice seeds.

## Conclusion

Treating seeds with water hyacinth decoctions and increasing its concentration significantly enhance germination rate and seed vigor index of traditional and modern rice varieties. Water hyacinth decoction concentrations at 75% is the most effective level to improve seed germination rate. Growth of plumule was primarily induced when soaking seeds with water hyacinth decoctions. Instead of using higher amount of seeds to augment the low germination rate, farmers may apply water hyacinth decoctions.

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