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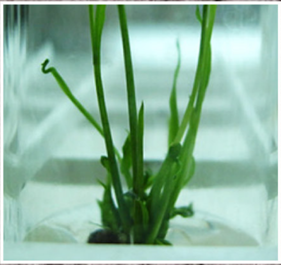

Philippine Rice Research Institute
Central Experiment Station
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# ABOUT THE COVER

As rice production is complex, all possible means to raise its productivity are needed to help ensure development in the rice sector. Rice technologies are being improved through research on drought, high-temperature, and lodging resistance; land leveling; organic nutrient sources; weed interference, and organic molluscicides. Alternative staples to rice are also studied for consumers to have healthier options on carbohydrate sources.

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## FULL PAPER



## RESEARCH NOTE

FULL PAPER

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# ENHANCING PHENOTYPIC AND GENETIC VARIABILITY IN DROUGHT-TOLERANT TRADITIONAL RICE VARIETY *SALUMPIKIT* THROUGH *IN VITRO* MUTAGENESIS

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## Abstract

*Salumpikit*, an upland traditional rice variety originating from the Philippines, has high tolerance to drought but has poor phenotype. *In vitro* mutagenesis (IVM), a technique of combining tissue culture and gamma irradiation, was used for this cultivar to induce genetic variability and to develop breeding lines with improved phenotypic acceptability in 2011 wet season. The activity resulted in enhanced genetic variability, which led to the identification of mutant lines with significant improvement in phenotypic agro-morphological traits exhibited during the crop's vegetative, reproductive, and maturity stages. Of the 484 mutant lines generated from IVM activity, 53 (11%) improved plants were selected in reference to the wildtype. Genotypic variability assessment of the mutant population using simple sequence repeat (SSR) markers showed distinctness of the genetic composition of the mutants from the wildtype. The variability assessment of the mutants proved the efficacy of IVM in inducing variability and generating improved breeding lines from drought-tolerant *Salumpikit,* that can be used as novel gene donors in breeding rice adapted to rainfed-drought prone environments.

**Keywords:** *In Vitro Mutagenesis, Wildtype, Mutants, Variation, Drought Tolerant, Genetic Diversity Index.*

## Introduction

Traditional rice varieties (TRVs) possess a wide and diverse gene pools of traits useful in enhancing crop's tolerance to drought, flooding, and salinity. *Salumpikit* is one of the most promising Philippine TRVs possessing a variety of tolerance to abiotic stresses. It is known for its high tolerance to drought stress and has been used as tolerant check for drought evaluations since 1970s (Datta and Seshu, 1982). However, this cultivar lacks desirable agronomic and grain traits acceptable to farmers for cultivation. Like other TRVs, it is late maturing and low yielding. It has open culm, short and non-dense panicle, and short-bold grains. Despite of its robust survival under drought, its undesirable traits make it less appealing and acceptable to farmers.

Induced mutation techniques in the past years have been used to improve crops, causing chromosomal or point mutations; changing the genetic and phenotypic composition of an organism (Sharma et al., 2013). Effects of physical mutagens is mostly breakage of the DNA double-strands, which increases in the potential of showing improved plant architecture and physiology (Fukai and Cooper, 1995). *In vitro* mutagenesis (IVM) is a combined method of irradiation and tissue culture to improve the qualitative and quantitative traits in plants against various biotic and abiotic stresses (Xu et al., 2012). Technique is widely used because of its advantages including high mutation frequency of almost eight times higher than any other mutation techniques, uniformity of treatment, and disease-free plants. It also requires less space to handle populations (Nagatomi and Degi, 2009). Plants generated from IVM can express both recessive and dominant mutations and fixation is rapid (Xu et al., 2012). Tissue culture in combination with physical or chemical mutagens is also extensively utilized to hasten generation and development of improved lines with desirable genotypes (Maluszynski et al., 1995).

This study utilized *in vitro* mutagenesis to enhance the phenotypic and genetic variability of *Salumpikit* to generate and develop improved breeding lines for drought stress tolerance with farmer and consumer acceptable agronomic traits.

## Materials and Methods

Mature seeds of the rice cultivar *Salumpikit* were used to produce callus pieces for gamma irradiation in 2011 wet season. The phenotypic traits of the traditional variety, generation advance, and evaluation of the mutant population derived from *Salumpikit* is presented in the schematic diagram (Figure 1a-e). Selection of lines was based on the improved phenotypic acceptability including maturity, plant height, and panicle and grain traits.

### *Seed Preparation, Sterilization, and Callus Induction*

Rough rice grains were dehulled using Satake rice tester (JLGJ2.5, SATAKE-Japan) and cleaned manually; removing broken, immature, and mix grains to maintain the purity of the genotype. Brown rice grains were sterilized with 50% (v/v) sodium hypochlorite (98% active ingredient), with agitation at 200 rpm for 30 minutes using Thermo Scientific MaxQ2000 orbital shaker (Thermo Scientific, Waltham, Massachusetts, USA). Seeds were then rinsed three times with sterilized distilled water. The procedure was repeated for another 30 min before blot dried in sterile petri plates inside the laminar flow hood. The dried seeds were cultured in callus induction medium (CIM) containing Murashige and Skoog (MS) basal salts (Murashige and Skoog, 1975) supplemented with 1 $mgml^{-1}$ each of naphthalene acetic acid, 2,4-dichlorophenoxyacetic acid, and 6-benzylaminopurine hormones; and hardened with 3 $gL^{-1}$ agar (Pronadisa, Belman Laboratories, Quezon City, Philippines) and 2 $gL^{-1}$ Phytagel (Sigma, Chemline Scientific, Quezon City, Philippines). A 30 ml culture media were dispensed in Gerber bottles and were sterilized in TOMY SX-7000 autoclave (Tomy Tech USA, California, USA) at 115 psi for 15 min at 115°C. The cultures were incubated in the dark at 25˚C±2˚C for two weeks until friable callus tissues were formed.

### *Callus Excision, Irradiation, and Regeneration*

Callus produced from the scutellar tissues of the cultured seeds were excised aseptically under a pre-sterilized laminar flow hood (Hitachi, PCV Clean Bench) using sterile forceps and scalpel and were inoculated into petri dishes containing 20 ml of half

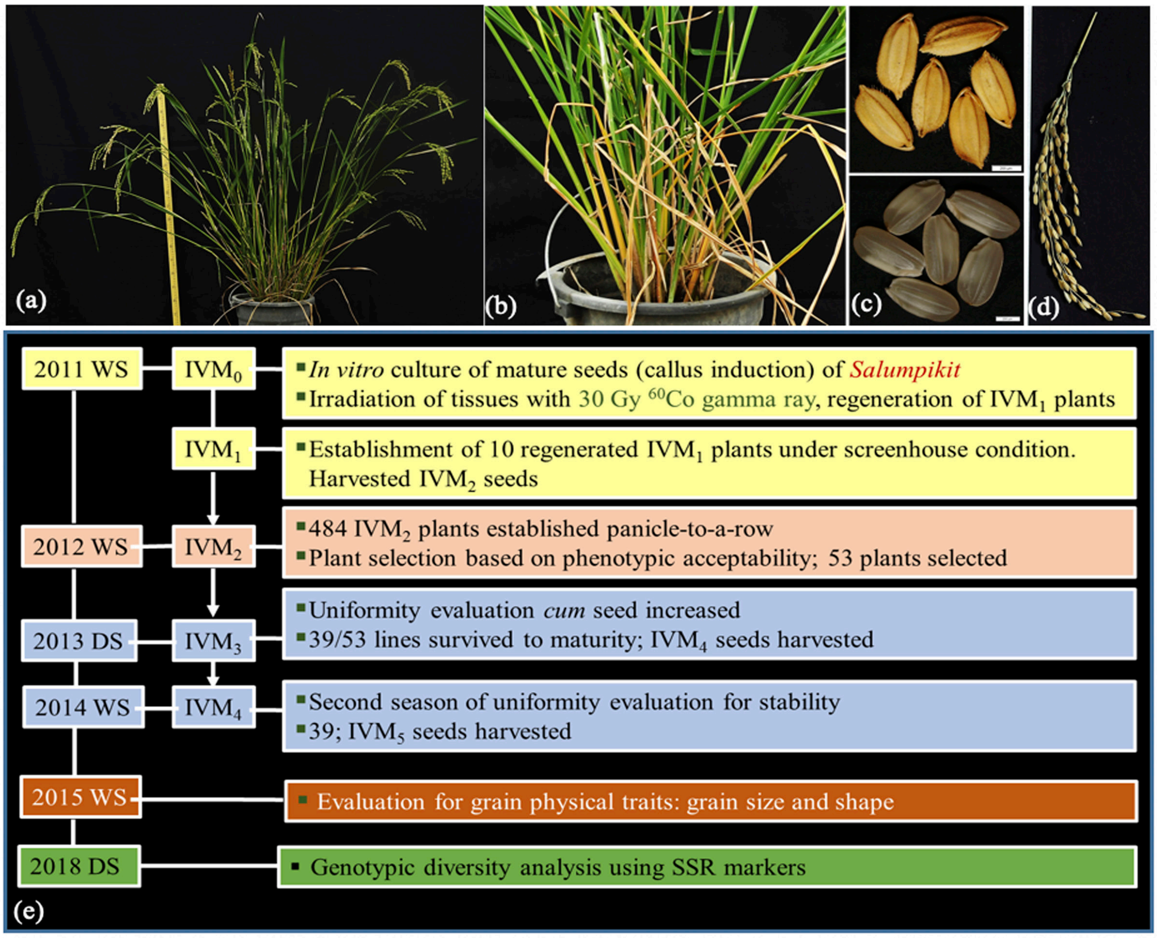



**Figure 1.** Agro-morphological, panicle and grain traits of Salumpikit (A-D) and the generation advance and evaluation of the mutant lines (E): A. Tall and late maturing; B. Open culm angle; C. short-intermediate grains; D. Sparse unbranched panicles. Note: WS wet season, DS dry season.

strength MS medium. The cultures were irradiated with 30 Gy dose of $^{60}Co$ gamma rays at the Philippine Nuclear Research Institute, Quezon City. The irradiated callus tissues were sub-cultured in MS regeneration medium (RM) within 24 h after irradiation, contained MS basal salts, and supplemented with NAA (0.5 mgml$^{-1}$) and Kinetin (2 mgml$^{-1}$). The cultures were incubated under light condition with 27˚C±2˚C temperature and 16 day/8-night h photo period for 4-6 weeks until shoots and roots were fully regenerated and developed. Plantlets were hardened for 3-5 days under laboratory condition and for 7 days under gradual sunlight exposure duration. Plantlets were then transplanted in the screen house for growing to maturity. The matured plants ($IVM_1$) produced the seeds, which were harvested and comprised the $IVM_2$ plant population and subjected to evaluation and selection.

### *Variability Evaluation of $IVM_2$ Plant Population for Agro-morphological Traits*

The $IVM_2$ seeds harvested from each $IVM_1$ plants were established panicle-to-a-row under field condition for agro-morphological trait variability evaluation. Each plant was characterized at vegetative and reproductive stage based on the characteristics published in the Descriptors for Rice (Bioversity, 2007). Descriptive statistics including frequency distribution, histograms, skewness and kurtosis (De Carlo, 1997), diversity index by Shannon-Weaver Index (Shannon and Weaver, 1949; Hutcheson, 1970; Redfern et al., 2012), and cluster analysis were used to describe phenotypic variations. Plants with improved agro-morphological traits compared with the wildtype were selected and evaluated for uniformity and stability up to $IVM_4$ generation.

$$\text{Diversity} = \sum_{i=1}^{n} P_i \log (P_i)$$

where*:
D = diversity index

$P_i$ = fraction of the entire population made up of variation i (proportion of variant i relative to total population size)
n = population size

### *Evaluation for Grain Size and Shape*

To assess the diversity of the selected mutant lines for grain size and shape, a sample of 10 milled rice grains were measured lengthwise from tip to base to determine the grain length (GL) and crosswise to determine the grain width (GW). Measurements were taken using the C3 digital caliper (Chicago Brand, Fremont, California). Grain shape (GS) was determined by getting the ratio of GL and GW. Classification of grain size and shape were based on the NCT Manual (2000).

### *Genetic Diversity Analysis of Selected Lines Using SSR Markers*

Leaf samples were collected for DNA extraction at seedling stage. The extraction of DNA was conducted using the established extraction method of Philippine Rice Research Institute (2007). There were 93 polymorphic SSR markers used to assess the diversity of the selected mutants in comparison with the wildtype. These SSR markers were randomly selected across the 12 chromosomes of the rice genome in a given genomic interval of 5 Mb.

### *Statistical Analysis*

Descriptive statistics and cluster analysis on phenotypic traits were used for each trait evaluated using Statistical Tool for Agriculture (STAR) version 2.0.1 (2013, IRRI, Philippines). Histograms of quantitative traits were generated using the IBM SPSS Statistics 20 (IBM, New York, USA). Clusters were analyzed by simple matching coefficient using NTSYS version 2.0 (Exeter Software, New Delhi, India).

## Results and Discussion

### *In Vitro Culture Response in Callus Induction and Regeneration Medium (Figure 2)*

Of the 120 dehulled rice grains cultured in callus induction medium in 2011 wet season, 53 (44.2%) seeds produced callus (Table 1) of which 34 (64%) callus pieces were irradiated with 30 Gy gamma rays. Regeneration efficiency of 41.2% (14 calli) were obtained and each of the calli regenerated at most two plantlets. Twenty-one $IVM_1$ plants were regenerated with 10 (48%) plants surviving maturity under greenhouse condition. From the 10 $IVM_1$ plants regenerated and survived to maturity, 484 $IVM_2$ plants were generated.

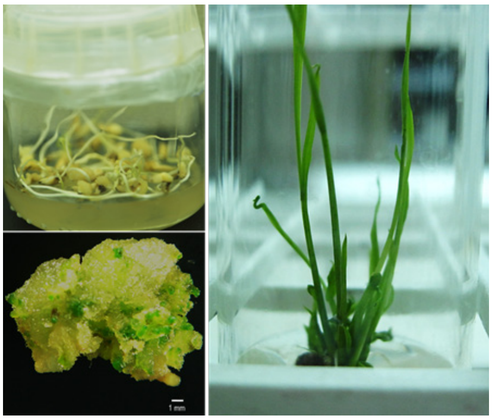


**Figure 2.** A. Callused seeds, 2 weeks in CIM; B. Friable-embryogenic callus; C. Regenerated plantlets.

**Table 1.** Seed culture response of *Salumpikit* in CIM and RM, 2011 WS, PhilRice.

| Response | | Percent/No. |
|---|---|---|
| No. of seed cultured (SC) | | 120 |
| No. of callused explant (CE) | | 53 |
| % CF (CE/SC) | | 44.2 |
| No. of Irradiated Calli (IC) | | 34 |
| Callus with regeneration | No. | 14 |
| | %/CE | 26.4 |
| | %/IC | 11.7 |
| No. of plants regenerated | | 21 |
| No. of regenerant/callus | | 2 |
| $IVM_1$ plants survived to maturity | No. | 10 |
| | %/PR | 47.6 |
| No. $IVM_2$ plants | | 484 |

### *Variability Evaluation of $IVM_2$ Plant Population for Agro-morphological Traits*

The 484 $IVM_2$ plants were generated and evaluated for variability in 12 morphological traits at vegetative and reproductive stages (Table 2). At vegetative stage, the mutants were variable in comparison with the wildtype, in which 78% had droopy blade angle. The mutants and the wildtype were similar in 5 other traits: leaf blade pubescence, leaf blade color, basal leaf sheath color, ligule color, and collar color. At reproductive stage, variation was observed in 6 (50%) traits; culm angle, flag leaf angle, panicle secondary branching, exsertion, and axis. Majority (27%) of the mutant plants had open culm angle similar with the wildtype. Majority (86%) of the $IVM_2$ plants had intermediate flag leaf compared with the descending angle of the wildtype, and the remaining 14% had either erect, horizontal, or mixed flag leaf angle. Majority (85%) of the mutants had panicle with secondary branching compared with the non-branching panicle of the wildtype. Fifteen percent of the $IVM_2$ plants had either light, heavy, or clustered panicle secondary branching (Figure 3). More variations were observed in panicle exsertion of the mutants. From the well-exserted panicles of the wildtype, four other variations were observed. Moreover, 97% of the mutants had droopy panicle axis, similar to the wildtype, while 3% had straight panicle axis. The mutants and the wildtype had compact panicle type.

In general, the derived mutants were variable in 7/12 (58%) morphological traits evaluated. High diversity was observed for flag leaf angle and panicle exsertion with a diversity index of 0.67 and 0.72,

**Table 2.** Frequency distribution and diversity index of the $IVM_2$ population for 6 morphological traits at vegetative and reproductive stages, 2012 wet season, PhilRice.

| No. | Traits | Classes | WT | $IVM_2$ plants, N=484 | | SWI | Evenness | Diversity* |
|---|---|---|---|---|---|---|---|---|
| | | | | no. | % | | | |
| *Vegetative stage* | | | | | | | | |
| 1 | Blade angle | Erect | | 107 | 22.1 | 2.98 | 0.48 | moderate |
| | | Droopy | √ | 377 | 77.9 | | | |
| | | Horizontal | | 0 | 0.0 | | | |
| *Reproductive stage* | | | | | | | | |
| 2 | Culm angle | Erect | | 123 | 25.4 | 2.68 | 0.43 | moderate |
| | | Intermediate | | 54 | 11.2 | | | |
| | | Open | √ | 132 | 27.3 | | | |
| | | Spreading | | 4 | 0.8 | | | |
| 3 | Flag leaf angle | Erect | | 53 | 11.0 | 4.09 | 0.67 | high |
| | | Intermediate | | 414 | 85.5 | | | |
| | | Horizontal | | 9 | 1.9 | | | |
| | | Descending | √ | 3 | 0.6 | | | |
| | | Mixture | | 5 | 1.0 | | | |
| 4 | Secondary branching | Absent | √ | 409 | 84.5 | 3.94 | 0.64 | moderate |
| | | Light | | 4 | 0.8 | | | |
| | | Heavy | | 56 | 11.6 | | | |
| | | Clustered | | 15 | 3.1 | | | |
| 5 | Panicle exsertion | Well | √ | 140 | 28.9 | 4.43 | 0.72 | high |
| | | Moderately | | 336 | 69.4 | | | |
| | | Just | | 1 | 0.2 | | | |
| | | Partly | | 1 | 0.2 | | | |
| | | Enclosed | | 6 | 1.2 | | | |
| 6 | Panicle axis | Straight | | 14 | 2.9 | 1.14 | 0.18 | low |
| | | Droopy | √ | 470 | 97.1 | | | |

WT – wild type SWI Shannon-Weaver Diversity Index
*Perere et al, 2012

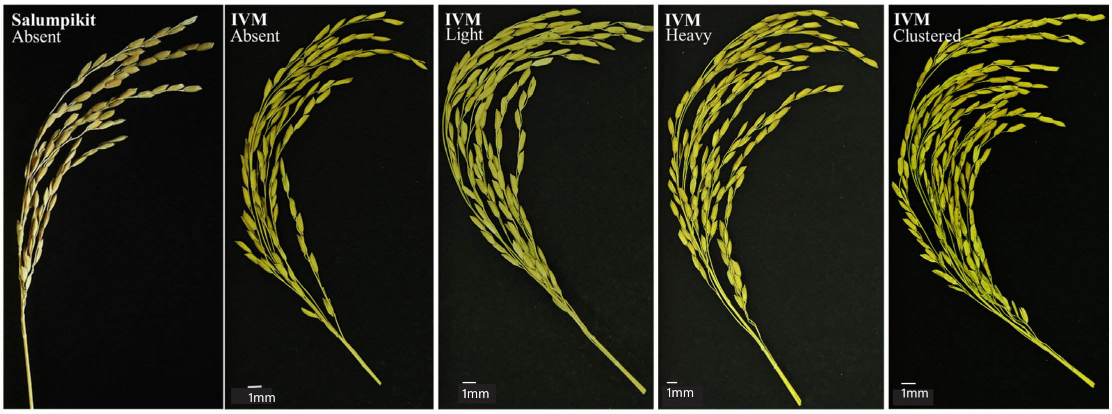



**Figure 3.** Variation in panicle secondary branching of the mutant lines in comparison with the wildtype.

respectively. Moderate diversity was observed from leaf blade angle, culm angle and panicle secondary branching, and low diversity in panicle axis.

### *Variability Evaluation for Agronomic Traits*

Mutant population was evaluated for variability in 5 agronomic traits: number of tillers at maximum tillering, days to heading, plant height at maturity, culm length, panicle length, and number of productive tillers (Table 3). Majority (96%) of the mutants was early maturing, while 4% was comparable with the wildtype. Comparable plant height with the wildtype was observed in 39% of the mutant population, while 18% had shorter plant height. Majority (41%) of the mutant plants had culm length comparable with the wildtype. Majority (67%) of the mutant plants had panicles shorter than the wildtype, while the remaining 33% had either comparable or longer panicles. Less tiller production was observed in the majority (66%) of the mutant plants, while the remaining 34% had either comparable or more tiller production.

A positive skewness (1.080) value was obtained from days to heading (Figure 4a) indicating that most of the mutants matured earlier in reference with the population mean (100 DAS). A bimodal histogram was obtained for plant height (Figure 4b) and culm length (Figure 4c) indicating that these traits have two distinct modes (most frequent occurring value): 95 cm and 151 cm for height and 75 cm and 125 cm for culm length. Histogram of panicle length (Figure 4d) and productive tiller (Figure 4e) were skewed to the left (negative skewness value) indicating that most (49% and 82%, respectively) of the mutants were taller than the population mean. Negative kurtosis was observed for days to heading (-0.581) indicating a platykurtic (more flat) distribution, which shows that no extreme values for heading days was observed and that the values are spread around the mean. Leptokurtic kurtosis was also observed for panicle length (1.897) and productive tiller (3.547) indicating that heading day values are concentrated near the population mean (Figure 4a-e). Variance Statistics of the mutant

**Table 3.** Frequency distribution of the $IVM_2$ population for the five agronomic traits, 2012 wet season, PhilRice.

| No. | Agronomic Trait | WT | Distribution of IVM2 Plants, N=484 | | |
|---|---|---|---|---|---|
| | | | Class* | no. | % |
| 1 | Days to heading (DAS) | 130 | Earlier | 465 | 96.1 |
| | | | Comparable | 19 | 3.9 |
| | | | Later | 0 | 0.0 |
| 2 | Plant height at maturity (cm) | 151 | Smaller | 206 | 42.6 |
| | | | Comparable | 192 | 39.7 |
| | | | Taller | 86 | 17.8 |
| 3 | Culm length (cm) | 121 | Shorter | 171 | 35.3 |
| | | | Comparable | 197 | 40.7 |
| | | | Longer | 116 | 24.0 |
| 4 | Panicle length (cm) | 30 | Shorter | 326 | 67.4 |
| | | | Comparable | 115 | 23.8 |
| | | | Longer | 44 | 9.1 |
| 5 | Productive tiller (no.) | 8 | Lesser | 320 | 66.1 |
| | | | Comparable | 44 | 9.1 |
| | | | Higher | 120 | 24.8 |

*Comparable = ±5% relative advantage in reference with the wildtype (WT)
DAS - days after seeding

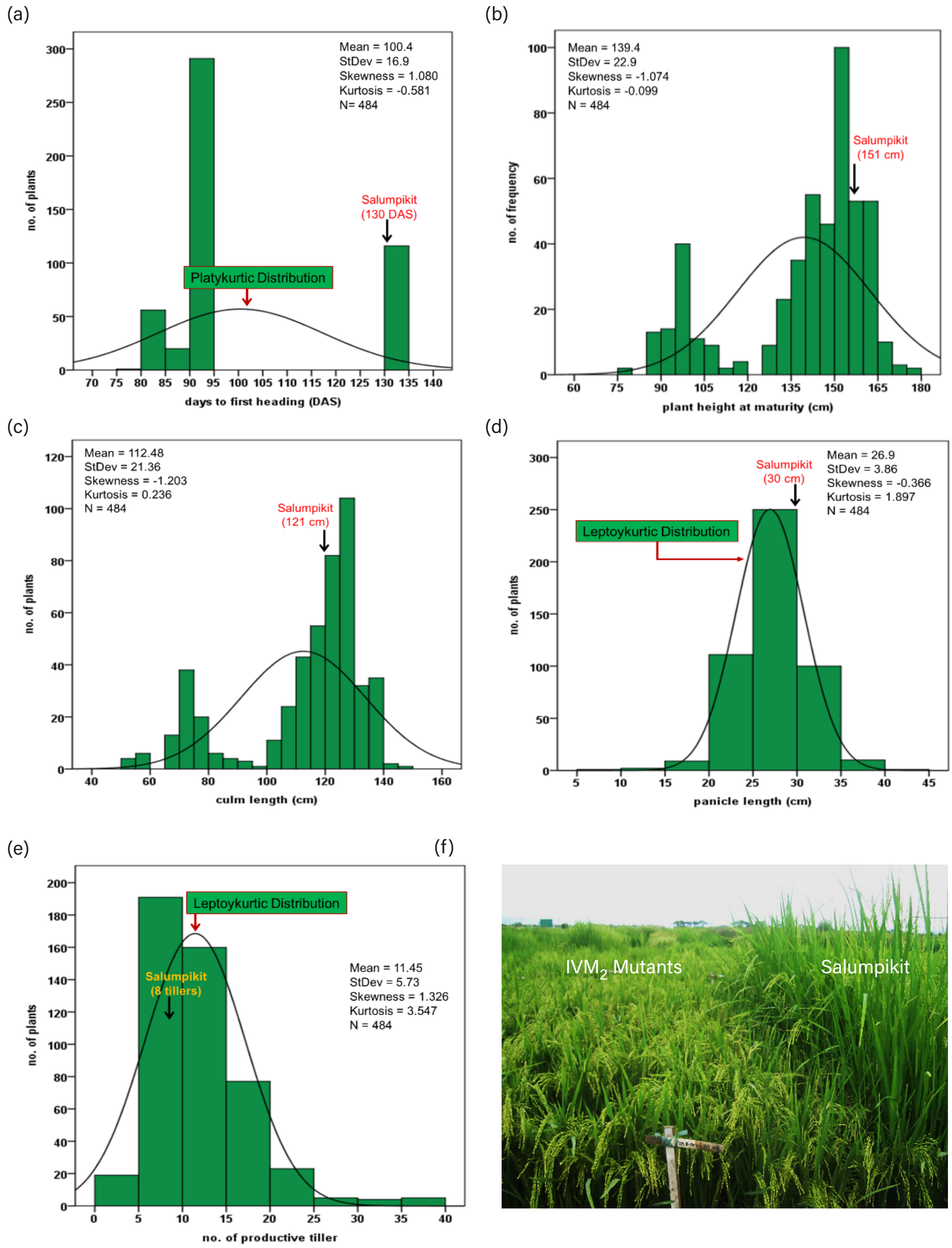



**Figure 4.** A. Frequency distribution of the $IVM_2$ mutant population for days to heading; B. Plant height; C. Culm length; D. Panicle length; E. Productive. F. Variation in plant height and maturity of the mutants in comparison with the wildtype, 2012 wet season, PhilRice, CES.

population for the five agronomic traits, in comparison with the wildtype is presented in Table 4.

### *Cluster Analysis of the $IVM_2$ Population Based on Agro-Morphological Traits*

Cluster analysis by genetic distance using agronomic and morphological traits to determine the relationship of the mutants to the wildtype resulted in the generation of a Dendrogram with 12 major clusters (Figure 5). Cluster 1 consists of the wildtype, *Salumpikit*, which was completely separated from the entire mutant population because of its distinct traits including spreading culm angle, droopy flag leaf, open panicle type, and no secondary branching. The cluster analysis indicated that the mutants were different from the wildtype in terms of agro-morphological characteristics. The remaining clusters were composed of the mutants that were further subdivided into several small clusters indicating which of the mutants were similar or different from one another. Of the 484

**Table 4**. Variance statistics of the mutant population for the five agronomic traits.

| Trait | Mutant Lines ($IVM_2$), N=484 | | | | | | |
|---|---|---|---|---|---|---|---|
| | Min-Max | Range | Mean | StdDev | CV | Skwn | Kurt |
| Heading days (DAS) | 78-130 | 52 | 100 | 17 | 16.9 | 1.080 | -0.581 |
| Plant height (cm) | 79-176 | 97 | 139 | 23 | 16.5 | -1.074 | -0.099 |
| Panicle length (cm) | 8-41 | 33 | 27 | 4 | 14.3 | -0.366 | 1.897 |
| Culm length (cm) | 53-145 | 92 | 112 | 21 | 19.0 | -1.203 | 0.236 |
| Productive tillers (no.) | 2-37 | 35 | 11 | 6 | 50.0 | 1.526 | 3.547 |

Note: Min – minimum, Max – Maximum, StdDev – standard deviation, CV – coefficient of variation, Skwn – skewness, Kurt - kurtosis

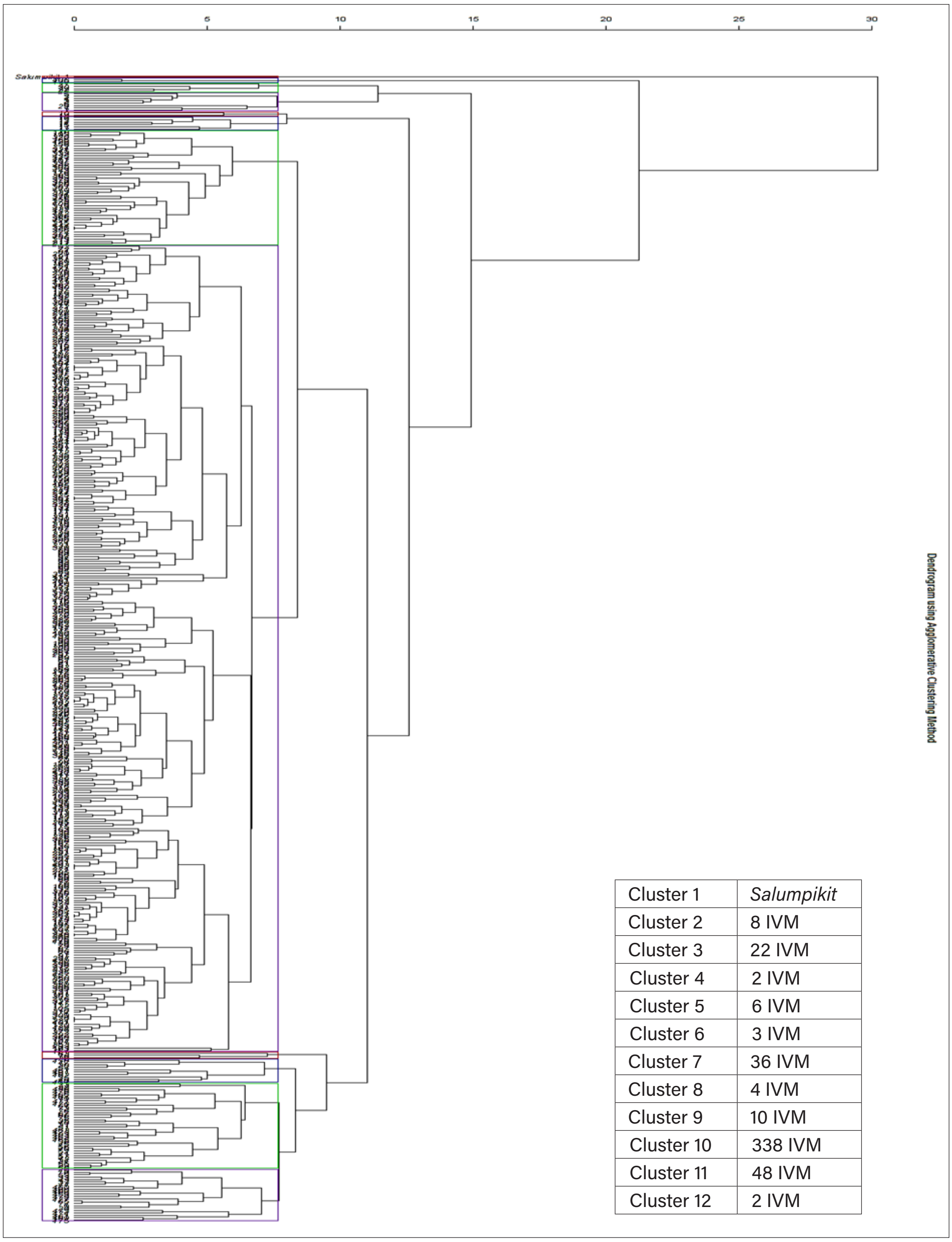



**Figure 5.** Dendrogram of the 484 $IVM_2$ plants showing the genetic relationship with the wildtype based on morphological and agronomic traits, 2012 wet season.

plants evaluated, 54 (11%) plants were improved in 11/12 (78%) agro-morphological traits evaluated.

Based on the agronomic and morphological traits, 53 (11%) plants were selected with improved traits and good phenotypic acceptability compared with the wildtype (Figure 6).

The selected lines were planted in 2013 dry season ($IVM_3$ generation) and 2013 wet season ($IVM_4$ generation) for uniformity and stability assessment. Results showed that lines were already uniform and stable (Table 5). Uniformity evaluation resulted in the selection of 39 lines based on phenotypic acceptability.

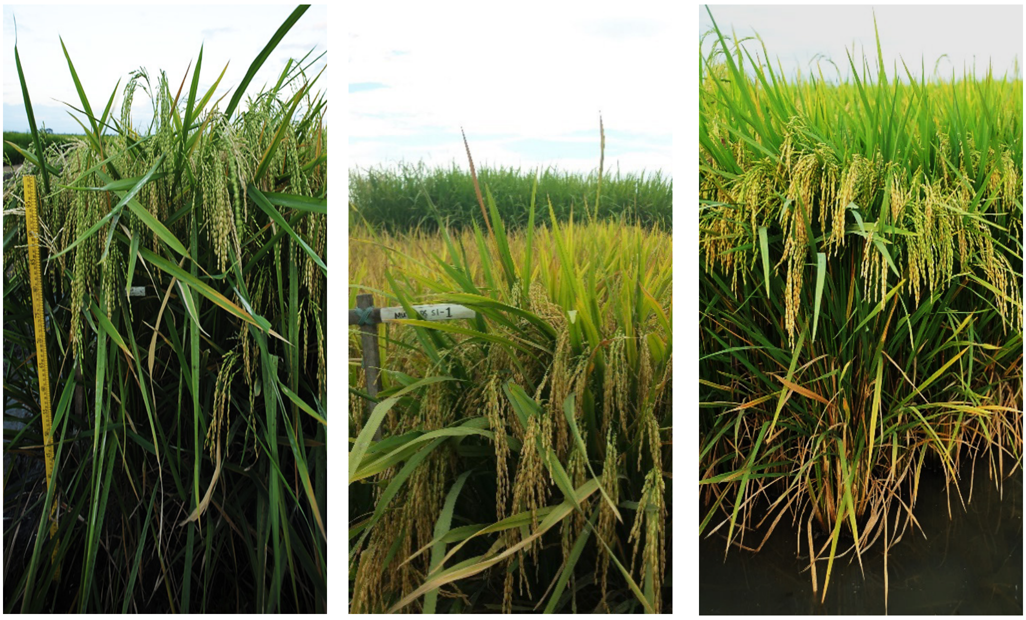
### *Evaluation for Grain Size and Shape*

Thirty-nine mutant lines were evaluated on grain size and shape. Reduced grain length by 13% (0.8 mm) to 20% (1.3 mm) was observed among 69% (27 lines) of the population shifting the grain classification from medium to short-sized grains (Figure 7a). The remaining lines had either retained the medium grain length or had increased, shifting the classification to long grains. In general, the grain shape (Figure 7b) of the selected mutants was improved from short to intermediate and slender, which is attributed to the increased in grain width (Figure 7c). Two (5%) lines had the acceptable grain size and shape of long and slender.

### *Genetic Diversity Assessment of the IVM Lines in Reference to the Wildtype*

Leaf samples of 53 promising lines were collected for DNA extraction to assess their genetic variability in comparison with the wildtype. Genetic diversity of the IVM lines was evaluated in 2018 dry season using 93 simple sequence repeat (SSR) markers. Of these markers, 46 (52%) were polymorphic, 30 (32%) monomorphic, and 15 (16%) had no amplification.

There were 148 alleles amplified using polymorphic markers. The number of alleles per marker varied from two to five alleles, averaging to three alleles per locus. The Polymorphism Information Content (PIC) values ranged from 0.0357 to 0.4889. Genetic diversity index (GDI) based on the SSR markers ranged from 0.0357 to 0.5679 (Figure 8).



Salumpikit    Mutant    Mutant

**Figure 6.** Selected plants with improved phenotypic acceptability compared with the wildtype.

**Table 5**. Variance statistics of the uniformity and stability assessment (for two seasons) of the mutant population for agronomic traits.

| | Agronomic Trait Min-Max | | Range | | Mean | | StDev | | CV | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 13DS $IVM_2$ | 13WS $IVM_3$ | 13DS $IVM_2$ | 13WS $IVM_3$ | 13DS $IVM_2$ | 13WS $IVM_3$ | 13DS $IVM_2$ | 13WS $IVM_3$ | 13DS $IVM_2$ | 13WS $IVM_3$ |
| Days to Heading (DAS) | 80-96 | 80-93 | 16 | 13 | 87 | 87 | 4.4 | 4.3 | 5.0 | 5.0 |
| Plant height (cm) | 86-101 | 87-101 | 15 | 14 | 94 | 94 | 3.0 | 3.1 | 3.2 | 3.3 |
| Culm length (cm) | 66-81 | 67-81 | 15 | 14 | 74 | 74 | 3.0 | 3.1 | 4.0 | 4.2 |

*DS* dry season, *WS* wet season *Min* minimum, *Max* maximum, *StDev* standard deviation, *CV* coefficient of variance

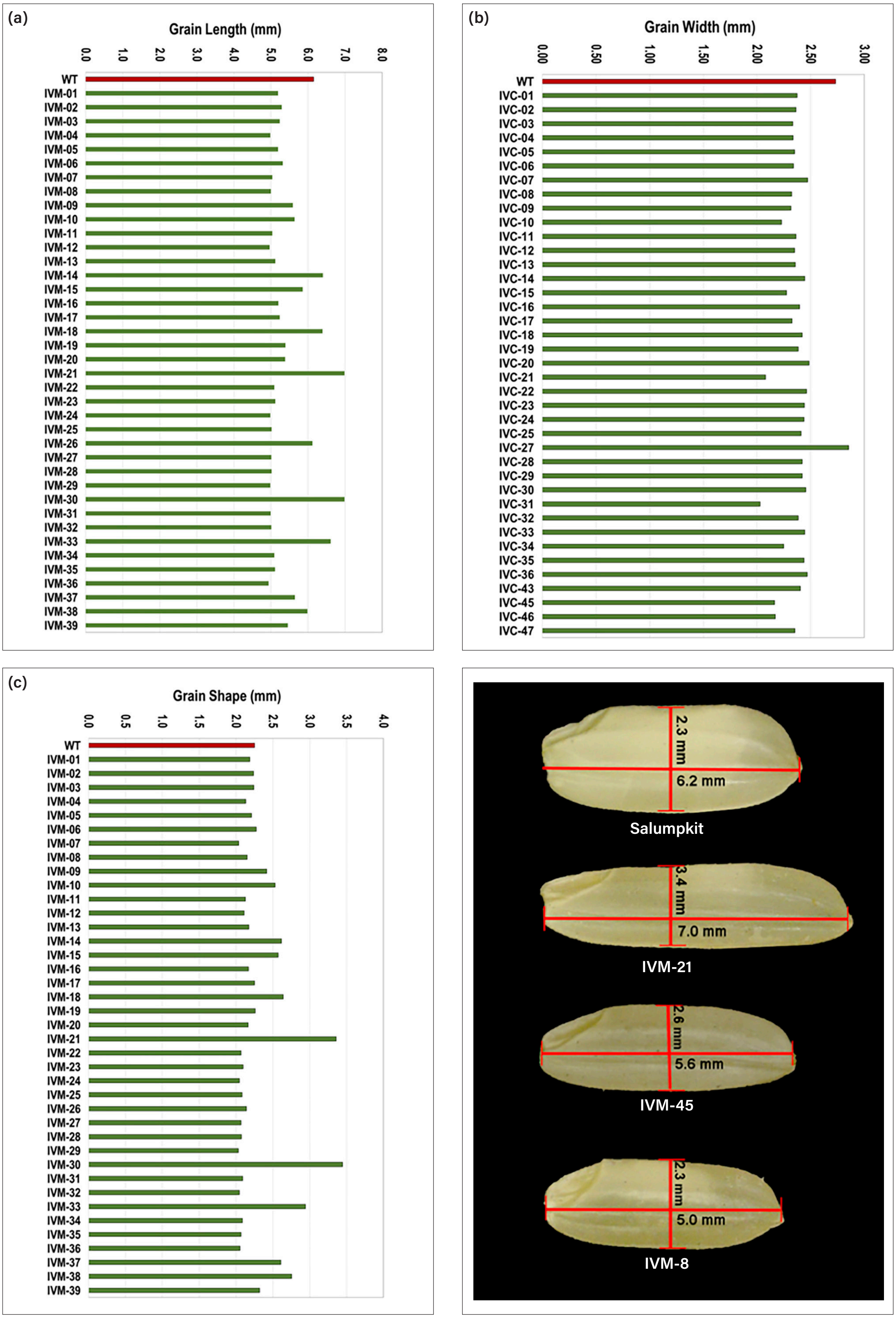



**Figure 7.** Grain length (a), width (b), and shape (c) of the selected mutant lines in comparison with the wildtype.

Cluster analysis using Simple Matching Coefficient of 46 polymorphic SSR markers separated the mutant lines from the wild type; generating two major clusters with a similarity of 50%. Cluster 1 was composed of 4 mutant lines and the wildtype, indicating a 61% similarity. Cluster 2 was composed of the remaining 49 mutants with a 52% variability in reference with the wildtype. Seven (13%) mutant lines separated into individual sub-clusters, indicating that these lines are molecularly unique from the rest of the mutant lines. Of the 39 surviving lines, 3 (8%) lines clustered with the wildtype, and 36 (92%) were clustered separately (Figure 9).

## Summary and Discussion

*In vitro* mutagenesis of drought-tolerant traditional rice variety, *Salumpikit* had induced mutations in 11 agro-morphological traits resulting in improved mutants with various combinations of desirable traits, which are important in rice crop improvement. Morphological traits of 39 (16%) mutant lines were observed during vegetative, reproductive, and maturity stage. These traits include leaf angle, culm angle, flag leaf angle, and panicle secondary branching. The mutants were also improved in terms of days to flowering, plant height, and number of

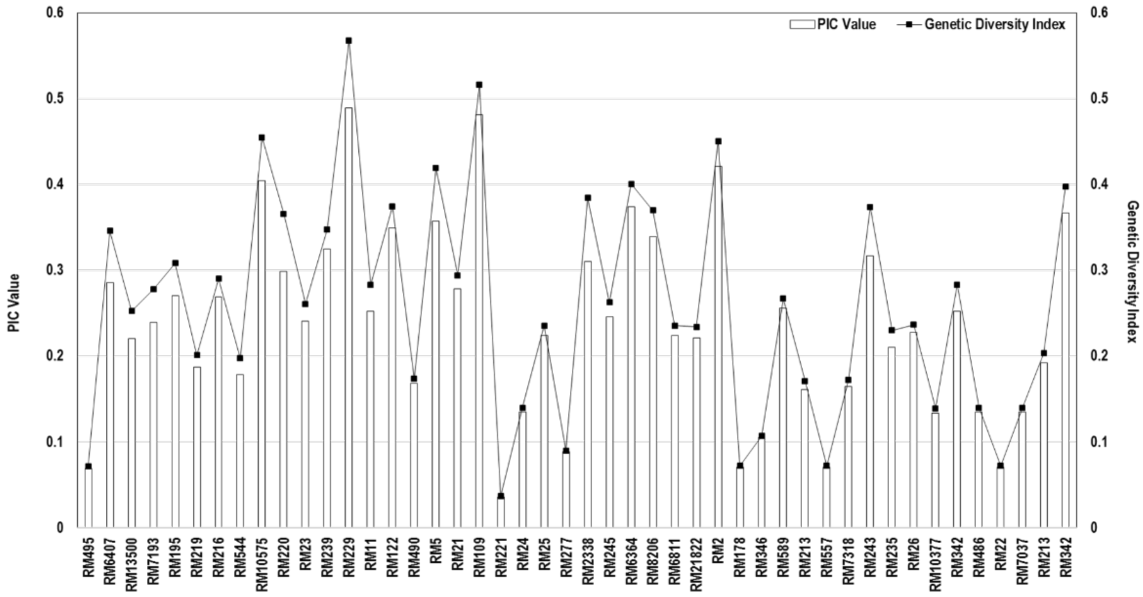



**Figure 8.** Polymorphism pattern obtained from the 46 SSR markers.

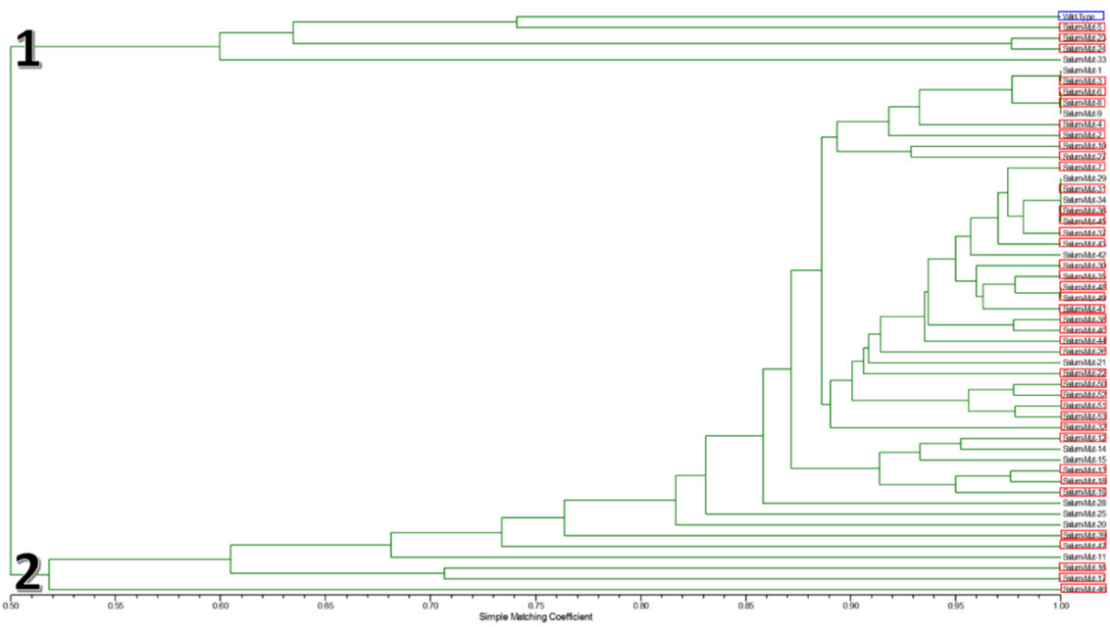



**Figure 9.** Cluster analysis by simple matching coefficient of the IVM-derived lines in reference with the wildtype, 2015.

yielding tillers. Phenotypic variability assessment also showed distinct diversity between the mutants and the wildtype. Furthermore, genotypic diversity assessment using SSR markers showed that the 39 selected IVM lines were distinctly different in reference with the wildtype. The result of this induced mutation strategy proved the efficiency of combining *in vitro* culture and gamma irradiation in inducing genetic variability leading to multiple mutations; thereby, improving phenotypic traits. The selected improved lines, once conferred to have retained the high drought tolerance as with the wildtype, can be used as gene donors for breeding rice genotypes adapted to rainfed-drought prone ecosystem.

## Acknowledgment

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FULL PAPER

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# POTENTIAL OF *Euphorbia hirta* ETHANOLIC EXTRACT AS MOLLUSCICIDE AGAINST *Pomacea canaliculata*

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## Abstract

*Pomacea canaliculata* (Golden apple snail) is one of the dominant rice pests in the Philippines due to the huge losses in the annual rice production as these snails consume rice seedlings. Synthetic molluscicides are used to control *Pomacea canaliculata;* however, these molluscicides have negative impact in the environment. In this study, the potential of ethanolic extracts of tawa-tawa (*Euphorbia hirta*) as molluscicide against *Pomacea canaliculata* was evaluated. *Euphorbia hirta* leaves were extracted with 95% ethanol for 48 h under normal room temperature. Extracted material was then evaporated through rotary evaporator in 38±5ºC and diluted to 2.8 ppm, 5.6 ppm, and 8.3 ppm concentrations. Golden apple snail was subjected to acclimatization for 10 days. Five set-ups were made: Experimental (T1, T2, and T3) and Controls (Positive and Negative). Twenty snails were allocated per set-up, which was then subjected to three trials at different time frames for precise results. Results showed that there is a high significant difference across treatments and controls. However, statistical analysis showed that concentrations 5.6 ppm and 8.3 ppm had p-value of 0.359 indicating that there is no significant difference between the two concentrations. Lethal dosages at 50% and 90% were calculated through Probit Analysis. Lethal dosage at 50% mortality of the snail population was found at 10.9 ppm concentration, while lethal dosage at 90% was recorded at 53.4 ppm. *Euphorbia hirta* possesses molluscicidal activity against *Pomacea canaliculata*. Higher concentrations of the solution leads to higher snail mortality.

**Keywords**: *Pomacea canaliculata, Euphorbia hirta, Niclosamide, Molluscicidal Activity.*

## Introduction

Golden apple snail (*Pomacea canaliculata* or GAS) is a dominant aquatic gastropod (Brito and Joshi, 2016) that originated from South America particularly in Argentina and Uruguay. From South America, it was introduced to other parts of the globe through aquarium trade in 1979 (Mochida, 1991; Halwart, 1994; Cowie, 2002; Joshi & Sebastian, 2006). It was introduced in Southeast Asian countries like Taiwan and Philippines as a potential food source for farmers in 1980s (Naylor, 1996). *P. canaliculata* can spread rapidly from agricultural areas to other areas through irrigation canals, flooding, and other natural water pathways where it may lead to serious ecosystem impact. Carlsson, Brönmark, and Hansson (2004) stated that "survey of natural wetlands in Thailand showed that high densities of the snail were associated with almost complete absence of aquatic plants, high nutrient concentrations, and high phytoplankton biomass, that is, a complete shift in both ecosystem state and function."

The study of dela Cruz et al. (2001) showed that the most destructive stage is when the length of the shell is from 10 mm to 40 mm. *Pomacea canaliculata* can live with or without water; burying in mud fields for up to six months then emerging when water is back in the fields. Furthermore, *Pomacea canaliculata* can cause a huge loss in rice production annually due to the amount of rice seedlings it can consume per day (Prabhakaran et al., 2017). According to the International Rice Research Institute (2012), *Pomacea canaliculata* can cause 1m$^2$ of field loss overnight—destroying almost 50% of farmers' yield, if control is not undertaken. Aside from being a major rice pest, *Pomacea canaliculata* is a vector of parasite, *Angiostrongyulus cantonensis*, which is linked to the cause of human eosinophilic meningitis and rat lungworm (Ranamukhaarachchi & Wickramasinghe, 2006). Thus, golden apple snail is potentially harmful to humans and biodiversity despite its nature as a biological weed control.

GAS remains as one of the dominant rice pests in the Philippines (Joshi et al., 2001), especially in the provinces of Samar, Ifugao, and Cagayan causing 1% to 40% of area loss (dela Cruz et al., 2000). Through the decades, Filipino researchers have been studying its possible solutions. To address the problems, different control methods are being practiced but not yet fully established as an effective means in eradicating the snail. Management options such as cultural/ mechanical control, biological control, and chemical controls have already been used by farmers although the reports on curative management of snails' invasion are still considered to be limited (Salleh et al., 2012). The use of synthetic "instant kill" molluscicides remains to be the most common snail control. Regardless of effectiveness, these molluscicides cause negative effects to the environment. Despite human intervention through different approaches, a solution that is affordable and practical on a wider scale is yet to be identified.

At present, Niclosamide is the only chemical molluscicide acceptable for operational use in snail control programs. Due to its high price, niclosamide is used on few local control programs. This was supported by the study of Klumpp and Chu (1987) in Iran, Egypt, and Ghana who stated that area-wide mollusciciding is relatively expensive, ineffective, and ecologically unsound. United States Environmental Protection Agency (1999) stated that niclosamide has a minimal acute dermal toxicity resulting in slight skin irritation and eye irritation. Although it has low toxicity to humans, it still causes ecological impacts. As niclosamide is applied to freshwater tributaries, it is expected to have impacts on the environment (WHO, 1997). Niclosamide's ecological effect to aquatic plants is toxic (0.04 to > 1,450 mg $L^{-1}$), while it is highly to very highly toxic on fish. McCullough (1992) supported this claim that niclosamide may lead to problems of toxicity; resulting in unsafety margins to other non-target flora and fauna. As such, plant-derived molluscicides are highly considered as better option for control because they contain saponins, flavonoid, steroids, tannins, and other secondary metabolites; similar active ingredients that could be used and synthesized as potential biodegradable molluscicide (Valverde et al., 2010).

In search of plant-derived molluscicides for biocontrol, several studies have used plants against *Pomacea canaliculata*. Picardal et al. (2018) utilized extract from garlic *(Allium sativum)* bulbs and found that 10 ppm and 8.75 ppm concentrations of the extract have comparable molluscicidal effect similar with niclosamide. Furthermore, Probit analysis showed that lethal dosage (LD) resulting in 50% and 90% snail mortality was recorded at 4.007 ppm and 7.602 ppm concentrations, respectively. In another study, "Molluscicidal activity of the aqueous extracts from *Solanum mammosum* L., *Sapindus saponaria* L. and *Jatropha curcas* L. *against Pomacea canaliculata*," it was found that the 100% concentration of *S. saponaria* aqueous extract had LD50 value of 24.04 ppm, while the 50%-50% mixture of *S. mammosum* and *S. saponaria* showed highest mortality with LD50 value of 17.78 ppm (Quijano et al., 2014). In the study of Taguiling (2015), combined extracts of *Sandoricum vidalii* fruit, *Harpulia arborea,* and *Parkia sp.* barks showed 100% mortality of *Pomacea canalicutata* in laboratory and field testing. The studies found that the plants' bioactive components such as alkaloids and saponins caused the mortality.

Tawa-tawa (*Euphorbia hirta),* an ornamental shrub in the Philippines, is traditionally used as an alternative for pharmaceutical drugs due to its healing property, which is induced by the milky juice that is produced when the leaves are being broken down. With its bioactive components, it is known for its contributions as an herbal medicine on numerous diseases such as asthma and dengue. According to Kumar et al. (2010), tawa-tawa possesses antibacterial, anthelmintic, anti-asthmatic, sedative, anti-spasmodic, anti-fertility, anti-fungal, and anti-malarial properties. Aside from that, it is positive in saponins, terpenoids, flavonoids, cardiac glycoside, phenolic compounds, steroids and alkaloids. These properties indicate that *Euphorbia hirta* may possess molluscicidal activity on *Pomacea canaliculate.* Thus, this study evaluated *Euphorbia hirta* as a potential molluscicide against *Pomacea canaliculata*. This paper also proposed a cost-efficient botanical molluscicide beneficial to the Filipino farmers.

## Materials and Methods

***Research Design***

The researchers conducted an experimental quantitative research on the potential of *Euphorbia hirta* ethanolic extract as a molluscicide against *Pomacea canaliculata*. Figure 1 represents the Randomized Block Experimental Design employed in this study. With 15 trials, 20 snails were allotted in each trial to avoid crowdedness in the environment; producing 300 snails in the experimentation process.

***Collection and Extraction of Tawa-tawa (Euphorbia hirta)***

Tawa-tawa was collected from Marilao, Bulacan and was brought to the Bureau of Plant Industry (BPI) for authentication. *Euphorbia hirta* was subjected to air drying for 14 days under the sun. Dried leaves were isolated from the other parts of the plant. Isolated parts were then crushed into fine powder using mortar and pestle. *Euphorbia hirta* leaves were grounded into powdered form and stored at room temperature. Its ethanol extract was obtained through maceration or by soaking 700 g of the powdered leaves in a 95% ethanol (100g of powder 500 $ml^{-1}$ of EtOH) solution for 48 h. Extracts were filtered and concentrated to dryness under reduced pressure using rotary evaporator at a temperature of 38±5ºC.

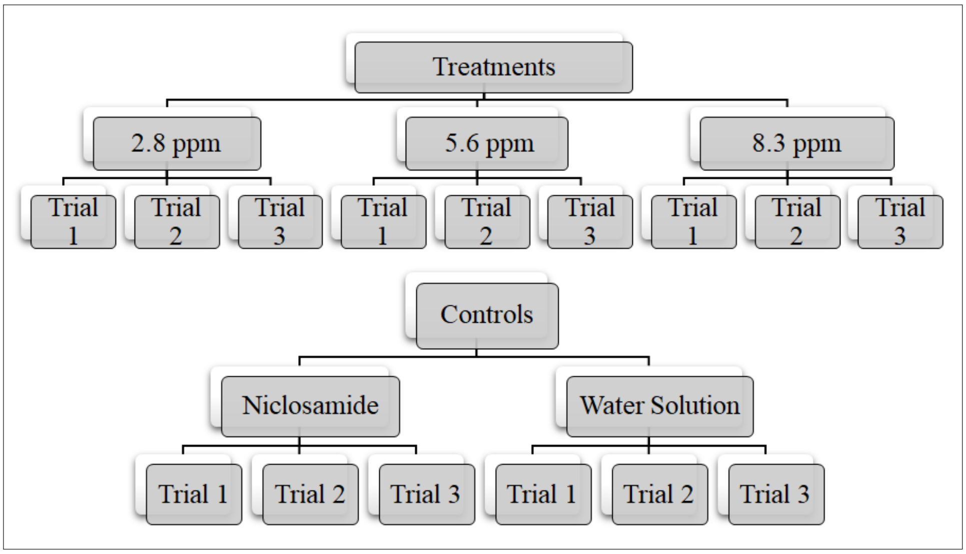


**Figure 1.** Complete Randomized Design of each treatment with equal number of trials.

***Formulation of Treatment and Controls***

The crude extracts of tawa-tawa (2.5 ml, 5ml, and 7.5 ml) were collected and mixed with distilled water to make a 900 ml solution yielding concentrations of 2.8 ppm, 5.6 ppm, and 8.3 ppm, respectively. The 900 ml solution in each concentration was divided into three and assigned as trial 1, trial 2, and trial 3. Bioassay procedure included negative and positive control. Distilled water served as the negative control for the snails while Niclosamide served as the positive control. The commercial snail control Niclosamide 70WP was utilized and prepared for its recommended dosage of 2.19g $L^{-1}$.

***Collection of Golden Apple Snail***

Golden apple snails were handpicked from a rice field in Paniqui, Tarlac. Snail samples were then brought to the Bureau of Fisheries and Aquatic Resources (BFAR) in Region III for authentication and verification. Four hundred snails collected from the province were subjected to acclimatization. Before the acclimatization process, snails were weighed using mini electronic digital scale. Smallest weight value was 8.5 g while the largest weight value was 18 g. Shell height was measured from the apex to the lower margin of aperture (Joshi et al., 2005). The snails were washed several times before being maintained in a container filled with dechlorinated water under diurnal lighting. Sexually active adult snails with a height ranging 3-5 cm and weigh 10-15 g were acclimatized following the acclimatization conditions prescribed by Joshi et al. (2005). The snails were fed with pechay leaves every day during the 10-day acclimatization period. Snails that remained active after the acclimatization were selected for the bioassay.

***Bioassay Procedure***

Standard procedures by WHO (1983) and Joshi et al. (2005) were followed. Each treatment including the two controls were conducted in 3 trials in different days to ensure validity. Snails were submerged in a rectangular container with a length of 20 cm, width of 16.2 cm, and depth of 9 cm containing 300 ml of the solution (T1, T2, T3, Niclosamide, and Distilled Water). Trial 1 started at the first 24 h; Trial 2, after 48 h; while Trial 3 after 72 h. The snails had recovery time for 1 h away from the solutions every 24 h. Qualitative observation was done through noting the pre and post mortem behavior of the snails in the 96-h observation duration. This included the changes in their physical characteristics (e.g., shell, operculum, and internal organs). Quantitative observation was done through tallying the number of dead snails during the experiment. Using needles, snails that did not exhibit any muscular contractions were considered dead. Samples were further analyzed after 96 h.

***Statistical Analysis***

Qualitative and quantitative data were gathered. Qualitative data included hyperactivity, presence of white sticky mucous, internal deterioration, and unusual changes in the operculum and their physical characteristics. Quantitative data included the mortality rate of snails. One-Way Anova was used to evaluate the significant difference among the treatments and controls. To determine whether which group is statistically significant or insignificant to another, the researchers used Post-hoc (Tukey's Honestly Significant Test) at 0.05 level of significance. Probit Analysis was used to determine the relationship between concentrations and percent mortality as well as lethal dosages.

## Results and Discussion

Data were gathered from the three trials of each treatment conducted within 96 h observation. Each trial per treatment contained 20 viable snails to avoid crowdedness in the environment that may affect mortality rate.

Figure 2 shows the average mortality rate of *Pomacea canaliculata* subjected to varying concentrations of *Euphorbia hirta* and controls after three trials during 96-h observation period. Among the three experimental groups, the solution with 8.3 ppm concentration exhibited the highest number of dead snails with a mortality rate of 45%. On the other hand, least number of dead snails is seen on 2.8 ppm concentration with a mortality rate of 15%.

Physical and behavioral changes occurred after the 24-h exposure of snails to *Euphorbia hirta* solutions. Snails were active during night days prior to the experiment, but after exposure to the solution, their hyperactivity decreased. Snails were inactive during the experiment; hiding in their shell, which is a normal mechanism for snails put under threatening environment. Some snails showed signs of itching through wiggling. The same observation was made by Picardal et al. (2018) who noted that snails showed muscular contraction that led to ataxia, convulsion, paralysis, and finally death affect the significant difference is the large difference between groups. Size of the operculum after 96 h decreased by almost 30% (Figure 3). The operculum of snails softened after 96 hours. Snail's outer structure showed sign of slimy and sticky feeling. The snails also lost rigidity along shells and produced unpleasant odor. These observations are probably due to the effect of being in contact with the solution for 96 h. A white sticky mucous was also seen near the snail's operculums after 24 h (Figure 4) indicating that chemicals have reached the snails' internal organs. Presence of this mucous indicated that *Euphorbia hirta*'s polar compounds have reacted to the snails' mucus membrane (Alves, 2013; Aljabarin et al., 2014 as cited by Edis et al., 2018). These physical remarks were also evident in the trials made with the positive control Niclosamide. The reactions of GAS upon contact with *Euphorbia hirta* solutions could be attributed to tawa-tawa's secondary metabolites (e.g., saponin, sterol, terpene, alkaloids, polyphenols, tannins, flavonoids, and mucilage) causing death within the snail's nervous system.

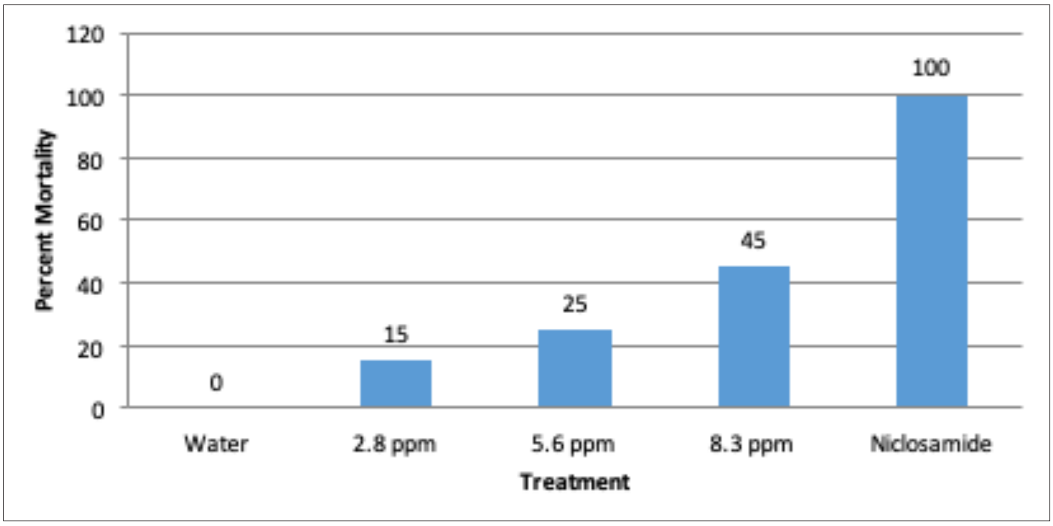


**Figure 2.** Average mortality rate of snails per treatment.

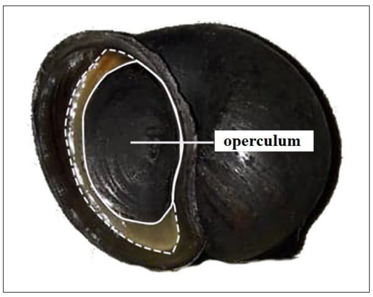


**Figure 3.** Change in the operculum size of *Pomacea canaliculata.*

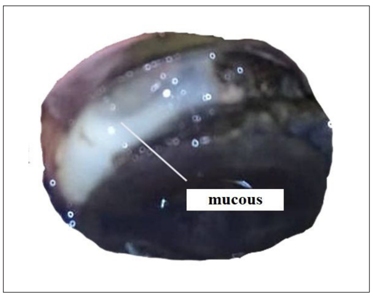


**Figure 4.** White sticky mucous found along the insides and operculum.

Significant difference across groups were found through One-way Analysis of Variance or ANOVA. Using SPSS at confidence level of 95%, p-value was calculated at 0.00 ($3.1519^{-11}$). High significant differences among treatment groups were noted at p-value less than 0.05. This means that the treatments (experimental groups and controls) are distinct and caused different mortality rates to *Pomacea canaliculata.* The null hypothesis, there is no difference in the mortality rates across groups, is rejected at 0.05 level of significance. One factor affecting the significant difference was the large difference between groups especially that the negative control (distilled water) resulted in a mortality rate of 0% compared to the positive control, which has 100% mortality rate.

Table 1 shows the pairwise comparison across groups using Post Hoc (Tukey's Honestly Significant Test). There is a significant difference between Treatment C (2.8 ppm) and Treatment D (5.6 ppm) as well as between Treatment C (2.8 ppm) and Treatment E (8.3 ppm). This implies that the effectiveness of the *Euphorbia hirta* as a molluscicide against *Pomacea canaliculata* increases as the concentration of the treatment increases. However, Treatment D (5.6 ppm) and Treatment E (8.3 ppm) showed a p-value of 0.359, which is greater than the 0.05 p-value, indicating that the concentrations 5.6 ppm and 8.3 ppm are not significant with each other. This means that the effectiveness of 5.6 ppm and 8.3 ppm concentrations as molluscicide against *Pomacea canaliculata* are comparable with each other.

Table 2 and Figure 5 present the results of regression analysis between the various concentrations of *Euphorbia hirta* and percent mortality of *Pomacea canaliculata.* The regression equation is = 1.8562X + 3.0712 with concentration of *Euphorbia hirta* as X variable and percent mortality of *Pomacea canaliculata* as Y variable. This indicates that with increase of one unit of concentration (X), the mortality of the *Pomacea canaliculata* increases by a unit of 3.0712. The extent to which concentration (X) predicts the mortality of the *Pomacea canaliculata* (Y) was found at 92.657% coefficient of determination. Furthermore, the r coefficient value is 0.963, which can be interpreted as "very high positive linear correlation". With this, it can be deduced that snail mortality is correlated and has a direct relationship with the concentration of solution. Increase in concentration will yield an increase in mortality rate.

This leads to the prediction of the minimum concentration that will yield 50% mortality ($LC_{50}$) and 90% mortality ($LC_{90}$). Regression analysis predicted that it is predicted that $LD_{50}$ would be at 10.90 ppm while $LD_{90}$ would be at 53.44 ppm. This suggests that 10.9 ppm concentration can kill 50% of the snail population, while 90% mortality can be achieved with 53.4 ppm concentration. The regression analysis also implies that snail mortality is dose dependent.

This study supports Picardal et al. (2018) who found a positive correlation between the increasing amount of concentration and the snail mortality

**Table 1.** Comparison across groups using Tukey's Honesty Significant Test.

| | A Negative Control | B Positive Control | C 2.8 ppm | D 5.6 ppm | E 8.3 ppm |
|---|---|---|---|---|---|
| A = negative control | - | 0.00 | 0.001 | 0.00 | 0.000 |
| B = positive control | | - | 0.000 | 0.000 | 0.000 |
| C = 2.8 ppm | | | - | 0.000 | 0.001 |
| D = 5.6 ppm | | | | - | 0.359* |
| E = 8.3 ppm | | | | | - |

*not significant at p-value of ≤ 0.05

**Table 2.** Calculated lethal dosage 50 and lethal dosage 90 for the *Euphorbia hirta* molluscicide.

| Lethal Doses of Tawa-tawa Molluscicide (ppm) | | $R^2$ | Chi-square Value | Slope Value |
|---|---|---|---|---|
| $LD_{50}$ | $LD_{90}$ | 0.92657 | 1.00 | 1.857 |
| 10.90 ppm | 53.44 ppm | | | |
| *95% LCL=10.57 ppm | *95% LCL=49.92 ppm | | | |
| *95% UCL=11.25 ppm | *95% UCL=57.51 ppm | | | |

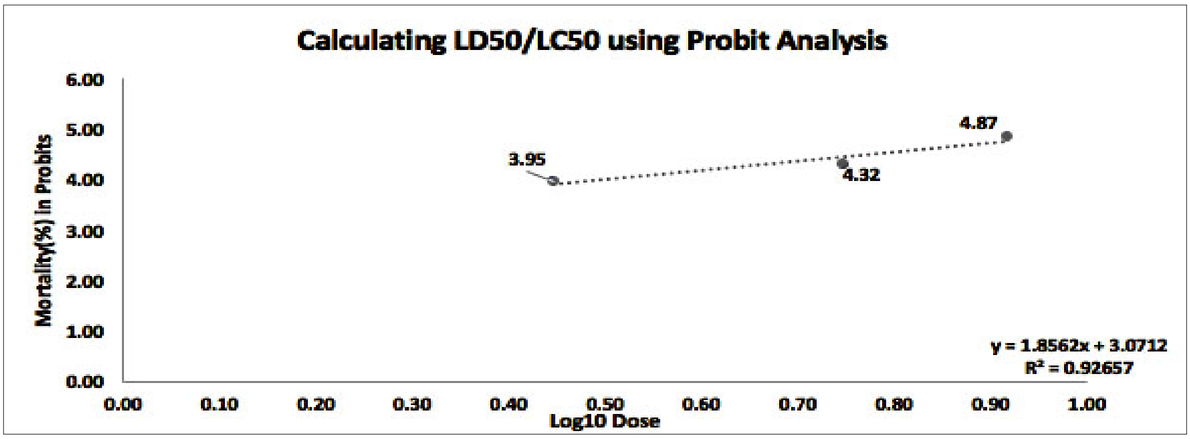


**Figure 5.** Graph of lethal dosage using Probit Analysis.

rate. This depicts that snail mortality depends of dosage; higher dose will yield higher mortality rate. Further observation found that after being exposed to the active plant extract, snails showed behavioral responses such as retraction inside their shell and crawling towards other snails. Changes in physical characteristics were also evident after 24 h exposure to the treatment. Snails' operculum also decreased in size and structure. Secretion of sticky substance (mucus) was also observed around the operculum indicating contact with *Euphorbia hirta's* chemical constituents. This kind of behavior was similar to the snails exposed in the positive control Niclosamide.

## Conclusion

Based on research findings, *Euphorbia hirta* possesses molluscicidal activity against *Pomacea canaliculata.* It can be used as a possible biological application, which is environmentally safe for controlling the infestation of *Pomacea canaliculata.* Small concentrations were used to comply with WHO's standard for molluscicides (concentration up to 20 ppm). Although concentrations used in the study had small mortality rate, treatments affected the snails in their physical aspects. Lethal dosages of 10.90 ppm ($LD_{50}$) and 53.94 ppm ($LD_{90}$) are the recommended concentration to kill 50% and 90% snail population.

For future research, it is recommended to use higher dosages such as 10.90 ppm and 53.95 ppm of *Euphorbia hirta* extract and test whether these concentrations can negatively affect the environment. Further exploration on the different parts of *Euphorbia hirta* such as stem, roots, and bud is also recommended. Use of different solvents in the preparation of extracts and effect of treatments to non-target organisms can also be explored. It is also recommended to explore more species from the family *Euphorbiaceae* and assess whether they possess molluscicidal activity against golden apple snail and to other snail species.

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# INTERDEPENDENT TRAIT SELECTION AMONG RICE (*Oryza sativa* L.) GENOTYPES FOR LODGING TOLERANCE UNDER DIRECT-SEEDED CONDITION THROUGH SEQUENTIAL MULTIVARIATE METHODOLOGIES

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## Abstract

Lodging is the bending over of rice culm caused by weak root anchorage, poor stem support, and unpredictable weather factors that reduce grain yield and seed quality. Push-resistance is a direct representation of rice lodging, which measures the plants' resistive force in the culm. This study categorized levels of push-resistance among 41 rice genotypes and identify lodging-tolerant ($L_t$) genotypes through subsequent multivariate analyses of phenotypic and anatomic traits. Seventy-two percent of genotypes screened and subjected to push-resistance had significantly comparable culm strength (0.83 – 1.08 kg $cm^2$) to the $L_t$ check, NSIC Rc 240 (1.01 kg $cm^2$). Only five genotypes had stronger culm (1.01 – 1.08 kg $cm^2$) than the lodging-susceptible ($L_s$) check, PSB Rc4 (0.77 kg $cm^2$). Bivariate analysis showed a significant association of push-resistance to 17 agro-morphological and three anatomic traits. Moreover, sequential use of multivariate procedure delineated NSIC Rc 300, Rc 360, Rc 396, and PR45299-14-3-2-B as $L_t$ genotypes. Direct-seeded rice with high level of push-resistance is crucial in sustaining increases in potential yield and lessening yield reduction caused by lodging.

**Keywords:** *Lodging-Tolerance, Direct-Seeded Rice, Stem Anatomy, Bivariate Analysis, Multivariate Analysis.*

## Introduction

Lodging is crop's permanent displacement from its upright position (Van Delden et. al., 2010) and manifested from sporadic to extensive coverage in farmers' field. In rice, it is mainly caused by the inherently weak phenotype of jointed, hollow culm, and dense grain-bearing panicle in the uppermost internode during the ripening phase. The occurrence of lodging in the Philippines is prevalent throughout the wet season (June-December) when lodging coincides with high rainfall intensity and tropical cyclones. Rainfall during this season covers 61-86% of the mean annual rainfall from 1991 to 2012 in Nueva Ecija, Philippines (PHILFSIS, 2019). Based on PAGASA (2014), the country experiences 19 tropical cyclones every year and no storm-free months (Corporal-Lodangco and Leslie, 2014). Wind speed during cyclone ranges from 30 to 220 kph (PAGASA, 2019), which is considerably stronger than the rate at which rice plant lodges. In general, first culm breaking started at 25 kph, while full culm breakdown occurred at 54-57 kph (Hitaka, 1968). High wind speed interrupts grain filling by bending and breaking culms, while rainfall specifically affects grain quality, which are all aftermaths of lodging.

Rice physiological processes are much affected by damages in culm morphology. The destruction of foliage structure leads to lower net photosynthetic rate, slower cellular growth (Setter et al., 1997), and lower dry matter production (Kobayashi and Hitaka, 1968). In broken culms, it completely inhibits the transport of water and nutrients through the vascular bundles, resulting in a reduction of assimilates for grain filling (Kashiwagi et. al., 2005). Furthermore, increased plant and grain moisture retention encourage fungal growth, and pest and disease occurrence highly injurious to the plant.

Lodging is common in transplanted and direct-seeded systems, in which a high degree of manifestation is recurrent in the direct seeding. Among morphological traits, plant height limits the occurrence of lodging and increase yield potential; however, susceptibility to lodging differs among cultivars with similar height (Ookawa and Ishihara, 1992; Easson et. al., 1993). Furthermore, thorough investigation of lodging in a direct-seeded system is crucial in sustaining the increasing farmer adaptation of transplanted to direct seeding practice for its low production cost (Manangkil et. al., 2013) and increase water-saving capability (Tabbal et. al., 2002).

Mechanical strength of the plant is mostly dependent on chemical and biochemical components of the cell wall (Kong et al., 2014). Sclerenchyma tissue, which functions as mechanical strength, surrounds vascular bundles and cell walls of vascular plants. Lignin also provides rigidity to the cell wall that improves mechanical stability of plants and tolerance to biotic and abiotic stresses (Frei, 2013).

Currently, percent lodging incidence and culm strength are the conventional screening techniques for rice lodging (IRRI, 2014). Data gathering in these classifiers is fast and easy but inadequate in providing applied and corresponding resistive force. Push-resistance, an index of lodging-tolerance can measure physical strength of basal culm using a force-gauge meter (Kashiwagi and Ishimaru, 2004), may be used as alternate technology for measuring resistive force.

Identifying traits related to lodging-tolerance through its morphology, anatomy, and push-resistance is necessary. Grain yield losses due to lodging can be as high as 57.5% (Alias et. al., 1989). These yield reductions when converted to price decline were estimated at \$0.0075-\$0.0119 per kg of rough rice market price in the USA (Salassi et. al., 2013) or about PhP0.60 per kg milled rice or PhP30 price reduction per 50 kg cavan in the Philippines. Mean annual losses reached PhP3.1 trillion worth of revenues from 2000 to 2012 in rice due to floods and tropical cyclones in the Philippines (PHILFSIS, 2019). In terms of quality, lodging a day earlier in the grain-filling stage can cause 0.15% decline of milled rice rate, an increase of 0.27% of chalky grain rate, and 0.02% protein content, which subsequently lower eating quality and market price of milled rice (Lang et. al., 2012). Lodging also causes difficulties in harvest operations, increases demand for grain drying, and consequently results in increased production cost (Hoshikawa and Wang, 1990).

In this study, 43 rice genotypes for push-resistance, agro-morphologic, and anatomic traits under direct-seeded condition were characterized. Traits in the plants' morphology and anatomy significant to lodging were determined and push-resistance of 41 rice genotypes to $L_t$ and $L_s$ checks were compared. Morphologic and anatomic traits contributing to push-resistance were also modified. Genotypes with lodging-tolerance were selected based on identified significant traits in plants' morphology and anatomy.

## Materials and Methods

### *Site Description*

The experiment was carried out in Philippine Rice Research Institute-Central Experiment Station (PhilRice-CES), Science City of Muñoz, Nueva Ecija (15°40'15" N, 120°53'37" E, 99.9 m altitude) during the main rice-growing season from January to early May in 2017. The site is naturally plain with a tropical monsoon Type-1 climate having two pronounced seasons; dry from November to April and wet during the rest of the year (PAGASA, 2017). The soil is a type of lowland soils classified as Maligaya series and texture of deep heavy clay, compact, sticky, and plastic (BSWM, 2017). The area has a mean temperature of 26.87 °C and a mean rainfall of 2.09 mm during the trial period (PhilRice, 2017).

### *Rice Materials*

Forty-three rice genotypes from six rice ecosystem comprised the set of treatments. This set-up was composed of 12 newly-bred lines for the direct-seeded environment, 20 irrigated lowland varieties, nine varieties for an adverse ecosystem, one $L_t$ check NSIC Rc 240, and one $L_s$ check PSB Rc4 (Table 1). Plant Breeding and Biotechnology Division of PhilRice-CES owns the seeds.

### *Experimental Design*

The field experiment followed a randomized complete block design (RCBD) with three replications. Treatments were laid-out in a systematic arrangement of plots from left to right. Entire experimental area is 1,133 $m^2$ divided into three blocks with each block measuring 44 m long and 8.25 m wide. Block comprised 43 plots separated further in two columns with dimension of 2 m x 4 m. Furrows were made using pull type 20 pins wooden rake with each plot having eight 4-linear m furrows and 0.25 m distance between furrows.

### *Direct Seeding*

Thirty-two grams of pre-germinated seeds were distributed evenly in an eight-row 4-linear m furrow after 24-h incubation. Before seeding, plot labels with proper identification mark in the leftmost corner of a hill on all treatments. The percent seedling emergence at 14 days after sowing determines the frequency of replanting.

### *Push-Resistance Method*

The push-resistance of the culm assessed using a force-gauge meter (HF50-100061, Handy, Taiwan) determined the strength of the basal part of the stem. Force-gauge meter was set perpendicularly to the plant 20 cm above soil surface based on the method

**Table 1.** List of genotype name, code, and type of rice growing environment.

| Genotype | | Ecosystem | Genotype | | Ecosystem |
|---|---|---|---|---|---|
| Code | Name | | Code | Name | |
| G1 | PR39142-10-3-2-1-1-B | DSR | G23 | NSIC Rc 238 | IL |
| G2 | PR39149-33-1-3-3-1-B | DSR | G24 | NSIC Rc 290 | SAL |
| G3 | PR39628-17-2-1-1-B | DSR | G25 | NSIC Rc 300 | IL |
| G4 | PR40334-61-1-1-B | DSR | G26 | NSIC Rc 302 | IL |
| G5 | PR40432-10-1-1-1-B-B | DSR | G27 | NSIC Rc 308 | IL |
| G6 | PR40432-14-2-1-B | DSR | G28 | NSIC Rc 324 | SAL |
| G7 | PR40432-17-3-1-2-B-B | DSR | G29 | NSIC Rc 352 | IL |
| G8 | PR43405-10-2-3-3-B | DSR | G30 | NSIC Rc 354 | IL |
| G9 | PR434325-25-2-1-1-1-B | DSR | G31 | NSIC Rc 356 | IL |
| G10 | PR43426-13-2-3-2-B-B | DSR | G32 | NSIC Rc 358 | IL |
| G11 | PR43433-21-2-1-1-1-B | DSR | G33 | NSIC Rc 360 | IL |
| G12 | PR45299-14-3-2-B | DSR | G34 | NSIC Rc 390 | SAL |
| G13 | NSIC Rc 11 | UPL | G35 | NSIC Rc 392 | SAL |
| G14 | NSIC Rc 25 | UPL | G36 | NSIC Rc 396 | IL |
| G15 | NSIC Rc 29 | UPL | G37 | NSIC Rc 402 | IL |
| G16 | NSIC Rc 194 | SUB | G38 | PSB Rc82 | IL |
| G17 | NSIC Rc 214 | IL | G39 | NSIC Rc 160 | IL |
| G18 | NSIC Rc 216 | IL | G40 | NSIC Rc 240$^{Lt}$ | IL |
| G19 | NSIC Rc 218 | IL | G41 | NSIC Rc 298 | IL |
| G20 | NSIC Rc 222 | IL | G42 | PSB Rc4$^{Ls}$ | IL |
| G21 | NSIC Rc 224 | IL | G43 | PSB Rc14 | RL |
| G22 | NSIC Rc 226 | IL | | | |

*G* Genotype, *DSR* Direct-seeded rice, *IL* Irrigated lowland, *RL* Rainfed lowland, *SAL* Saline, *SUB* Submergence, *UPL* Upland, *Lt* Lodging-tolerant check, *Ls* Lodging-susceptible check

reported by Kashiwagi and Ishimaru (2004) at 20 days after full heading. Wood board measuring 0.40 m length x 0.40 m height x 0.05 m wide served as guideboard in data collection. Push-resistance of five representative plants were measured until the test plant had inclined vertical 45° angle. The value was expressed in kilogram-force per square centimeter (kg cm$^2$), which indicated sturdiness of the basal stem and root anchorage (Fig. 1).

### *Phenotypic Evaluation*

The 43 rice genotypes exhibited 19 Qualitative ($Q_l$) agro-morphologic characters (Table 2). These characters include: auricle color, basal leaf sheath color, collar color, culm habit, early and late flag leaf attitude, leaf blade anthocyanin coloration, leaf blade attitude, leaf blade intensity of green color, leaf blade pubescence, leaf sheath anthocyanin coloration,

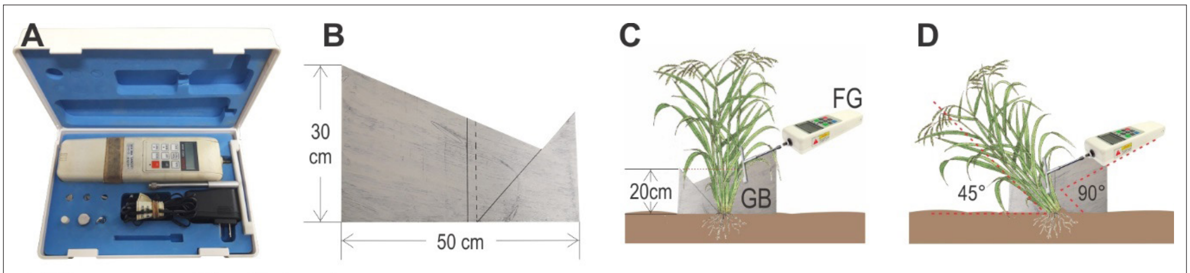



**Figure 1.** Push-resistance measurement of the lower stem: **A.** Handy force-gauge HF50-100061 can measure resistive force of up to 50 kg; **B.** Wooden board with a dimension of 0.30 m x 0.50 m x 0.05 m used to guide the force-gauge in data collection; **C.** Force-gauge and guide board at starting point in push resistance data collection; **D.** Data on push resistance collected at 45 degrees angle pushed for 5 sec. Resistive force of five plants per treatment were measured.

**Table 2**. Level of the diversity of seven categorical characters compared with NSIC Rc 240 ($L_t$ check).

| Qualitative Trait | NSIC Rc 240 | Observed Phenotypic Class | Class | | H′ | Level of Diversity* |
|---|---|---|---|---|---|---|
| | | | No | % | | |
| Leaf blade intensity of green color | Dark | No green color visible | 0 | 0 | 0.32 | Low |
| | | Light | 30 | 69.8 | | |
| | | Medium | 11 | 25.5 | | |
| | | Dark | 2 | 4.7 | | |
| Ligule color | Whitish | Absent | 0 | 0 | 0.16 | Low |
| | | Whitish | 38 | 88.4 | | |
| | | Yellowish green | 5 | 11.6 | | |
| | | Purple | 0 | 0 | | |
| | | Light purple | 0 | 0 | | |
| | | Purple lines | 0 | 0 | | |
| Flag leaf attitude | Semi-erect | Erect | 9 | 20.9 | 0.22 | Low |
| | | Semi-erect | 34 | 79.1 | | |
| | | Horizontal | 0 | 0 | | |
| | | Descending | 0 | 0 | | |
| Culm habit | Semi-erect | Erect | 10 | 23.3 | 0.24 | Low |
| | | Semi-erect | 33 | 76.7 | | |
| | | Open | 0 | 0 | | |
| | | Spreading | 0 | 0 | | |
| | | Procumbent | 0 | 0 | | |
| Panicle main axis attitude | Strongly drooping | Upright | 0 | 0 | 0.27 | Low |
| | | Semi-upright | 0 | 0 | | |
| | | Slightly drooping | 30 | 69.8 | | |
| | | Strongly drooping | 13 | 30.2 | | |
| Panicle secondary branching | Dense | Absent | 0 | 0 | 0.16 | Low |
| | | Sparse | 32 | 74.4 | | |
| | | Dense | 11 | 25.6 | | |
| | | Clustered | 0 | 0 | | |
| Panicle exsertion | Moderately well exserted | Enclosed | 0 | 0 | 0.20 | Low |
| | | Partly exserted | 1 | 2.4 | | |
| | | Just exserted | 5 | 11.6 | | |
| | | Moderately well exserted | 37 | 86.0 | | |
| | | Well exserted | 0 | 0 | | |

* Source: Jamago and Cortes 2012

ligule color, ligule shape, panicle branches attitude, panicle exsertion, panicle main axis attitude, panicle secondary branching, panicle shattering, and panicle threshability.

The genotype's 23 Quantitative ($Q_n$) agro-morphologic measurements are presented in Table 3: culm length, culm number per linear meter, diameters of 1st to 5th internodes, leaf blade width and length, length of 1st to 5th internodes, ligule length, flag leaf width and length, heading, maturity, panicle length of main axis, panicle number per linear meter, plant height at maturity, and seedling height conforming to descriptions in the Bioversity International Descriptors for Wild and Cultivated Rice 2007 at vegetative, reproductive, and ripening stages.

### *Anatomical Analysis*

Radial segments of approximately 20 µm cell thick from the 5th internode of the main culm excluding leaf sheath were collected 10 days after 100% flowering, stained with 0.2 ml (1% Safranin-O; 30% EOH) solution, air-dried for 45 min in ambient temperature (Johansen 1940), and examined under a light microscope (CX41, Olympus, Japan). Captured images were in Tagged Image File Format (TIFF), which is better in preserving detailed images and uncompressed data. Olympus DP2-BSW application measures the number and area of abaxial, median, and adaxial vascular bundles using low dimension images, while the thickness of sclerenchyma ring and culm wall gauge were captured in high dimension

**Table 3**. Data of the 43 rice genotypes at ripening phase under direct seeded condition (seedling height, leaf characteristics, stem length and diameter, panicle number and length, grain length, and grain width).

| Trt No | Variety Name / Line Designation | SHT (cm) | Leaf Blade (cm) | | Ligule Length (mm) | Flag Leaf (cm) | | Internode Diameter (mm) | | | | | Internode Length (cm) | | | | | Panicle | | Grain (mm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | LBL | LBW | | FLL | FLW | D1 IN | D2 IN | D3 IN | D4 IN | D5 IN | L1 IN | L2 IN | L3 IN | L4 IN | L5 IN | PNLM | PANL (cm) | GL | GW |
| 1 | PR39142-10-3-2-1-1-B | 33 | 45.4 | 1.4 | 17.64 | 28.2 | 1.6 | 5.14 | 4.49 | 3.88 | 3.29 | 2.19 | 3.6 | 6.4 | 12.6 | 20.8 | 36.3 | 96 | 23 | 8.21 | 2.17 |
| 2 | PR39149-33-1-3-3-1-B | 33 | 34.9 | 1.2 | 14.48 | 20.8 | 1.4 | 4.50 | 4.12 | 3.58 | 3.22 | 2.03 | 4.0 | 6.9 | 10.3 | 15.7 | 32.0 | 101 | 21 | 8.89 | 2.13 |
| 3 | PR39628-17-2-1-1-B | 38 | 40.8 | 1.3 | 17.40 | 27.1 | 1.5 | 4.64 | 4.02 | 3.59 | 3.36 | 2.06 | 3.0 | 6.2 | 10.4 | 16.3 | 29.9 | 110 | 24 | 9.83 | 2.18 |
| 4 | PR40334-61-1-1-B | 29 | 38.0 | 1.2 | 15.49 | 28.0 | 1.4 | 4.12 | 3.85 | 3.60 | 3.08 | 1.79 | 2.7 | 5.6 | 8.0 | 14.6 | 29.7 | 71 | 22 | 8.65 | 2.12 |
| 5 | PR40432-10-1-1-1-B-B | 31 | 40.5 | 1.4 | 16.79 | 31.0 | 1.5 | 4.83 | 4.34 | 3.92 | 3.58 | 1.81 | 4.2 | 6.4 | 8.0 | 16.8 | 30.2 | 90 | 23 | 9.35 | 2.15 |
| 6 | PR40432-14-2-1-B | 40 | 39.5 | 1.5 | 12.87 | 26.2 | 1.6 | 4.44 | 4.19 | 3.62 | 3.27 | 2.00 | 6.4 | 9.6 | 10.7 | 15.8 | 31.4 | 80 | 23 | 9.83 | 2.74 |
| 7 | PR40432-17-3-1-2-B-B | 29 | 33.5 | 1.2 | 12.09 | 23.9 | 1.2 | 3.90 | 3.46 | 3.02 | 3.02 | 1.71 | 4.2 | 6.3 | 7.8 | 14.9 | 26.2 | 109 | 21 | 9.22 | 2.12 |
| 8 | PR43405-10-2-3-3-B | 35 | 42.0 | 1.5 | 16.80 | 29.2 | 1.6 | 5.54 | 5.02 | 4.53 | 4.17 | 2.59 | 3.3 | 7.7 | 10.4 | 17.2 | 35.8 | 74 | 23 | 8.83 | 2.19 |
| 9 | PR43425-25-2-1-1-1-B | 35 | 37.7 | 1.4 | 22.64 | 27.1 | 1.4 | 4.33 | 3.81 | 3.47 | 3.21 | 1.54 | 3.6 | 7.2 | 9.1 | 16.3 | 28.9 | 97 | 24 | 10.17 | 2.18 |
| 10 | PR43426-13-2-3-2-B-B | 37 | 42.1 | 1.5 | 16.26 | 26.0 | 1.7 | 6.12 | 5.64 | 4.90 | 4.16 | 2.20 | 3.4 | 7.9 | 12.3 | 17.1 | 32.9 | 88 | 24 | 9.25 | 2.09 |
| 11 | PR43433-21-2-1-1-1-B | 36 | 46.7 | 1.7 | 18.96 | 27.6 | 2.0 | 4.91 | 4.90 | 3.96 | 3.84 | 2.30 | 3.4 | 6.7 | 10.8 | 17.4 | 27.9 | 80 | 19 | 9.31 | 2.20 |
| 12 | PR45299-14-3-2-B | 34 | 36.5 | 1.8 | 12.93 | 24.8 | 1.9 | 5.98 | 5.05 | 4.33 | 3.81 | 2.33 | 7.8 | 12.8 | 14.7 | 19.6 | 31.2 | 92 | 21 | 8.91 | 2.14 |
| 13 | NSIC Rc 11 | 33 | 38.1 | 1.3 | 14.54 | 23.6 | 1.5 | 4.56 | 3.94 | 3.57 | 3.24 | 1.82 | 4.8 | 8.1 | 11.6 | 18.1 | 31.7 | 86 | 24 | 9.44 | 2.24 |
| 14 | NSIC Rc 25 | 32 | 36.6 | 1.2 | 13.17 | 26.2 | 1.5 | 4.22 | 3.48 | 3.21 | 3.04 | 2.08 | 2.5 | 6.0 | 12.7 | 23.4 | 33.7 | 122 | 23 | 8.54 | 2.13 |
| 15 | NSIC Rc 29 | 31 | 41.1 | 1.2 | 17.23 | 29.2 | 1.4 | 4.40 | 3.82 | 3.59 | 3.14 | 2.09 | 3.2 | 5.3 | 9.8 | 18.6 | 33.0 | 89 | 25 | 9.33 | 2.14 |
| 16 | NSIC Rc 194 | 30 | 32.9 | 1.1 | 15.85 | 23.1 | 1.2 | 4.34 | 3.99 | 3.58 | 3.12 | 1.75 | 2.5 | 5.1 | 10.2 | 15.3 | 29.6 | 90 | 21 | 9.52 | 2.21 |
| 17 | NSIC Rc 214 | 28 | 39.4 | 1.1 | 18.11 | 33.3 | 1.3 | 4.49 | 4.17 | 3.88 | 3.40 | 2.35 | 2.1 | 3.9 | 7.5 | 18.1 | 32.0 | 100 | 25 | 9.23 | 2.11 |
| 18 | NSIC Rc 216 | 30 | 38.9 | 1.2 | 17.44 | 29.1 | 1.3 | 4.47 | 3.94 | 3.60 | 3.14 | 1.87 | 4.0 | 6.3 | 9.0 | 16.5 | 33.9 | 87 | 25 | 9.41 | 2.23 |
| 19 | NSIC Rc 218 | 33 | 41.8 | 1.4 | 20.86 | 25.2 | 1.5 | 5.45 | 4.78 | 4.37 | 3.84 | 2.18 | 4.0 | 8.0 | 11.2 | 18.0 | 31.2 | 68 | 23 | 9.63 | 2.20 |
| 20 | NSIC Rc 222 | 30 | 41.3 | 1.2 | 15.71 | 29.2 | 1.3 | 5.04 | 4.31 | 3.77 | 3.37 | 1.99 | 3.4 | 6.0 | 10.2 | 16.9 | 37.4 | 78 | 25 | 9.22 | 2.15 |
| 21 | NSIC Rc 224 | 32 | 44.5 | 1.3 | 21.34 | 36.4 | 1.5 | 4.47 | 4.32 | 3.98 | 3.51 | 2.12 | 3.0 | 6.4 | 9.0 | 14.4 | 33.1 | 80 | 29 | 9.26 | 2.12 |
| 22 | NSIC Rc 226 | 32 | 35.2 | 1.3 | 14.01 | 25.1 | 1.4 | 4.60 | 4.08 | 3.73 | 3.39 | 2.21 | 7.0 | 10.7 | 12.7 | 17.2 | 33.6 | 87 | 25 | 9.12 | 2.66 |
| 23 | NSIC Rc 238 | 30 | 42.4 | 1.2 | 17.03 | 22.1 | 1.2 | 5.19 | 4.96 | 4.27 | 3.71 | 2.32 | 4.1 | 8.2 | 11.8 | 17.2 | 32.9 | 98 | 25 | 8.86 | 2.22 |
| 24 | NSIC Rc 290 | 28 | 34.4 | 1.2 | 14.94 | 24.0 | 1.3 | 4.74 | 4.18 | 3.96 | 3.08 | 2.27 | 3.5 | 7.7 | 10.6 | 15.4 | 30.8 | 96 | 23 | 8.57 | 2.11 |
| 25 | NSIC Rc 300 | 31 | 49.6 | 1.3 | 26.27 | 36.0 | 1.6 | 4.96 | 4.92 | 4.69 | 4.08 | 1.95 | 2.1 | 4.6 | 7.7 | 16.6 | 37.6 | 76 | 26 | 9.09 | 2.18 |
| 26 | NSIC Rc 302 | 30 | 40.9 | 1.2 | 17.19 | 24.7 | 1.3 | 4.21 | 3.85 | 3.73 | 3.21 | 1.88 | 2.6 | 5.8 | 8.6 | 15.7 | 33.6 | 87 | 24 | 9.72 | 2.29 |
| 27 | NSIC Rc 308 | 32 | 34.6 | 1.2 | 12.32 | 24.8 | 1.4 | 3.86 | 3.21 | 2.80 | 2.36 | 1.47 | 3.9 | 6.9 | 7.9 | 15.2 | 27.5 | 108 | 22 | 9.09 | 2.15 |
| 28 | NSIC Rc 324 | 33 | 45.2 | 1.3 | 18.83 | 30.3 | 1.5 | 4.87 | 4.04 | 4.14 | 3.51 | 1.69 | 5.2 | 9.3 | 12.3 | 20.8 | 34.1 | 82 | 26 | 9.96 | 2.16 |
| 29 | NSIC Rc 352 | 31 | 34.5 | 1.1 | 12.75 | 19.7 | 1.2 | 4.01 | 3.48 | 3.01 | 2.76 | 1.35 | 3.3 | 6.5 | 11.6 | 17.1 | 31.1 | 102 | 22 | 9.48 | 2.12 |
| 30 | NSIC Rc 354 | 32 | 47.7 | 1.3 | 16.60 | 31.3 | 1.6 | 4.32 | 3.84 | 3.52 | 3.05 | 1.81 | 2.7 | 6.3 | 10.5 | 17.0 | 33.8 | 85 | 23 | 9.01 | 2.19 |
| 31 | NSIC Rc 356 | 28 | 35.6 | 1.0 | 16.08 | 27.4 | 1.1 | 4.08 | 3.56 | 3.28 | 3.28 | 1.64 | 3.2 | 5.9 | 8.8 | 16.3 | 30.5 | 108 | 23 | 9.20 | 2.12 |
| 32 | NSIC Rc 358 | 31 | 36.1 | 1.2 | 16.14 | 25.9 | 1.2 | 4.43 | 4.06 | 3.71 | 3.14 | 2.03 | 3.6 | 5.4 | 8.0 | 16.6 | 30.0 | 71 | 24 | 8.79 | 2.07 |
| 33 | NSIC Rc 360 | 30 | 44.0 | 1.3 | 22.35 | 34.0 | 1.6 | 4.95 | 4.51 | 4.08 | 3.26 | 2.11 | 3.4 | 6.8 | 10.5 | 17.1 | 34.4 | 57 | 28 | 9.44 | 2.19 |
| 34 | NSIC Rc 390 | 32 | 33.9 | 1.2 | 16.61 | 23.5 | 1.4 | 4.27 | 3.75 | 3.24 | 2.76 | 1.56 | 3.5 | 7.4 | 12.6 | 19.2 | 29.3 | 119 | 24 | 9.25 | 2.18 |
| 35 | NSIC Rc 392 | 30 | 38.0 | 1.2 | 19.95 | 26.2 | 1.3 | 4.12 | 3.93 | 3.48 | 3.16 | 1.82 | 1.9 | 4.4 | 7.7 | 16.3 | 31.0 | 92 | 24 | 9.51 | 2.17 |
| 36 | NSIC Rc 396 | 29 | 43.3 | 1.3 | 16.84 | 32.5 | 1.5 | 5.15 | 4.74 | 4.23 | 3.44 | 2.21 | 2.2 | 5.1 | 8.1 | 16.0 | 31.1 | 82 | 24 | 8.55 | 2.20 |
| 37 | NSIC Rc 402 | 34 | 44.7 | 1.3 | 22.10 | 30.4 | 1.4 | 5.12 | 4.66 | 3.99 | 3.38 | 2.07 | 2.4 | 4.4 | 10.1 | 18.2 | 31.8 | 69 | 27 | 8.94 | 2.17 |
| 38 | PSB Rc82 | 31 | 36.7 | 1.0 | 13.90 | 24.5 | 1.1 | 4.30 | 3.56 | 2.98 | 3.01 | 1.70 | 3.2 | 6.1 | 11.8 | 16.1 | 29.5 | 120 | 21 | 8.84 | 2.17 |
| 39 | NSIC Rc 160 | 30 | 44.8 | 1.4 | 21.06 | 31.5 | 1.6 | 3.84 | 3.88 | 3.41 | 3.06 | 1.72 | 2.3 | 5.2 | 9.7 | 18.4 | 33.4 | 91 | 24 | 9.59 | 2.17 |
| 40 | NSIC Rc 240 | 32 | 42.4 | 1.4 | 19.28 | 35.7 | 1.7 | 5.04 | 4.74 | 4.30 | 3.89 | 2.44 | 2.5 | 5.1 | 10.7 | 18.2 | 35.3 | 73 | 26 | 8.72 | 2.30 |
| 41 | NSIC Rc 298 | 29 | 38.0 | 1.2 | 15.49 | 26.2 | 1.3 | 5.26 | 4.53 | 4.24 | 3.77 | 2.20 | 3.8 | 6.9 | 9.0 | 16.4 | 33.5 | 110 | 23 | 9.30 | 2.18 |
| 42 | PSB Rc4 | 29 | 43.9 | 1.0 | 15.78 | 33.0 | 1.2 | 3.81 | 3.59 | 3.16 | 2.97 | 2.11 | 2.5 | 5.1 | 10.8 | 16.9 | 31.4 | 75 | 23 | 9.17 | 2.30 |
| 43 | PSB Rc14 | 28 | 35.1 | 1.1 | 16.92 | 22.8 | 1.2 | 3.66 | 2.93 | 2.69 | 2.64 | 1.36 | 3.0 | 4.8 | 7.2 | 13.8 | 28.5 | 132 | 18 | 8.99 | 2.10 |

***Sht*** seedling height; ***lbl*** leaf blade length; ***lbw*** leaf blade width; ***fll*** flag leaf length; ***flw*** flag leaf width; ***d1in*** diameter of 1st internode; ***d2in*** diameter of 2nd internode; ***d3in*** diameter of 3rd internode; ***d4in*** diameter of 4th internode; ***d5in*** diameter of 5th internode; ***l1in*** length of 1st internode; ***l2in*** length of 2nd internode; ***l3in*** length of 3rd internode; ***l4in*** length of 4th internode; ***l5in*** length of 5th internode; ***pnlm*** panicle number per linear meter; ***panl*** panicle length; ***gl*** grain length; ***gw*** grain width.

image (Fig. 2). Image Color Summarizer v.0.76 (Krzywinski, 2017) processed and analyzed levels of lignin via pixels of colors ensuing k-means clustering of five major color groups with its corresponding percent number of pixels (Fig. 3).

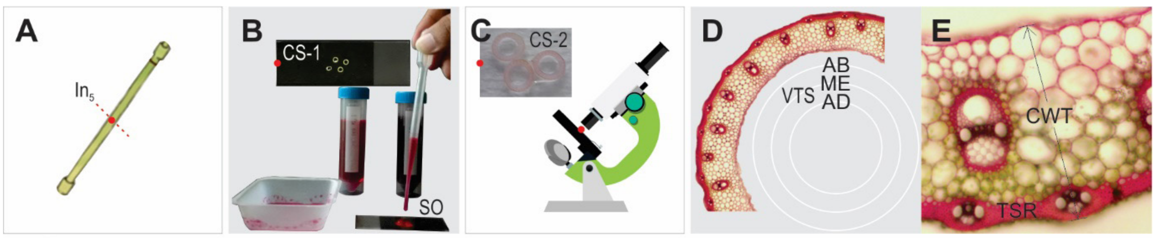
### *Statistical Analysis*

Nominal ($Q_l$) and ratio ($Q_n$) are two data types entirety of the analysis. $Q_l$ observations follow descriptions in the Bioversity International Descriptors for Wild and Cultivated Rice (2007) and describe through Shannon-Weaver Diversity Index (*H'*) to recognize variations among $Q_l$ traits in contrary with the lodging-tolerant check NSIC Rc 240. Arbitrary scale adapted from Jamago and Cortes (2012) categorizes computed indices into maximum (*H'* = 1.00), high (*H'* = 0.76-0.99), moderate (*H'* = 0.46-0.75), low diversity (*H'* = 0.01-0.45) and no diversity or non-variable (*H'* = 0.00), and then computed based on phenotypic frequency using *H'* formula (Shannon, 1948):

$$H' = -\sum_{i=1}^{s} pi\,(logpi)$$

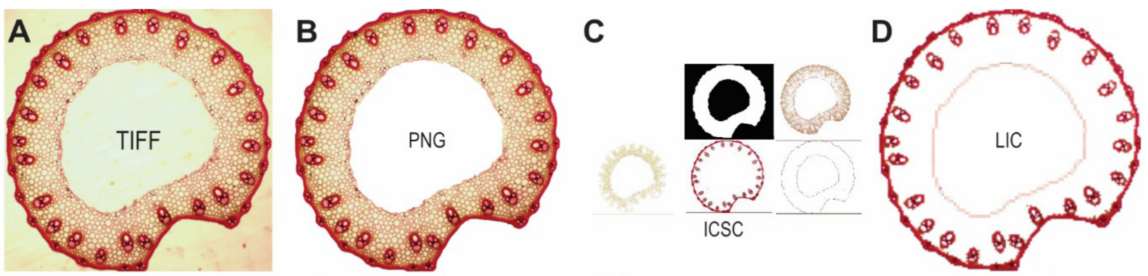
where ***H'*** = the Shannon-weaver diversity index; ***s*** = total number of species in the community; ***i*** – *ith* species; ***pi*** = the proportion of species ***i*** relative to the total number of species; ***logpi*** = logarithm of this proportion; the result is added across species and multiplied by -1 (Beals et al., 2000). Furthermore, Multiple Correspondence Analysis determines the diversity of the 19 $Q_l$ traits (Greenacre and Blasius, 2006; Abdi and Valentin, 2007).

$Q_n$ data were arranged accordingly in General Linear Model (GLM) while Two-way Analysis of Variance (ANOVA) determined the interaction of several independent and dependent variables. Duncan's Multiple Range Test (DMRT) at 0.05 level of significance group mean differences




*$In_5$* $5^{th}$ internode, *CS-1* cross section before staining, *SO* safranin-O solution, *CS-2* cross section after staining, *VTS* vascular tissue system, *AB* abaxial vascular bundle, *ME* median vascular bundle, *AD* adaxial vascular bundle, *CWT* culm wall thickness, *TSR* thickness of sclerenchymatous ring

**Figure 2**. Anatomic evaluation: **A.** $In_5$ sample collected ten days after anthesis; **B.** Four 20 µm thick sections stained with 0.2 ml Safranin-O solution; **C.** Stained sections viewed under low and high magnification; **D.** Arrangement of vascular bundles; **E.** High Power Objective image used in measuring thickness and area of cells.




*TIFF* tagged image file format, *PNG* portable network graphics, *ICSC* image color statistics and clustering, *LIC* lignified image cluster

**Figure 3.** Determination of percent lignin content using image color summarizer: **A.** Microscope generated image in TIFF; **B.** TIFF image converted to PNG; **C.** Pixels of colors were grouped by k-means clustering through five major groups with corresponding percent number of pixels; **D.** Color cluster with red-pinkish brown pixels.

among treatments. Dunnett's t-tests compared the performance of treatments against $L_t$ (NSIC Rc 240) and $L_s$ (PSB Rc4) control (R Core Team, 2018). The bivariate analysis was predicted through Pearson correlation (*r*), while multivariate analyses identified relationships among traits directly in Principal Component (PCA) and cluster analyses. Rotation in PCA revolves obliquely in the form of Promax with Kaizer normalization. Furthermore, cluster analysis in Ward's method in Euclidian Distance distinguished $L_t$ and $L_s$ genotypes based on correlated traits. XLSTAT (2017) was used for bivariate and multivariate analyses.

## Results

### *Push-resistance at the Lower Stem of Rice*

Push-resistance of lodging tolerant check was measured at 1.02 kg cm$^2$ while the susceptible check was 0.77 kg cm$^2$ with a mean of 0.89 kg cm$^2$ across all test genotypes. Push-resistance varies among the 43 rice genotypes in which NSIC Rc 240 $L_t$ check can withstand an average resistance of 1.02 kg cm$^2$, significantly higher than the $L_s$ check PSB Rc4 with 0.77 kg cm$^2$ push resistance (Table 4). Thirty-one (72%) of 43 genotypes have push-resistance (0.83 – 1.08 kg cm$^2$) comparable with NSIC Rc 240 while six genotypes (1.01 – 1.08 kg cm$^2$) had significantly better culm strength than the susceptible check, PSB Rc4 (Fig. 4). It was observed that resistance to lodging was high during plant maturity.

### *Phenotypic Traits*

#### Relationships of 19 $Q_l$ Agro-morphologic Characters to Lodging

Table 2 shows that eleven (58%) of 19 $Q_l$ traits exhibited no diversity among 43 genotypes. All test genotypes including lodging checks showed green basal leaf sheath color, no leaf blade anthocyanin, erect leaf blade, intermediate pubescence, yellow-green auricle, light green collar, 2-cleft ligule, semi-erect panicle branches, moderate shattering, and intermediate panicle threshability. Similarities among these traits showed that line selection schemes across various environments do not significantly different indicating that preferences to certain traits among rice breeders during progeny selection were comparable.

In terms of leaf descriptors, majority (70%) have light green leaf blade while 11 (25%) genotypes have medium intensity. NSIC Rc 396 and Rc 240 showed dark intensity. Almost all (89%) of test genotypes have yellow-green ligule while five (11%) genotypes displayed white ligule. Leaf blade color intensity and ligule colors have *H'* of 0.32 and 0.16 indicating low levels of diversity. Twenty-one percent of 43 rice genotypes showed erect flag leaf while 34 (79%) rice genotypes exhibited semi-erect type, similar with the resistant check, NSIC Rc 240. However, at maturity, PR39149-33-1-3-3-1-B, PR39628-17-2-1-1-B, PR43433-21-2-1-1-1-B and PR45299-14-3-2-B, NSIC Rc 194, Rc 218, Rc 226, Rc 290, and susceptible variety (PSB Rc4) were observed to change from erect to semi-erect flag leaf due to wind force and pressure. Furthermore, diversity remains to be poor at 0.22 *H'*. Ten (23%) of 43 rice genotypes displayed erect culm habit: PR39149-33-1-3-3-1-B, PR39628-17-2-1-1-B, PR43433-21-2-1-1-1-B, PR45299-14-3-2-B, NSIC Rc 194, Rc 218, Rc 226, Rc 290, PSB Rc82, and PSB Rc4, while the remaining 33 rice genotypes have semi-erect culm habit. Culm habit showed *H'* of 0.24. Poor panicle diversity was manifested in the main axis attitude (*H'* 0.27), in which 68% of test genotypes were slightly droopingwhile 32% had a strongly drooping type.


**Figure 4**. Phenotypes of the six highly $L_t$ genotypes: **A.** PR43426-13-2-3-2-B; **B.** NSIC Rc 396, **C.** PR39142-10-3-2-1-1-B; **D.** NSIC Rc 29; **E.** NSIC Rc 240; **F.** PR45299-14-3-2-B; **G.** Proportions of intermediately $L_t$; **H.** $L_s$ genotypes out of the 43 rice genotypes evaluated for push-resistance under direct-seeded condition.

**Table 4.** Push resistance of 43 rice genotypes against resistant check, NSIC Rc 240.

| Treatment No. | Variety Name / Line Designation | Push Resistance (kgf) | Treament No. | Variety Name | Push Resistance (kgf) |
|---|---|---|---|---|---|
| 1 | PR39142-10-3-2-1-1-B | 1.05 | 23 | NSIC Rc 238 | 0.83 |
| 2 | PR39149-33-1-3-3-1-B | 0.86 | 24 | NSIC Rc 290 | 0.85 |
| 3 | PR39628-17-2-1-1-B | 0.89 | 25 | NSIC Rc 300 | 0.91 |
| 4 | PR40334-61-1-1-B | 0.78# | 26 | NSIC Rc 302 | 0.82# |
| 5 | PR40432-10-1-1-1-B-B | 0.98 | 27 | NSIC Rc 308 | 0.81# |
| 6 | PR40432-14-2-1-B | 0.81# | 28 | NSIC Rc 324 | 0.83 |
| 7 | PR40432-17-3-1-2-B-B | 0.82# | 29 | NSIC Rc 352 | 0.82# |
| 8 | PR43405-10-2-3-3-B | 0.93 | 30 | NSIC Rc 354 | 0.98 |
| 9 | PR43425-25-2-1-1-1-B | 0.94 | 31 | NSIC Rc 356 | 0.88 |
| 10 | PR43426-13-2-3-2-B-B | 1.08 | 32 | NSIC Rc 358 | 0.82# |
| 11 | PR43433-21-2-1-1-1-B | 0.85 | 33 | NSIC Rc 360 | 0.93 |
| 12 | PR45299-14-3-2-B | 1.01 | 34 | NSIC Rc 390 | 0.81# |
| 13 | NSIC Rc 11 | 0.86 | 35 | NSIC Rc 392 | 0.85 |
| 14 | NSIC Rc 25 | 0.85 | 36 | NSIC Rc 396 | 1.06 |
| 15 | NSIC Rc 29 | 1.03 | 37 | NSIC Rc 402 | 0.91 |
| 16 | NSIC Rc 194 | 0.86 | 38 | PSB Rc 82 | 0.74# |
| 17 | NSIC Rc 214 | 0.91 | 39 | NSIC Rc 160 | 0.98 |
| 18 | NSIC Rc 216 | 0.87 | 40 | NSIC Rc 240 (check) | 1.02 |
| 19 | NSIC Rc 218 SR | 0.91 | 41 | NSIC Rc 298 | 0.83 |
| 20 | NSIC Rc 222 | 0.89 | 42 | PSB Rc 4 | 0.77# |
| 21 | NSIC Rc 224 | 0.94 | 43 | PSB Rc 14 | 0.78# |
| 22 | NSIC Rc 226 | 0.92 | | | |

**gmean** (0.89 kgf); **pvalue** (<.0001); **$r^2$** (0.62); **cv** (8.50); **MSD 5%** (0.20)
# Rice selections significantly lower than NSIC Rc 240 at 0.05 probability levels

Furthermore, 73% of rice genotypes had sparse secondary branching while 27% of genotypes demonstrated dense branching (*H'* 0.16). PR43433-21-2-1-1-1-B had partly exserted panicle while 11% of test genotypes had just exserted. Majority (84%) of genotypes had moderately well-exserted panicle with *H'* of 0.20 (Table 2).

### *Relationship of 23 $Q_n$ Agro-morphologic Measurements to Lodging*

In the direct-seeded condition, all $Q_n$ traits showed significant variations. Out of these 23 characters, 15 significant rice descriptors presented a noticeable relationship to lodging. Systematic reduction of internode girth was clear from the basal ($1^{st}$) to terminal ($5^{th}$) internodes. PR43426-13-2-3-2-B-B exhibited the widest $1^{st}$, $2^{nd}$, and $4^{th}$ internodes at 6.12, 5.64, and 4.16 mm respectively, and 14% wider than NSIC Rc 240 (5.04-3.89 mm) and 35% thicker than PSB Rc4 (3.81-2.97 mm). Internode length delineated a similar trend reduction in size like in internode diameter, in which lengths tend to go higher from the basal to terminal internodes. The $1^{st}$-$3^{rd}$ internode were closer in length while the $4^{th}$ internode was closer in length to the $1^{st}$ three internodes than the $5^{th}$ internode. Length of $5^{th}$ internode started at 26 cm regardless of internode length. Majority of the modern-bred rice varieties are semi-dwarf except for NSIC Rc 324, which is intermediate. NSIC Rc 392, Rc 214, Rc 300, and Rc 396 showed the shortest basal internode of 1.9, 2.1, 2.1, and 2.2 cm, respectively. NSIC Rc 300 registered in the shortest $1^{st}$ to $3^{rd}$ internode while having the longest $5^{th}$ internode of 37.6 cm (Fig. 5). NSIC Rc 224 recorded the most extended panicle of 29 cm followed by Rc 360 (28 cm), Rc 402 (27 cm), and Rc 300 (26 cm).

Culm length ranged from 60 to 92 cm while plant height was at 78-118 cm. Test genotypes were relatively short with mean culm length and plant height of 72 cm and 95 cm, respectively.

All genotypes were intermediate in leaf blade length with NSIC Rc 300 exhibiting the most extended leaf blade of 49.6 cm while PhilRice-bred lines PR45299-14-3-2-B had the widest leaf blade width of 1.8 cm. For the flag leaf length and width, NSIC Rc 224 showed the longest flag leaf of 36.4 cm while PR43433-21-2-1-1-1-B obtained the widest flag leaf at 2.0 cm. NSIC Rc 300 recorded the longest ligule of 26.27 mm while PR40432-17-3-1-2-B-B had the shortest ligule of 12.09 mm. PSB Rc14 exhibited the highest number of culms at 149 tillers while NSIC Rc 240 and Rc 402 had the lowest number of tillers (78).

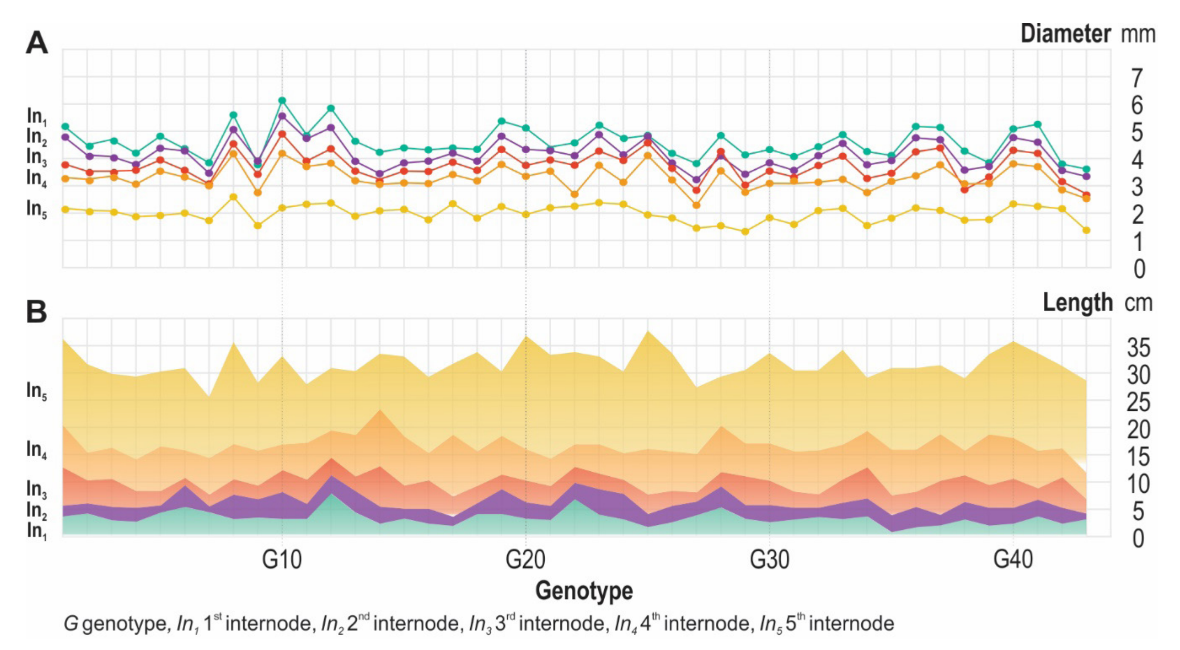



**Figure 5**. Internode diameter and length of the 43 rice genotypes: **A.** systematic reduction of thickness from the basal to the terminal internode; **B.** internode length tends to increase from the basal towards the terminal internode.

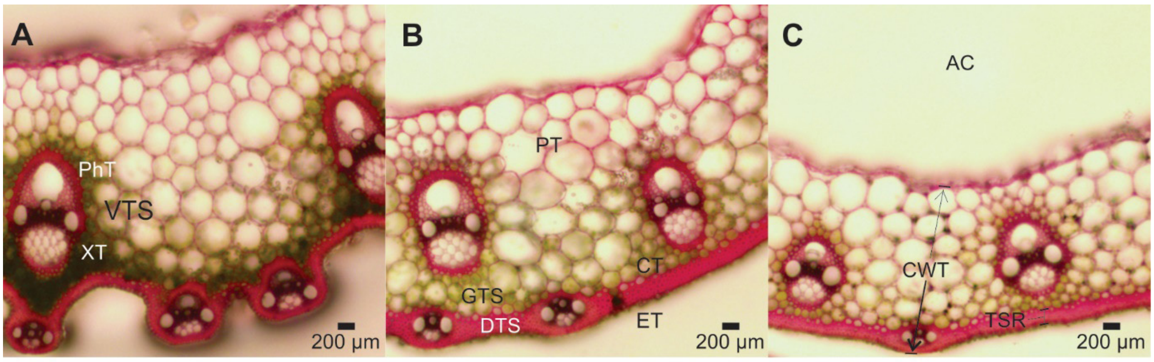
Grain yield across genotypes ranged from 1.69 to 7.34 t/ha with a mean yield of 5.08 t/ha. NSIC Rc 160 (7.34 t/ha), NSIC Rc 222 (7.16 t/ha), and PR40432-17-3-1-2-B-B (6.79 t/ha) were the highest-yielding test genotypes and with a significantly higher yield than the resistant check, NSIC Rc 240 (5.85 t/ha).

### *Characteristics of Anatomic Traits in the 5th Internode*

The main deviation in the structure of rice stems rest on the proportion and spatial arrangement of vascular and ground tissues. In the internal regions of the ground tissue, Number of Adaxial (NAD) vascular bundles of 43 rice genotypes ranged from 9 to 17 with NSIC Rc 224 registering the highest frequency and Rc 194 with the least NAD. Moreover, the Area of Adaxial (AAD) span from 27.2 to 47.0 x 103 µm2 with five rice genotypes: NSIC Rc 308 (47.0 x 103 µm2), Rc 396 (44.0 x 103 µm2), Rc 224 (42.8 x 103 µm2), Rc 29 (42.5 x 103 µm2) and Rc 302 (42.1 x 103 µm2) had significantly comparable AAD with NSIC Rc 240 (45.0 x 103 µm2). From the ground tissue to the dermal tissue embed low (2-5) Number of Median (NME) vascular bundles present in most rice genotypes, while high (8-9) NME exist in NSIC Rc 214, Rc 300, Rc 396, Rc 402, and PR40432-17-3-1-2-B-B, which is noticeably higher than NSIC Rc 240 (6). Moreover, the size of the median-vascular bundle ranged from 18.3 to 30.3 x 103 µm2. Protruding in the dermal tissue of the stem set the smallest vascular bundles, the abaxial. High (17-27) Number of Abaxial (NAB) was detected in all genotypes. Only NSIC Rc 29 significantly outnumbered the lodging-tolerant check NSIC Rc 240 (22) by 7 NAB. Moreover, NSIC Rc 214, Rc 224, Rc 360, and Rc 390 displayed NAB comparable with Rc 240. Area of Abaxial (AAB) range from 3.82 to 9.80 x 103 µm2 with NSIC Rc 29 (9.80 x 103 µm2) registering the largest AAB comparable with NSIC Rc 240 (9.17 x 103 µm2). Figure 6 elucidates the perceptible phenotypes of a highly Lt, intermediate Lt, and Ls genotypes.

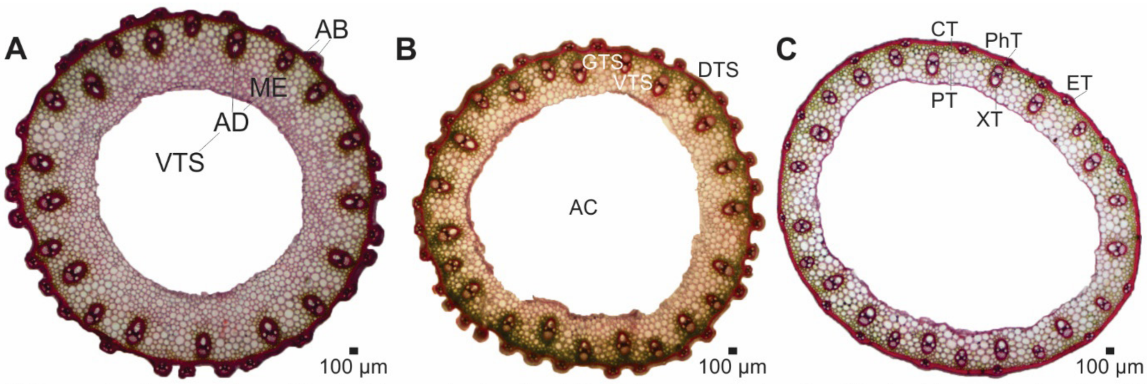
Culm wall thickness (CWT) surrounds and regulates the distributions of anatomical characters whereas Thickness of Sclerenchymatous ring (TSR) protects plant organs from stretching, bending, weight and pressure, without excessive damage to the thin-walled softer cells (Esau, 1965). Significant variations among the 43 genotypes' include CWT ranging from 398.28 to 741.20 µm, with PR43433-21-2-1-1-1-B outstandingly showed profuse CWT and provided 35% wider cellular space than the Lt check, NSIC Rc 240 (479.50 µm). TSR ranged from 24.50 to 49.80 µm, in which PR39149-33-1-3-3-1-B and NSIC Rc 390 had the thickest TSR. They also showed 46% added wall protection than Rc 240 (26.70 µm) (Fig. 7).

Lignified tissues in the 5th internode appear bright red when Safranin O (basic dye, positively charge) stains lignin-rich (acidic, negatively charge) cell walls (Kutscha and Gray 1972; Bond et al. 2008). Twenty-three of the 43 genotypes showed significantly high lignin than the $L_t$ check NSIC Rc 240 (16.29%) and average percent lignin of 19.83. NSIC Rc 396 (24.03%) outperformed all genotypes in the content of lignin (Figure 8).




*VTS* vascular tissue system, *PhT* phloem tissue, *XT* xylem tissue, , *GTS*, ground tissue system, *DTS* dermal tissue system, *CT* chlorenchyma tissue, *ET* epidermal tissue, *PT* parenchyma tissue, *CWT* culm wall thickness, *TSR* thickness of sclerenchymatous ring, *AC* aerenchymous center

**Figure 6**. Light micrographs of vascular bundles in the middle part of the 5$^{th}$ internode. Transverse sections of **A.** highly $L_t$ (PR43426-13-2-3-2-B-B), **B.** intermediate $L_t$ (NSIC Rc 222), and **C.** $L_s$ (PSB Rc4) genotypes at 10 days after 100% flowering. Scale bar, 100µm.




*VTS* vascular tissue system, *AD* adaxial vascular bundle, *ME* median vascular bundle, *AB* abaxial vascular bundle, *AC* aerenchymous center, *GTS*, ground tissue system, *DTS* dermal tissue system, *CT* chlorenchyma tissue, *PhT* phloem tissue, *ET* epidermal tissue, *PT* parenchyma tissue, *XT* xylem tissue

**Figure 7**. Light micrographs of the thickness of the culm wall and sclerenchymatous ring in the middle part of the 5$^{th}$ internode. Transverse sections in a high dimension of **A.** PR43433-21-2-1-1-1-B with the widest CWT, **B.** NSIC Rc 240, $L_t$ check, and **C.** PSB Rc4, $L_s$ check. Scale bar, 200 µm.

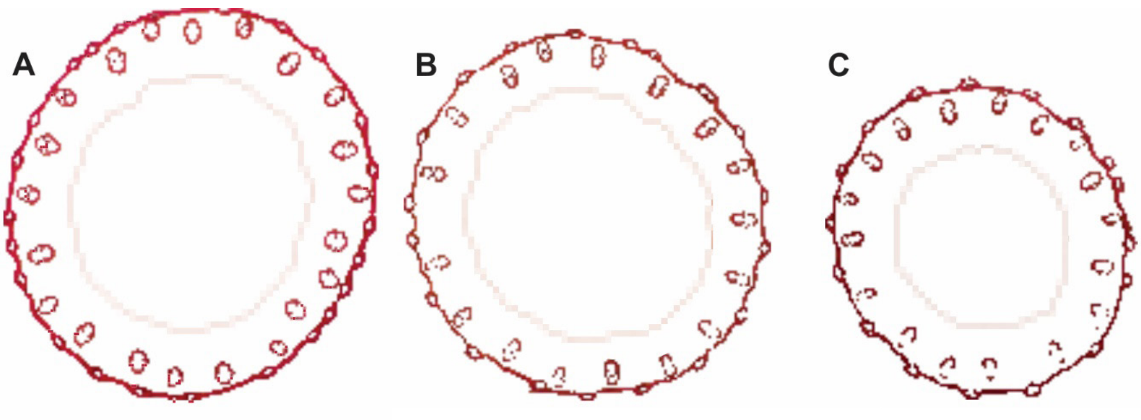



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**Figure 8**. Lignified portion of the micrographs captured in the middle portion of the 5th internode. Accumulation of lignin in the lignified-rich vascular bundles, culm wall, and sclerenchymatous ring. Transverse section **A.** $L_t$ test genotype (NSIC Rc 396), **B.** $L_t$ check (NSIC Rc 240), and **C.** $L_s$ check (PSB Rc4) in approximately 20 µm thick cell.

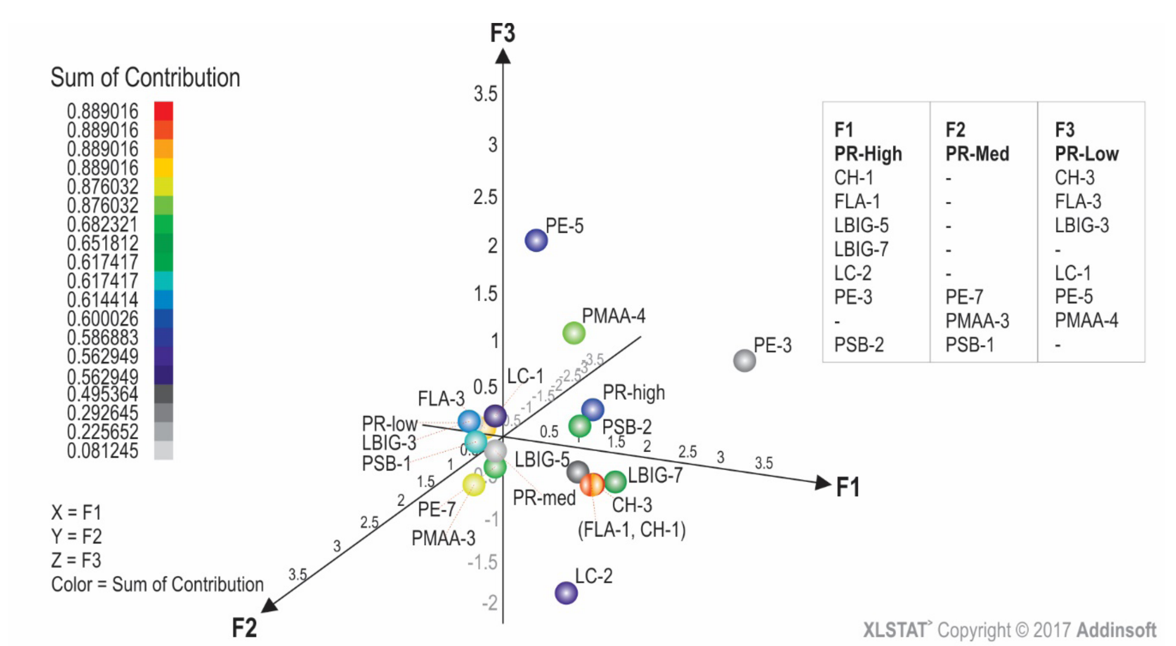
## Discussion

Lodging contributes to yield loss especially in direct-seeded rice production system (Won et al., 1998). In the direct-seeded field, root lodging is likely to occur than stem lodging (Oladokun and Ennos, 2006) because of the nature of crop establishment. Although determining the physical strength of root is the appropriate screening for lodging (Miyasaka, 1971) in direct seeded rice, horizontal pushing of the stem mimics rice lodging far better than vertical root-pull tests (Smit et al., 2000). In this study, the stem strength has been used as an index of lodging-tolerance like in wheat (Hai et al., 2005) and corn (Xue et al., 2017).

$Q_n$ data in three sets of observations for push-resistance, phenotypic traits, and anatomic characters were arranged on a conventional linear regression model for a continuous response variable given constant categorical predictors. Analysis of variance of all traits showed significant *p-value* (0.0018 - <0.0001), an acceptable coefficient of variation (*CV*) per trait (2.60 – 42.14), and reasonable coefficient of determination ($r^2$) per trait (0.50 – 0.92).

$L_t$ rice genotypes showed remarkably higher push-resistance than $L_s$ genotypes (Terashima et al., 2003; Yoshinaga, 2005; Kashiwagi et al., 2008). NSIC Rc 240, the $L_t$ check, can withstand an average resistance of 1.01 kg cm$^{-2}$, which is significantly higher than PSB Rc4 (0.77 kg cm$^{-2}$), the $L_s$ check. PR43426-13-2-3-2-B-B (1.08 kg cm$^{-2}$), NSIC Rc 396 (1.06 kg cm$^{-2}$), and PR39142-10-3-2-1-1-B (1.05 kg cm$^2$) exhibited the highest push-resistances indicative of a stronger culm (Joarder et al., 2006) and higher root anchorage (Abe and Morita, 1994) than all test genotypes.

Lodging tolerance is a combination of several structural characteristics of rice pre-formed in the surface of its morphology and underlying anatomical body. These positive traits act as a physical barrier and natural defense of rice against high rainfall intensity and strong wind. $L_t$ genotypes possess both the features of $Q_l$ and $Q_n$ traits. Shannon-Weaver Diversity Index (*H'*) was used to determine diversity among test entries and assess the patterns of agronomically important phenotypic variations concerning lodging among major $Q_l$ traits. Seven $Q_l$ characters showed a slight variation in the characteristics of test genotypes. However, *H'* specifies a low level of diversity when compared with $L_t$ check NSIC Rc 240 indicating that the dissimilarity found are not sufficient to conclude the differences and determine the phenotype of a $L_t$ genotype. These $Q_l$ traits and transformed data of push-resistance (low ≤0.82 kg cm$^2$; med 0.83-1.00 kg cm$^2$; high ≥1.01 kg cm$^2$) were then subjected to multiple correspondence analysis (MCA) to determine the genotypes with similar profiles. The % inertia value of 10 factors accounted for 100% of the total variation. The adjusted first factor accounted for 45.42% while the 2$^{nd}$ and 3$^{rd}$ factors represented 20.58% and 7.43% of the total variations. Figure 9 shows the correspondence analysis on three-dimensional plot, which explains 73.43% of the total variation and the divisions of categorical traits in three factors.



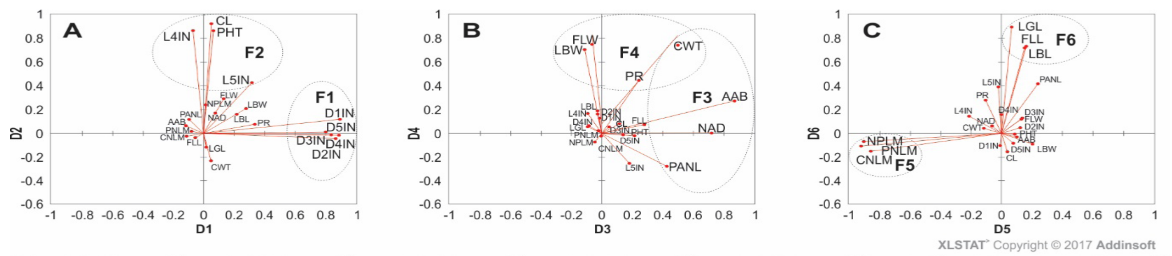

*PR-High* push resistance high, *PR-Med* push resistance medium, *PR-Low* push resistance low, *CH-1* culm habit erect, *CH-3* culm habit semi-erect, *FLA-1* flag leaf attitude erect, *FLA-3* flag leaf attitude semi-erect, *LBIG-3* leaf blade intensity of green color light, *LBIG-5* leaf blade intensity of green color medium, *LBIG-7* leaf blade intensity of green color dark, *LC-1* ligule color whitish, *LC-2* ligule color yellowish green, *PE-3* panicle exsertion partly exserted, *PE-5* panicle exsertion just exserted, *PE-7* panicle exsertion moderately well exserted, *PMAA-3* panicle main axis attitude slightly drooping, *PMAA-4* panicle main axis attitude strongly drooping, *PSB-1* panicle secondary branching sparse, *PSB-2* panicle secondary branching dense

**Figure 9.** Correspondence analysis plot of nominal traits projecting perpendicularly onto the horizontal and vertical axis.

In Multiple Correspondence Analysis, all variables have the same status. Therefore, it can be seen as an overall dimension toward lodging-tolerance with a relatively high push-resistance and relatively low push-resistance. The seven agronomic traits in terms of its quality were distributed according to its principal coordinates. Squared cosine explains the contribution of a component to the squared distance of the observation origin. It corresponds to the angle of the squared cosine from the right triangle made with the origin, observation, and projection on the component (Abdi and Williams, 2010). The 1st dimension represents the seven codes of $Q_l$ traits associated with high push-resistance: erect culm habit, erect flag leaf attitude, medium to dark leaf blade intensity of green color, yellowish-green ligule color, partly-exserted panicle exsertion, and dense panicle secondary branching. The 2nd dimension epitomizes medium push-resistance define in three codes of $Q_l$ traits: the moderately well-exserted panicle exsertion, the slightly drooping panicle main axis attitude, and the sparse panicle secondary branching. The 3rd dimension provides the undesirable $Q_l$ traits related with low push-resistance per se express in six codes of $Q_l$ traits: the semi-erect culm habit and flag leaf attitude, the light leaf blade intensity of green color, the whitish ligule color, the just exserted panicle exsertion, and the strongly drooping panicle main axis attitude.

Correlation ($r$) is useful in determining the overall linear association between any two independent variables. Selection based on the detailed knowledge of magnitude and direction of the association between traits of interest and its attributes is critical in identifying the principal characters, which can be exploited for crop improvement through a suitable breeding program (Rabindra Babu et al., 2012). Strong positive $r$ existed in push-resistance to the diameter of 2nd internode ($r$ 0.58), diameter of 1st internode ($r$ 0.57), diameter of 3rd internode ($r$ 0.57), flag leaf width ($r$ 0.57), and leaf blade width ($r$ 0.54). Medium positive associations were present in push resistance and diameter of 4th internode ($r$ 0.49), flag leaf length ($r$ 0.45), culm wall thickness ($r$ 0.44), diameter of 5th internode ($r$ 0.44), leaf blade length ($r$ 0.44), length of 5th internode ($r$ 0.42), number of abaxial vascular bundles ($r$ 0.38), plant height ($r$ 0.35), panicle length ($r$ 0.34), ligule length ($r$ 0.33), number of adaxial vascular bundles ($r$ 0.31), length of 4th internode ($r$ 0.30), and culm length ($r$ 0.30). Medium negative $r$ was accounted between push-resistance and culm number per linear meter ($r$ -0.37) and panicle number per linear meter ($r$ -0.31). A significant $r$ does not indicate causality but a common linkage in a sequence of events. The coefficient of determination ($r^2$) is more useful than $r$ as it gives a more plausible statistical explanation of the relationship between push-resistance and phenotypic traits. In $r$ analysis, strong $r$ occurs on push-resistance and the diameter of 2nd internode, however, $r^2$ is equal only to 0.33 signifying 33% of the variability linked the relationship of the two factors. The remaining 67% is the contribution of other factors that influence push-resistance but not measured in the analysis, which is one of the limitations of Bivariate techniques (Goodwin and Leech, 2006).

Morphologic, agronomic, and anatomic traits with significant $r$ to push-resistance continue through Principal Component Analysis (PCA) following Promax rotation (Abdi, 2003) for better visualization and identification of hidden categories in multiple independent variables (Smith, 2002). PCA is a method for reducing the dimensionality of datasets (Devarajan, 2016); improving interpretability while at the same time minimizing information loss (Jolliffe and Cadima, 2016). It is a multivariate technique that allows summarizing the systematic patterns of variations in the data (Sanchez, 2012). Rotation of eigenvectors (factors) is essential to achieve a simple structure (Bryant and Yarnold, 1995), in which correlated traits to push-resistance follow the oblique rotation. Majority of the factor correlations exceed 0.32, which warrants greater than 10% overlap in variance among factors, enough variance to warrant oblique rotation (Tabachnick and Fiddell, 2013). The resulting factor loadings after Promax rotation satisfied the five requirements set by Thurstone (1947) to achieve a simple structure. The analysis resulted in 22 factors with a cumulative variance of 100%, in which six factors were retained based on the proportion of variance or eigenvalues greater than 1 to retain positive variability (Kaiser, 1960). The first six principal components accounted for 84.68% of the total variation among genotypes. The 1st factor had factor loading of 0.80-0.89 and set of the diameter of 1st to 5th internodes. The 2nd factor with a factor loading of 0.42-0.92 linked the length of 4th and 5th internode, culm length, and plant height. The 3rd factor associated panicle length, culm wall thickness, and number of adaxial and abaxial vascular bundles with factor loadings of 0.42-0.87. The 4th factor (0.45-0.74) connected push-resistance, leaf blade width, flag leaf width, and culm wall thickness. In the 5th factor, culm and panicle number per linear meter had factor loadings from -0.85 to -0.91, while in the 6th factor (0.71-0.89) leaf blade length, ligule length, and flag leaf length were integrated (Fig. 10).




*F1* factor 1, *D1IN* diameter of 1st internode, *D2IN* diameter of 2nd internode, *D3IN* diameter of 3rd internode, *D4IN* diameter of 4th internode, *D5IN* diameter of 5th internode, *F2* factor 2, *CL* culm length, *L4IN* length of 4th internode, *L5IN* length of 5th internode, *PHT* plant height, *F3* factor 3, *AAB* area of abaxial vascular bundle, *CWT* culm wall thickness, *NAD* number of adaxial vascular bundle, *PANL* panicle length, *F4* factor 4, *FLW* flag leaf width, *LBW* leaf blade width, *PR* push resistance, *F5* factor 5, *NPLM* number of panicle per linear meter, *PNLM* panicle number per linear meter, *CNLM* culm number per linear meter, *F6* factor 6, *FLL* flag leaf length, *LBL* leaf blade length, *LGL* ligule length

**Figure 10**. PCA Biplot of push-resistance and 21 correlated traits showing six groups after PROMAX rotation: **A.** Distribution of factors in dimension 1 and 2; **B.** Projection of factors in dimensions 3 and 4; **C.** Dissimilarity of factors in dimensions 5 and 6.

Through PCA, the contributions of the diameter of internodes from the basal (1$^{st}$ internode) to the terminal (5$^{th}$ internode) in a lodging-tolerance trait were highlighted. The ideal plant height and the distributions of its length in the culm, 4$^{th}$ internode, and 5$^{th}$ internode were expressed in lodging-tolerance. The compress data identified the closeness of panicle length to anatomic traits in the culm wall thickness, and number of abaxial and adaxial vascular bundles, which are related to the lodging-tolerance trait.

It is essential to identify levels of similarity and the dissimilarity of rice genotypes and determine its levels of tolerance to lodging. Cluster analysis method allows assembling similar items belonging to the same class (XLSTAT, 2017) and partitions the data sets into subsets according to its Euclidian distance (Lee et al., 2012; Madhulatha, 2012). Push-resistance and correlated traits data were centered, reduced, and truncated to avoid group scaling effects and show entropy based homogenous groups. Forty-three rice genotypes were grouped using Ward's method in Euclidian distance (0.33), which divided the genotypes into four clusters: 1st cluster consisted of 9 genotypes, 2nd cluster with 13 test entries, 3rd cluster with four accessions, and 4th cluster with 17 cultivars (Fig. 11). Cluster III obtained highest mean values of push-resistance, leaf blade length and width, ligule length, flag leaf length and width, diameter of 1st to 5th internode, length of 4th and 5th internode, panicle and culm length, plant height, number of adaxial and abaxial vascular bundles and culm wall thickness with he lowest in terms of the number of culm and panicle per linear meter. Thus, cluster III became the Lt cluster, followed by clusters IV and II, while cluster I is the most Ls cluster. Cluster III was composed of NSIC Rc 360 (genotype 33), NSIC Rc 396 (G36), PR45299-14-3-2-B (G12), and NSIC Rc 300 (G25) with average push-resistance of 0.98 kg cm$^2$.

Moreover, cluster IV with category of intermediate tolerance to lodging had mean push-resistance of 0.93 kg cm2, which included NSIC Rc 218 (G19), Rc 402 (G37), Rc 240 (G40), PR43405-10-2-3-3-B (G8), Rc 354 (G30), Rc 160 (G39), Rc 11 (G13), PR39142-10-3-2-1-1-B (G1), PR43426-13-2-3-2-B-B (G5), Rc 29 (G15), Rc 194 (G16), Rc 302 (G26), Rc 224 (G21), Rc 222 (G20), Rc 358 (G32), PR40432-14-2-1-B (G6), and PR43426-13-2-3-2-B-B (G10).

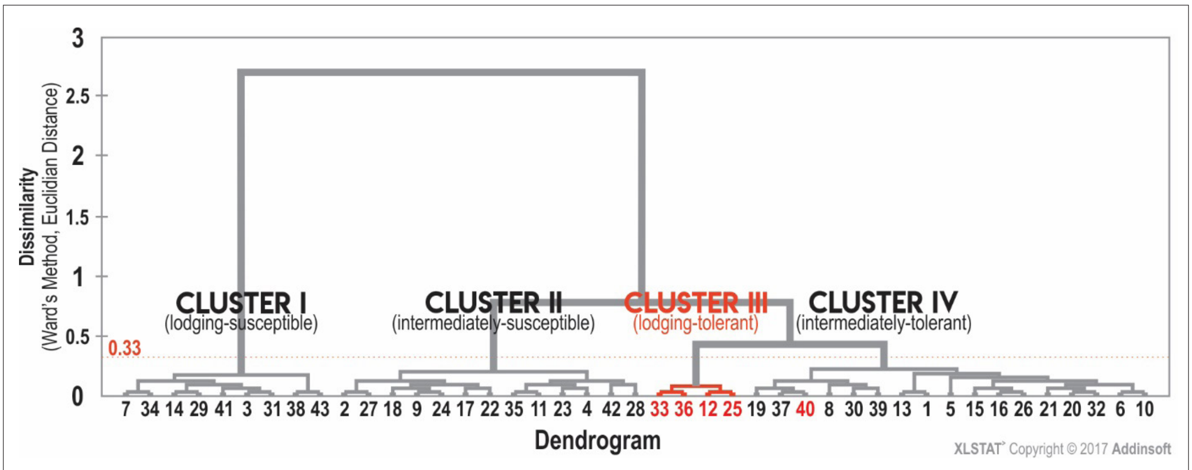



**Figure 11**. The distribution of 43 rice genotypes into four types of cluster based on push-resistance and correlated traits using Ward's method in Euclidian Distance.

In cluster IV, rice genotypes 6, 26, and 32 had low push-resistances of 0.81-0.82 kg $cm^2$ but was still included in this series. It is assumed that other unmeasured factors hindered its push-resistance expression. Cluster II (intermediate susceptibility, mean push-resistance of 0.85 kg $cm^2$) genotypes was composed of PR39149-33-1-3-3-1-B (G2), NSIC Rc 308 (G27), Rc 216 (G18), PR43425-25-2-1-1-1-B (G9), NSIC Rc 290 (G24), Rc 214 (G17), Rc 226 (G22), Rc 392 (G35), PR43433-21-2-1-1-1-B (G11), NSIC Rc 238 (G23), PR40334-61-1-1-B (G4), PSB Rc4 (G42), and NSIC Rc 324 (G28). PR434325-25-2-1-1-1-B (G9) had high push-resistance of 0.94 kg $cm^2$. However, G9 was considered an outlier as other rice traits excluded in the study could be possibly contributing to its lodging-tolerance; thus, warranting further investigation. Cluster I (susceptible, mean push-resistance of 0.82 kg $cm^2$) included PR40432-17-3-1-2-B-B (G7), NSIC Rc 390 (G34), Rc 25 (G14), Rc 352 (G29), Rc 298 (G41), PR39628-17-2-1-1-B (G3), NSIC Rc 356 (G31), PSB Rc 82 (G38), and Rc 14 (G43), which had lower push-resistance than the $L_s$ check, PSB Rc4 (G42). The dendrogram showed maximum Euclidian distance is present between cluster III and cluster I indicating two groups of rice genotypes with different levels of tolerance to lodging. Cluster analysis presents a complete view of the variation present among 43 rice genotypes and provides rice breeders a more consistent approach in improving genetic diversity and combination.

Furthermore, NSIC Rc 300, Rc 360, Rc 396, and PR45299-14-3-2-B were identified as Lt genotypes with high mean values of push-resistance (1.0 kg cm2), leaf blade length (43.4 cm), leaf blade width (1.4 cm), ligule length (19.6 mm), flag leaf length (31.8 cm), flag leaf width (1.7 cm), diameter of 1st internode (5.3 mm), diameter of 2nd internode (4.8 mm), diameter of 3rd internode (4.3 mm), diameter of 4th internode (3.7 mm), diameter of 5th internode (2.2 mm), length of 5th internode (33.6 cm), number of abaxial vascular bundle (24), culm wall thickness (608.5 µm), average values of length of 4th internode (17.3 cm), panicle length (24.8 cm), culm length (73 cm), plant height (97.8 cm), and number of adaxial vascular bundle (13).

## Conclusion

Preliminary investigation on the relationship of push-resistance, phenotypic traits, and anatomical characteristics showed information in increasing tolerance to lodging and improving screening techniques in the direct-seeded system. Based on results of the study, genotypes with a resistive force of 1.01-1.08 kg $cm^2$ had push-resistance comparable with NSIC Rc 240 and had better culm strength than PSB Rc4; indicating high tolerance to lodging during maturity. Rice genotypes with high lodging tolerance are supported by simultaneous increases in the leaf area (leaf blade, ligule, flag leaf), diameter of the stem (diameter of the $1^{st}$ to $5^{th}$ internodes), extension of the upper internodes (length of $4^{th}$ to $5^{th}$ internode, culm length, plant height and panicle length), and anatomical structures (number of abaxial and adaxial vascular bundles, culm wall thickness). It was noted that the increase in the number of culm has a negative effect on strength and elasticity of the stem as the number increases above 100 tillers per linear meter. Rice genotype with upright culm habit flag leaf, medium to dark green leaf blade, yellowish-green ligule color, partly-exserted panicle exsertion, and dense panicle secondary branching is a phenotype of a highly $L_t$ plant.

Studies to further support lodging should focus on the interaction of culm, leaf sheath, panicle weight, and root. A multi-environment testing sites should be conducted to assess the effect of environment to these traits, which can provide additionl information in breeding activites for the direct-seeded rice. Identification of these fundamental traits will hasten the development of lodging tolerant cultivars through conventional breeding for highly heritable desirable traits and even marker-assisted breeding.

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FULL PAPER

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# POLLEN FERTILITY AND YIELD PARAMETERS OF RICE (*Oryza sativa* L.) UNDER HIGH TEMPERATURE ENVIRONMENTS

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## Abtract

Development of new varieties with high temperature tolerance combined with desirable traits is important in addressing climate change impact in rice production. Progeny derived from crossing high-yielding varieties with heat-tolerant donors were evaluated to determine the effect of high temperature on pollen fertility, yield, and yield components. The study was conducted in two locations with varying temperature regimes, Los Baños (LB) and Nueva Ecija. The experiment in Nueva Ecija was established in the field (NE-F) and glasshouse (NE-GH). Maximum temperatures ranged 35.43-38.40ºC that generally reduced pollen fertility by as much as 40% in susceptible entries. Genotypes with 80% pollen fertility beyond 38°C were identified. Genotypes with stable pollen fertility were: PR44505A12-1-3, PR42222-3-1, and PR42222-20-3. The genotypes exhibited high percent pollen fertility (PPF) of more than 80% similar to the tolerant check variety N22. PR44500B17-3-3 and PR44505A19-27-1 had high (>80%) PPF in LB and NE-F. In terms of grain yield, 12 genotypes were consistent high yielders across locations. The number of panicles $m^{-2}$ was reduced under maximum temperatures of 33ºC and higher during vegetative stage. Other yield components such as spikelets $panicle^{-1}$, percent grain filling, and 1000-seed weight were not affected by high temperature although some genotypes were identified to perform under high temperature of more than 35ºC; thus, they were included in the selection. Three more genotypes, PR44505A14-15-2, PR42222-3-2, and PR44505A26-5-3, were selected as potential heat-tolerant materials for evaluation and improvement.

**Keywords:** *High Temperature, Pollen Fertility, Yield, Yield Component.*

## Introduction

Climate change brought about by gradual global warming is currently one of the major concerns in agricultural production. The earth's temperature is predicted to increase by an average of 2-4ºC by the end of the 21st century (IPCC, 2007) due to both anthropogenic and natural factors (Eitzinger et al., 2010). In the Philippines, records at International Rice Research Institute (IRRI) showed that annual mean maximum and minimum temperatures have increased by 0.35°C and 1.13°C, respectively, in 1979-2003 (Peng et al., 2004). The major contributing factor to this increase in temperature is the emission of greenhouse gases (GHG) such as: carbon dioxide (CO2), methane (CH4), and nitrous oxide (N2O) (Maraseni et al., 2009; Smith and Olesen, 2010).

The temperature increase due to global warming could enhance agriculture production in some temperate countries, while reduction would probably be felt in most Asian countries (Rosenzweig and Parry, 1994) including the Philippines where current temperature is close to optimum for rice productivity (Zhang et al., 2013; Aghamolki et al., 2014), and further increase in the temperature would be detrimental to yield. Future rice will be grown in much warmer environments (Battisti and Taylor, 2009) with a greater likelihood of high temperatures coinciding with heat-sensitive processes during the reproductive stage. Nearly half of the world's population depends on rice, and an increase in rice production by 0.6-0.9% annually until 2050 is needed to meet the demand (Carriger and Vallee, 2007). One of the most effective ways of mitigating the effect of climate change is the deployment of high temperature-tolerant cultivars (Manigbas and Sebastian, 2007). In breeding stress tolerant crops, information on the effect of temperature to rice growth and development is necessary (Wahid et al., 2007). At organismic level, it is important to study the effect of temperature on rice yield through its pollen behavior and response of yield parameters.

Several researchers have reported detailed information on the effect of high temperature during vegetative (Yoshida 1978; Yoshida 1981), reproductive, and anthesis stage (Jagadish et al., 2007; Jagadish et al., 2010) and grain formation

(Morita, 2004) of rice. Previous studies reported the development of heat tolerant lines using N22 as donor parent, which is one of the most heat tolerant genotypes in chamber and field experiments (Jagadish et al., 2010; Ye et al., 2012; Poli et al., 2013; Manigbas et al., 2014). Breeding for heat tolerance in rice at the Philippine Rice Research Institute (PhilRice) started in 2010 in collaboration with other countries in Southeast Asia. This partnership aimed to develop heat-tolerant rice, establish a protocol for screening heat-tolerant genotypes, and identify location specific varieties that can adapt and tolerate heat stress (Manigbas et al., 2014). Despite these efforts, heat-tolerant varieties are yet to be released in the Philippines.

This study was conducted to quantify the pollen fertility and yield formation of backcross inbred lines (BILs) generated from the cross combination between popular Philippine and Korean cultivars (recurrent parents) and heat tolerant donors in response to high temperature. It is hypothesized that high amount of pollen grains dehisced to stigma determines the capability of the rice plant to tolerate high temperatures during flowering. Information generated in this area will be helpful in understanding the mechanism of high temperature tolerance and identification of key traits to select in breeding programs lines. Additionally, identified lines from the cross between a widely-grown, high-yielding varieties and heat tolerant donors will be used as an important genetic source in developing heat tolerant varieties for Philippine condition.

## Materials and Methods

### *Time and Place of Study*

The study was conducted from January to May 2014 in two locations representing two temperature regimes: Science City of Muñoz, Nueva Ecija and Los Baños, Laguna. The experiment in Nueva Ecija was established in two separate set-ups: in the field (NE-F) and glasshouse (NE-GH). The entries were grouped based on their number of days to flowering stage and were seeded to coincide with the occurrence of high temperatures. Grospe et al. (2016) conducted similar study on screening QTL for high-temperature tolerance in rice.

### *Genotypes Tested in the Experiment*

Progenies of three populations derived from the method described in Figure 1 were tested. The progenies were generated by crossing a local popular rice variety NSIC Rc 160 and introduced South Korean varieties *Hanareumbyeo* and *Gayabyeo* as recurrent parents with heat-tolerant varieties N22 and Dular as donors' parents for heat tolerance. NSIC Rc 160 is a widely grown *indica* variety in the Philippines while *Hanareumbyeo* and *Gayabyeo* are Tongil type (92% *indica* and 8% japonica background) varieties from South Korea. The recurrent parents were identified as susceptible to high temperature particularly at reproductive stage. Tolerant (N22 and Dular), intermediate (IR64 and Milyang 23), and susceptible (IR52) genotypes to high temperature were used as check varieties including the parents of each population. The progeny designation of each individual line is presented in Table 1.

### *Field Experiments*

Field experiments in Nueva Ecija (NE-F) and Los Baños (LB) were established in 2014 dry season (DS). Fifteen plants per entry were transplanted using one seedling per hill at 20 cm x 20 cm spacing. The experiment was laid out in randomized complete block design (RCBD) with four replications. The temperature and relative humidity in Nueva Ecija were measured at 1 m above the canopy and within-canopy layer every 2 min for 24 h daily and 7 days a week using an automated Micrometeorological Instrument for Near Canopy Environment of Rice (MINCER). Data on temperature and relative

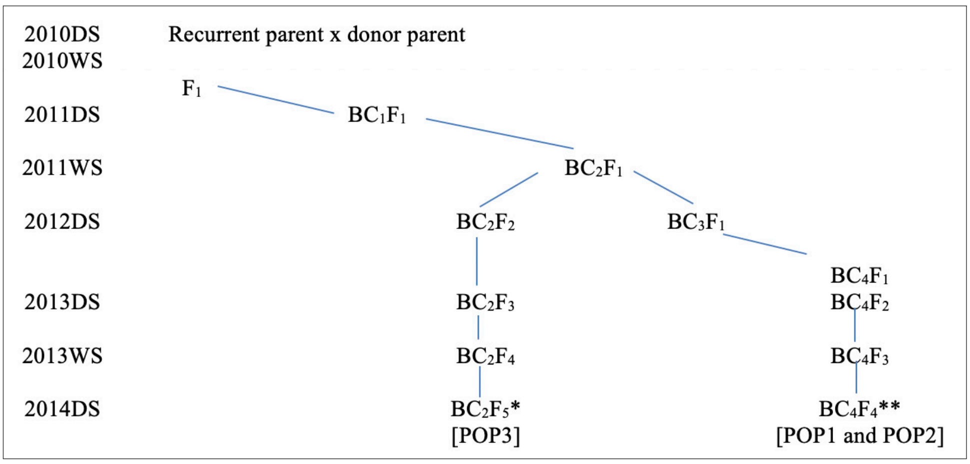


**Figure 1.** Composition of the pedigree nursery of the backcross inbred lines (BILs) tested in the study. (POP1-NSIC Rc160/Dular; POP2-Hanareumbyeo/N22; POP3-Gayabyeo/N22). (Manigbas et al., 2014).

**Table 1**. Progeny designation of the lines tested in the experiment.

| | POP1 NSIC Rc 160/ Dular | POP2 Hanareumbyeo/ N22 | POP3 Gayabyeo/ N22 |
|---|---|---|---|
| 1 | PR44500A21-5-2 | PR44505A12-1-3 | PR42222-2-1 |
| 2 | PR44500A21-6-2 | PR44505A12-4-1 | PR42222-2-2 |
| 3 | PR44500A21-6-3 | PR44505A12-8-1 | PR42222-2-3 |
| 4 | PR44500A21-7-1 | PR44505A14-11-1 | PR42222-3-1 |
| 5 | PR44500A6-2-1 | PR44505 | PR42222-3-2 |
| 6 | PR44500A6-4-3 | PR44505A14-15-1 | PR42222-7-1 |
| 7 | PR44500A6-5-2 | PR44505A14-15-2 | PR42222-7-2 |
| 8 | PR44500A6-5-3 | PR44505 | PR42222-33-3 |
| 9 | PR44500A8-2-1 | PR44505A16-14-1 | PR42222-18-3 |
| 10 | PR44500A8-3-3 | PR44505A16-14-2 | PR42222-20-1 |
| 11 | PR44500B13-10-2 | PR44505A16-5-2 | PR42222-20-2 |
| 12 | PR44500B13-10-3 | PR44505A16-7-2 | PR42222-20-3 |
| 13 | PR44500B15-5-1 | PR44505A19-21-1 | PR42222-21-1 |
| 14 | PR44500B17-2-1 | PR44505A19-22-2 | PR42222-21-2 |
| 15 | PR44500B17-3-3 | PR44505A19-24-1 | PR42222-21-3 |
| 16 | PR44500B17-5-2 | PR44505A19-25-2 | PR42222-25-1 |
| 17 | PR44500B4-10-2 | PR44505A19-27-1 | PR42222-25-2 |
| 18 | PR44500B4-3-1 | PR44505A26-5-2 | PR42222-33-1 |
| 19 | PR44500B4-6-1 | PR44505A26-5-3 | PR42222-33-2 |
| 20 | PR44500B9-2-2 | PR44505-B738(5) | PR42222-33-3 |

humidity in Los Baños were collected from the PAGASA Agro-meteorological Station located at Pili Drive, UPLB Campus, Los Baños, Laguna. Standard land preparation operations for lowland irrigated rice were conducted. Recommended fertilizer application rates for Nueva Ecija (120N, $60P_2O_5$, $60K_2O$) and Los Baños (140N, $40P_2O_5$, $40K_2O$) per hectare bases were used. Optimum control on pests and diseases were implemented.

### *Glasshouse Experiment*

The glasshouse experiment in Nueva Ecija (NE-GH) was laid out in RCBD with four replications with 10 plants per entry planted at one plant per hill using 20 cm x 20 cm spacing. The temperature and relative humidity were measured using MINCER. In glasshouse experiment, wooden plant boxes measuring 267 cm x 77 cm were prepared and filled with soil. Water depth of 3-5 cm was maintained until the hard dough stage. Optimum control on pests and diseases were implemented.

### *Pollen Microscopic Observation*

PPF was determined using the protocol as described in the Standard Evaluation System (SES). Five plants were randomly selected from each entry. Main tiller spikelets were collected and fixed in 70% ethyl alcohol. Pollen grains were stained with 1% iodine potassium iodide ($I_2KI$) solution. Stained pollens were considered as fertile while the unstained were recorded as sterile.

### *Yield and Agronomic Parameters*

Five plants per entry were randomly selected for the measurement of yield components such as the number of panicles per hill, number of filled and unfilled spikelets per panicle, and 1000-seed weight (g). Grain yield per plant (g $m^{-2}$) was determined by weighing the dried grains from 5 randomly selected plants from each entry and adjusted to 14% moisture content. Yield component parameters were taken from the following: (1) number of panicles per hill, (2) number of spikelets per panicle, (3) percent filled spikelets, and (4) 1000-seed weight (g). Grain yield per plant (g $m^{-2}$) was estimated by sampling five hills from each entry adjusted to 14% moisture content. Data were analyzed using the Statistical Tool for Agricultural Research version 2.0.1. Treatment means were compared using Tukey's Honest Significant Difference at 5% level. Analysis was done using Pearson correlation.

## Results

### *Temperature During Flowering*

The temperature (minimum, maximum, and mean) during flowering in different locations is presented in Table 2. Each site exhibited varying maximum temperatures ranging from 35.4 ± 0.64 to 38.4 ± 1.10°C. The maximum temperature under LB condition was lower (35.4ºC) than NE-F (36.5). The maximum temperature in NE-GH was 38.4ºC. Overall, the variation in maximum temperature during flowering was around 1°C.

**Table 2.** Minimum, maximum, and mean temperature in different experimental sites during flowering. 2014 dry season.

| Location | Temperature (°C) | | |
|---|---|---|---|
| | Minimum | Maximum | Mean |
| Los Banos - Field (LB)* | 24.19 ± 0.94 | 35.43 ± 0.64 | 29.84 ± 0.70 |
| Nueva Ecija - Field (NE-F)** | 24.09 ± 1.00 | 36.53 ± 0.84 | 29.43 ± 0.68 |
| Nueva Ecija - Glasshouse (NE-GH)** | 24.79 ± 0.56 | 38.40 ± 0.90 | 29.69 ± 0.89 |

*PAGASA Agro-meteorological Station Los Baños, Laguna
**MINCER 1 m above canopy Muñoz, Nueva Ecija

### *Relationship of Pollen Fertility and Temperature*

PPF of the progeny derived from different populations in response to maximum, minimum, and mean temperatures during flowering was quantified. The relationship between PPF with maximum temperature and minimum temperatures was negative and significant (Table 3). On the other hand, PPF had no significant relationship with the mean temperature.

**Table 3.** The relationship between temperature (minimum, maximum, and mean) and pollen fertility.

| Parameter | Pollen Fertility |
|---|---|
| Pollen fertility | - |
| Minimum temperature | -0.248* |
| Maximum temperature | -0.590** |
| Mean temperature | -0.072ns |

The maximum temperature during the reproductive stage under the field (LB and NE-F) and screen house (NE-GH) was plotted against the PPF in each of the breeding lines (Figure 2). The results generally showed a negative response of PPF to increasing maximum temperatures at a temperature range 34-39°C. Some lines had 80% pollen fertility at maximum temperature greater than 38°C indicating high temperature tolerance. The lines showed PPF reduction by 37% in NE-GH relative to LB. This reduction was attributed to an increase of more than 2°C in the maximum temperature in NE-GH. About 5% decrease in PPF was also observed in NE-GH relative to NE-F when the difference in maximum temperatures increased to 1.8ºC (Table 4). Progenies of the cross between Hanareumbyeo/N22 had the highest mean pollen fertility (54.6%) among populations under NE-GH. In LB and NE-F, progenies of Gayabyeo/N22 had the highest PPF at 75.1 and 80.2%, respectively.

### *Performance of Checks and Genotypes with Stable PPF under High Temperature*

In general, high PPF was observed in tolerant and intermediate check varieties (i.e., source of genes for high temperature tolerance); N22, Dular, IR64, and Milyang 23 under different maximum temperature in LB, NE-F, and NE-GH. The susceptible check, IR52, showed intolerant to high temperature under three environments (Table 4).

Several genotypes from each population were identified to have a stable PPF across locations with different maximum temperature regimes (Table 5). These lines were PR44505A-12-1, PR42222-3-1, and PR42222-20-3, which showed high PPF ranging 80-

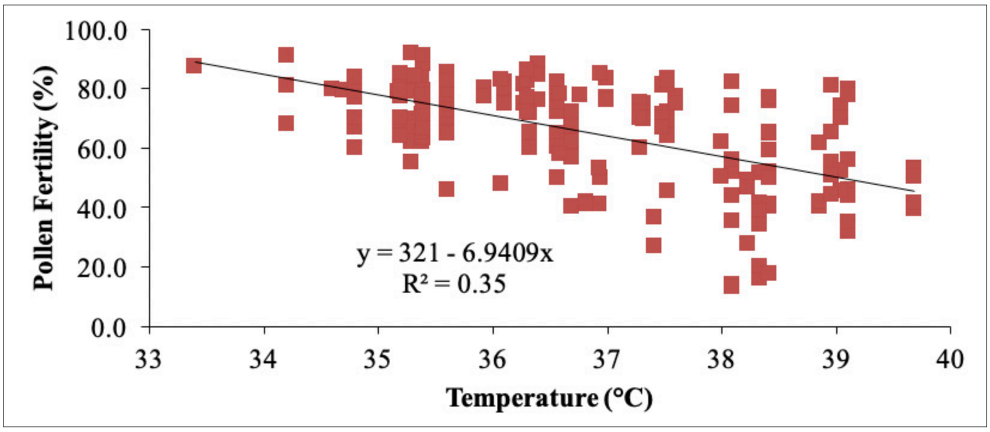


**Figure 2.** The relationship between pollen fertility (%) and maximum temperature dynamics during the reproductive stage.

**Table 4.** The percent pollen fertility of three BILs populations under different maximum temperature regimes.

| Genotype | Cross/Parent | LB | NE-F | NE-GH |
|---|---|---|---|---|
| POP1 | NSIC Rc 160*4/Dular | 72.4 ± 9.6$^{de}$ | 67.5 ± 11.7$^{bc}$ | 41.7 ± 16.2$^{d}$ |
| POP2 | Hanareumbyeo*4/N22 | 76.5 ± 6.5$^{bcd}$ | 68.0 ± 8.9$^{bc}$ | 54.6 ± 16.8$^{bc}$ |
| POP3 | Gayabyeo*2/N22 | 80.5 ± 5.6$^{abc}$ | 75.5 ± 7.8$^{ab}$ | 48.9 ± 15.6$^{cd}$ |
| NSIC Rc 160 | Parent | 84.0$^{abc}$ | 85.0$^{a}$ | 41.0$^{d}$ |
| Hanareumbyeo | Parent | 75.0$^{cd}$ | 75.0$^{ab}$ | 62.0$_{b}$ |
| Gayabyeo | Parent | 85.0$^{ab}$ | 80.3$^{a}$ | 26.8$^{e}$ |
| N22 (03911) | Tolerant | 87.5$^{a}$ | 80.0$^{a}$ | 83.0$^{a}$ |
| Dular | Tolerant | 79.8$^{abcd}$ | 79.0$^{a}$ | 77.8$^{a}$ |
| IR64 | Intermediate | 77.8$^{bcd}$ | 77.2$^{ab}$ | 78.3$_{a}$ |
| Milyang 23 | Intermediate | 76.8$^{bcd}$ | 74.8$^{ab}$ | 57.7$_{b}$ |
| IR52 | Susceptible | 64.8$^{e}$ | 60.0$^{c}$ | 45.3$_{d}$ |

**Table 5.** List of genotypes identified to show stable percent pollen fertility across locations. 2014 dry season.

| Genotype | Cross/Parent | Pollen Fertility (%)* | | |
|---|---|---|---|---|
| | | LB | NE-F | NE-GH |
| PR44500B15-5-1 | NSIC Rc 160/Dular | 72.3 k-r | 72.0 g-k | 74.0 de |
| PR44505A12-1-3 | Hanareumbyeo/N22 | 84.8 a-f | 82.0 bc | 82.0 ab |
| PR44505A19-24-1 | Hanareumbyeo/N22 | 75.4 i-o | 72.0 g-k | 70.2 ef |
| PR42222-3-1 | Gayabyeo/N22 | 81.0 c-j | 81.0 b-e | 79.7 a-d |
| PR42222-20-3 | Gayabyeo/N22 | 81.5 c-j | 81.0 b-e | 81.0 a-c |
| PR44500B17-3-3 | NSIC Rc 160/Dular | 83.8 a-f | 83.0 bc | 75.7 c-e |
| PR44505A12-4-1 | Hanareumbyeo/N22 | 70.0 n-s | 64.0 n-q | 65.2 fg |
| PR44505A14-15-2 | Hanareumbyeo/N22 | 70.0 n-s | 69.0 i-n | 64.7 f-h |
| PR44505A19-27-1 | Hanareumbyeo/N22 | 91.3 a | 83.0 bc | 77.0 b-d |
| NSIC Rc 160 | Parent | 84.0 a-f | 75.0 e-i | 41.0 v-z |
| Hanareumbyeo | Parent | 75.0 h-o | 80.0 b-f | 62.0 g-i |
| Gayabyeo | Parent | 85.0 a-f | 80.0 b-f | 26.7 g-i |
| N22 | Tolerant | 87.5 a-d | 85.0 b | 83.0 a |
| Dular | Tolerant | 79.8 d-k | 79.0 b-f | 77.7 a-d |
| IR64 | Intermediate | 77.8 f-n | 77.0 c-g | 78.2 a-d |
| Milyang 23 | Intermediate | 76.8 g-n | 75.0 f-j | 57.7 j-l |
| IR52 | Susceptible | 64.8 r-t | 60.0 q-s | 45.2 r-w |

*In a column, means followed by the same letter/s are not significantly different at 5% level by HSD.

84% and within same PPF level of the most tolerant check variety, N22, under field and glasshouse conditions. Other lines, which showed high PPF at high maximum temperatures were PR44500B17-3-3 and PR44505A19-27-1 both for LB and NE-F. Most of the genotypes that showed high PPF across locations were derived from the cross of Hanareumbyeo/N22, Gayabyeo/N22, and NSIC Rc 160/Dular.

### *Selection Based on Grain Yield*

The mean plant grain yield (g m$^{-2}$) taken from five plants of each population is summarized in Table 6. Progenies from each population with the highest yield from each location were selected based on the mean performance of the genotypes at each site. The site with the highest average yield was the field set-up at NE-F (641 m m$^{-2}$) followed by LB (491 g m$^{-2}$) and NE-GH (292 g m$^{-2}$). Based on the means, yield level of genotypes selected were set at 600 g m$^{-2}$ for NE-F, 500 g m$^{-2}$ for LB, and 300 g m$^{-2}$ for NE-GH. Only the genotypes listed as top yielders across three locations were considered, and corresponding pollen fertility of each selected line is summarized. Twelve lines were selected from the 60 evaluated genotypes. The selected materials were consistently the top performers across locations with yields ranging 505.5-642.2 g m$^{-2}$ in LB, 602.1-777.9 g m$^{-2}$ in NE-F, and 301.6- 392.2 g m$^{-2}$ in NE-GH. It was observed that among the top yielders in LB and NE-F, only five entries from each location had a pollen fertility of greater than 80%, only one entry was identified to have pollen fertility of 80% in NE-GH. Under field conditions of Los Baños and Nueva Ecija, Pollen Fertility (PF) ranged 64-81%, while the tested genotypes/lines inside the glasshouse had PF as low as 27%. Only PR42222-20-3 had more than 80% PF across locations.

**Table 6.** Mean plant grain yield (g m$^{-2}$) of breeding populations, parents and check varieties across location. 2014 dry season.

| Genotype | Plant Grain Yield (g m$^{-2}$) | | |
|---|---|---|---|
| | LB | NE-F | NE-GH |
| POP1 | 437.2 | 619.5 | 373.9 |
| POP2 | 479.6 | 655.7 | 274.5 |
| POP3 | 557.5 | 650.6 | 228.6 |
| Mean | 491.0 | 641.0 | 292.0 |
| NSIC Rc 160 | 387.0 | 605.9 | 217.0 |
| Hanareumbyeo | 545.1 | 696.2 | 217.2 |
| Gayabyeo | 537.2 | 609.3 | 261.3 |
| N22 (03911) | 322.4 | 424.6 | 291.2 |
| Dular | 270.6 | 451.0 | 198.6 |
| IR64 | 483.0 | 580.0 | 233.8 |
| Milyang 23 | 257.7 | 280.2 | 227.5 |
| IR52 | 116.6 | 244.9 | 146.4 |

### *Relationship Between Plant Grain Yield and Pollen Fertility*

There was a positive correlation between pollen fertility (%) and plant grain yield (g m$^{-2}$) of the selected lines (Figure 3). It was noted that entries with the highest pollen fertility were not necessarily the top yielders although PR42222-3-1 performed consistently across location in terms of pollen fertility and yield. Many of the selected lines, which exhibited 80% pollen fertility had grain yield of more than 500 g m$^{-2}$. Lines with less than 60% fertility generally yielded around 300 g m$^{-2}$.

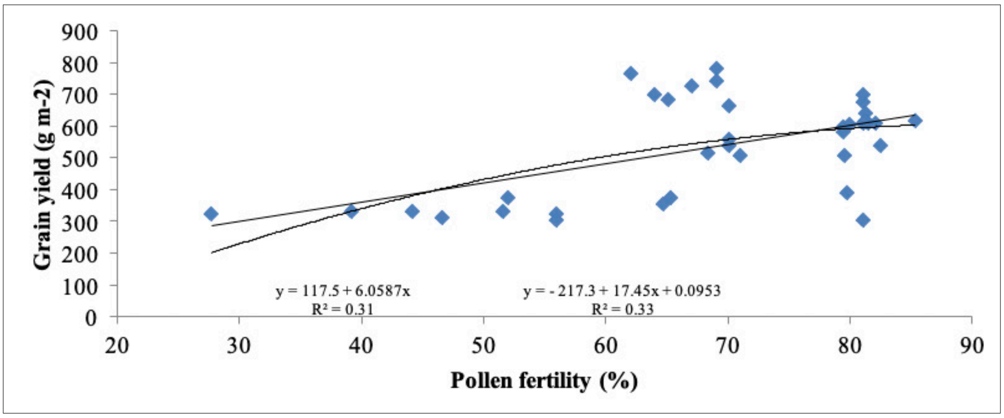


**Figure 3**. Relationship between pollen fertility and plant grain yield obtained from selected rice genotypes that flowered under maximum temperature of 35-39°C.

***Yield Components as Affected by Temperature***

Temperatures during the critical stage affecting specific yield component were recorded. The selection parameters below were made for each yield component based on the response of the genotypes to maximum temperature. The selection of the genotypes based on panicle $m^{-2}$ was done at maximum temperature ranging 31-33°C and 33-35°C. For the other yield components such as spikelets $panicle^{-1}$, percent grain filling, and 1000-seed weight, selection was done under maximum temperature range of 35-38°C and 38-39°C.

***Number of Panicles*** $m^{-2}$. The number of panicles per $m^{-2}$ decreased at maximum temperature 31-34°C (Figure 4). From 31–33°C, some genotypes had more than 500 panicles $m^{-2}$ while most of the entries had 300-400 panicles $m^{-2}$. Temperature beyond 33°C greatly reduced the number of panicles to 100-200. Yoshida (1981) reported that the threshold temperature during tillering is 32°C and reduction of tillers can be observed beyond this point. Most of the lines had 250-500 panicles per $m^{-2}$ at temperatures between 26–27°C. The same trend of response from the mean and maximum temperatures was observed.

Genotypes (Figure 4) having at least 500 panicles $m^{-2}$ (boxed) under temperatures 31-33°C were selected. Most of the selected genotypes were progenies of Gayabyeo/N22. Among the selected 14 genotypes, PR42222-33-2 had the highest number of panicles $m^{-2}$ (699), which was relatively higher than the tolerant check varieties. PR44505A-14-15-2 and PR44505A-11-1, progenies of Hanareumbyeo/N22, had around 550 panicles $m^{-2}$. All of the selected lines were under NE-F condition. Genotypes with more than 150 panicles $m^{-2}$ were also selected under temperatures 33-35°C (encircled in Figure 4). Half of the 18 selected genotypes were progenies of the cross Hanareumbyeo/N22. PR44500A21-5-2 and PR42222-3-2 showed the highest number of panicles $m^{-2}$ under this condition. The selected genotypes had higher number of panicles $m^{-2}$ than the check tolerant varieties. The selected genotypes were all under glasshouse condition.

***Spikelets Panicle***$^{-1}$. The number of spikelets per panicle reduced as temperature increased by 34–39°C (Figure 5). Several genotypes were identified to have more than 250 spikelets per panicle at maximum temperatures ranging from 35 to 38°C (boxed in Figure 5). Beyond 38°C, the number of spikelets per panicle was reduced to <200. Genotypes that can produce >150 spikelets per panicle beyond 38°C may have tolerance to high temperature.

In Figure 5, genotypes with at least 250 spikelets per panicle at maximum temperature 35-38°C were selected. Relative to the tolerant check N22, three genotypes (PR44505A14-15-1, PR42222-33-3, and PR44500B-17-5-2) had higher spikelet number per panicle. Most of the genotypes selected were under NE-F condition. Under maximum temperatures of more than 38°C (encircled in Figure 5), genotypes with >150 spikelets per panicle were selected. PR42222-25-1 and PR42222-3-2 had the highest number of spikelets per panicle even they were exposed to temperature of >39°C. The selections under this condition had a relatively higher number of spikelets per panicle than the tolerant check. It was noted that check varieties such as Hanareumbyeo and IR64 had very low number of spikelets per panicle.

***Percent Grain Filling.*** Generally, period of grain filling is shortened by high temperature, which negatively affects this yield component (Yoshida, 1981). Percent grain filling generally shows a negative trend in relation to mean and maximum temperature. Several lines had 85-90% grain filling at maximum temperature of 35-38°C. Several lines were also identified to have grain filling of more than 75% beyond 38°C. Genotypes with more than 85% grain filling were selected at 35-38°C (boxed in Figure 5). Selected genotypes were exposed to up to 38°C under the different locations. More than 20 genotypes,

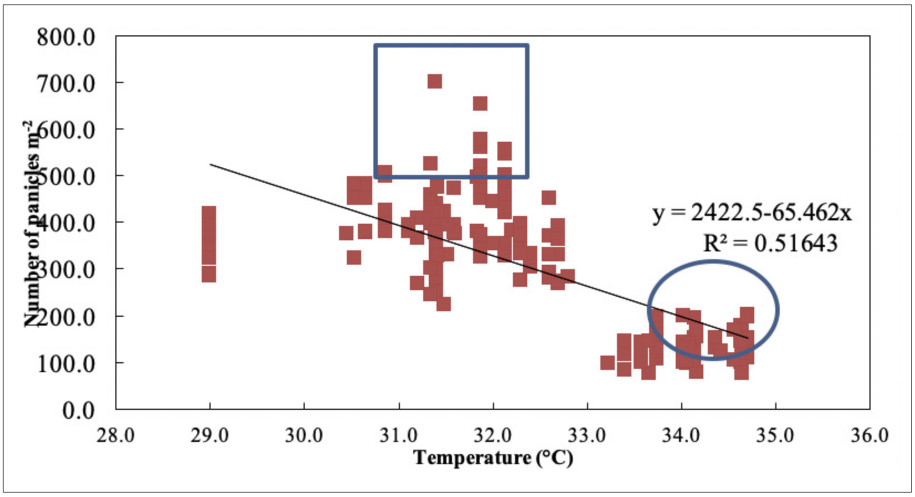


**Figure 4.** The number of panicles as affected by mean and maximum temperature during vegetative stage.

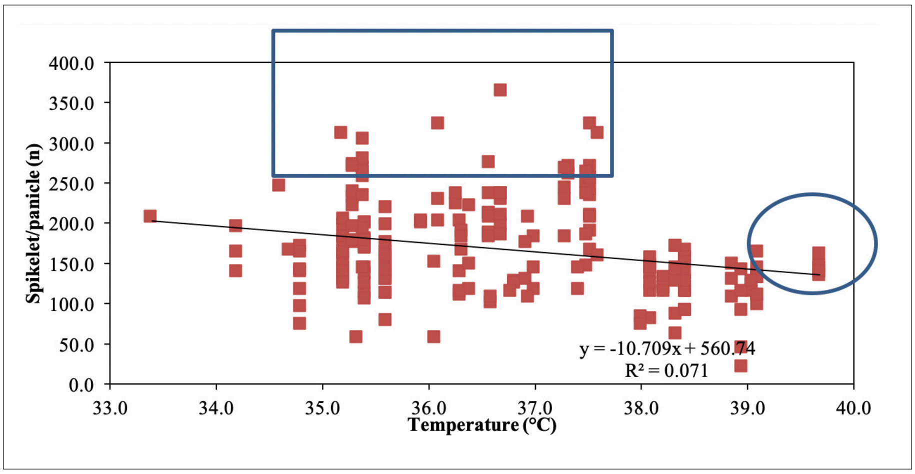


**Figure 5.** Spikelets panicle-1 as affected mean and maximum temperature during the reproductive stage.

composed of progenies with N22 were selected. PR44500A7-5-3 from NSIC Rc 160/Dular cross had the highest grain filling at 91.4% among the selections even at 36.4°C (under LB condition). It was observed that this genotype had more than 85% grain filling under field and glasshouse conditions. Two genotypes, PR44505A14-15-2 from Hanareumbyeo/N22 cross and PR42222-3-1 (Gayabyeo/N22), also showed high percentage of grain filling in different locations, while other genotypes had high grain filling in only one site. These lines have the potential to exhibit high percent grain filling under different high temperature environments.

Grain filling decreased by 75% at maximum temperatures >38°C. Even at high temperature, grain filling of PR42222-25-1 was 92%, relatively higher than N22 and Dular under NE-GH condition. Grain filling of the susceptible check was 85.4%. Other genotypes, PR44500B4-10-2, PR44505A19-22-2, PR44505A19-24-1, and PR44505A16-5-2 had grain filling of more than 80%, which is comparable with the tolerant checks, even at 39°C. Intermediate checks had a grain filling percentage of more than 75% under NE-F and NE-GH condition including the parent check Hanareumbyeo.

***1000-seed Weight Generally.*** 1000-seed weight is a very stable varietal trait. The influence of mean temperature is more noticeable than maximum temperature. Some genotypes had 1000-seed weight of more than 25 g at 35-38°C. A downward trend was observed after 38°C, but some genotypes were found to have at least 21 g of 1000 seed weight up to nearly 39°C. Around 25 genotypes had more than 25 g 1000 seed weight with almost half of the selected entries progenies of Hanareumbyeo/N22. Most of the selected entries were under NE-F while 7 entries were considered under LB. Only PR44505A26-5-2 was chosen under NE-GH condition. PR44505A8-3-3 (NSIC Rc 160/Dular) and PR42222-21-1 (Gayabyeo/N22) had the highest grain weight at 28.4 and 28.1 g, respectively.

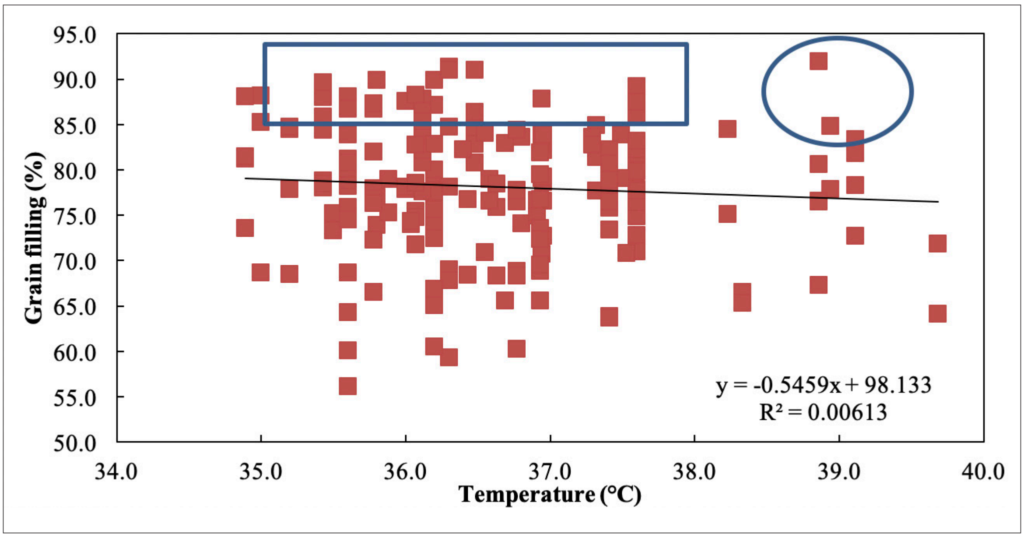


**Figure 6.** Percent grain filling as affected by mean and maximum temperature during maturity.

Genotypes selected under 38°C (encircled in Figure 6) were identified. The 1000-seed weight was reduced from 25-21 g under this condition. Most of the genotypes selected were derived from the cross Hanareumbyeo/N22 under NE-GH experiment. PR44505A19-27-1 (Hanareumbyeo/N22) had the highest 1000 grain weight under this condition (24.1 g). PR44500B4-10-2 (NSIC Rc 160/Dular) and PR42222-20-1 (Gayabyeo/N22) had 1000-seed weight of 23.5 and 23.0 g, respectively. The rest of the selections had less than 22 g 1000-seed weight. Among the check varieties, Hanareumbyeo recorded the highest number of 1000-seed weight at 25.4 g, which was relatively higher than all of the entries selected under the defined condition. Although PR44505A19-24-1 and PR44505A19-22-2 recorded only 1000-seed weight of 21.9 and 20.6 g, respectively, these lines were subjected to more than 39°C.

## Discussion

Maximum temperature during flowering in different locations varied by 1°C with the lowest recorded at LB condition with 35.43°C. The highest maximum temperatures were recorded at NE-GH with 38.40°C.The minimum and mean temperature was almost similar in all locations. Maximum temperature highly influenced the pollen fertility of the rice lines. Minimum temperature to some extent has negative effect on PPF, while the mean temperature has no significant effect on the evaluated parameter. A negative response of the rice lines to increasing maximum temperatures was observed from 34 to 39°C. Some lines had 80% pollen fertility beyond 38°C, which have the potential to withstand high maximum temperatures. PPF reduction by 37% from LB to NE-GH were observed that can be attributed to an increase of more than 2°C in the maximum temperature. Under NE-GH, progenies of the cross between Hanareumbyeo/N22 had the highest mean pollen fertility among populations. Under field conditions, LB and NE-F lines derived from Gayabyeo/N22 had the highest PPF. Jagadish et al. (2007; 2010) reported that pollen was negatively affected when exposed to at least 35°C.

Genotypes identified at high PPF with 70-85% across locations under field conditions were PR44500B15-5-1, PR44505A-12-1-3, PR44505A19-24-1, PR42222-3-1, and PR42222-20-3. The parent checks had high PPF under LB condition but low at 18% under NE-GH condition. IR64, an intermediate check, had high PPF across locations. Most of the identified genotypes that showed high PPF across locations were derived from Hanareumbyeo/N22. Dular, also a heat-tolerant check, had same performance with N22 in terms of PPF.

Most of the selections with more than 80% pollen fertility were from the progenies of Gayabyeo/N22. N22 was reported to have greater tolerance to high temperature (Redona et al., 2007). PR42222-20-3 and PR42222-3-1 had high pollen fertility similar with N22 at temperature higher than 35°C. The population with N22 as heat tolerant parent had relative advantage over the population with Dular as another tolerant parent. PR44505A12-1-3 and PR42222-20-3 had more than 80% pollen fertility under glasshouse condition. Jagadish et al. (2007; 2010) reported that pollen was negatively affected when exposed to at least 35°C. However, this study focused only on the response of rice lines to maximum temperature. Other temperature conditions such as the minimum and mean temperatures were not considered. Although some studies reported that spikelet sterility increases in response to daily maximum temperature (Matthews

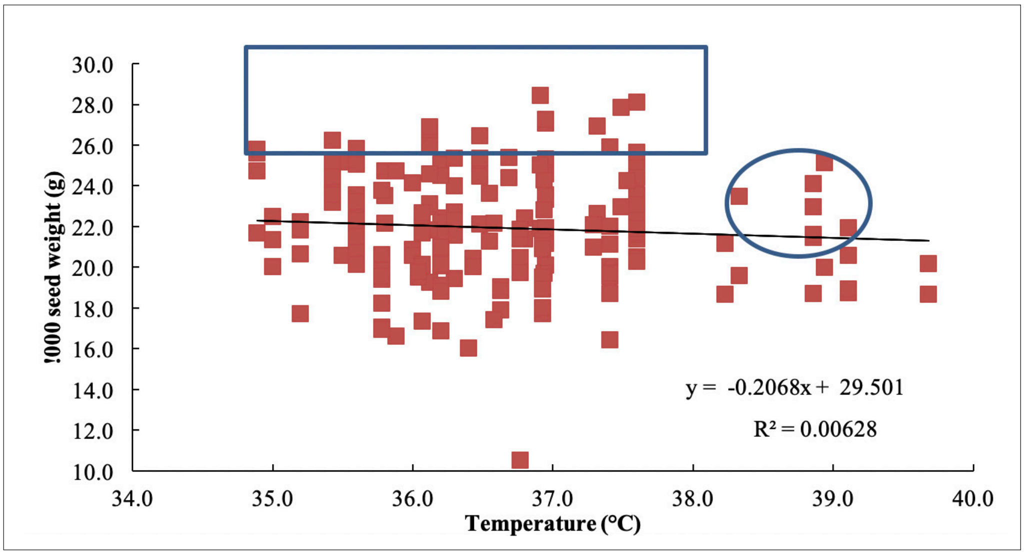


**Figure 7.** 1000-seed weight as affected by mean and maximum temperature during grain filling.

et al., 1995; Horie et al., 1996; Nakagawa et al., 2002), it is important to further investigate if the measured pollen fertility can be translated to crop productivity by analyzing yield and yield components.

The site with highest average yield was NE-F with 641 g $m^{-2}$ followed by LB with 491 g $m^{-2}$ and NE-GH with 292 g $m^{-2}$. Based on the means, yield level of genotypes selected was set at 600 g $m^{-2}$ for NE-F, 500 g $m^{-2}$ for LB, and 300 g $m^{-2}$ for NE-GH. Only genotypes listed as top yielders across three locations were considered. Twelve lines were selected from 60 genotypes. The selections were consistently the top performers across locations with yields ranging 505.5-642.2 g $m^{-2}$ in LB, 602.1-777.9 g $m^{-2}$ in NE-F, and 301.6- 392.2 g $m^{-2}$ in NE-GH condition. It was observed that among the top yielders at LB and NE-F, only five entries from each location had a pollen fertility of greater than 80%. One entry recorded a pollen fertility of 80% in NE-GH. Under field condition in Los Baños and Nueva Ecija, PF ranged 64-81%, while the genotypes inside the glasshouse had 27% PF. Only PR42222-20-3 had more than 80% PF across locations.

The relationship between PPF and grain yield (g $m^{-2}$) of the selected genotypes is generally positive. It was observed that entries with high pollen fertility were not necessarily top yielders except PR42222-3-1, which consistently showed high pollen fertility and yield across locations. Most of the selections with pollen fertility of at least 60% had grain yield of more than 500 g $m^{-2}$. Genotypes with less than 60% fertility generally yielded 300 g $m^{-2}$.

Temperatures during the critical stage at flowering, which affects yield were automatically recorded. Selection parameters were made for each yield component based on the response of the genotypes to maximum temperature. The selection of the genotypes based on panicle $m^{-2}$ was done at maximum temperature of 31-33°C and 33-35°C. For yield components such as spikelets panicle$^{-1}$, percent grain filling, and 1000-seed weight, selection was conducted under maximum temperature of 35-38°C and 38-39C.

The number of panicles per $m^{-2}$ decreased at temperatures 31-34°C. From 31–33°C, some genotypes had more than 500 panicles $m^{-2}$ at maximum temperature, while most of the entries clustered at 300-400 panicles $m^{-2}$. Maximum temperature beyond 33°C greatly reduced the number of panicles to 100-200. Yoshida (1981) reported that the threshold temperature during tillering is 32°C and reduction of tillers can be observed beyond this point.

A downward trend on the number of spikelets per panicle was observed from maximum temperatures of 34–39°C. Several genotypes were identified to have more than 250 spikelets per panicle at maximum temperatures ranging 35-38°C. Beyond 38°C, the number of spikelets per panicle was reduced to less than 200. Genotypes, which can produce more than 150 spikelets per panicle beyond 38°C, may have tolerance to high temperature.

Generally, the period of grain filling is shortened by high temperature, which negatively affects this yield component (Yoshida, 1981). Percent grain filling generally shows a negative trend in relation to mean and maximum temperature. Several lines had 85-90% grain filling at maximum temperatures 35-38°C. Several lines were also identified to have grain filling of more than 75% beyond 38°C. Genotypes with more than 85% grain filling were selected at 35-38°C. Grain filling decreased by 75% at temperatures beyond 38°C. Even at high temperature, grain filling of PR42222-25-1 was 92%, relatively higher than N22 and Dular under NE-GH condition. It was observed that the grain filling of the susceptible check was 85.4%.

Seed weight is generally a very stable varietal trait. The influence of mean temperature is more noticeable than maximum temperature on seed weight. Some genotypes had 1000-seed weight of more than 25 g at maximum temperature from 35-38°C. A downward trend was observed after 38°C, but some genotypes were found to have at least 21 g of 1000 seed weight at 39°C. Genotypes selected at 38°C were identified. The 1000-seed weight was reduced from 25 g to 21 g under this condition. Most of the genotypes selected were derived from Hanareumbyeo/N22 under NE-GH experiment. PR44505A19-27-1 (Hanareumbyeo/N22) had the highest 1000 grain weight under this condition with 24.1g. PR44500B4-10-2 (NSIC Rc 160/Dular) and PR42222-20-1 (Gayabyeo/N22) had 1000-seed weight of 23.5 g and 23. g, respectively.

Based on selection parameters (Table 7), PR44505A14-15-2 was the best genotype among the progenies in the three populations. It has the highest yield at NE-F and one of the best yielders in LB and NE-GH conditions. The number of panicles $m^{-2}$, percent grain filling, and 1000 seed weight were high. PR42222-3-2 was next in rank. This genotype, coming from Gayabyeo/N22 cross had 764 g $m^{-2}$ grain yield at NE-F with 500 and 300 g $m^{-2}$ yield in LB and NE-GH, respectively. The number of panicle $m^{-2}$, spikelet $panicle^{-1}$, and percent grain filling of this genotype were also high.

## Conclusion

The effects of high temperature on yield and yield component of genotypes were determined. Pollen fertility was reduced at temperatures between 35.4-38.4°C. Some genotypes however, exhibited 80% pollen fertility beyond 38°C indicating tolerance to tolerate high temperatures. Genotypes with stable pollen fertility were: PR44500B15-5-1, PR44505A12-1-3, PR44505A19-24-1, PR42222-3-1, and PR42222-20-3. These genotypes exhibited high percent pollen fertility from 72 to 85%, similar to the tolerant check varieties N22 and Dular. Other selections, which showed potential to maintain high PPF at high temperatures were PR44500B17-3-3, PR44505A12-4-1, PR44505A14-15-2, and PR44505A19-27-1. The parent checks, on the other hand, had high PPF under LB condition at 88%, but dropped to as low as 18% under high temperature NE-GH condition. IR64, the intermediate check, showed high PPF across locations. Twelve genotypes were found to be consistent high yielders. There was a decrease in the number of panicles $m^{-2}$ during the vegetative stage at temperatures 33-35°C.

Five genotypes were identified based on percent pollen fertility, yield, and yield components PR44505A14-15-2, PR42222-3-2, PR42222-3-1, PR42222-20-3, and PR44505A26-5-3, which are potential heat-tolerant breeding lines for further evaluation and improvement. These genotypes had higher pollen fertility and yield under high temperature conditions.

**Table 7**. List of selected genotypes based on pollen fertility, yield, and yield components.

| Genotype | Pollen Fertility (%) | | | Grain Yield $M^{-2}$ (g) | | | Panicle $M^{-2}$ | Spikelet $Panicle^{-1}$ | Grain Filling (%) | 1000-Seed Weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| | LB | CES | GH | $LB^a$ | $CES^b$ | $GH^c$ | | | | |
| HT12757 | 68.4 | 65.0 | 56.0 | 517.9 | 687.5 | 326.4 | | | | |
| HT13222 | 70.0 | 64.0 | 65.2 | 539.2 | 695.6 | 376.9 | | | + | |
| HT13255 | 71.0 | 67.0 | 51.5 | 506.3 | 728.8 | 332.0 | | + | | |
| HT13256 | 70.0 | 69.0 | 64.7 | 561.4 | 777.9 | 356.3 | + | | + | + |
| HT13410 | 81.3 | 69.0 | 52.0 | 642.2 | 743.1 | 376.5 | | + | | |
| HT13879 | 79.3 | 80.0 | 27.7 | 583.5 | 602.1 | 323.2 | | | | |
| HT13882 | 81.3 | 81.0 | 79.7 | 616.1 | 610.9 | 392.2 | + | | + | |
| HT13883 | 79.5 | 62.0 | 56.0 | 505.5 | 764.3 | 301.6 | + | + | + | |
| HT13905 | 81.5 | 81.0 | 81.0 | 608.8 | 678.8 | 306.3 | | + | + | |
| HT13909 | 79.3 | 70.0 | 39.2 | 599.2 | 665.3 | 330.5 | | | + | + |
| HT13910 | 85.3 | 81.0 | 44.2 | 615.3 | 695.0 | 335.9 | | | | |
| HT13914 | 82.5 | 82.0 | 46.5 | 536.2 | 611.2 | 309.4 | | + | | |
| Selection basis | | | | $^a$>500 g $m^{-2}$<br>$^b$>600 g $m^{-2}$<br>$^c$>300 g $m^{-2}$ | | | >500 panicles $m^{-2}$ at maximum temperature between 31-33°C | >200 spikelets $panicles^{-1}$ at maximum temperature between 35-38°C | >85% at maximum temperature between 35-38°C | >25 g at maximum temperature between 35-38°C |

Notes: Genotypes selected are the top yielders across locations with corresponding pollen fertility
(+) – Genotype attained the value of yield component specified in the last row
Genotypes were ranked based on yield performance across location and relative performance in terms of yield
Ranking – 1-highest-10-lowest

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FULL PAPER

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# INTERFERENCE OF *Commelina diffusa* BURM. F. ON RICE GROWTH AND YIELD

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## Abstract

An experiment was conducted from August to December 2018 at Central Luzon State University in Nueva Ecija, Philippines to determine the effects of *C. diffusa* (COMDI) interference on rice growth and yield under saturated condition. Plastic pots with transplanted rice (TPR) or direct-seeded rice (DSR) were cultivated with COMDI at 0, 1, 3, 5, and 7 densities. Each pot was replicated five times and arranged in RCBD. Agronomic, yield components, and grain yield (GY) of rice were recorded. All the data were subjected to ANOVA while treatments means were compared through LSD and TTEST at 5% level of significance. Correlation and simple linear regression were also used to relate and create predictive models for GY. COMDI significantly affected the agronomic, yield components, and GY of TPR and DSR. GY reductions at 1, 3, 5, and 7 COMDI densities were 5.4, 8.4, 17.2, and 22.4% for TPR, while 9.8, 18.6, 26.5, and 34% for DSR. Density and biomass of COMDI were strongly-negatively correlated to GY. The final predictive models were $GY_{TPR}$ = -0.8769 (COMDI density) + 27.866 and $GY_{TPR}$ = -0.0381 (COMDI shoot dry weight) + 28.394 for TPR; $GY_{DSR}$ = -1.2985 (COMDI density) + 27.248 and $GY_{DSR}$ = -0.0573 (COMDI shoot dry weight) + 28.1 for DSR. Results suggest that COMDI can reduce rice yield under saturated condition.

**Keywords:** *Commelina diffusa, COMDI, Commelinaceae, Interference, NSIC Rc 222, Rice-Weed Competition, Spreading Dayflower.*

## Introduction

Pests are one of the important factors to consider when growing rice. Occurring at high population and inflicting significant damages or injuries, pests can significantly reduce the quantity and quality of harvested grains if not properly managed (Kumar et al., 2017; Mataia and Francisco, 2008; Adalla and Magsino, 2006; Raymundo, 2006; Savary et al., 2000; Ampong-Nyarko and De Datta, 1991). Weeds are one of the major groups of pests that farmers always consider in selecting and implementing appropriate pest management strategies and techniques (Beltran et al., 2016; Donayre et al., 2014; Moody et al., 1997). In fact, this group of pests ranked fourth as the most considered pests of rice farmers of 10 Asian countries (Heong and Escalada, 1997). Topping the weeds in the rank are lepidopterous leaf feeders, stemborers, and brown plant hoppers. When growth is uncontrollable, potential yield is reduced by 48-55% in irrigated lowland, 51-74% in rainfed lowland, and 96% in the upland (Ampong-Nyarko and De Datta, 1991).

*Commelina diffusa* Burm. f. is an annual or perennial plant belonging to Family Commelinaceae. It is commonly called “spreading dayflower” in English literature with COMDI as the official code (EPPO, 2019; Caton et al., 2010). Moody et al. (2014) described it as a plant with smooth, fleshy, creeping, and ascending stems, and rooting at the nodes. Its characteristics have been differentiated from its close relative (*C. benghalensis* L.) through its blue, funnel-shaped flowers; inflorescence that is contained by a folded leaf-like bract; and lanceolate or oblong-lanceolate leaves with stem clasping sheaths (Pancho and Obien, 1995). It propagates either by seeds (1000 seeds $plant^{-1}$) or through stem fragments with ready roots at the nodes (Galinato et al., 1999).

*C. diffusa*, known to many farmers as alikbangon, gatilang, or kulasi, is one of the common weeds of rice in the Philippines particularly in rainfed and upland areas (Donayre et al., 2018; Fried et al., 2017; Rao et al., 2017; Galinato et al., 1999; Moody, 1989; Pablico and Moody, 1987). It is also a weed in fields planted with corn, banana, onion, and other vegetables (Donayre et al., 2019; Fabro and Barcial, 2015; Magsino, 2015; Baltazar et al., 1999). A study in United States showed that *C. diffusa*, allowed to grow for season-long period at a density of 22 plants $m^{-2}$, reduced rough rice yield of drill-seeded paddy rice by 18% (Smith, 1984). In separate study conducted in Brazil, presence of *C. diffusa* on two bean grain (*Phaseolus vulgaris* L.) varieties significantly reduced the number of leaves and chlorophyll contents in the leaves as well as the nitrogen, phosphorus, and iron contents of the grains (Oliveira et al., 2017).

Knowledge on the competitive ability of a weed against a certain crop is one of the requirements of successful and effective weed management. This is because it helps farmers and extension workers to decide whether a weed is to be controlled (Swanton et al., 2015). Having enough knowledge on this weed's ability also helps select appropriate management techniques in case the weed, at defined critical density, needs to be controlled.

Proof on the impact of *C. diffusa* interference on yields of major crops is very limited and its effect on rice growth and yield in the Philippine condition is still unknown. This paper hypothesized that (a) *C. diffusa*, when allowed to grow at certain density until maturity, will reduce growth and yield of both transplanted and direct-seeded rice under saturated condition, and (b) there will be difference on yield reductions between transplanted and direct-seeded rice.

## Materials and Methods

### *Location and Materials*

The research was conducted at the experiment area of the Crop Protection Department, College of Agriculture, Central Luzon State University, Science City of Muñoz, Nueva Ecija in the Philippines from August to December 2018. NSIC Rc 222 (registered class), obtained from the Philippine Rice Research Institute (PhilRice), was used as test rice variety for this experiment because it is widely planted in irrigated and rainfed-lowland areas. Preparation of planting materials of NSIC Rc 222 for transplanting and direct-seeding was conducted following the standard procedure of PhilRice (2007). Healthy stolons of *C. diffusa* and composite soil (Maligaya soil series) were collected from the department's nearby rice fields.

### *Experimental Design*

The experimental unit of this study was a plastic pot (25 cm diameter wide and 20 cm deep) filled with 5 kg sterilized, moist soil. Each pot was planted with either one 21-day old transplanted rice or three-day old pre-germinated rice seed of NSIC Rc 222. Cut healthy stolons of *C. diffusa* (5 cm length stolon$^{-1}$) were simultaneously planted in pots at 0, 1, 3, 5, and 7 densities. Rice and *C. diffusa* were grown until maturity. All plants were nourished with synthetic fertilizers based on recommended rates. Water was also supplied in each pot and maintained at saturation level whenever necessary. An additive design was utilized in this experiment to determine the outcome of *C. diffusa* - rice competition (Swanton et al., 2015). In this design, the density of rice was held constant while the density of *C. diffusa* was continuously increased.

### *Data Collected*

Height of rice plant was measured using a meter stick while number of tillers and leaves plant$^{-1}$ were manually counted at 60 days after transplanting/ direct-seeding. Shoot-dry weights of rice and *C. diffusa* plant$^{-1}$ were determined by drying the fresh weights inside an oven for 48 h at 70°C and weighing the dried biomasses on the digital weighing balance. Yield components (number of panicles plant$^{-1}$, number of grains panicle$^{-1}$, percentage filled spikelets plant$^{-1}$, and 1000-grain weight) were manually counted and measured after harvest. Grain yield plant$^{-1}$ was calculated from yield components (Yoshida, 1981). Percentage reductions on agronomic, yield components, and grain yield (GY) of rice were calculated using the equation YL (%) = $[(GY_0 - GY_1)/GY_0]*100$, where, $GY_0$ as the mean values at 0 weed density and $GY_1$ as the mean values at 1, 3, 5, and 7 weed densities.

### *Statistical Analysis*

Data were subjected to analysis of variance (ANOVA) using STAR 201. Fishers' LSD was used to compare the treatments means of agronomic characteristics, yield components, and grain yields of both transplanted and direct-seeded rice at 5% level of significance (Gomez and Gomez, 1984). Likewise, t-test was utilized to compare the grain yield reductions between transplanted and direct-seeded rice at different *C. diffusa* densities (Steinberg, 2008). A Pearson-product moment correlation coefficient was also computed to determine the strength and direction of relationship between *C. diffusa* variables (density and shoot dry weight) and grain yield of rice. A simple linear model (Y = *b*x + a), on the other hand, was fitted to *C. diffusa* variables and grain yields of rice to create prediction models.

## Results and Discussion

### *Transplanted Rice*

No significant differences were observed on height, number of tillers, number of leaves, number of panicles, and 1000-grain weight of transplanted rice (TPR) grown at different densities of *C. diffusa* (Table 1). Significant differences, on the other hand, were observed on shoot dry weight, number of grains panicle$^{-1}$, percentage of filled spikelets, and grain yield. Shoot dry weight of TPR was 107.1 g plant$^{-1}$ when *C. diffusa* was at 0 density pot$^{-1}$ while 91.6, 77.6, 66.3, and 47.2 g plant$^{-1}$ when the weed was at 1, 3, 5, and 7 densities pot$^{-1}$, respectively. There were significant differences on shoot dry weight between densities. More number of grains panicle$^{-1}$ were also observed when the weed was at 0 density pot$^{-1}$. However, number of grains panicle$^{-1}$ were not significantly different between 0 and 1 density pot$^{-1}$ (130.2 and 125.9 grains panicle$^{-1}$); and between 1, 3, 5, and 7 densities pot$^{-1}$ (26.5, 25.7, 23.2, and 21.8 grains panicle$^{-1}$). No significant differences were observed on filled spikelets between 0 and 1 density pot$^{-1}$ (99.1 and 98.0%) and between 1, 3, 5, and 7 densities pot$^{-1}$ (98.0, 96.1, 93.2, and 89.3%). Grain yield (GY) of transplanted rice was also highest (28.1 g plant$^{-1}$) when the weed was at 0 density pot$^{-1}$. However, there were no significant differences between 0, 1, and 3 densities pot$^{-1}$ (28.1, 26.5, and 25.7 g plant$^{-1}$); and between 3, 5, and 7 densities pot$^{-1}$ (25.7, 23.2, and 21.8 g plant$^{-1}$).

### *Direct-Seeded Rice*

*C. diffusa* significantly affected all the growth, yield components, and grain yield of direct-seeded rice (DSR) except for the number of panicles plant$^{-1}$. All values were highest at 0 density pot$^{-1}$ while lowest when the weed was at 7 densities pot$^{-1}$. Height of rice was 73.2cm at 0 density pot$^{-1}$ while 68.4, 68, and 67.6cm when the weed was present at 1, 3, 5, and 7 densities pot$^{-1}$, respectively. No significant differences on height of rice when the weed was present. No significant differences on number of tillers between 0 and 1 density pot$^{-1}$ (14.2 and 13.2 tillers plant$^{-1}$); and between 3 and 4 densities pot$^{-1}$ (11.4 and 10.6 tillers plant$^{-1}$). Least number of tillers (7.0 tillers plant$^{-1}$) was observed when the weed was present at 7 densities pot$^{-1}$. Most number of leaves was recorded at 0 densities pot$^{-1}$ (12.4 leaves plant$^{-1}$). No significant differences on number of leaves between 1, 3, and 5 densities pot$^{-1}$ (10.6, 9.8, and 9.6 leaves plant$^{-1}$); and between 3, 5, and 7 densities pot$^{-1}$. Least number of leaves was at 7 densities pot$^{-1}$ (9.4 leaves plant$^{-1}$). There were significant differences on shoot dry weight between weed density. Most number of panicles was produced at 0 density pot$^{-1}$ (8.2 panicles plant$^{-1}$) followed by 1 density pot$^{-1}$ (7.2 panicles plant$^{-1}$). No significant differences on number of panicles between 3 and 5 densities pot$^{-1}$ (6.0 and 5.4 panicles plant$^{-1}$). Rice with *C. diffusa* at 7 densities pot$^{-1}$ produced the least number of panicles (3.6 panicles plant$^{-1}$). No significant differences on number of filled spikelets between 0 and 1 density pot$^{-1}$ (99.2 and 97.0% filled spikelets). There were significant differences on filled spikelets between 1, 3, 5, and 7 densities pot$^{-1}$ (97, 92.3, 97.9, and 78.1% filled spikelets). One-thousand grain weight was lowest at 0 density pot$^{-1}$ while highest at 7 densities pot$^{-1}$. No significant differences on 1000-grain weight between 0 and 1 density pot$^{-1}$ (26.5 and 27.1 g$^{-1000\ seeds}$) and between 3 and 5 densities pot$^{-1}$ (32.1 and 33.9 g$^{-1000\ seeds}$). Although, GY was highest at 0 density pot$^{-1}$ (28.1 g plant$^{-1}$), its values had no significant difference at 1 density pot$^{-1}$ (25.3 g plant$^{-1}$). No significant differences were also observed on GY between 1 and 3 densities pot$^{-1}$, between 3 and 5 densities pot$^{-1}$, and between 5 and 7 densities pot$^{-1}$. Lowest GY of DSR was at 7 densities pot$^{-1}$.

### *Yield Reduction*

Except for 1000-grain weight, reduction on agronomic, yield components, and yield parameters of rice due to competition by *C. diffusa* were generally

**Table 1**. Agronomic characteristics, yield components, and grain yields of rice as affected by different densities of *Commelina diffusa* L.

| COMDI (density pot$^{-1}$) | Height (cm plant$^{-1}$) | No. of Tillers plant$^{-1}$ | No. of Leaves plant$^{-1}$ | Shoot Dry Weight (g plant$^{-1}$) | No. of Panicles plant$^{-1}$ | No. of Grains panicle$^{-1}$ | Filled Spikelets (%) | 1000 Grain Weight (g) | Grain Yield Plant$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| Transplanted rice | | | | | | | | | |
| 0 | 90.4 a | 14.2 a | 18.0 a | 107.1 a | 8.2 a | 130.2 a | 99.1 a | 26.4 a | 28.1 a |
| 1 | 88.8 a | 13.8 a | 16.0 a | 91.6 b | 7.8 a | 125.9 ab | 98.0 a | 27.4 a | 26.5 ab |
| 3 | 87.4 a | 13.6 a | 17.0 a | 77.6 c | 7.6 a | 124.0 b | 96.1 b | 28.1 a | 25.7 abc |
| 5 | 86.2 a | 13.4 a | 15.8 a | 66.3 d | 7.4 a | 121.5 b | 93.2 c | 27.6 a | 23.2 bc |
| 7 | 87.2 a | 12.8 a | 15.2 a | 47.2 e | 6.8 a | 122.5 b | 89.3 d | 29.4 a | 21.8 c |
| P | .051 ns | .960 ns | .595 ns | .000** | .960 ns | .019* | .000** | .423 ns | .036* |
| Direct-seeded rice | | | | | | | | | |
| 0 | 73.2 a | 14.2 a | 12.4 a | 117.2 a | 8.2 a | 129.5 a | 99.2 a | 26.5 c | 28.1 a |
| 1 | 68.4 b | 13.2 a | 10.6 b | 83.4 b | 7.2 b | 133.1 a | 97.0 a | 27.1 c | 25.3 ab |
| 3 | 68.0 b | 11.4 b | 9.8 bc | 66.1 c | 6.0 c | 128.1 a | 92.3 b | 32.1 b | 22.9 bc |
| 5 | 68.0 b | 10.6 b | 9.6 bc | 56.0 d | 5.4 c | 127.3 a | 87.9 c | 33.9 b | 20.7 cd |
| 7 | 67.6 b | 7.0 c | 9.4 c | 35.6 e | 3.6 d | 134.4 a | 78.1 d | 49.9 a | 18.5 d |
| P | .000** | .000** | .000** | .000** | .000** | .594 ns | .000** | .000** | .001** |

COMDI – *C. diffusa*; *P <.05, **P <.005, P <.0005, ns- not significant; means with the same letters are not significantly different at .05 level of significance using Fischer's LSD lowest at 1 density pot$^{-1}$ while highest at 7 densities pot$^{-1}$ (Table 2). High percentages of reduction were observed on shoot dry-weight of TPR (14.5-55.9%) while number of tillers (7-50.7%), shoot dry-weight (28.7- 69.6%), and number of panicles (12.2-56.1%) were recorded for DSR. Reductions on grain yield ranged from 5.4 to 22.4% for TPR while 9.8-34% for DSR. Although reductions on TPR were lower than in DSR, TTEST showed that grain yield of TPR had no significant difference with DSR at 1, 3, and 5 densities pot$^{-1}$ except at 7 densities pot$^{-1}$ (Table 3). Interference of other weed species and their effects on growths and yields of rice were also reported by other researchers in the country. For example, Donayre and Endino-Tayson (2015) reported that *Hydrolea zelanica* (L.) Vahl. at 1:1, 1:3, 1:5, 1:7, 1:10, and 1:20 crop:weed ratios significantly reduced the yields of transplanted rice by 18.99, 24.68, 23.80, 35.46, 51.72, and 55.90%. Chauhan (2013), in a study on the effects of two weedy rice variants on the growth and yield of two rice cultivars, also reported that grain yield was reduced by 30 and 47%, and 66 and 81%, when rice had to compete with weedy rice variants' 1 and 2 at population densities of 4 and 8 weedy rice pot$^{-1}$. De Grano (2008) also reported that season competition of *Limnnocharis fava* (L.) Buch. at 10, 20, 25, and 30 densities m$^{-2}$ reduced the yield of transplanted rice by 18.4, 22.5, 30.2, and 48%. At 50-60 densities, yield reduction was 63.4% under natural infestation in the field. Rao and Moody (1992) in their study on competition between *Echinochloa glabrescens* Munro ex Hook. f. and transplanted rice reported 11.9 and 12.8% reductions on height, 60.5 and 62.5% reductions on number of tillers, and 59.1 and 51.5% reductions on maximum leaf area index. Reductions were also registered at 77 and 81% on total dry weight and 90 and 94% on yield when all rice plants had 100% infestation of the weed. Lubigan and Moody (1990) also reported that *Ischaemum rugosum* Salisb at densities of 5 and 10 plants m$^{-2}$ were enough to cause significant yield reduction in transplanted rice in both seasons.

**Table 2.** Reductions (%) on agronomic, yield components, and grain yield parameters of rice as affected by different densities of *Commelina diffusa* L.

| Rice:COMDI Ratio | Height | No. of Tillers Plant$^{-1}$ | No. of Leaves Plant$^{-1}$ | Shoot Dry Weight | No. of Panicles Plant$^{-1}$ | No. of Grains Panicle$^{-1}$ | Filled Spikelets | 1000 Grain Weight | Grain Yield Plant$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| Transplanted rice | | | | | | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1.8 | 2.8 | 11.1 | 14.5 | 4.9 | 3.2 | 1.1 | -3.7 | 5.4 |
| 3 | 3.3 | 4.2 | 5.6 | 27.6 | 7.3 | 4.7 | 3.0 | -6.5 | 8.4 |
| 5 | 4.6 | 5.6 | 12.2 | 38.1 | 9.8 | 6.7 | 6.0 | -4.6 | 17.2 |
| 7 | 3.5 | 9.9 | 15.6 | 55.9 | 17.1 | 5.9 | 9.9 | -11.3 | 22.4 |
| Direct-seeded rice | | | | | | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 6.6 | 7.0 | 14.5 | 28.7 | 12.2 | -2.7 | 2.2 | -2.3 | 9.8 |
| 3 | 7.1 | 19.7 | 21.0 | 43.6 | 26.8 | 1.1 | 6.9 | -20.8 | 18.6 |
| 5 | 7.1 | 25.4 | 22.6 | 52.2 | 34.1 | 1.7 | 11.3 | -27.9 | 26.5 |
| 7 | 7.7 | 50.7 | 24.2 | 69.6 | 56.1 | -3.7 | 21.2 | -88.0 | 34.0 |

**Table 3.** *P*-values as results of comparison between grain yield reductions of transplanted and direct-seeded rice.

| COMDI Density | Direct-Seeded | | | |
|---|---|---|---|---|
| | 1 | 3 | 5 | 7 |
| Transplanted | | | | |
| 1 | 0.737$^{ns}$ | | | |
| 3 | | 0.484 $^{ns}$ | | |
| 5 | | | 0.406 $^{ns}$ | |
| 7 | | | | 0.027$^{*}$ |

Comparison of means were computed through TTEST; **P*< .05, ns – not significant at 5% level of significance

Grain yield reduction in transplanted rice was generally lower than in direct-seeded rice. Ampong-Nyarko and De Datta (1991) explained that transplanted rice has low potential to yield loss due to its head start over the weeds. Meanwhile, direct-seeded rice has high potential to yield losses because its germinating seeds grow together with weeds (Casimero, 2004). The absence of early flooding to suppress weeds during the initial growth phase, absence of seedling size to compete with weeds, and the uneven stand that provides space weeds to grow further aggravate the vulnerability of direct-seeded rice to weed competition and yield losses (Kumar et al., 2017; Chauhan, 2012).

***Correlation and Regression***

Density and shoot dry weight of *C. diffusa* were strongly, negatively correlated to grain yields of TPR (r = -0.990, -0.987) and DSR (r = -0.987, -0.965). Simple linear regression analysis also indicated that 98.02 and 97.49% grain yield of TPR and 97.41 and 99.64% grain yield of DSR were influenced by the density and shoot dry weight of *C. diffusa* (Figure 1). Further analysis showed that the density and shoot dry weight of *C. diffusa* were significant predictors and contributors to the grain yield of rice at 5% level of significance (density: $p$ = .0012 TPR, $p$ = .0018 DSR) (shoot dry weight: $p$ = .0017 TPR, $p$ = .0078 DSR). The final predictive models for TPR were GY = -0.8769 (*C. diffusa* density pot$^{-1}$) + 27.866 and GY = -0.0381 (*C. diffusa* shoot dry weight in g plant$^{-1}$) + 28.394. For the direct-seeded, GY = -1.2985 (*C. diffusa* density pot$^{-1}$) + 27.248 and GY = -0.0573 (*C. diffusa* shoot dry weight in g plant$^{-1}$) + 28.1.

Knowledge on the weed's competitive ability helps decide whether control has to be implemented to suppress its growth. In this study, *C. diffusa* significantly reduced the growth and grain yield of rice under saturated condition implying that control must be implemented whenever it grows with rice at minimum of 5 densities. In controlling *C. diffusa* and other perennial weeds of rainfed rice, practicing field sanitation, thorough land preparation, handweeding, and chemical control were regarded as best options by Donayre et al. (2018) and PhilRice (2001, 2007). They emphasized that practicing field sanitation such as keeping the seedling nurseries, irrigation canals, and field bunds clean and weed-free help prevent entries of volunteer weed seeds and asexual propagules. Furthermore, thorough land preparation and practicing stale-seedbed technique bury weeds under the soil, separate shoots from roots, encourage germination of dormant seeds, desiccate shoots, and exhaust carbohydrate reserves of perennial weeds. In addition, manual weeding and herbicide application are very effective and efficient in removing and eradicating weeds that grow within rows and hills of rice.

## Conclusion and Recommendation

*C. diffusa* at 1, 3, 5, and 7 densities, interfered the growth and yield of rice. When grown until crop's maturity, it significantly reduced the grain by 5.4-22.4% for transplanted and 9.8-34% for direct-seeded rice. The results indicate that *C. diffusa* is truly a weed of rice under saturated condition. There are several options on how to manage the weed. However, careful selection, planning, and implementation must be done to achieve effective, economical, and environmentally-sound weed management. To grasp more knowledge on ecology, further researches are recommended particularly on its interference ability under different times of irrigation (early and late flooding), fertilizer rates (nitrogen), and rice varieties (short and tall or early and late maturing). Predictive models under field conditions and the different weed management techniques through a holistic approach must be evaluated.

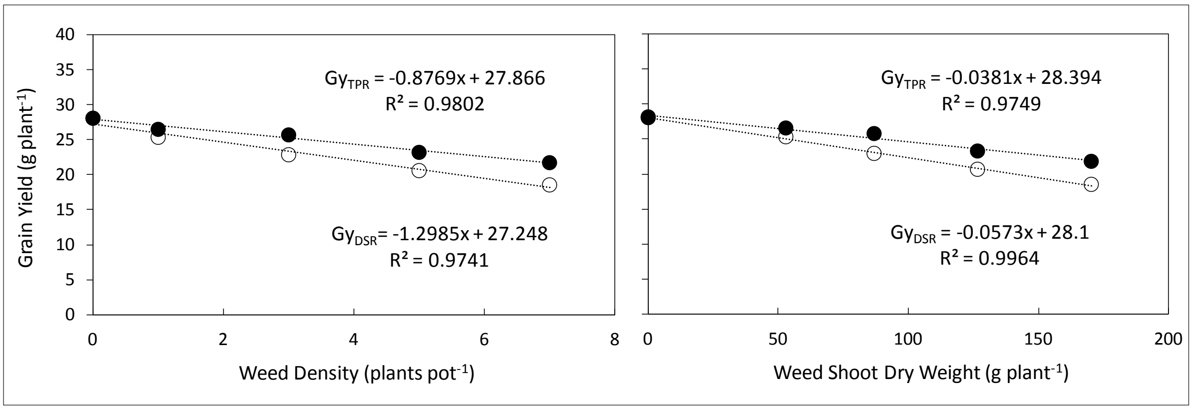


**Figure 1**. Correlation and simple linear regression between density and weight of *Commelina diffusa* and grain yields of rice (transplanted rice - black circles, direct-seeded rice - white circles).

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# QUALITY CHARACTERISTICS AND CONSUMER ACCEPTABILITY OF RICE: *ADLAI* BLEND

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## Abstract

*Adlai* (*Coixlacryma-jobi L.*) was recently promoted by the government as alternative to rice. This study evaluated the cooking and eating quality and consumer acceptability of rice:*adlai* blends. Three *adlai* varieties, namely *Ginampay, Gulian*, and *Tapol*, were substituted to NSIC Rc 160, a popular rice variety, at different ratios (100:0, 75:25, 50:50, 25:75, and 0:100 rice:*adlai*). Higher gelatinization property of *adlai*, regardless of variety, resulted in longer cooking time by 1.69-6.73 min compared with pure rice. Laboratory sensory evaluation showed that pure *Ginampay* and all its blend forms had overall likings comparable with rice. *Gulian* and *Tapol* gave overall likings comparable with rice at 25:75 and 50:50 ratios, respectively. In terms of consumer acceptability, the 50:50 rice: *Ginampay* blend was preferred over the other two blends. It received the same rating and rank scores as of pure rice. The blends were preferred in the following order: pure rice ≥ rice:*Ginampay* ≥ rice:*Tapol* ≥ rice:*Gulian.* Meanwhile, blending rice with *adlai*, regardless of variety, resulted in significant increase in protein content (11.19 - 11.52%) and fat (1.02 - 1.22%) of the blends. This study showed that *Ginampay* can be substituted to rice at 50:50 ratio as source of carbohydrates, providing consumer the same eating satisfaction with higher amount of protein and fats.

**Keywords***: Adlai, Consumer Acceptability, Proximate Composition, Rice Blend, Sensory Evaluation.*

## Introduction

Among the agricultural commodities, rice remains to be the major source of calories among Filipinos. The seemingly dependence of Filipinos to rice requires reliable supply. However, the continuous growth of population has been identified as one of the factors contributing to rice shortage. In 2017, rice contributed 1,156.59 g of calories per day per individual. Meanwhile, the country's average per capita consumption of rice is about 110 kg per year (PSA, 2018). One way of ensuring that Filipinos obtain enough calories without depending on rice is through the consumption of other food crops that can provide at least some of the caloric and other nutritional requirements of the population.

Crops such as corn, cassava, sweet potato, and *adlai* are now being advocated as alternative staples to rice. Through its Food Staples Self-Sufficiency Program, the Philippine's Department of Agriculture (DA) regards the consumption of these staple alternatives as a measure to reduce rice intake and consequently, address food security.

Among the alternative staple food crops being campaigned by the government, *adlai* is currently the least known in the country. *Adlai* or *katigbi* (*Coix lacryma-jobi* L.), also called "Job's tears" and Chinese pearl barley, has been grown and consumed mostly in Southern Philippines, although it is more popular in neighboring Asian countries, particularly in India and China, where cultivation of the crop was believed to have started since the ancient times (Lim, 2013). In the Philippines, four varieties are being cultivated: *Gulian* (white), *Tapol* (purple), *Ginampay* (brown), and *Pulot* (white-glutinous) (*Adlai,* n.d.). However, *adlai* is still relatively unknown to many Filipinos. Availability of information on its nutritional and potential health advantages would therefore encourage more consumption and utilization of *adlai*, which is championed by the DA through the Bureau of Agricultural Research (BAR).

Nutritional value of *adlai* was reported to be comparable, or even better, than rice. Analysis of the Food and Nutrition Research Institute (FNRI) (*Adlai*, n.d.) showed that *adlai* provides almost the same amount of energy as milled rice (Juliano, 2003) at 356 kcal 100 $g^{-1}$, but its protein and total fat content are higher (*Adlai* n.d.; Dechkunchon and Thongngam, 2007). Protein is an important component of staple crops because this serves as a primary source of nutrient particularly to underprivileged populations.

To be an effective substitute to rice, grain quality of *adlai* must also satisfy consumer requirements. For rice, cooked grains that are cohesive and soft even on cooling are favored by most Filipino consumers. These are affected by the intrinsic physicochemical characteristics of rice, specifically its starch fractions, and also by cooking conditions. Hence, the cooking quality of different local *adlai* varieties must be established and proximate property and consumer acceptability determined.

In view of the scarce information on the varieties of *adlai* locally grown in the country, investigation of the quality profile of Philippine *adlai* varieties is therefore imperative. In addition, validation of the nutritive quality of local *adlai* varieties would help intensify current efforts in promoting *adlai* as alternative caloric source. This study aimed to establish the cooking parameters of three *adlai* varieties, identify the best *adlai* variety and its ratio to rice by sensory evaluation, and characterize the nutritional value of the rice:*adlai* blends.

## Materials and Methods

### *Source of Rice and Adlai*

NSIC Rc 160, a popular low-amylose rice variety in the Philippines, was sourced from the Philippine Rice Research Institute (PhilRice) in Science City of Muñoz, Nueva Ecija. Meanwhile, the three varieties of *adlai*, namely *Ginampay*, *Gulian*, and *Tapol* were obtained from the Department of Agriculture - Cagayan Valley Integrated Agricultural Research Center (DA-CVIARC) in Ilagan City, Isabela, Philippines (Figure 1).

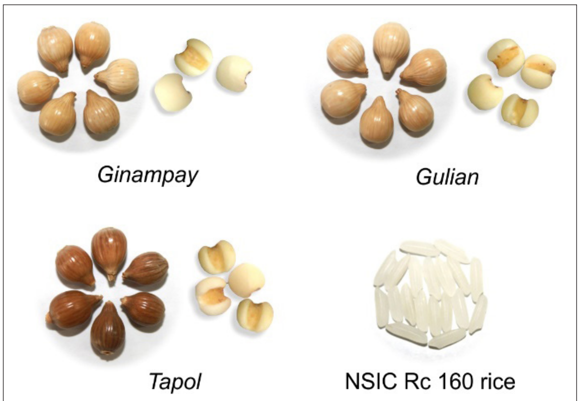


**Figure 1.** Three varieties of *adlai* and NSIC Rc 160.

### *Experimental Treatments*

Table 1 shows the different rice to *adlai* ratios used in the experiment. Prior to cooking, *Ginampay*, *Gulian,* and *Tapol*, and their blend forms were soaked in water for 30 min to increase water penetration and absorption.

**Table 1.** Treatments used in the experiments.

| Ratio of rice to *adlai* | |
|---|---|
| Rice (NSIC Rc 160) | *Adlai** |
| 100 | 0 |
| 75 | 25 |
| 50 | 50 |
| 25 | 75 |
| 0 | 100 |

**Ginampay, Gulian, or Tapol*

### *Determination of Amylose Content, Cooking Quality, and Gelatinization Property*

Amylose content of raw NSIC Rc 160 and the three *adlai* varieties and the cooking qualities (optimum cooking water, weight increase, height increase, and cooking time) of rice:*adlai* blend forms were determined based on the National Cooperative Testing (NCT) Manual for Rice (NCT, 1997).

Gelatinization property of the samples was determined based on the modified method of Manaois (2007) using a differential scanning calorimeter (DSC) (TA Q100, TA Instruments, Newcastle, DE). Floured forms of the sample (10 mg) were weighed into a steel DSC pan, added with 20 µL of distilled water, sealed, and equilibrated at room temperature for at least 1 h. Heating was carried out from 35°C to 140°C at a rate of 5°C $min^{-1}$. Gelatinization temperature was calculated using the Universal Analysis 2000 Software (version 4.5A, TA Instruments-Waters LLC, New Castle, DE). DSC runs were done in duplicate.

### *Sensory Evaluation*

*Laboratory*. Eleven panelists from the Rice Chemistry and Food Science Division of PhilRice were invited to participate in the laboratory-panel sensory evaluation. Three separate sessions were conducted, one *adlai* variety per session. Cooked samples were coded randomly and presented to the panelist to be evaluated using hedonic scale sensory score card for rice (NCT 1997). As the objective of this study was to directly compare *adlai* to rice, the following attributes commonly used for rice were gathered: aroma, off-odor, color, gloss, cohesiveness, tenderness, smoothness, taste, off-taste, and overall liking.

*Consumer*. Sixty PhilRice employees (18-54 years old) were randomly recruited as consumer panelists. They were presented with coded samples and asked to rate the products based on their preference and acceptability.

***Determination of Proximate Composition***

Crude protein, crude ash, crude fat, dietary fiber, and carbohydrates of the cooked samples were determined based on standard methods (AOAC, 2000). The total carbohydrate content was determined by difference.

***Statistical Analysis***

All analyses were done in duplicate. Analysis of variance and mean comparison analysis using Tukey's HSD test were analyzed using SPSS for Windows version 20 statistical software package (IBM SPSS Statistics, Armonk, NY). The level of significance used was $p$=0.05.

## Results and Discussion

Amylose content, gelatinization property, and cooking quality of rice and *adlai* varieties were evaluated. Amylose content and gelatinization property are the major factors affecting the cooking and eating qualities of rice. Based on amylose content, rice can either be classified as waxy (0-2.0%), very low (2.1-10.0%), low (10.1-17.0%), intermediate (17.1-22.0%), or high (22.1% and above). Rice with high amylose content tends to cook hard and dry, while low-amylose rices have softer and stickier cooked grains. Waxy rice, which has little or no amylose, is also referred to as glutinous rice. In the Philippines, rice varieties with low-intermediate amylose content are generally preferred. Table 2 shows the amylose content and classification of rice and *adlai* samples. Consistent with NCT data, NSIC Rc 160 had amylose content of 13.50% equivalent to low. On the other hand, the three *adlai* varieties were classified as very low amylose types (5.08-7.89%) (Table 2). The amylose content of three *adlai* varieties were lower than the values reported by Li and Corke (1999) for job's tears, which ranged from 15.9 to 25.8%.

**Table 2**. Amylose content of rice and *adlai* varieties.

| Sample | Amylose Content (%) | Amylose Classification* |
|---|---|---|
| NSIC Rc 160 | 13.50 | Low |
| *Ginampay* | 7.27 | Very low |
| *Gulian* | 7.89 | Very low |
| *Tapol* | 5.08 | Very low |

*low (10.1 – 17.0%); very low (2.1 – 10.0%)

Meanwhile, gelatinization temperature of starch granules provides index of the cooking time of rice. Rice with low or intermediate gelatinization temperature cooks faster. Meanwhile, varieties with high gelatinization temperature require more water and time for cooking and become excessively soft when overcooked and elongate less. The three adlai samples had gelatinization temperature (74.49-75.92°C), which is significantly higher than NSIC Rc 160 (71.71°C). A positive correlation was observed between gelatinization temperature and cooking time of samples. Samples high in gelatinization temperature had a longer cooking time (Table 3).

**Table 3**. Gelatinization property and cooking time of rice and *adlai* varieties.

| Sample | Gelatinization temperature (°C) | Cooking time (min) |
|---|---|---|
| NSIC Rc 160 | 71.71c | 14.84b |
| *Ginampay* | 74.92b | 20.49a |
| *Gulian* | 74.49b | 21.39a |
| *Tapol* | 75.54a | 20.42a |

The higher gelatinization property of *adlai* resulted in longer cooking time of rice blended with 75% *Ginampay* and *Tapol* as compared with pure rice. For rice:*Gulian*, blending rice with only 25% *Gulian* already produced significantly longer cooking time. In terms of weight and height increase, blending rice with lower *Ginampay* ratio (25%) resulted in significant increase in height (66%) compared with pure rice. All blend forms were significantly heavier than pure rice by 31- 32%. Meanwhile, blending rice with either *Gulian* or *Tapol* at any ratio did not give additional increase in height but significantly produced heavier meals (Table 4, 5, and 6). Despite longer cooking time, rice:*Ginampay* could provide more food due to the synergistic effect of increased height and weight on the volume of the blend.

**Table 4**. Cooking quality of rice:*Ginampay* blends.

| Rice: *Ginampay* Ratio | Cooking Time (min) | Height Increase (%) | Weight Increase (%) |
|---|---|---|---|
| 100:0 | $14.84^{b}$ | $158.65^{cd}$ | $101.34^{b}$ |
| 75:25 | $16.32^{b}$ | $262.73^{a}$ | $133.48^{a}$ |
| 50:50 | $17.68^{b}$ | $206.63^{bc}$ | $131.46^{a}$ |
| 25:75 | $19.82^{a}$ | $214.85^{ab}$ | $132.33^{a}$ |
| 0:100 | $20.49^{a}$ | $154.44^{d}$ | $124.63^{a}$ |

Mean values with the same letter within a column are not significantly different at p=0.05.

**Table 5**. Cooking quality of rice:*Gulian* blends.

| Rice:*Gulian* Ratio | Cooking Time (min) | Height Increase (%) | Weight Increase (%) |
|---|---|---|---|
| 100:0 | $14.84^{c}$ | $158.65^{ab}$ | $101.34^{b}$ |
| 75:25 | $17.67^{b}$ | $166.67^{a}$ | $144.71^{a}$ |
| 50:50 | $17.89^{b}$ | $112.15^{ab}$ | $139.67^{a}$ |
| 25:75 | $18.25^{b}$ | $101.61^{b}$ | $135.06^{a}$ |
| 0:100 | $21.39^{a}$ | $98.18^{b}$ | $132.81^{a}$ |

Mean values with the same letter within a column are not significantly different at p=0.05.

**Table 6**. Cooking quality of rice:*Tapol* blends.

| Rice:*Tapol* Ratio | Cooking Time (min) | Height Increase (%) | Weight Increase (%) |
|---|---|---|---|
| 100:0 | $14.84^{b}$ | $158.65^{a}$ | $101.34^{c}$ |
| 75:25 | $18.16^{ab}$ | $131.51^{a}$ | $142.59^{a}$ |
| 50:50 | $19.27^{ab}$ | $120.00^{a}$ | $138.73^{a}$ |
| 25:75 | $22.07^{a}$ | $138.15^{a}$ | $131.82^{ab}$ |
| 0:100 | $20.42^{a}$ | $97.31^{a}$ | $119.17^{b}$ |

Mean values with the same letter within a column are not significantly different at p=0.05.

### *Sensory Properties*

*Laboratory*. The sensory properties of pure rice, *adlai*, and their blend forms were evaluated by 11 participants. In Table 7, all the rice:*Ginampay* blends received comparable scores for aroma, color, gloss, texture (cohesiveness, tenderness, and smoothness), and taste with pure rice. Even the pure *Ginampay* had generally the same sensory properties as of the pure rice. No off-odor and off-taste were perceived among the samples. Pure *Ginampay* and all its blend forms received overall liking comparable with the pure rice.

The rice:*Gulian* blends also garnered score for aroma, gloss, texture, and taste comparable with pure rice. However, blend with 75% *Gulian* produced grayish meal but remained comparable with pure rice in terms of overall likings. No off-odor and off-taste were detected among the samples (Table 8).

In Table 9, all the rice:*Tapol* blends received comparable scores with rice for aroma, gloss, texture, and taste. Blend with 75% *Tapol* had grayish appearance compared with pure rice and other

**Table 7**. Mean laboratory sensory scores of cooked rice:*Ginampay* blend.

| Sensory Attributes[1] | Rice:*Ginampay* Ratio | | | | |
|---|---|---|---|---|---|
| | 100:00 | 75:25 | 50:50 | 25:75 | 00:100 |
| Aroma | $1.45{\pm}0.66^{a}$ | $1.55{\pm}0.50^{a}$ | $1.55{\pm}0.66^{a}$ | $1.64{\pm}0.77^{a}$ | $1.64{\pm}0.77^{a}$ |
| Off-odor | $1.00{\pm}0.00^{a}$ | $1.00{\pm}0.00^{a}$ | $1.00{\pm}0.00^{a}$ | $1.09{\pm}0.29^{a}$ | $1.09{\pm}0.29^{a}$ |
| Color | $2.36{\pm}0.48^{ab}$ | $2.55{\pm}0.50^{a}$ | $2.00{\pm}0.60^{ab}$ | $1.91{\pm}0.67^{ab}$ | $1.73{\pm}0.62^{b}$ |
| Gloss | $2.64{\pm}0.48^{a}$ | $2.82{\pm}0.57^{a}$ | $2.36{\pm}0.64^{a}$ | $2.27{\pm}0.62^{a}$ | $2.09{\pm}0.79^{a}$ |
| Cohesiveness | $2.82{\pm}0.57^{a}$ | $3.00{\pm}0.43^{a}$ | $2.73{\pm}0.62^{a}$ | $2.45{\pm}0.66^{ab}$ | $1.82{\pm}0.57^{b}$ |
| Tenderness | $3.09{\pm}0.51^{a}$ | $3.00{\pm}0.60^{a}$ | $3.09{\pm}0.51^{a}$ | $3.09{\pm}0.29^{a}$ | $2.73{\pm}0.62^{a}$ |
| Smoothness | $2.82{\pm}0.57^{a}$ | $3.09{\pm}0.67^{a}$ | $2.91{\pm}0.51^{a}$ | $2.82{\pm}0.57^{a}$ | $2.55{\pm}0.50^{a}$ |
| Taste | $2.09{\pm}0.67^{a}$ | $2.27{\pm}0.96^{a}$ | $2.00{\pm}0.74^{a}$ | $2.09{\pm}0.79^{a}$ | $1.91{\pm}0.67^{a}$ |
| Off-taste | $1.00{\pm}0.00^{a}$ | $1.00{\pm}0.00^{a}$ | $1.00{\pm}0.00^{a}$ | $1.09{\pm}0.29^{a}$ | $1.18{\pm}0.39^{a}$ |
| Overall liking | $3.45{\pm}0.50^{ab}$ | $4.09{\pm}0.67^{a}$ | $3.55{\pm}0.66^{ab}$ | $3.36{\pm}0.48^{ab}$ | $2.73{\pm}0.86^{b}$ |

Mean values with the same letter within a row are not significantly different at $p$=0.05 ($n$=11)
[1]aroma (1=none, 5=extremely aromatic), off-odor and off-taste (1=none, 5=extremely perceptible),color (1=gray/cream, 5=extremely white), gloss (1=dull, 5=extremely glossy), cohesiveness (1=separated, 5=extremely cohesive), tenderness (1=hard, 5= extremely tender), smoothness (1=rough, 5=extremely smooth), taste (1=bland, 5=extremely tasty), and over-all liking (1=dislike very much, 5=like very much).

**Table 8**. Mean laboratory sensory scores of cooked rice:*Gulian* blend.

| Sensory Attributes[1] | Rice:*Gulian* Ratio | | | | |
|---|---|---|---|---|---|
| | 100:00 | 75:25 | 50:50 | 25:75 | 00:100 |
| Aroma | $1.45{\pm}0.66^{a}$ | $1.45{\pm}0.66^{a}$ | $1.55{\pm}0.50^{a}$ | $1.36{\pm}0.48^{a}$ | $1.27{\pm}0.45^{a}$ |
| Off-odor | $1.00{\pm}0.00^{a}$ | $1.00{\pm}0.00^{a}$ | $1.00{\pm}0.00^{a}$ | $1.09{\pm}0.29^{a}$ | $1.18{\pm}0.39^{a}$ |
| Color | $2.45{\pm}0.50^{a}$ | $2.18{\pm}0.57^{ab}$ | $2.00{\pm}0.60^{ab}$ | $1.64{\pm}0.48^{b}$ | $1.64{\pm}0.48^{b}$ |
| Gloss | $2.91{\pm}0.51^{a}$ | $3.00{\pm}0.43^{a}$ | $2.73{\pm}0.62^{a}$ | $2.73{\pm}0.62^{a}$ | $2.36{\pm}0.77^{a}$ |
| Cohesiveness | $3.18{\pm}0.39^{a}$ | $3.09{\pm}0.29^{a}$ | $3.00{\pm}0.43^{a}$ | $2.73{\pm}0.62^{ab}$ | $2.27{\pm}0.86^{b}$ |
| Tenderness | $3.18{\pm}0.39^{a}$ | $3.09{\pm}0.51^{a}$ | $3.00{\pm}0.43^{a}$ | $2.91{\pm}0.67^{a}$ | $2.73{\pm}0.86^{a}$ |
| Smoothness | $2.82{\pm}0.39^{a}$ | $3.09{\pm}0.51^{a}$ | $2.91{\pm}0.51^{a}$ | $2.82{\pm}0.57^{a}$ | $2.64{\pm}0.77^{a}$ |
| Taste | $1.91{\pm}0.79^{a}$ | $2.00{\pm}0.85^{a}$ | $2.18{\pm}0.72^{a}$ | $1.91{\pm}0.67^{a}$ | $1.82{\pm}0.57^{a}$ |
| Off-taste | $1.00{\pm}0.00^{a}$ | $1.14{\pm}0.31^{a}$ | $1.18{\pm}0.39^{a}$ | $1.23{\pm}0.39^{a}$ | $1.27{\pm}0.45^{a}$ |
| Overall liking | $3.45{\pm}0.66^{a}$ | $3.27{\pm}0.45^{a}$ | $3.36{\pm}0.64^{a}$ | $2.82{\pm}0.57^{ab}$ | $2.45{\pm}0.78^{b}$ |

Mean values with the same letter within a row are not significantly different at $p$=0.05 ($n$=11)
[1]aroma (1=none, 5=extremely aromatic), off-odor and off-taste (1=none, 5=extremely perceptible),color (1=gray/cream, 5=extremely white), gloss (1=dull, 5=extremely glossy), cohesiveness (1=separated, 5=extremely cohesive), tenderness (1=hard, 5= extremely tender), smoothness (1=rough, 5=extremely smooth), taste (1=bland, 5=extremely tasty), and over-all liking (1=dislike very much, 5=like very much).

**Table 9**. Mean laboratory sensory scores of cooked rice:*Tapol* blend.

| Sensory Attributes[1] | Rice:*Tapol* Ratio | | | | |
|---|---|---|---|---|---|
| | 100:00 | 75:25 | 50:50 | 25:75 | 00:100 |
| Aroma | 1.64±0.64[a] | 1.55±0.66[a] | 1.27±0.45[a] | 1.27±0.45[a] | 1.18±0.39[a] |
| Off-odor | 1.00±0.00[a] | 1.09±0.29[a] | 1.00±0.00[a] | 1.23±0.39[a] | 1.18±0.39[a] |
| Color | 2.73±0.62[a] | 2.45±0.66[ab] | 2.27±0.45[ab] | 1.91±0.67[b] | 1.73±0.62[b] |
| Gloss | 3.00±0.43[a] | 2.82±0.57[a] | 2.91±0.51[a] | 2.64±0.48[a] | 2.36±0.88[a] |
| Cohesiveness | 2.91±0.37[a] | 2.91±0.51[a] | 2.82±0.39[a] | 2.55±0.50[ab] | 2.18±0.72[b] |
| Tenderness | 2.91±0.51[a] | 2.91±0.29[a] | 2.82±0.57[a] | 2.64±0.88[a] | 2.27±0.75[a] |
| Smoothness | 2.82±0.57[a] | 2.73±0.45[a] | 2.91±0.67[a] | 2.64±0.64[a] | 2.27±0.62[a] |
| Taste | 1.82±0.83[a] | 1.91±0.67[a] | 1.82±0.57[a] | 1.55±0.50[a] | 1.45±0.50[a] |
| Off-taste | 1.00±0.00[a] | 1.18±0.39[a] | 1.18±0.39[a] | 1.45±0.66[a] | 1.64±0.77[a] |
| Overall liking | 3.82±0.57[a] | 3.55±0.66[a] | 3.27±0.45[ab] | 2.73±0.62[b] | 2.55±0.66[b] |

Mean values with the same letter within a row are not significantly different at $p$=0.05 ($n$=11)
[1]aroma (1=none, 5=extremely aromatic), off-odor and off-taste (1=none, 5=extremely perceptible),color (1=gray/cream, 5=extremely white), gloss (1=dull, 5=extremely glossy), cohesiveness (1=separated, 5=extremely cohesive), tenderness (1=hard, 5= extremely tender), smoothness (1=rough, 5=extremely smooth), taste (1=bland, 5=extremely tasty), and over-all liking (1=dislike very much, 5=like very much).

blend forms. Off-odor and off-taste were also not perceived among the samples. Meals with 25-50% *Tapol* obtained comparable score for overall liking with pure rice. Higher *Tapol* proportion resulted in lower score.

In general, it can be observed that the low-amylose NSIC Rc 160 received higher scores for tenderness and smoothness than the three *adlai* varieties, despite the latter having an amylose content equivalent to very low. It can be deduced that the amylose property of *adlai* could be different from rice. Sensory evaluation also showed that *Ginampay* can be substituted to rice at any ratio but any of the three varieties can be blended with rice at 50% substitution.

*Consumer*. To determine the preference of the consumers and acceptability of the samples, the three rice:*adlai* blends at 50:50 ratio were subjected to consumer test (Figure 2). Sixty rice consumer representing 18-54 years old and composed of 32% male and 68% female evaluated the samples. Table 10 summarizes the results of the preference and consumer acceptance tests. Percentage acceptability of the control and rice:*adlai* blends ranged 71.67-85%. Among the *adlai* samples, 50:50 rice: *Ginampay* blend was preferred over rice:*Gulian* and rice:*Tapol* blends. It received ratings and rank score comparable with the pure rice. In terms of ranking, the blends were preferred in the following order: pure rice ≥ rice: *Ginampay* ≥ rice: *Tapol* ≥ rice: *Gulian.*


**Figure 2.** Cooked rice:adlai blends at 50:50 ratio.

**Table 10**. Preference score and consumer acceptance of rice:*adlai* blends.

| Item | Mean Sensory Scores | | | |
|---|---|---|---|---|
| | 50:50 rice:*Ginampay* | 50:50 rice:*Gulian* | 50:50 rice:*Tapol* | pure NSIC Rc 160 (Control) |
| *Adults (18-54 yr, n=60)* | | | | |
| % Acceptability[a] | 85.00[a] | 71.67[a] | 73.33[a] | 83.33[a] |
| Rating[b] | 3.22[ab] | 2.97[a] | 2.93[a] | 3.48[b] |
| Rank Score[c] | 2.23[b] | 2.87[a] | 2.85[a] | 2.03[b] |
| Ranking[c] | 2 | 4 | 3 | 1 |

[a]Based on percentage of positive responses
[b]1= poor, 2=fair, 3=good, 4=very good, 5=excellent
[c]1= highest, 4= lowest

***Proximate Composition***

Proximate composition is an estimation of the relative nutritional value of a particular food which includes protein, ash, fat, and dietary fiber. Polished rice usually has 5.8-7.1% crude protein while *adlai* contains 13.1% (Juliano 2010; FNRI, 1997). In addition to its nutritional value, protein contributes to the textural property of rice. Meanwhile, ash represents the total mineral content in foods. Ash content in rice usually ranges from 0.3 to 0.8%. *Adlai*, on the other hand, contains 0.9% ash. Fats and oils are concentrated sources of energy and transport fat-soluble in vitamins in the blood. *Adlai* has higher amount of fat (2.5%) than polished rice (0.3-0.6%). Dietary fiber in rice usually ranges from 0.7 to 2.3% dietary fiber, which is considerably lower than *adlai* (3.7%).

Table 11 presents the proximate composition of all the rice:*adlai* blends at 50:50 ratio. The three *adlai* varieties had significantly higher protein content (12.15-12.91%) than pure rice (8.97%). The values are consistent with data published by the FNRI (1997) in the Philippine Food Composition Table. The higher protein content of *adlai* caused a significant increase in the protein content of all the rice:*adlai* blends. In terms of ash content, only *Tapol* and its blend form produced comparable amount (0.63 and 0.67%, respectively) with pure rice (0.95%). Both *Ginampay* and *Gulian* and their blend forms had lower ash content than the reported values (0.9%). Meanwhile, blending rice with *adlai*, regardless of the variety, significantly increased the fat content of the blend samples by 1.02-1.22%. Dietary fiber content of the blends (1.60-2.10%) was generally comparable with pure rice (1.95%). Pure *adlai* samples had considerably lower amount of carbohydrates compared with rice. However, when rice was blended with *Ginampay*, the carbohydrate content was compensated. Some of the proximate properties of the pure *adlai* varieties were similar with the Thai's white *adlai* as reported by Dechkunchon and Thongngam (2007).

## Conclusion

Factors like gelatinization temperature and ratio of *adlai* to rice affect the cooking and eating quality of rice:*adlai* blends. The high gelatinization property *adlai* was positively correlated with longer cooking time of the rice:*adlai* blends, but the effect depends on the variety and ratio of *adlai*. All the three *adlai* varieties produced heavier meal when blended with rice at any ratio, but the rice:*Ginampay* blend gave

**Table 11**. Proximate composition (%) of rice, *adlai*, and rice:*adlai* (50:50) blends.

| Sample | Crude Protein | Crude Ash | Crude Fat | Dietary Fiber | Carbohydrates |
|---|---|---|---|---|---|
| Rice[1] | 8.97±0.01[d] | 0.95±0.02[a] | 0.32±0.00[c] | 1.95±0.21[bc] | 82.67±0.07[a] |
| Rice:*Ginampay* | 11.52±0.15[bc] | 0.52±0.01[b] | 1.02±0.01[b] | 1.60±0.14[c] | 81.67±0.03[a] |
| *Ginampay* | 12.15±0.35[ab] | 0.49±0.02[b] | 1.89±0.05[a] | 2.35±0.21[ab] | 74.05±0.46[d] |
| Rice:*Gulian* | 11.19±0.08[c] | 0.47±0.01[b] | 1.22±0.10[b] | 1.75±0.07[bc] | 76.75±0.21[c] |
| *Gulian* | 12.38±0.06[a] | 0.45±0.01[b] | 1.96±0.21[a] | 2.20±0.14[bc] | 73.83±0.28[d] |
| Rice:*Tapol* | 11.46±0.20[bc] | 0.67±0.26[ab] | 1.10±0.36[b] | 2.10±0.14[bc] | 78.41±0.38[b] |
| *Tapol* | 12.91±0.30[a] | 0.63±0.05[ab] | 1.99±0.04[a] | 2.95±0.21[a] | 73.50±0.29[d] |

Mean values with the same letter within a column are not significantly different at $p$=0.05 ($n$=2)
[1] NSIC Rc 160

an additional volume that could be translated to more food. *Ginampay* and all its blend forms also produced similar sensory property with pure rice. Consumer acceptability showed that the 50:50 rice:*Ginampay* blend was the most preferred and accepted by the consumers than the *Gulian* and *Tapol* blends. This study showed that *Ginampay* can be substituted to rice at 50:50 ratio as source of carbohydrates, providing the consumer the same eating satisfaction and higher amount of protein and fats. Further studies on the shelf-life of raw and cooked *adlai* are recommended.

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# RICE FARMERS' VULNERABILITY TO DROUGHT HAZARDS AND THEIR AUTONOMOUS ADAPTATION STRATEGIES IN CENTRAL LUZON, PHILIPPINES

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## Abstract

This paper provides empirical analysis on the question: does agriculture-based adaptation strategies improve the resilience of rice farming households? This was answered through a survey of 109 rice farmers in Central Luzon, Philippines. The study used the concept of vulnerability to expected poverty (VEP) to assess farmers' level of vulnerability and employed econometric models to analyze the survey data. Econometric results showed that autonomous adaptation strategies reduce farmers vulnerability to expected poverty not to drought events, but rather to flooding events. Drought events may not have long term consequences on households' vulnerability due to their infrequent occurrences. This also shows that agriculture-based adaptation strategies make households resilient against multiple hazards. Results also indicate the potential role of varietal improvement programs particularly the development of stress tolerant varieties, as a viable, effective, and equitable planned adaptation strategy. The study also emphasizes the need to capacitate extension agents so they can perform an expanded role in advising farmers not only on increasing production but also on improving resiliency against climate hazards. Gender should also be considered in future planned adaptation strategies as it plays a factor in vulnerability assessment and adaptation strategy choice analysis.

**Keywords:** *Autonomous Agriculture-Based Adaptation, Drought, Stress-Tolerant Rice Varieties, Vulnerability to Expected Poverty, Women Empowerment Index, Gender and Adaptation Strategies, Multiple Climate Hazards.*

## Introduction

Climatic variability is one of the major sources of risks and uncertainties in agriculture. Drought is one of the hazards associated with climate change, which has unfavorable impact on households. Typified as limited rainfall that is substantially below the established "normal" value in an area, its occurrence can lead to adverse consequences on human welfare. Over the years, the global extent of drought has significantly widened with climate change identified as a key factor. Serious occurrences of drought affect a large percentage of the world's land area, which more than doubled from the 1970s to the early 2000s (Isendahl and Schmidt, 2006; NCAR and UCAR, 2005). Based on climate models, future projections of drought point to a very significant proportion of the global land surface being affected by extreme levels of the hazard. Consequently, this effect will have substantial impact and will pose adaptation challenges (Burke et al., 2006). In crop production, drought is one of the most important yield-limiting factors (Passioura, 2007). Many agricultural pests are likely to progress and farmers are forced to produce crops under a scenario of reduced fresh water supply due to drought (Grayson, 2013).

Drought is extensive throughout Southeast Asia with highest frequencies of occurrences in Laos, Philippines, Indonesia, and Thailand. The Philippines, in general, is vulnerable to drought spells with high probability (0.41), but still lower than Indonesia (0.46) and Thailand (0.45) (Pandey et al., 2007). A large extent of the country's area has been affected by sporadic drought, the most extensive and most severe of which occurred in 1997-1998, affecting almost 70% of the country. Unlike typhoons and flooding that occur frequently and in distinct areas, drought occurs with a less discernible pattern both in time and space (Department of Agriculture et al., 2010). Thus, it is quite hard to predict when and where the effects of drought would be most destructive. Drought has higher degree of uncertainty than other climate-related hazards such as flooding. The most affected and damaged sector is usually agriculture, in particular, the rice and corn industries. Estimates for three drought episodes (1982-1983, 1991-1992, and 1997-1998) revealed total production losses for both crops amounting to about PhP18.2 billion (US$ 445 million) (Department of Agriculture et al., 2010).

Another study estimated the damage to rice farming to reach an average of PhP2.4M (US$59.9 million) per year from 2007 to 2010 (Israel, 2012).

While there have been studies that looked at the impact of drought and the coping mechanisms associated with it in other Southeast Asian countries (Pandey et al., 2007), only a few had analyzed cases in the Philippines, in particular its implications to the Filipino rice farmers. Studying specific cases is important as results from site-specific assessments can yield effective policies. As Grayson (2013) pointed out, "no universal answer is applicable"; although climate change hazards are global in origin and nature, the most effective responses are context-specific.

This study provides local information through a specific case study of how drought episodes affect Filipino rice farmers and how they respond to this hazard autonomously. In particular, this study answers, "Does agriculture-based autonomous adaptation strategies improve the resilience of rice farming households particularly in two provinces in the Philippines?" Auxiliary to this, are the following research questions: a. How does drought events fare against other weather (i.e., flooding) and non-weather-related farm shocks (i.e., rodent/insect, weeds, and other pests) in affecting the vulnerability of rice farming households?; b. What factors affect rice farming households' vulnerability to expected poverty?; and c. What factors encourage/discourage these households to implement agriculture-based adaptation strategies against drought? It is hoped that the results would provide clear answers to these questions and basis for crafting planned agro-technology interventions that can reduce and manage risks associated with drought in the rice farming communities.

The three main analyses involved in the study were hazard and impact analysis, vulnerability assessment, and analysis of adaptation strategies. Hazard or risk analysis was qualitative in nature. In particular, reduction in rice yield and other damages identified by the farmers from the worst and latest cases of drought were deduced from farmers' perceptions.

For vulnerability assessment, the interest is largely on the impact of drought using a measure that is related to household poverty level and on farmers' adaptation strategies. Thus, analysis is framed on the vulnerability to expected poverty (VEP) concept. The estimated VEP or probability was used as a measure of the household's vulnerability brought about by drought events. This computed VEP measures were then regressed with other variables such as gender, income, and on the estimated probability of using agriculture-based adaptation strategies. This way, associations between some critical variables and household vulnerability were explored. The probability of using agriculture-based adaptation strategies were estimated from a probit regression on the decision to implement farm-based adaptation strategies against demographic, farm characteristics, and institutional (i.e., visits of extension agents) variables. The institutional variable was also used as an overidentifying or instrumental variable to identify the effects of adaptation, along with other key determinants, on the Vulnerability Index of rice farming households. A Tobit regression was used to establish association between these variables and the Vulnerability to Expected Poverty Index.

## Methods

### *Survey Details and Survey Sites*

The study covered sites in two provinces (Tarlac and Pangasinan) in Central Luzon, Philippines. These were selected based on information provided by the local government units (LGUs) in drought-prone areas and project sites of the Consortium for Unfavorable Rice Environments - International Rice Research Institute (CURE-IRRI). The sites in Tarlac included three villages from two municipalities while those in Pangasinan included two villages from one municipality with a large proportion of drought-prone areas. Sample size was proportional to the number of drought-affected rice farmers in the project site. For each site, a target of 20% of the total rice farmers in drought-prone areas was set. Each farmer was selected using simple random sampling. There were 109 farmer-respondents: 67 from Tarlac and 42 from Pangasinan.

Heads of households were mostly male only 10% of households had female household heads, a reflection of the dominant patriarchal structure of Philippine households. On average, household heads had 10 years of education; thus, they have at least completed the primary level of schooling with some years spent in the secondary level. Average farming experience was 22 years and average age of household heads was 52, implying that household heads have been farming for about half of their lives. This is consistent with the finding that work is mostly found inside the village with 76% of household members working within the village and few household members employed abroad.

Households in the study areas, on average, earned an annual income of PhP277,616 (US$6,532) a year before the survey. Respondent household in Tarlac earned more (PhP323,082 or US$7,602) than those in Pangasinan (PhP205,089 or US$4,826); however, the difference was not statistically significant. Data on gender-related decision-making activities in the households were also gathered. Husbands mainly decide on agriculture-related activities while women decide largely on non-agricultural matters such as income allocation and spending on child education. The result on agriculture-related decision-making, however, seems unrepresentative of the whole country as FAO (1996) has noted that women also participate in farming decisions to a great extent. These include decisions on seed selection, harvesting, and threshing.

Irrigation is available for most of the cultivated plots with an average of irrigated 0.97 ha. Considering that the mean parcel size is 1.75 ha, then 55% of cultivated land on average has access to irrigation facilities. Farms in Pangasinan are 460 m away from the irrigation source while farms in Tarlac are only 3 m away. Majority of the farmers were not aware of the existence of drought-tolerant varieties. However, when asked whether they want to try these varieties in the future, 85% of the respondents answered positively. This means that there is a high willingness among farmers to try and grow these varieties.

Net income from rice cultivation accounted, on average, for 34% of total household income. As shown in Table 1, there were more respondents from Tarlac, although marginally, they are more dependent on rice farming (36%) than rice farmer-respondents in Pangasinan (32%). Wet season rice income was significantly higher (at 1%) than dry season income. This pattern was also observed at the provincial level. There was no observed difference in wet season rice income between the respondents from the two provinces. However, mean rice income during the dry season for Tarlac households were significantly higher (at 1%) than Pangasinan households.

**Table 1.** Rice income profile, by study site and season

| | Province | | |
|---|---|---|---|
| | Pangasinan (n=42) | Tarlac (n=67) | Whole Sample (n=109) |
| Wet Season (PhP) | 36,874 | 40,008 | 38,874 |
| Dry Season (PhP) | 7,988 | 30,634 | 22,443 |
| Total (PhP) | 37,7238 | 63,339 | 53,282 |
| % of Total Income | 34 | 40 | 40 |

Table 2 shows that rice-rice is the most common cropping pattern in Tarlac as reported by 44% of the farmers, followed by rice-fallow (32%), rice-rice-mongo (14%), rice-camote (3%), rice-peanut (3%), and rice-vegetables (2%). This means that in dry season, some farmers practiced land fallowing while some planted non-rice crops that are tolerant to drought. This is to avoid significant losses during possible drought events. In contrast, there are only three cropping patterns practiced in Pangasinan with 42% are rice-corn, 33% rice-rice and 26% rice-fallow. Corn is considered the second most important crop in Pangasinan with 42% of the farm households planting in the dry season. This is logical as corn requires less water than rice. This can also partly account for the significantly lower net returns from dry season rice farming for these farming households. Alternative crops such as maize, vegetables, and other dry-season crops are usually cultivated in late August (Mitin, 2009).

**Table 2.** Cropping calendar and cropping pattern, by study site.

| | Tarlac (n=67) | Pangasinan (n=42) | Whole Sample (n=109) |
|---|---|---|---|
| Cropping pattern (%) | | | |
| Rice-Rice | 44 | 33 | 39 |
| Rice-Fallow | 32 | 26 | 29 |
| Rice-Corn | 3 | 42 | 18 |
| Rice-Rice-Monggo | 14 | - | 8 |
| Rice-Camote | 3 | - | 2 |
| Rice-Peanut | 3 | - | 2 |
| Rice-Vegetables | 2 | - | 1 |
| Rice Cropping calendar | | | |
| Wet Season | Jul-Oct | Jul-Oct | Jul-Oct |
| Dry Season | Nov-Feb | Dec-Mar | Dec-Mar |

### *Approach to Estimating Household Vulnerability and Assessing Adaptation Strategies*

As discussed earlier, the study used VEP to measure vulnerability. Aside, from its appeal from being directly related to poverty, this approach was adopted and selected from among the different econometric approaches in measuring vulnerability [e.g., vulnerability as low expected utility (VEU) and vulnerability as uninsured exposure to risk (VER)] because it fits the cross-section data collected for the study. Further, VEP was chosen over index-based approaches because VEU and VER are often very sensitive to changes in arbitrary weights assigned to different categorization of variables.

The expected poverty approach defines vulnerability as the probability that a household will fall into poverty given certain shocks. Poverty is a situation where the consumption of a household falls below a minimum threshold level. To compute for the VEP per household, the method by Chaudhuri et al. (2002) was liberally adopted. Estimation of VEP starts with estimating a consumption determination function. In estimating for this function, characteristics of the household, household head, and farm cultivated were considered as regressors. Gender-related components such as women empowerment index (WEI) (Paris et al., 2010; Hossain and Bose, 2004) were also considered. The WEI is a survey-based index developed to assess the decision-making authority of the wife in relation to the husband in agriculture and non-agriculture activities. An index value closer to 5 indicates that women are empowered; that closer to 1 reflects male dominance in decision-making (Hossain and Bose, 2004).

The consumption determination function is of the following form:

$$\ln C = \beta' X + \varepsilon \qquad (1)$$

where

ln C is the logarithm of consumption expenditure,
X is a matrix of regressors, and
ε is an error term.

The usual assumption on the error term is e~iid N (0,s). Estimation of the consumption determination function[1] would either be through feasible generalized least square (FGLS) — a common estimation procedure to correct for heteroscedasticity, which is observed in consumption data, or robust regression. The expected or estimated consumption level is then computed. The probability of the estimated consumption falling below a critical level will be computed using the following formula under the normal distribution assumption

$$V_h = Pr(ln\, C_h < ln\, z\, |X_h) = \phi(\frac{ln\, z - \hat{\beta} X}{\sqrt{\theta' X}}) \qquad (2)$$

where

$V_h$ is the vulnerability index/measure,

ln z is the critical or minimum household consumption,

$\hat{\beta}X\hat{\beta}X$ is the estimated consumption from the FGLS or robust regression estimation, and

ΦΦ is the cumulative normal function.

Note that the denominator (which is the standard deviation of the consumption determination function) for the cumulative normal function is already corrected for potential heteroscedasticity — that is, the variance is estimated to be also a function of a set of variables.

The estimated probability was used as a measure of the household's vulnerability due to drought events. The computed probability can be interpreted as the probability of consumption falling below a critical level due to shocks.

Probit regression was used in modeling the determinants of household decision to adapt to drought. Households would, in all likelihood, employ a combination of strategies or choose joint adaptation strategies. One econometric model that reflects joint choices and that was initially planned for use in this study is the multivariate probit regression. However, because there were only a few households that chose to employ adaptation strategies, further disaggregation of the data did not yield robust results. Thus, simple probit regression was used.

***Household Vulnerability and Use of Adaptation Strategies: A Tobit Regression***

To determine whether agriculture-based adaptation strategies do affect household vulnerability (or resilience), regression of vulnerability measure ($V_h$) against the use of agriculture-based adaptation strategies and other control variables was generated. There are econometric issues in this regression. First, the dependent variable $V_h$ is constrained to be between 0 and 1. Ordinary least squares may yield predictions for the dependent variable beyond this range. Second, adaptation is potentially endogenous and maybe correlated with the error term. To address these issues, Tobit regression using the predicted probability of adaptation which we obtain from the previously discussed probit regression of the determinants of use of agriculture-based adaptation strategies was employed. Visits of extension agents as an overidentifying variable was counted because it affects the probability of adaptation but not the Vulnerability to Expected Poverty measure. Predicted probability was measured with the variables representing drought, flooding, and other agricultural hazards. These interacted variables are of primary interest because they capture the mitigating effect of adaptation strategies. Finally, bootstrapped standard errors for the estimated coefficients was employed.

## Results and Discussion

Farmers' reported occurrences of drought in their area were largely based on their recall and perception. At the time of the survey, the latest drought occurrence recalled by respondents seemed to have occurred quite recently in 2008, 2010, and 2011. On the other hand, respondents remember worst drought events to have occurred in 2008, 2004, 2003, and 2002. Recall of drought events, however, did not seem to coincide with government recorded drought events. In 2007 and 2010, both provinces had reported drought occurrences but very few respondents were able to recall drought events during these years. Of the 35 respondents who recall the exact year of the drought events, only 2 (6%) cited 2007 as a drought year while only 7 (20%) recalled drought happening in 2010. Five or 14% of these respondents reported drought occurring in 2008. However, no drought affecting the two provinces was officially reported in 2008 (Israel, 2012). The dry spell that hit Pangasinan in 2007 lasted from June to August (Mitin, 2009).

1 For brevity, discussion of the results was not included in this paper. It is available upon request from the authors.

***Hazard and Impact Analysis***

Respondents were mostly affected by drought events in the study sites. Table 3 shows that 52% of the respondents said their crops or farms were affected by drought while only 38% (41 households) indicated that they were affected by flooding and other non-weather-related shocks. In Tarlac, both flooding (34 households or 51%) and drought (32 or 48%) events caused crop problems for rice farming households. In contrast, respondents from Pangasinan felt affected more by drought (25 or 60%) than flooding (7 or 17%) and non-weather-related farm shocks such as weed and pest (insects and rodents) infestations.

**Table 3**. Proportion of households experiencing drought, flooding, and non-weather-related farm shocks, by study site.

| Stress | Province | | Whole Sample (n=109) |
|---|---|---|---|
| | Pangasinan (n=42) | Tarlac (n=67) | |
| Drought | 25 (60%) | 32 (48%) | 57 (52%) |
| Flooding | 7 (17%) | 34 (51%) | 41 (38%) |
| Non-weather farm shocks (e.g., rodent/ pest and weeds) | 14 (33%) | 27 (40%) | 41 (38%) |

Based on farmers' answers, the highest probability of drought occurs around February-March. Rainfall is generally low during dry months (February-April) in the country and drought may occur in some areas (Lansigan et al., 2000). In Tarlac and Pangasinan, 43% and 30% of households, respectively, reported that drought often occurs during these months. Drought was also recorded to have occurred in the country from July to November but not in the study sites.

Respondents were also asked regarding their perception and experience on the severity, length, and frequency of drought episodes during the last 20 years and their perceived projections for the next 20 years. During the past 20 years, less than half of the farmer-respondents perceived that severity (43%), length (49%), and frequency (38%) of drought events have been increasing. Thus, the more dominant perception was that drought spells are getting longer during the past 20 years rather than being more severe and more frequent. Farmers in Pangasinan seemed to be more optimistic about drought events because only a handful of respondents felt that drought spells have become more frequent (28%), longer (33%), and more damaging (19%) in the last two decades. Farmers also shared their perception about future drought episodes. Contrary to their perceived history of drought events, respondents indicated that future events of droughts would be more frequent (62%), longer (51%), and would have more deleterious impact (57%). Thus, farmers' perceptions point to a greater threat from drought in the future, more on frequency rather than on duration or degree of damage. However, only 10-15% of the respondents said their assessment with certainty as climate is quite erratic.

The responses on the impacts of drought are presented in Table 4. The analysis was focused on agricultural impacts, in particular, on yield and total production. There was a 31% reduction in production during the recent cases of drought and 48% reduction during the perceived worst drought occurrence. On the other hand, yield declined by 33% and 41% for the latest and worst occurrence of drought, respectively. A larger relative decline in both yield and total production occurred in Pangasinan, which can be

**Table 4.** Impacts of drought, by study site.

| | Province | | Whole Sample (n=109) |
|---|---|---|---|
| | Pangasinan (n=42) | Tarlac (n=67) | |
| Area planted during normal times | 0.54 | 0.43 | 0.47 |
| Area planted during latest drought | 0.49 | 0.43 | 0.45 |
| Area planted during worst drought | 0.22 | 0.33 | 0.29 |
| Area harvested during normal times | 0.54 | 0.39 | 0.44 |
| Area harvested during latest drought | 0.46 | 0.38 | 0.41 |
| Area harvested during worst drought | 0.19 | 0.28 | 0.25 |
| Total production during normal times | 476.56 | 606.98 | 560.09 |
| Total production during latest drought | 138.28 | 524.72 | 385.78 |
| Total production during worst drought | 63.59 | 420.26 | 292.02 |
| Yield during normal times | 477.66 | 264.25 | 340.98 |
| Yield during latest drought | 175.94 | 256.96 | 227.83 |
| Yield during worst drought | 150.63 | 228.93 | 200.78 |
| Value of loss in livestock during the latest drought | 670.33 | - | 670.33 |
| Value of loss in livestock during the worst drought | 669.67 | - | 669.67 |

explained by the differences in severity of drought occurrence between the two provinces.

Another possible impact of drought is the unavailability of rice. This is especially true if farmers are net buyers or rice consumers. Very few farmers reported to have experienced rice shortages. Only 16 farmers or 15% of the total sample reported having experienced rice shortages. Furthermore, the number of months with rice shortages during normal and drought years was statistically the same for these farmers. Thus, drought, at least for these areas, does not seem to affect household consumption of rice.

***Vulnerability Assessment***

The vulnerability assessment using VEP relies on the predicted value of consumption falling below a minimum threshold level. The predicted consumption was obtained through robust regression. The results of this estimation are available upon request from the authors.

***Estimated Vulnerability Index***

The vulnerability of households to expected poverty due to various shocks is captured through the computed or estimated vulnerability index (VI). For the whole sample, there was a 78% mean probability that rice-farming households will fall below the minimum daily per capita consumption of US$1.25. The mean VEP for Pangasinan respondents was estimated to be at 81% while 75% for the Tarlac respondents. Although the mean VIs for the two provinces were numerically different, a simple t-test proved that the difference was not statistically significant.

The distribution of mean VIs was further analyzed across varying degrees of vulnerability and study sites. Degrees of vulnerability were based on terciles of the data, i.e., the first tercile (0- 0.73) represents low vulnerability, second tercile (0.731-0.97) moderate vulnerability, and the third tercile (> 0.971) as highly vulnerable. Based on this degree classification, most of the respondents in Tarlac were classified under low vulnerability, while for majority of Pangasinan respondents were classified as moderately vulnerable. A crucial question here is whether the VEP measure is somewhat related to drought events or whether what is being captured is simply the existing poverty situation of the farmers, even without the impact of drought and other stresses. This means that the probability of farming households to be poor in general may not be necessarily associated with drought episodes.

To answer this question, the mean VIs of households that experienced drought and those that did not were compared for both provinces and the whole sample (Table 6). For farmer-respondents from Pangasinan, association between drought and the mean VI were not statistically significant. Thus, for this subsample, there was a very weak association between drought and expected poverty. These data captured the vulnerability of households to expected poverty (i.e., their innate susceptibility to being poor apart from whether they have been affected by drought). Note that the earlier discussion on household characteristics also showed that Pangasinan respondents have a lower level of annual household income. Thus, this corroborates the observation that they may be innately poor regardless of the degree of hazard.

**Table 5**. Distribution of vulnerability index, by degree of vulnerability and study site.

| Degree of Vulnerability | Province | | Whole Sample (n=109) |
|---|---|---|---|
| | Pangasinan (n=42) | Tarlac (n=67) | |
| Low vulnerability (0<VI≤0.73) | 11 (26%) | 26 (39%) | 37 (34%) |
| Moderately vulnerable (0.73<VI I≤0.97) | 16 (38%) | 20 (30%) | 36 (33%) |
| Highly vulnerable (VI>0.97) | 15 (35%) | 21 (31%) | 36 (33%) |
| Total | 42 | 67 | 109 |

**Table 6**. Mean vulnerability index, by drought experience and study site.

| | Province | | All |
|---|---|---|---|
| | Pangasinan | Tarlac | |
| Did not experience drought | 0.76 (n=17) | 0.67** (n=35) | 0.70*** (n=52) |
| Experienced drought | 0.84 (n=25) | 0.84** (n=21) | 0.84*** (n=57) |
| Whole sample | 0.81 (n=42) | 0.75 (n=67) | 0.78 (N=109) |

The situation of Tarlac households was different. There was a significant difference in the probability of falling into poverty between the two types of households. VEP increased by 17 percentage points when households were affected by drought events. Thus, Tarlac households are indeed susceptible to drought. The same can be said for the whole sample. The statistically significant difference is noticeable in mean VI between households affected by drought events and those that were not affected. On average, for the whole sample, households who experienced the effects of drought were 14% points more likely to fall into expected poverty.

***Vulnerability, Farm Size, and Income Categories***

This section looks at the qualitative relationship between the estimated mean VI and key farming and socioeconomic characteristics of the households. In particular, association between farm size, income categories, and the degree of vulnerability were studied to have a deeper understanding of the agrarian and socioeconomic nature of the most vulnerable groups. This analysis considered only the 57 households affected by drought episodes.

Farmers with large landholdings are often better-off and are thus in a better position to cope with the deleterious impacts of drought. However, from Table 7, most households affected by drought were highly vulnerable, regardless of farm size. Across the three farm sizes, it is observed that the highest proportion of drought-affected households are in the highly vulnerable category (i.e., 53% for marginal sized farms, 43% for medium sized farms, and 58% for large farms). Within each vulnerability degree category, the highest proportion of drought-affected households fall within the large farm category. For drought-affected households with low vulnerability to expected poverty, 32% are classified as large farm owners. Similarly, 58% of highly vulnerable sampled households were large farm owners. On the other hand, the farm size distribution seems to be different for moderately vulnerable households. For households in this vulnerability degree category, majority of rice farmers were considered as medium farm size owners.

A more direct correlate of the household's capacity to bear shocks is income. Patterns were analyzed between income quartiles and the degree of VEP due to drought and other farm related shocks. For the whole sample, drought-affected households were somewhat equally distributed across the income quartiles. Differences in VEP distribution is more evident within the vulnerability degree categories. Majority of highly-vulnerable respondents fall in the mid quartiles, i.e., quartiles 2 (56%) and 3 (60%). Households classified under low vulnerability to expected poverty, were mostly in the 4$^{th}$ quartile (42%). Finally, moderately vulnerable drought-affected rice farmers were concentrated in the 1$^{st}$ quartile (43%). Like the previous discussion, no clear discernable pattern is seen between the relationship in income quartiles and vulnerability degrees; thus, quantitative Tobit regression supplements the qualitative discussion.

**Table 7**. Degrees of vulnerability for drought-affected households, by farm size category.

| Farm Size | Degree of Vulnerability | | | Total |
|---|---|---|---|---|
| | Low Vulnerability | Moderately Vulnerable | Highly Vulnerable | |
| Marginal (< 1 ha) | 4 (24%) | 4 (24%) | 9 (53%) | 17 |
| Medium (1 to 2 ha) | 4 (19%) | 8 (38%) | 9 (43%) | 21 |
| Large (> 2 ha) | 6 (32%) | 2 (11%) | 11 (58%) | 19 |
| Total | 14 (24%) | 14 (24%) | 29 (51%) | 57 |

**Table 8**. Degrees of vulnerability for drought affected households, by income quantile.

| Income Quantile (PhP) | Degree of Vulnerability | | | Total |
|---|---|---|---|---|
| | Low Vulnerability | Moderately Vulnerable | Highly Vulnerable | |
| Quantile 1 (≤54,000) | 2 (14%) | 6 (43%) | 6 (43%) | 14 |
| Quantile 2 (54,001 - 126,613) | 4 (25%) | 3 (19%) | 9 (56%) | 16 |
| Quantile 3 (126,61 - 230,092) | 3 (20%) | 3 (20%) | 9 (60%) | 15 |
| Quantile 4 (>230,092) | 5 (42%) | 2 (17%) | 5 (42%) | 12 |
| Whole sample | 14 (25%) | 14 (25%) | 29 (51%) | 57 |

***Households' Choice of Agriculture-Based Adaptation Strategies Against Drought***

Only 29 (51%) of drought-affected households used some form of agriculture-based adaptation strategy to drought. Farmer respondents did not adapt for a variety of reasons. Forty percent (40%) of the respondents indicated lack of information about the appropriate adaptation options as their primary reason. The second most common reason for non-adaptation is the lack of information regarding long-term climate change (38%). This corroborates the earlier finding that farmers are not very certain about future trends of drought episodes.

Table 9 shows the common agriculture-based adaptation strategies employed by rice farming households in the two study sites. Top three most commonly employed adaptation strategy can generally be categorized as either adjustments in cultivation practices or installation of farm infrastructure. For all drought-affected households, the most common strategy is the shifting of planting dates. This was done by 19% of all drought affected households. Planting rice is often delayed because seedlings are short and small due to limited water (Mitin, 2009).

Adoption of new rice variety was also a common practice as 14% of all drought-affected farmers employed this strategy. This shows the potential of rice varietal improvements to be part of a broader planned adaptation strategy against drought. Respondents indicated their willingness to grow drought-tolerant varieties. This is not surprising as the impact of drought is primarily reduced yield. Furthermore, literature points to the importance of drought-tolerant varieties or technologies in general in crafting plausible solutions to ease the effects of drought.

In eastern India, a higher social return was seen on drought-tolerant rice research and dissemination (Gautam, 2009). Technologies focusing on stress tolerance are central to improving the plight of farmers in unfavorable environments as these technologies help stabilize rice yield and reduce farmers' vulnerability to shocks and stresses (Pandey and Bhandari, 2009; Pandey et al., 2012). The choice of cultivating modern varieties over traditional ones resulted in a very convincing increase in yield (Pandey et al., 2012). It was found that the use of drought-tolerant varieties can increase yield and result in lower rice prices under varying climate scenarios generated from a climate model. This, in turn, could translate into improved food security and better nutritional outcome for households in the future (Mottaleb et al., 2012).

Specific adaptation practices also differed across study sites. Farmer-respondents in Pangasinan modified their cultivation practices through shifting of planting dates (16%), diversification of crops (12%) and farming activities (16%), and increase used of chemical inputs and fertilizers (12%). Infrastructure improvements were done through construction of water storing facilities (20%). These types of infrastructures were the most widely used adaptation strategy against drought in this study area.

**Table 9.** Distribution of agriculture specific adaptation practices among drought affected households, by province.

| Agriculture Specific Adaptation Practice | Pangasinan (n=25) | Tarlac (n=32) | Whole Sample (n=57) |
|---|---|---|---|
| 1. Crop diversification (mixed/multi-cropping) | 12% | 22% | 18% |
| 2. Crop rotation | 4% | 22% | 14% |
| 3. Shift/different planting dates | 16% | 22% | 19% |
| 4. Water storage (i.e., construction of wells, dams, impoundments, and rainwater harvesting) | 20% | 9% | 14% |
| 5. Improve irrigation systems (i.e., water-saving irrigation systems) | 4% | 19% | 12% |
| 6. Adoption of water-saving technologies (i.e., alternate wetting and drying irrigation) | 4% | 6% | 5% |
| 7. Shift from single to double cropping | 4% | 0% | 2% |
| 8. Change amount of land under cultivation (i.e., increase land area) | 4% | 6% | 5% |
| 9. Adoption of new rice varieties | 12% | 16% | 14% |
| 10. Implement soil conservation techniques (terracing to conserve soil and moisture) | 0% | 0% | 0% |
| 11. Integrate other farming activities (i.e., cattle raising and vegetable planting) | 16% | 6% | 11% |
| 12. Buy insurance | 0% | 3% | 2% |
| 13. Increase fertilizer and chemical inputs (i.e., pesticides) | 12% | 25% | 19% |

In contrast, Tarlac farmer-respondents opted to improve their irrigation systems (19%). Irrigation, aside from the adoption of high-quality seeds, is a primary factor that helped raise the level of rice yields in the Philippines throughout the years across regional, seasonal, or ecosystem boundaries (Mataia et al., 2011). Modification in cultivation practices came in the form of crop diversification (22%), crop rotation (22%), shifting of planting dates (22%), and increased used of inputs (25%). Unlike respondents in Pangasinan, it was more common among Tarlac rice farmers to increase input use in response to droughts than improve farm infrastructures. For the whole sample, the distribution of adaptation strategies are similar with the Pangasinan farmers. However, the proportions are different. It was more common to modify cultivation practices through shifting of rice planting dates (19%) than water storage improvements (14%).

To better understand the choice of agriculture-based adaptation strategies of the households, the sample was further disaggregated by degree of vulnerability, farm size categories, and female dominance in agricultural and non-agricultural decision making. Table 10 shows the results for the degree of vulnerability disaggregation. For the whole sample, it seems that the distribution of agriculture-based adaptation practices is more dispersed for highly vulnerable drought-affected households relative to those that had low vulnerability. Low-vulnerable farmers tend to use only a few agriculture related adaptation strategies. Furthermore, it can be seen that highly-vulnerable farmers favor more modifications in their farming or cultivation practices. This is in contrast to mildly-vulnerable farmers who favor farm infrastructure improvements like building water storage (36%). The differences in the range of adaptation practices across vulnerability categories are more evident at the provincial level. For both provinces, low-vulnerable households tend to use only a few adaptation practices.

Table 11 shows the distribution of agriculture-based adaptation strategies as it relates to gender dominance in agricultural and non-agricultural decision making. For drought-affected households, the husband dominates in both agricultural and non-agricultural decision-making. In 42 households (or 74% of drought-affected households), the husband decides on non-agricultural matters. Agricultural related decisions were mostly by husbands in 56 (98%) households. Thus, male dominance is noted in both decision-making spheres. Table 11 also shows that husbands prefer to modify crop planting dates as an adaptation strategy against drought. This strategy is also chosen in 24% and 20% of households in which husbands dominate non-agricultural and agricultural decision making, respectively. In contrast, wife chose crop diversification (20%) and establishment of water storage (100%) as adaptation strategies against drought.

The choice of adaptation strategies was also related to the size of landholdings of drought-affected households. Drought-affected households with large landholdings generally employed more adaptation strategies than households with marginal and medium sized landholdings. While this is maybe evident for the whole sample, this observation is more pronounced at the provincial level, in particular for drought-affected rice farmers in Pangasinan. Commonly used adaptation strategy includes modification of planting dates for both marginal (24%) and large (26%) landed farmers. For farmers with large holdings, increase in input usage and crop diversification was also equally widely practiced. At the provincial level, the adoption of new rice varieties was identified as a common adaptation strategy. Twenty percent of farmers with marginal landholdings and 23% of farmers with medium landholdings practiced this strategy in Pangasinan and Tarlac, respectively.

***Determinants of Choice of Adaptation Strategies***

The choice to employ agriculture-based adaptation strategies against drought can be influenced by numerous factors. Knowing these factors is of interest from policy and methodological perspective. As discussed earlier, predicted probability of adaptation is used as determinant of vulnerability of rice farming households. The probit regression on possible determinants of adaptation against drought are presented in Table 13. One of the drivers of autonomous adaptation is the drought event itself. Rice farmers are more likely to employ adaptation strategies if they have been affected by drought. This simply means that farmers are rational and do respond to threats autonomously. The marginal effects for this variable showed that the households experiencing the impacts of drought are 50 percentage points more likely to adapt than households that have not experienced drought. Among the variables, experience has the largest significant marginal effect, implying that this is, ceteris paribus, the strongest factor determining the choice whether to adapt. Likewise, the proportion of female household members also increases the likelihood of employing a number of agriculture-based adaptation strategies. A 1% increase in the number of female household members results into a 39% point increase in the likelihood of practicing agriculture-based adaptation practices. Visits by extension worker (i.e., the overidentifying variable), however, is associated with lower probability of use of agriculture-based adaptation strategies against drought. Calculated marginal effects show that the likelihood of employing adaptation strategies is lower

**Table 10.** Distribution of agriculture specific adaptation practices among drought-affected households, by province and degree of vulnerability.

| Adaption Practice | Pangasinan | | | Tarlac | | | Whole Sample | | |
|---|---|---|---|---|---|---|---|---|---|
| | Low Vulnerability (n=6) | Mild Vulnerability (n=7) | High Vulnerability (n=12) | Low Vulnerability (n=8) | Mild Vulnerability (n=7) | High Vulnerability (n=17) | Low Vulnerability (n=14%) | Mild Vulnerability (n=14) | High Vulnerability (n=29) |
| 1. Crop diversification (mixed/multi cropping) | 33% | 14% | 0% | 13% | 14% | 29% | 21% | 14% | 17% |
| 2. Crop rotation | 0% | 14% | 0% | 13% | 43% | 18% | 7% | 29% | 10% |
| 3. Shift/different planting dates | 17% | 29% | 8% | 0% | 29% | 29% | 7% | 29% | 21% |
| 4. Water storage (i.e. construction of wells, dams, impoundments, and rain-water harvesting) | 0% | 57% | 8% | 0% | 14% | 12% | 0% | 36% | 10% |
| 5. improve irrigation systems (i.e. water saving irrigation systems) | 0% | 14% | 0% | 25% | 14% | 18% | 14% | 14% | 10% |
| 6. Adoption of water-saving technologies (i.e., alternate wetting and drying irrigation) | 0% | 14% | 0% | 0% | 14% | 6% | 0% | 14% | 3% |
| 7. Shift from single to double cropping | 0% | 14% | 0% | 0% | 0% | 0% | 0% | 7% | 0% |
| 8. Change amount of land under cultivation (i.e. increase land area) | 17% | 0% | 0% | 13% | 0% | 6% | 14% | 0% | 3% |
| 9. Adoption of new rice verities | 0% | 29% | 8% | 0% | 29% | 18% | 0% | 29% | 14% |
| 10. Implement soil conservation techniques (terracing to conserve soil and moisture) | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| 11. Integrate other farming activities (i.e., cattle raising and vegetable planting) | 17% | 29% | 8% | 0% | 0% | 12% | 7% | 14% | 10% |
| 12. Buy insurance | 0% | 0% | 0% | 0% | 0% | 6% | 0% | 0% | 3% |
| 13. Increase fertilizer and chemical inputs (i.e., pesticides) | 0% | 29% | 8% | 0% | 29% | 35% | 0% | 29% | 24% |

**Table 11**. Distribution of agriculture specific adaptation practices among drought affected households, by province and husband/ wife dominance in agriculture and non-agriculture decision-making.

| Adaptation Practice | Husband Dominant in Non-Agriculture Decision-Making (n=42) | Wife Dominant in Non-Agriculture Decision-Making (n=15) | Husband Dominant in Agriculture Decision-Making (n=56) | Wife Dominant in Agriculture Decision-Making (n=1) |
|---|---|---|---|---|
| 1. Crop diversification (mixed/multi cropping) | 17% | 20% | 18% | 0% |
| 2. Crop rotation | 17% | 7% | 14% | 0% |
| 3. Shift/different planting dates | 24% | 7% | 20% | 0% |
| 4. Water storage (i.e., construction of wells, dams, impoundments and rain water harvesting) | 14% | 13% | 13% | 100% |
| 5. Improve irrigation systems (i.e., water-saving irrigation systems) | 12% | 13% | 13% | 0% |
| 6. Adoption of water-saving technologies (i.e., alternate wetting and drying irrigation) | 5% | 7% | 5% | 0% |
| 7. Shift from single to double cropping | 2% | 0% | 2% | 0% |
| 8. Change amount of land under cultivation (i.e., increase land area) | 7% | 0% | 5% | 0% |
| 9. Adoption of new rice varieties | 17% | 7% | 14% | 0% |
| 10. Implement soil conservation techniques (terracing to conserve soil and moisture) | 0% | 0% | 0% | 0% |
| 11. Integrate other farming activities (i.e., cattle raising and vegetable planting) | 10% | 13% | 9% | 100% |
| 12. Buy insurance | 2% | 0% | 2% | 0% |
| 13. Increase fertilizer and chemical inputs (i.e., pesticides) | 24% | 7% | 20% | 0% |

**Table 12**. Distribution of agriculture specific adaptation practices among drought affected households, by province and farm size.

| Adaption Practice | Pangasinan | | | Tarlac | | | Whole Sample | | |
|---|---|---|---|---|---|---|---|---|---|
| | Marginal (n=10) | Medium (n=28) | Large (n=7) | Marginal (n=7) | Medium (n=13) | Large (n=12) | Marginal (n=17) | Medium (n=21) | Large (n=19) |
| 1. Crop diversification (mixed/multi cropping) | 0% | 13% | 29% | 14% | 23% | 25% | 6% | 19% | 26% |
| 2. Crop rotation | 0% | 0% | 14% | 14% | 31% | 17% | 6% | 19% | 16% |
| 3. Shift/different planting dates | 20% | 13% | 14% | 29% | 8% | 33% | 24% | 10% | 26% |
| 4. Water storage (i.e., construction of wells, dams, impoundments, rain water harvesting) | 20% | 25% | 14% | 14% | 8% | 8% | 18% | 14% | 11% |
| 5. Improve irrigation systems (i.e., water-saving irrigation systems) | 0% | 0% | 14% | 29% | 23% | 8% | 12% | 14% | 11% |
| 6. Adoption of water saving technologies (i.e., alternate wetting and drying irigation) | 0% | 0% | 14% | 0% | 15% | 0% | 0% | 10% | 5% |
| 7. Shift from single to double cropping | 0% | 0% | 14% | 0% | 0% | 0% | 0% | 0% | 5% |
| 8. Change amount of land under cultivation (i.e., increase land area) | 0% | 0% | 14% | 0% | 8% | 8% | 0% | 5% | 11% |
| 9. Adoption of new rice varities | 20% | 0% | 14% | 0% | 23% | 17% | 12% | 14% | 16% |
| 10. Implement soil conservation techniques (terracing to conserve soil and moisture) | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| 11. Integrate other farming activities (i.e., cattle raising and vegetable planting) | 10% | 13% | 29% | 0% | 0% | 17% | 6% | 5% | 21% |
| 12. Buy insurance | 0% | 0% | 0% | 0% | 0% | 8% | 0% | 0% | 5% |
| 13. Increase fertilizer and chemical inputs (i.e., pesticides) | 20% | 0% | 14% | 14% | 23% | 33% | 18% | 14% | 26% |

**Table 13**. Probit regression on the determinants of adaptation against drought.

| Independent Variable | Coefficient | Standard Error |
|---|---|---|
| Age of household head | -0.083 | 0.139 |
| (Age of household head)^2 | 0.001 | 0.001 |
| Gender of household head | -0.855 | 0.526 |
| Years of schooling of household head | -0.082 | 0.071 |
| Dependency ratio | -0.078 | 0.244 |
| % of female household members | 1.868* | 1.126 |
| Number of household members with off-farm work | -0.336 | 0.281 |
| Wife dominant in non-agricultural decision-making | -0.142 | 0.425 |
| Wife dominant in agricultural decision-making | -0.16 | 0.629 |
| Type of house material | -0.176 | 0.197 |
| Medium farm size (1-2 ha) | 0.181 | 0.486 |
| Large farm size (> 2 ha) | 0.2 | 0.448 |
| Household experienced worst drought in 2004 | -0.757 | 0.575 |
| Household experienced worst drought in 2003 | -0.252 | 0.962 |
| Household experienced worst drought in 2002 | 0.461 | 0.616 |
| Crops affected by drought | 2.681*** | 0.577 |
| Crops affected by non-weather related farm shocks | 0.537 | 0.427 |
| Crops affected by flooding | 0.622 | 0.45 |
| % of household income from rice cultivation | -0.786 | 0.746 |
| Has access to irrigation during dry season | -0.081 | 0.833 |
| Household with access to credit | 0.538 | 0.66 |
| Provincial dummy | 0.078 | 0.068 |
| Household visited by extension worker | -1.077** | 0.543 |
| Constant | -4.052 | 6.428 |

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$

by 29% points for households who were visited by an agricultural extension worker. This is surprising as agricultural extension workers are supposedly a source of knowledge and farm advise. These agents traditionally provide knowledge and technology to increase production and incomes of farmers. However, in the advent of increasing concern for the impacts of climate change, their role should have expanded to providing advice on adaptation and other resilience-improving measures and technology. However, probit regression results suggest that extension agents may, on the contrary, impede or lower the likelihood of adaptation by rice farmers.

### *Agriculture Based Adaptation and Vulnerability Index (VI)*

Before looking at the determinants of rice farming households' Vulnerability Index, possible correlation was first analyzed between adaptation and vulnerability through simple cross-tabulation. This would indicate whether the two variables are associated. For the whole sample, significant difference was not established between the mean VI of adapting and non-adapting rice farming households. At the provincial level, however, marginally significant difference was observed only for rice farmer respondents from the province of Pangasinan. Farmers who used agriculture-based adaptation strategies were less likely to fall into expected poverty by 16 percentage points for households in Pangasinan.

**Table 14.** Agriculture-based adaptation and mean vulnerability index (VI) among drought affected households, by study site.

| | Province | | Whole Sample |
|---|---|---|---|
| | Pangasinan | Tarlac | |
| Did not use agriculture-based adaptation | 0.91* (N=15) | 0.80 (N=13) | 0.86 (N=28) |
| Used agricultural-based adaptation | 0.75 (N=10) | 0.87 (N=19) | 0.83 (N=29) |
| Total | 25 | 32 | 57 |

### *Determinants of Vulnerability Index*

Identifying factors that help determine the vulnerability level of households were done through a Tobit regression and the results of this regression are shown in Table 15. There were gender-related variables such as gender of household head and wife dominance in agricultural and non-agricultural decision making that affected the estimated VIs. In particular, male headed households were associated with lower estimated VIs. Similar studies in rural India support this finding in that women were found to contribute more to increasing the probability of poverty (Jha et al., 2012). Women in the rural areas tend to

**Table 15**. Results of Tobit regression of the determinants of VI.

| Independent Variable | Coefficient | Standard Error |
|---|---|---|
| Age of household head | -0.002 | 0.009 |
| (Age of household head)^2 | 0.000 | 0.000 |
| Gender of household head | -0.185*** | 0.056 |
| Years of schooling of household head | -0.001 | 0.005 |
| Dependency ratio | 0.061*** | 0.015 |
| % of female household members | 0.072 | 0.060 |
| Number of household members with off-farm work | 0.154*** | 0.017 |
| Wife dominant in non-agricultural decision-making | -0.190*** | 0.024 |
| Wife Dominant in agricultural decision-making | -0.409*** | 0.054 |
| Type of house material | -0.122*** | 0.011 |
| Medium farm size (1-2 ha) | -0.001 | 0.028 |
| Large farm size (> 2 ha) | -0.105*** | 0.03 |
| Household experienced worst drought in 2004 | -0.083* | 0.043 |
| Household experienced worst drought in 2003 | -0.003 | 0.051 |
| Household experienced worst drought in 2002 | -0.160*** | 0.042 |
| Crops affected by drought | 0.041 | 0.051 |
| Estimated probability of adaptation | -0.463 | 0.806 |
| Estimated probability of adaptation x crops affected by drought | 0.571 | 0.749 |
| Crops affected by non-weather related farm shocks | -0.026 | 0.031 |
| Estimated probability of adaptation x crops affected by non-weather-related shocks | -0.069 | 0.076 |
| Crops affected by flooding | 0.157*** | 0.034 |
| Estimated probability of adaptation x crops affected by flooding | -0.161** | 0.067 |
| % of household income coming from rice cultivation | 0.286*** | 0.039 |
| Has access to irrigation during dry season | -0.031 | 0.036 |
| Household with access to credit | -0.026 | 0.036 |
| Provincial dummy | -0.012*** | 0.004 |
| Constant | 2.078*** | 0.367 |

concentrate on household chores and oftentimes, labor market opportunities for women in the countryside are limited as work and farming are traditionally reserved for men. The World Bank (2009) also reported that women are generally less resilient in coping with shocks because they have relatively limited access to credit, inputs, and extension services. In India, gender disparity in employment is evident as women receive lower wages than men in casual labor (Holmes et al., 2010). On the other hand, wife dominance in both agricultural and non-agricultural decision-making spheres are correlated with lower vulnerability to expected poverty.

Also seen from the Tobit results is that the dependency ratio has a positive and significant coefficient. This means that vulnerability increases as more household members are in the 0-14 and above 65 age groups. Thus, households with more unemployed members are more vulnerable to expected poverty. In general, a larger family size, even with family members not categorized as dependents, is found to be strongly associated with high vulnerability to poverty (Orbeta, 2005). The finding that "the larger the dependency ratio of a household is, the more vulnerable the household is to poverty" agrees with results from similar studies in areas such as Central Asia, India, Timor-Leste, and rural southwest Nigeria (Jha and Dang, 2008; Jha and Dang, 2009; Jha et al., 2012; Adepoju and Yusuf, 2012).

The number of members with off-farm work also has a positive and significant coefficient. Calculated marginal effects showed that an additional household member working off-farm increases vulnerability by 15 percentage points. Off-farm work are usually part of the income diversification strategies of households. However, regression results showed that, at least for the sampled households, this strategy is not a very effective insurance mechanism. This is maybe because these rice farming households have little skill other than rice farming; thus, ending up with very menial jobs that does not gain high income.

Households with houses built with more permanent materials were also found to have lower vulnerability indices. Marginal effects showed that they are less likely to fall below the poverty threshold by 12 percentage points than farmers with dwellings built with temporary and weak materials. Housing materials is often a proxy for both wealth and adaptive capacity of households. Hence, it is as expected associated with a household's lower vulnerability to expected poverty. Similar logic applies to landholdings. Households with larger landholdings are often also wealthier and in a better capacity to self-insure against idiosyncratic and systemic shocks. Thus, they can smoothen their consumption better. Households owning farms around 2 ha or larger are less likely to fall into expected poverty by around 12 percentage points relative to households with marginal farm sizes.

Coefficients of dummy variables representing drought episodes in 2004 and in 2002 were also found to be significant and negative. This may mean that from farmers' perception, these drought episodes were less severe compared in 2008, which is the base drought event. Thus, they lead to lower vulnerability relative to drought occurrence in 2008. Drought impacts on crops, however, was found to be an insignificant determinant of the household's Vulnerability Index. Consequently, the interaction term between crop impacts and the estimated probability of adaptation was also not associated with the household's Vulnerability to Expected Poverty. These results may be related to the nature of drought as a climate change-related hazard. Specifically, droughts are extreme but often sparsely recurring events. Therefore, it is possible that the farmers can recover gradually from its impact, i.e., there is no sustained or lasting effect on the vulnerability level of rice farming households.

In contrast, frequently recurring climate change-related hazards like flooding have a significant positive association with the Vulnerability Index. The VI of flood-affected farmers were higher by 15% points. Thus, compared with drought events, flooding events is associated more with increased vulnerability to expected poverty. The frequent recurrence of floods might make it harder for households to recover from its negative crop impacts. Note, however, that the Tobit results also show that adaptation against drought is also effective against flooding. The interaction term between crops being affected by flood and the estimated probability of adaptation has a negative and significant coefficient. Marginal effects for this variable show that VI is reduced by 16% points. Therefore, the net effect of increased probability of adaptation is a decline in the probability of falling into poverty by 1% points. This shows that adaptation strategies of rice farming households can potentially manage risk from multiple climate change related hazards. Thus, agro-technological strategies must be adaptive to multiple types of climate-related hazards.

Table 15 also shows that households with income largely from rice cultivation are more likely to fall into expected poverty. This can be related to the earlier finding that the main impact of drought is on reduced yield. Thus, households that are highly dependent on rice farming (i.e., those that derive a majority of total income from rice farming) are also more vulnerable.

Vulnerability to expected poverty was also found to be dependent on the provincial dummy. In particular, Tarlac respondents had a lower likelihood of falling into expected poverty by 1% points than those from Pangasinan. This result is consistent with earlier discussion pertaining to Tarlac respondents having higher annual rice income and were mostly classified as having low vulnerability to expected poverty. Thus, there are intrinsic provincial differences in the likelihood of being susceptible to poverty apart from the impact of extreme events.

## Conclusion and Recommendations

This study sought to answer, "Does agriculture-based autonomous adaptation strategies improve the resilience of rice farming households in Pangasinan and Tarlac, Philippines?" The discussion presented in this paper highlights the implications of the study's key results on crafting programs and policies that can increase rice farmers resiliency against climate-related hazards. Policy directions that could be identified from the synthesis can pave the way for a more targeted development and dissemination of agro-based adaptation strategies.

One of the limitations of the study includes the small sample size. Future similar studies may consider bigger samples for wider assessment of vulnerability to hazards and poverty. Despite this limitation, the study has shown significant results within the acceptable sampling frame and methodological design that can provide insights into policy and program reforms.

This study focused on agriculture-based adaptation strategies. The household survey showed that the most common response to drought among the surveyed rice farmers was shifting of planting dates and crop diversification. Notably, adoption of new rice varieties was also a preferred strategy against drought. Households who were highly vulnerable to expected poverty were also found to employ more adaptation strategies against drought. This observation, along with the fact that experiencing drought is the primary determinant of using agriculture-based adaptation strategies, point to a high degree of rationality among farmers in their decision to self-insure against drought.

Choice of adaptation strategy also varied by size of landholdings and gender dominance in household decision making. Adoption of new rice varieties as an adaptation strategy were more common among medium and marginal landed rice farmers. Therefore, varietal improvement programs must consider resiliency and social equity. Introduction of stress-tolerant modern rice varieties can be effective, acceptable, and equitable planned adaptation strategy to reduce vulnerability against drought. The literature further supports the importance of drought-tolerant varieties or technologies, in general, to ease the effects of the hazard.

Dominance in household decision-making was also seen to be related to the choice of adaptation strategy. Qualitative analysis showed that if the husband is the dominant decision maker in both the agricultural and non-agricultural decision-making spheres, shifting of planting dates was the preferred adaptation strategy. On the other hand, wife dominance in household decision-making would prefer crop diversification and establishment of water storage facilities as adaptation strategy. The differences in preferred strategies imply the need to consider women's preferences in designing adaptation strategies for them to be acceptable. Enhancing the role of women and ensuring their participation in agricultural decision-making and programs can also be part of complementary support schemes to increase resilience of households.

Use of agriculture-based adaptation strategies were found to be constrained mostly by information. The often-cited reason by respondents for non-adaptation was the lack of information on adaptation strategies and on the long-term climate conditions. The probit regression results showed that visits of extension agents reduce the likelihood of employing agriculture-based adaptation strategies. It can be surmised that extension agents' orientation is still anchored on the traditional objective of enhancing farm production. There is a need to reorient and equip extension agents so they can perform a wider role in increasing farmer resiliency amidst climate change. Better access to short- and long-term weather and climate information can be an important starting point. Building capacity of extension agents to interpret and identify weather and climate knowledge products that are available is also essential. As the preferred adaptation strategies of rice farmers are shifting of planting dates and crop diversification, processing these weather and climate information into adaptive crop calendars and cropping patterns would also improve the advising capacity of extension agents.

Vulnerability assessment through the use of the Vulnerability to Expected Poverty (VEP) was also conducted in this study. While the qualitative analysis on adaptation choices yielded distinct trends, the trends in VEP and its association with different driving factors were less discernible. Hence, deeper insights were more readily obtained through the Tobit regression on the determinants of the VEP or the Vulnerability Index. Improvements in the VI were found to be associated with the household's wealth level as proxied or captured by the size of his landholdings and the type of housing materials. In particular, farmers with larger landholdings and with houses built with more permanent materials had lower VI levels.

Similar to the adaptation choice analysis, wife's dominance in both the agricultural and non-agricultural decision-making reduced households VI. However, households headed by females were associated with higher VI or are more vulnerable to expected poverty. This may be due to conditions that women in the study sites had no access to work outside of the farm and the house. While greater participation of women inside the household reduces the household's vulnerability to expected poverty, however, limited economic opportunities outside of the household may reducing their participation. Enhancing the role of women in income diversification and making agricultural decisions can therefore enhance the resiliency of households. They should be offered equal opportunities to attend agricultural training and to have access to livelihood projects that can contribute to household income diversification.

The impact of climate-related hazards and the effectiveness of agriculture-based adaptation strategies are related to the nature of the hazards. Study results showed that drought, because of its intermittent or infrequent but extreme nature, had no effect on the vulnerability of the households to expected poverty. Households have time to recover in between these infrequent drought events. On the other hand, results showed that flooding, which affects crop production, increases the vulnerability of households to expected poverty. In contrast to drought, floods are frequent extreme events. Therefore, rice farming households are more prone to poverty brought about by floods. Surprisingly, adaptation strategies aimed at mitigating the impacts of drought also mitigated the impacts of floods. Thus, agriculture-based adaptation strategies increase resiliency of households in the two study areas, but not for drought events. Furthermore, these adaptation strategies make rice farmers resilient to multiple climate-related hazards.

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FULL PAPER

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# LEARNING BY DOING: CHALLENGES AND OPPORTUNITIES OF UNDERGRADUATE STUDENTS IN AGRICULTURE AT PHILRICE

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## Abstract

Learning does not only happen within the four corners of the classroom. This study evaluated the contributions of internship programs of local and international higher education institutions (HEI) implemented in the Plant Breeding and Biotechnology Division (PBBD) of Philippine Rice Research Institute (PhilRice). Specifically, this study evaluated the students' capacity of engagement in field work, contributions to the hybrid rice breeding program, and learnings from linking with HEI using active feedback mechanisms, key informant analysis, and tracer methods. From 2012 to 2017, PBBD accommodated 40 agriculture students from Nueva Ecija University of Science and Technology (NEUST), Don Mariano Marcos Memorial State University (DMMMSU), Central Luzon State University (CLSU), Mindoro State College of Agriculture (MINSCAT), and Khon Kaen University (KKU-Thailand) in the conduct of their thesis, major practice, and data analyses on hybrid rice breeding and seed production. The students were provided tasks relevant to their specialization. Majority of the students rated their experience as excellent and relevant to their studies and future career (60%) and most of the appreciative students are currently practicing agriculturist in private and government service. The learning-by-doing approach enabled them to practice and grasped processes involved in their tasks. Strong partnership between research agencies and HEI could provide important support to the future professionals of this country.

**Keywords**: *Engagement, Internship, Linkages, Research Institutions, State Universities and Colleges (SUCs).*

## Introduction

It has long been established that experiential education helps provide young professionals with balanced learning of theories and their application. Acquiring new knowledge and bringing out the students' creativity through internships, major practices, on the job trainings, thesis, and conducting special problems enable students to be more equipped and confident to pursue their chosen career. Service-learning or outside-the-classroom approach is an informal way of learning that cuts across a variety of disciplines, with or without direct connection to formal education. Outside-the-classroom approach that has direct connection to formal education involves formal and structured teaching design and corresponds to the students' specialization (Furco, 2008).

The concept of experiential learning dates back to the early periods of Medieval Europe. It was developed as vocational training through apprenticeships for 5-7 years of service. The apprenticeships were used as a pathway for students to get into a trade guild, which is an association of specialists in a specific field of work. While vocational apprenticeships and "journeyman" programs are still offered in fields in which this style of instruction originated, college students today participate in internship, on-the-job training (OJT), or practicum, related to their chosen curriculum.

In the late 1970s and 1980s, there was a surge of college faculty members establishing internship programs after hearing about success stories of these teaching styles from other institutions. Universities took the lead in making internships more appealing to and productive for students by giving course credit. Most early university internship programs were established in business and medicine. After the increase in new interest in these curricula, most universities established internship programs in psychology and social work. These early programs served as a way for students to try out possible future careers without the commitment of having to work a full-time job (Jackel et al., 2011).

There are few publications on the nature of the academically structured practicum, on-the-job-training (OJT) or internship. Most students felt their OJT was a great experience that prepared them for a career (Dieffenbach et al., 2011). Apart from a requirement for graduation, taking theory into practice is also advantageous for future employers as it involves less training time for their prospective new staff (Dieffenbach et al., 2011; Furco, 2008).

The Philippines has many internship programs in private and public institutions. However, there are few publications discussing the influence of internship experience on students' career selection. A paper described tourism and hospitality industry (Salatan, 2016) while the other on pharmacy (Carrido et al, 2015). This paper tackles the agriculture sector highlighting partnership between Philippine Rice Research Institute (PhilRice) and State University and Colleges (SUCs) as a case study. This is the first formal study on the impact of internship in the students' career path and as a model in forming the next generation of agriculture research, development, and extension workers in the country and in the world.

PhilRice is a government corporate entity (Classification E) under the Department of Agriculture. It was created through Executive Order 1061 on November 5, 1985 (as amended) to help develop high-yielding and cost-reducing technologies so farmers can produce enough rice for the Filipinos. With the vision "Rice-Secure Philippines," PhilRice adheres to equip the Filipino rice farmers and the Philippine rice industry to be competitive through research for development. PhilRice fully coordinates its research work to its seven branch stations, satellite stations, and networks comprising 60 members nationwide. PhilRice is certified on ISO 9001:2015 (Quality Management), ISO 14001:2015 (Environmental Management), and OHSAS 18001:2007 (Occupational Health and Safety Assessment Series).

PhilRice Plant Breeding and Biotechnology Division (PBBD) accommodates students under major practice, thesis, internship, and OJT on cultural management of crops for breeding inbred and hybrid rice, seed production technologies, special problems, and data gathering and analysis. Active in engaging interns, PhilRice PBBD needs to document students' and teachers' learnings and career after graduation, and identify obstacles and best practices. This paper also evaluated the contributions of internship programs in the agriculture sector.

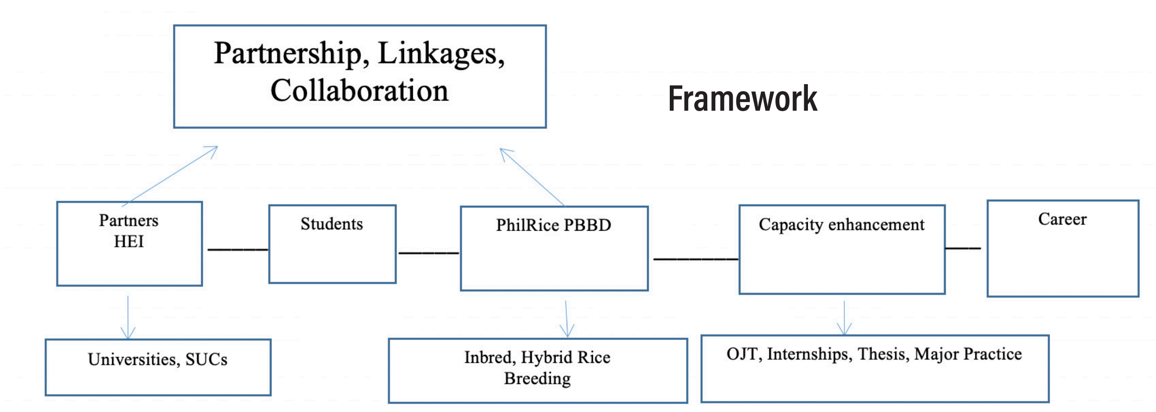
## Objectives of the Study

This study aimed to (1) document the students' preferred ways of field engagement; (2) identify the contributions of the students and the internship program to the hybrid rice breeding program; (3) trace students after the internship program; and (4) determine the learnings, challenges, and opportunities in collaborating with Higher Education Institution (HEIs) for internship programs.

## Methodology

A descriptive, correlational research design was employed using survey, key-informant interviews, tracer method, and participant observation as methods of data collection. Online survey was conducted for students who underwent internship programs in PhilRice PBBD from 2012 to 2018. Advisers and instructors who were assigned to oversee the students served as the participants of the key-informant interviews. PhilRice researchers conducted participants' observations and schedules during their internship, including students' exit presentations. A simple tracer method was also conducted to know the whereabouts of the students who underwent internship.

Respondents consisted of three teachers and 40 graduate students from universities in Thailand and Philippines. Four of them were interns from Khon Kaen University (KKU), 18 major practice and thesis students from Central Luzon State University (CLSU), and 18 OJT students from Nueva Ecija University of Science and Technology (NEUST), Mindoro State College of Agriculture and Technology (MinSCAT), and Don Mariano Marcos Memorial State University (DMMMSU). Students were categorized as follows:



***Internship***. Khon Kaen University, Thailand, dispatched their students to a four month or a season-long involvement in hybrid rice breeding and seed production.

***Major practice***. A season-long involvement of students with specific study related to the normal operation of the hybrid rice breeding and seed production group.

***Thesis.*** A season-long involvement of the student conducting applied or basic research related to hybrid rice breeding and seed production.

***On the job training***. A 30-working days given to students involving series of lectures and hands-on training on growth stages of rice.

***Data Analysis***

Data were collected and encoded in Microsoft Office Excel 2007© for statistical analysis. Each school was given a code for confidentiality. IBM Statistical Package for Social Sciences© Statistics version 21 was used for correlation and descriptive analysis. Descriptive statistical analysis, frequency, mean, and standard deviation, were employed to evaluate the different variables in the study. Pearson's Correlation Coefficient was used to investigate the relationship among independent and dependent variables.

## Results and Discussion

***Students' Methods of Field Engagement***

Most of the students (83%) preferred hands-on activities over lectures. Meanwhile, majority of the students (70%) found it more relevant if they would engage in the entire farming operation process rather than handling one specific activity. This activity limited the students' exposure because they only stayed for 30 working days.

**a. *Conduct of student programs***

As part of their orientation, students were informed of the division's breeding program in rice ecosystems including rainfed, upland, cool elevated, drought, saline, submergence, high temperature, and irrigated lowland for inbred and hybrid rice. They were also toured around the facilities and experimental areas. Students were also briefed on the impact and importance of developing rice varieties of our country. The division head or senior breeder of the division usually led the orientation. Students were then introduced to the assigned researchers, and the interrelatedness of processes were explained. Series of lectures and actual application were provided, and students were encouraged to ask questions. After lectures, students were turned over to the hybrid rice breeding group for their specific studies.

**b. *Internships, major practice, thesis, and on the job training***

The four Thai intern students from Khon Kaen University were immersed to the core activities of the hybrid breeding project such as nucleus seed multiplication, evaluation of paired crosses, breeder and foundation seed production, and flowering synchronization. The students were also taught on gathering and analyzing data. Progress of their studies were monitored regularly.

The topics of nine major practice students from CLSU were based on the routine activities of the breeders, specifically in producing F1 hybrid seed or the A x R seed production. This was done to engage them in actual field work in producing hybrid rice seeds. The students were required to be in the field with core workers from sowing until harvesting. They participated in activities conducted in critical stages of the crop during fertilizer application, primordial sampling, boot collection, gathering initial heading and flowering, leaf clipping, GA3 application, pollination, and data gathering. The written report was monitored monthly or whenever there are suggestions and clarifications.

Topics of CLSU thesis students were conceptualized by their school and PhilRice adviser through focused group discussion conducted before implementation. The nine students were required to visit their set-up at least three days every week from sowing to harvesting. The students were also taught how to gather data and interpret results.

The 18 OJT students from different universities participated in lecture and field work. However, they were not immersed in all stages of the rice crop as their training duration was only 30 working days. OJT students were also given a special problem for them to appreciate research work, which results they presented before they culminate the training.

Based on the results, 83% of the students noted that the most relevant part of the training was their engagement in actual field operations. Jayson, a major practice student of CLSU said,

> *"The most relevant part of major practice is to be engaged in field activities and communicating with other researchers and laborers about the techniques and strategies in hybrid seed production."*

Meanwhile, Sylvia of CLSU said,

> *"For me, hands-on was the most relevant part of the training. It tells me whether I can pursue research work or not."*

These statements showed that learning-by-doing approach is an effective tool for the students conducting studies at PBBD. These findings support Sides et al. (2017) who concluded that internships provide an opportunity to "learn the process of self-generating skill."

All students agreed that internship, OJT, thesis, and major practice in PBBD were very helpful. Alpha of MinSCAT noted,

> *"Yes, OJT was helpful because it enhanced my understanding and capabilities that I could use in my work. It also equipped me with the crucial skills and knowledge. It also provided me the venue to learn from some of the best professionals in agriculture industry."*

### *Contribution of Students on Hybrid Rice Breeding Program*

According to PBBD's study leaders who handled the students, division projects had benefitted from the study programs. As part of the team, students assisted the researchers in their day-to-day activities in field and laboratories, including slide preparations, pollen evaluations, pair crossing, rouging, leaf clipping, supplemental pollination, bagging of panicles, data gathering, and conducting post-harvest activities. Thus, projects saved resources as they complemented the needed human resources during the peak of breeding work. As discussed by Divine et al. (2006), employers benefit from internship because it provides them with risk-free-trial access to potential future employees, while schools strengthen their connections to the community. Students also helped in the promotion of the public hybrids and in adoption of the varieties in their schools and communities.

## *Tracer Method*

Among the 40 students involved in the study coming from the four schools, 70% of them were females and 30% were males (Figure 1). This could state some gender imbalance for students taking up agriculture as dominantly female.

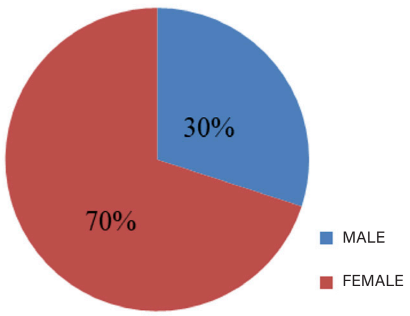



**Figure 1.** Gender of former students who attended the PBBD internships over the past year.

Majority of the students came from CLSU (52%) which is about 20 km away from PhilRice; followed by NEUST (15%), DMMMSU (13%), and MinSCAT and KKU (10%) (Figure 2).

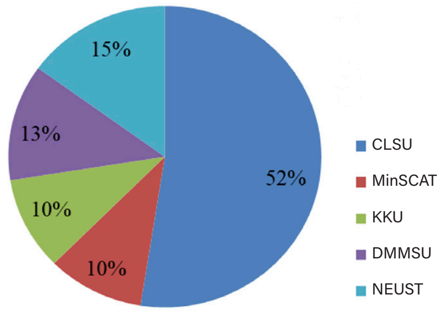



**Figure 2.** Student distribution among the four universities in the study.

Ninety percent of the students are already employed (Figure 3) with 75% of them joining the agriculture sector (Figure 4). They all agreed that conducting studies at PhilRice helped them gain employment and that the program was useful in their work. The students said that skills such as land preparation, DNA extraction, data analyses, post-harvest operation, and writing and communication skills, which they learned from the internship program, had helped them land a job in government and private companies. For the unemployed, 5% continued their academic studies, while the remaining 5% are still job searching (Figure 3).

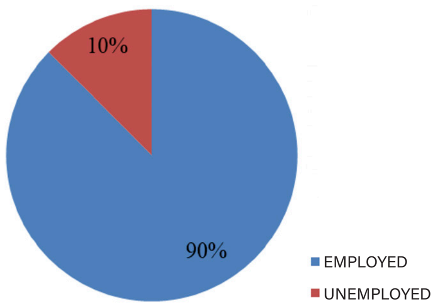



**Figure 3.** Employment status of the former students involved in this study.

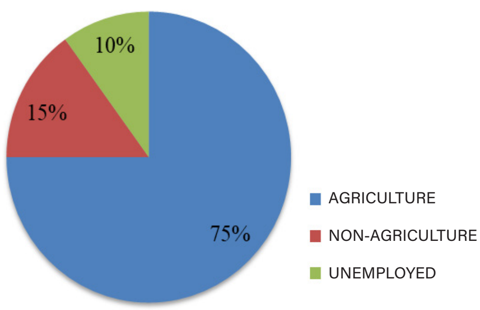



**Figure 4.** Percentage of former students who landed jobs in agriculture and non-agriculture sector.

Among the 90% employed, 55% of them are working in government institutions with mandate in agriculture, while 20% are connected to private agricultural companies (Figure 5). Based on the students' program categories, high percentages of students who landed on agriculture-related jobs came from thesis and major practice students (89%).

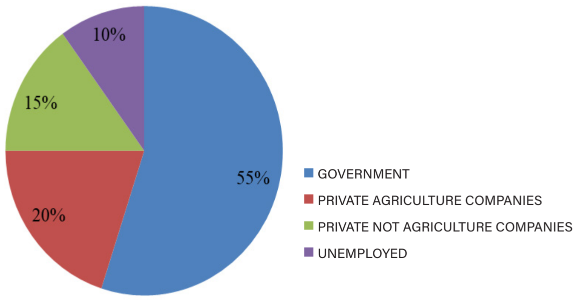



**Figure 5.** Percentage of employed former students involved in the study.

Of the students who participated in the internship program at PBBD- Hybrid Rice Breeding group, 60% responded to the online survey. More than half (60%) expressed their improvement on their skills in field operations. Thomas, a major practice student said:

> *"Involvement in actual activities from land preparation until postharvest made me realized that being agriculturist is fun. The activities also engaged me in gaining new knowledge and learning on proper management practices, which can help attain high yield."*

Other than enhanced technical skills, 41% of the students agreed that their motivation towards research were improved. According to them, they have learned to be independent, resourceful, and flexible. They learned to manage their time and think critically especially analyzing data. Meanwhile, some students (25%) improved their proficiency in the English language.

### *Learning from Linking with HEI Using Active Feedback Mechanism*

At the culmination of their training, students submitted their outputs through narrative report, power point presentations, and manuscript. Reports were checked thoroughly by the assigned PhilRice advisers while study results were presented in an exit seminar. PhilRice researchers, school advisers, and student representatives attended the seminar, which also served as a venue to validate study results. Researchers contributed to the discussion by clarifying some processes or adding information to the studies conducted. Part of their grade, which was submitted to their schools, came from selected researchers.

School advisers also gave their feedback about the program. Challenges, opportunities, and importance of collaborations were also tackled during the presentation. This feedback can be used to assess areas of strength and weakness; thus, influencing the school's curriculum and/or preparation of students to improve workplace performance (Divine et al., 2006).

Most of the school representatives expressed their gratitude to PhilRice for their students' accomplishment. They also said that the partnership should continue because students need the exposure to be competitive. Angie, DMMMSU OJT adviser said:

> *"Most of us here at school were amazed when the students presented their outputs. They are very confident in presenting their reports; indicating their training went well. Many of our incoming fourth year students already gave their intentions to be PhilRice OJTs, but we only have limited slots."*

According to a CLSU adviser, the collaboration facilitated students' training with competent professionals. The adviser also said that students became more equipped and capable when they graduate, and are expected to be productive in their future work. This statement supports Gavigan (2010) and O'Neil (2010) concluding that students also gain professional knowledge about workplace behaviors and are better prepared for future work or graduate school. School advisers also noted during interviews that PhilRice has advanced facilities and that the place is very conducive for learning because staff are research-oriented and friendly.

Meanwhile, some of the challenges met by students included "too much academic load or number of units" during the conduct of the thesis and major practice. This constrained the students from attending some of the field work. Students also needed time to study more about their work as they have limited background on rice production and rice research.

According to one respondent, the curriculum must be upgraded to meet the current trends and issues in agriculture. Some students also needed time to adjust on using equipment and learning terminologies that are new to them. Some schools also noted that programs must be scheduled before harvesting season so the students can practice different cultural management at different growth stages. As the OJTs only had a maximum of 240 hours, they had very short period to learn the entire process of plant breeding, which left most of the crop management and hybrid rice breeding process unlearned. Meanwhile, Thai students met challenges on adjusting with the terminology used in plant breeding and Philippine rice production.

Opportunities also came out during the interview with the advisers. According to Joy, one of the CLSU thesis adviser said:

> *"Creating a strong research network that taps the young minds to come up with relevant and timely technologies can benefit the farmers and the nation. We need research institutions like PhilRice as a partner to fill the gaps that academe cannot provide to the students."*

As result of the training, two students presented their thesis results to the Crop Science Society of the Philippines (CSSP) scientific conference in Legazpi City, Albay in 2008. This is a good encouragement for students to continue conducting research not only in the local level but in international forum. These experiences also motivate students to pursue their career in agriculture.

## Summary and Conclusions

Most of the students preferred hands-on exercises because they can easily understand the topics when complemented with experiential learning. This supports the learning-by-doing approach as an effective strategy for interns, OJTs, thesis, and major practice students.

Most of the students noted that engagement in actual field operations was the most relevant part of the OJT, internship, major practice, and thesis because they are immersed the entire process of hybrid rice breeding and seed production.

Students' engagement in Hybrid Rice Program helped researchers gather and process data easier and faster and saved on resources. However, further study must be conducted to establish greater impact and contribution of internship, thesis, OJT, and major practice programs.

Too much academic load for the major practice and thesis students, and lack of exposure to some stages of rice were among the challenges noted during the program.

Creating new linkages and having advantage on being employed were among the opportunities for the HEI and students who conducted their research at PhilRice.

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# IMPACT OF LASER LAND LEVELING IN RAINFED LOWLAND AREAS IN ILOILO

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## Abstract

The study was conducted in three different farmers' fields in rainfed lowland areas of Calaboa Este, Sta. Barbara, Iloilo. The fields were leveled using a laser attached to a four-wheel tractor and manual leveling for farmers' practices. Average soil depth cut ranged 2-8 cm for the three sites. Different plots were established to determine the separate effects of laser leveling on nutrient and weed management where a leveled field with farmer's practice was treated as control. The trials also evaluated the effect of laser land leveling, nutrient management, and weed management on rice grain yield. The plots for the two treatments measured 4 x 5 m and were replicated four times. The standard deviations (SD) of elevation were computed to evaluate field levels through accomplished topographic survey on laser leveled and non-laser leveled field. The SD of elevation on unleveled fields were 2.61, 9.36, and 6.54 cm while that of leveled field were 1.95, 2.01, and 1.61 cm. Average rice yield in the laser leveled plot was higher by 15.7% than non-laser leveled plots when combined with good nutrient management. Laser leveled plot had also Higher average grain yield by 11.7% was also recorded when complemented with good weed management.

**Keywords:** *Laser Land Leveling Technology, Nutrient Management, Rainfed Lowland, Weed Management.*

## Introduction

Land leveling was proven to bring about uniform crop growth, better weed and golden apple snail control, and increased nutrient and water efficiency. Uneven soil surface topography widely affects rice crop production essentially because a well-leveled field is a prerequisite to good water and crop management. On the other hand, non-leveled fields result in uneven water distribution, which means that more water is needed to fill the entire field for land preparation and crop establishment. This could also lead to poor drainage that can affect rice grain yields. Field that is not efficiently leveled has uneven crop stands, increased weed burdens, and uneven maturing of crops (IRRI, 2013).

In the Philippines, most rice fields are have uneven elevations of plots, which limit the application of controlled irrigation and stable irrigation water flow. Uses of farm machinery are not also efficient in fragmented rice fields because small plots consume more operating time due to frequent headland turning.

Through land leveling, several plots are combined together to create a favorable environment for rice production. Field plots are fragmented and irregularly shaped because there is no available equipment for efficient leveling. Farmers level their fields using handtractor and carabaos. Land leveling through laser-guided leveling technology has numerous advantages in improving agricultural production. Laser leveling of agricultural land is a resource conservation technology that improves water use efficiency (Goswami and Nishad, 2015) and fertilizer and agrochemicals applications (Qurat et al., 2015). It also enhances environmental quality and crop yields (Jat et al., 2006). Jat et al. found that laser leveled field had reduced irrigation input to 5-8% for flooded rice (100-150mm) compared with traditional leveling in northwest India and Pakistan (Jat et al., 2006, Kahlown et al. 2006). Rickman (2002) and Jat (2004) also reported a 5-7% increase in cropped area due to precise land leveling. On-farm evaluation of laser leveling resulted in a 5.5% gain in rice productivity in the first year. Rickman (2002) observed up to a 24% increase in yield due to precise leveling.

Laser leveling moves the soil from the higher points of the field to the lower points with higher accuracy in a very effective way compared with manual leveling. This kind of land leveling involves a topographic survey of the field before and after the operations to record the high and low spots for systematic route analysis. The field diagram and the mean height of the field are used to determine the effective movement of soil from the high to low areas for better performance of the laser-controlled bucket connected to a tractor or laser leveling system (CSISA, 2014).

Rainfed lowland rice is characterized as transplanted rice grown in well leveled, bunded fields that are usually flooded with rainwater. Precipitation water in these areas needs to be stored and utilized efficiently to take advantage of its availability and unpredicted occurrences. In the Philippines, rainfed lowland rice accounts for almost 40% of the riceland (Mackfill et al., 1996). In Region 6, where this study was conducted, rainfed areas comprised about 51.77% of the region's total paddy rice production (PSA, 2017). Rainfed areas contributed 27% increase in rice production in 2000-2010 (PhilRice, 2015). Hence, increasing yield in the rainfed areas will result in more abundant Philippine rice production.

Experiments conducted in different localities in south-east Asia established that in rainfed environments, every 1 cm variation in rice field levelness reduces yield by 260 kg of grain (Rickman, 2002). This implies that level of the rice field has direct effect on the use of irrigation water and efficiency of agrochemicals and fertilizers.

This study compared the effect of laser and traditional manual leveling on rice grain yield. Leveling practices were combined with good nutrient and weed management. Verifying previous results in the Philippine condition, this study was conducted in Iloilo rice farms where fields are uneven and fragmented. As such, concerns on water flow and weed control are widely experienced in these areas.

## Materials and Methods

### *Site Description*

Trials were established in farmers' fields located in three locations in Calaboa Este, Sta. Barbara, Iloilo, which represent the toposequence of the rainfed lowlands in the village (Figure 1). Sites were leveled using the laser guided land leveler (Figure 2). A manually-leveled field, with the same size as the field trials, was used as control. Location of field trials were as follows:

**Site 1**: Farmer 1 (lower toposequence) – Located in the lower part (9.66m above sea level; 10º48'44" N 122 º 35'12" E) of the village. It has a good water source for supplemental irrigation during the wet season and in summer. The soil is light clay; thus, water in the paddy does not drain easily. The total area of the laser field was 2463 $m^2$.

**Site 2**: Famer 2 (middle toposequence) – Located in the middle toposequence (10.3 m above sea level; 10º 48'48" N 122 º 35'11" E) of the village. It has supplemental irrigation from a shallow tube well. The soil is heavy clay loam, which can retain irrigation water for a few days. The total area of the laser field was 2838 $m^2$.

**Site 3**: Farmer 3 (upper toposequence) – Located in the upper area (43.8 m above sea level; 10º


**Figure 1.** Study sites.

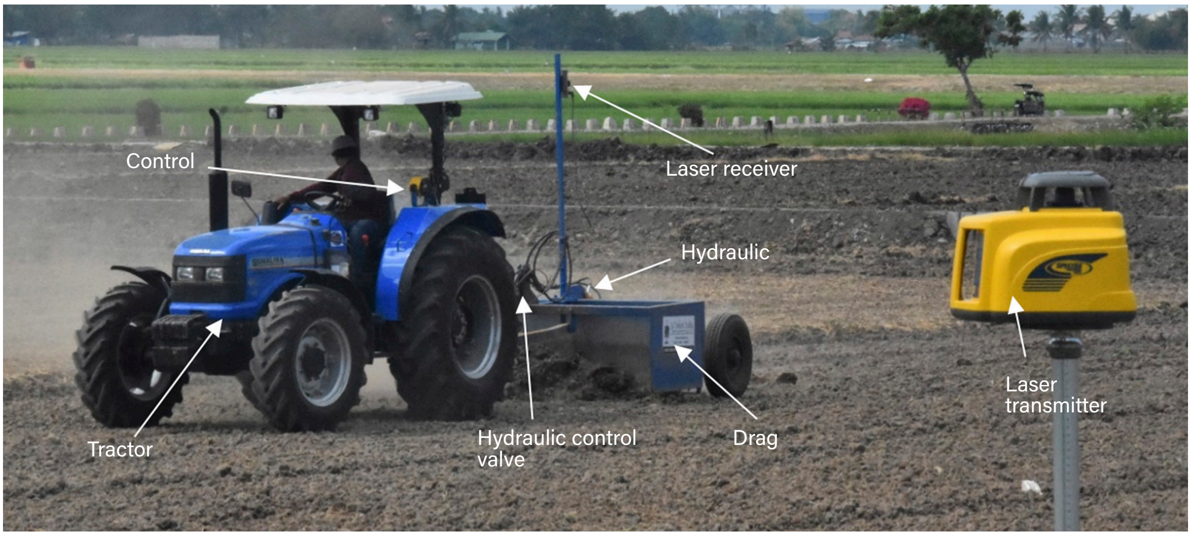


**Figure 2.** Laser land leveling operation system.

49'08" N 122 ° 34'40" E) of the village. The field has limited water access and dries up quickly as the soil is sandy loam. This field is far from the creek where farmers access water for irrigation. The total area of the laser field was 2440 m$^2$.

### *Topographic Survey*

Before the fields were plowed, topographic survey was done to record the high and low areas in the field. Readings were obtained from 10x10 m grid. The mean height of the field was determined by taking the sum of all the readings and dividing by the number of readings taken. Moving soil from the high and low spots was determined through an accomplished field diagram and the computed mean height.

### *Plowing the Field*

The area was plowed and pulverized using disc plow and rotavator to soften the soil surface and residues to make easier soil movement in and out of the drag bucket. Another round of plowing was needed when previously plowed soil on the higher surface was fully scraped during laser leveling.

### *Laser Leveling Components*

Laser leveling system has components such as four wheel tractor and plow, drag bucket, laser transmitter, laser receiver, control box, and hydraulic control system (Figure 3). The laser- controlled bucket was positioned at the determined mean field height of the


**Figure 3.** Components of a Laser land leveling system.

field where its cutting blade was set slightly above ground level (1-2 cm). The tractor was operated in a circular motion from high to low areas of the field until the entire field is leveled.

***Resurvey the Field After Laser Leveling***

After leveling, the field was resurveyed using the laser transmission system and receiver to determine if the level of precision was attained. Readings were also obtained from a 10 x 10 m grid of the previous surveys.

***Field Efficiency***

The theoretical time to level the field was calculated by knowing the depth of cut from the cut/fill map, dimensions of the field, volume of soil needing to be moved by the bucket, and tractor operating speed. Assumptions were made to the bucket fill (%), bucket fill efficiency (%), and tractor speed (km/h). Field efficiency was calculated using the actual operating time and the computed theoretical time.

$$Eff = (Ta / Tt) \times 100$$

Where:

Eff= efficiency, %

Ta = actual operating time, h

Tt = theoretical operating time, h

***Establishment of Field Experiment***

Field experiments were established in three different locations with leveling practices as treatments. Farmer's leveling practice was carried out using a wooden plank drawn by either a hand tractor or a cattle. Each treatment has four sampling areas measuring 4 x 5 m. Each trial was planted with rice of the same variety based on the farmer's choice of variety. Wet direct seeding was used as establishment method.

***Weed Management, Nutrient Management, and Other Practices***

Rice production practices on water, fertilizer, and weed management were similar to all plots and were based on farmer's practice. As the trials were conducted on the farmer's field, variety planted and weed and nutrient management were based on their preference. Table 1 shows the farmer's practices.

***Grain Yield***

At physical maturity during the time of harvest, samples were obtained from designated sampling areas. The harvested samples were threshed, cleaned, dried, and weighed. Final grain yield (GY) was adjusted to 14% moisture content.

***Statistical Analysis***

The data gathered was analyzed using a Completely Randomized Design (CRD) and Analysis of Variance (ANOVA) at 5% level of significance.

**Table 1**. Farmer's practices of the three sites.

| Sites | Variety planted | Nutrient Management (NPK) | Weed Management | Water Source |
|---|---|---|---|---|
| 1 | NSIC Rc 150 | 102-10-10 | Pretilachlor 0.6 kg ai/ha fb fenoxaprop ethyl (69 g ai/ha) 12 DAS+ cyhalofop butyl (0 g ai/ha) 28 DAS fb spot weeding 35-40 DAS | Rainfall + supplemental irrigation |
| 2 | NSIC Rc 216 | 77-37-7 | Butachlor + propanil (0.3 + 0.3 kg ai/ha fb fenoxaprop ethyl (69 g ai/ha) + bispyribac sodium (10 g ai/ha) 40 DAS fb spot weeding | Rainfall + supplemental irrigation |
| 3 | NSIC Rc 216 | 75-18-18 | (0.8 kg ai/ha) 3 DAS fb byspiribac sodium (10 g ai/ha) 18 DAS fb byspiribac sodium (10 g ai/ha) 28 DAS fb spot weeding 45 DAS | Rainfall only |

**Table 2**. Comparison of elevation difference of three sites before and after leveling.

| Sites | Elevation Difference Before Leveling (cm) | Elevation Difference After Leveling (cm) |
|---|---|---|
| 1 | ± 2.61 | ±1.95 |
| 2 | ± 9.36 | ± 2.01 |
| 3 | ± 6.54 | ± 1.61 |

## Results

### *Topographic Survey*

### *Before and After Leveling*

All field trials were originally composed of two plots but were converted into one plot after laser leveling. Before leveling, the three site's elevation differences were 2.61 cm, 9.36 cm, and 6.54 cm (Table 2). After leveling, all trials reached the ± 2 cm level of precision, which is the acceptable field level requirement under Palaycheck rice production system. Figures 1-3 shows the elevation of the sites computed through topographic survey for Sites 1-3, respectively. Figure 4 shows the condition of the three sites before and after leveling.

### *Field Efficiency*

The computed field efficiency ranged 41-51% as shown in Table 3. It was observed that the biggest area, though it also had the highest depth of soil to be cut, attained the highest field efficiency. This maybe because machine field efficiency tends to be higher in larger plots because of lesser headland turnings compared to smaller plots.


**Figure 4.** Comparison of mean elevation (cm) in Site 1 before and after land laser leveling.

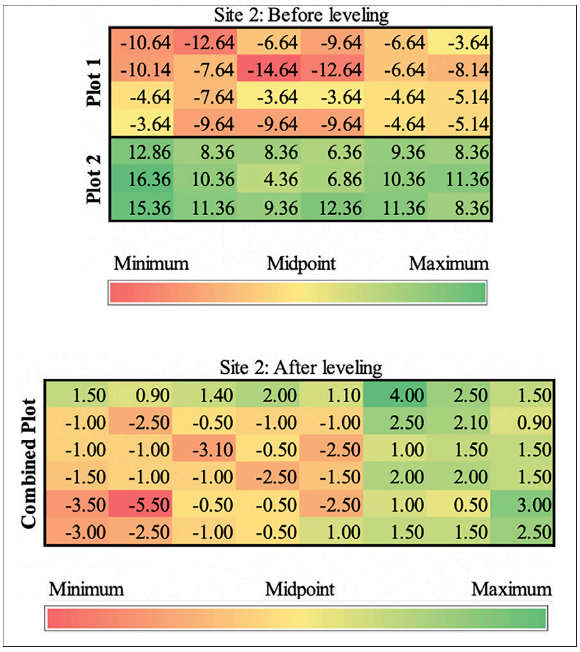


**Figure 5.** Comparison of mean elevation (cm) in Site 2 before and after land laser leveling.

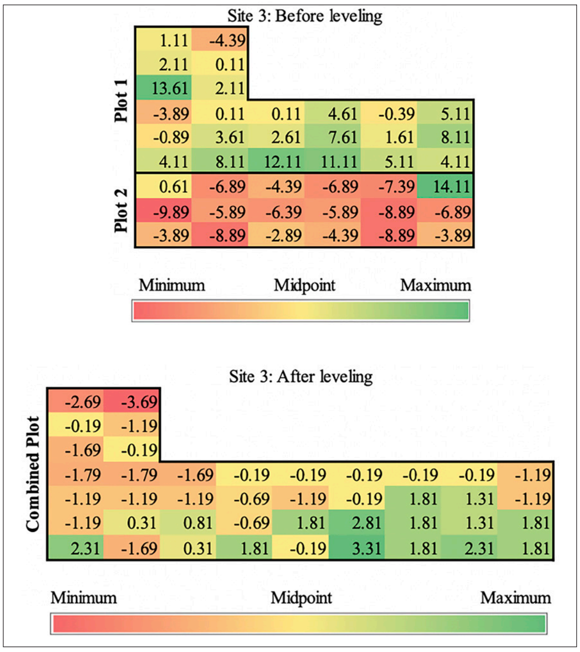


**Figure 6.** Comparison of mean elevation (cm) in Site 3 before and after land laser leveling.

**Table 3**. Field efficiency computations.

| Variable | Calculations/Assumptions | Site 1 | Site 2 | Site 3 |
|---|---|---|---|---|
| Area, A($m^2$) | None | 2462.70 | 2837.92 | 2440.48 |
| Depth of soil to be cut, D (m) | Calculated average | 0.026 | 0.094 | 0.065 |
| Bucket dimensions, BD (LxWxH) | none | 3 x1 x1m | 3 x1 x1m | 3 x1 x1m |
| Bucket fill, BF & BF eff.,BFE (%) | assume 50% | 50% | 50% | 50% |
| Tractor speed, S (kph) | assume 7 kph | 7 kph | 7 kph | 7 kph |
| Vol. of soil to be moved, Vm ($m^3$) | A/2 x D (m) | 32.20 | 132.82 | 79.86 |
| Vol. soil in bucket, Vb ($m^3$) | BD x BF x BFE | 0.75 | 0.75 | 0.75 |
| Number of trips required ($N_T$) | (Vm/Vb) /2 | 86 | 354 | 213 |
| Average trip length, L (m) | 50% of the length field | 37.61 | 28.02 | 31.85 |
| Total distance traveled, D (m) | $N_T$ x L | 3229.31 | 9922.34 | 6782.97 |
| Theoretical time, $T_t$ (h) | D/S | 0.46 | 1.42 | 0.97 |
| Actual time, $T_a$ (h) | none | 1.13 | 2.78 | 2.08 |
| Field efficiency (%) | $T_1$ / $T_2$ | 40.71 | 50.93 | 46.51 |

### *Effect of Laser Leveling and Nutrient Management*

Higher grain yield was recorded in the all laser leveled field than in farmer's leveled field. Yield difference ranged from 5.6% to 25.7% or 0.4-0.76 t $ha^{-1}$. The results showed that more efficient fertilizers were utilized by rice plants at laser leveled fields making it more productive and evenly grown plants within the field plots. On the other hand, a not well leveled fields resulted in uneven effect of fertilizers throughout the plots leading to lower yield.

**Table 4.** Grain yield (t $ha^{-1}$) of laser leveled field with nutrient management relative to farmer's leveled field.

| Site | Laser Leveled | Farmer's Leveled | Yield Difference |
|---|---|---|---|
| 1 (77-37-7) | 8.14 | 7.58 | 0.57 (7.52%) |
| 2 (102-10-10) | 7.50 | 7.10 | 0.40 (5.60%) |
| 3 (75-18-18) | 3.71 | 2.95 | 0.76 (25.7%) |

### *Effect of Laser Leveling and Weed Management*

Higher grain yield were harvested from all laser leveled fields than in farmer's leveled field. Yield difference ranged from 0.63 to 22.71% or 0.04-0.57 t $ha^{-1}$. Field plots that were laser leveled had higher yields because of lesser weed occurrences, which resulted in lesser nutrient competitions and better plant growth.

**Table 5.** Grain yield (t/ha) of laser leveled field with weed management relative to farmer's leveled field.

| Site | Laser Leveled | Farmer's Leveled | Yield Difference |
|---|---|---|---|
| Site 1 | 7.05 | 6.90 | 0.15 (2.17%) |
| Site 2 | 6.38 | 6.34 | 0.04 (0.63%) |
| Site 3 | 3.07 | 2.51 | 0.57 (22.71%) |

## Discussion

### *Topographic Survey*

With the use of a laser-guided land leveler, smaller plots with varying elevations can be combined to form a plot with relatively uniform elevation, which could prove to be beneficial to plant growth and higher machine efficiency. A study of Shuhao (2005) showed that larger farm size reduces labor, seed, and herbicide and pesticide costs, but not those of fertilizers, oxen, and tractors.

### *Field Efficiency*

The elevation difference of laser leveled field were lower than farmer's leveled field (using wooden plank) and it attained the ±2 cm level of precision requirements of the Key Check 2 PalayCheck System©. The field efficiency of laser guided leveler used in less than 3000 $m^2$ ranged 41-51% and though the largest field had also the highest depth of soil to be cut it attained the highest field efficiency. This is because machine field efficiency tends to be higher in larger plots. Wadud and White (2000) as cited by Shuhao (2005) mentioned that on average farmers with greater plot size or less land fragmentation operates at a higher level of technical efficiency.

### *Effect of Laser Leveling Complemented with Nutrient and Weed Management*

Laser leveling complemented with nutrient management or weed management showed desirable effects on grain yield. Laser land leveling combined with good nutrient management increased yield by at most 25% (0.76 t/ha) in all farmer's fields. On the other hand, laser land leveling performed with good weed management increased yield by at most 22.71% (0.57 t/ha) in the three farmer's fields. Though there is yield increase, analysis of variance (Appendix Tables 1 & 2) showed that the variation among treatments is not statistically different. Therefore, it can be assumed that laser-guided leveling does not directly affect the yield.

## Conclusion

The study was conducted in three rainfed sites in Iloilo to compare grain yield gathered from plots that were levelled through laser and manual means. The practices were combined with good nutrient and weed management. Results showed that there is no significant variation among grain yields. Though laser-guided land leveling did not directly affect the yield, study showed that it can address land fragmentation.

## Recommendation

The laser-guided land leveling is claimed in other countries like India to enhance water application efficiency. It saves water by 25% and is a gateway for mechanization. It is therefore recommended that future studies should consider gathering water data and field efficiency of rice production machineries such as mechanical transplanter. Laser-guided land leveler technology is still in the initial stages of adaption in the country; therefore, it is recommended to further conduct studies to prove its potential and adaptability to the specific conditions of the country.

## Acknowledgments

The Rice Engineering and Mechanization Division (REMD) of the Philippine Rice Research Institute and the Water-Efficient and Risk Mitigation Technologies for Enhancing Rice Production in Irrigated and Rainfed Environments (WateRice) Project are recognized for the conduct of this study. The Rice Program of the Department of Agriculture (DA) thru the DA-Bureau of Agricultural Research.

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# Appendix Tables

**Appendix Table 1.** ANOVA on the effect of leveling practice on nutrient management.

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between groups | 0.49307 | 1 | 0.49307 | 0.08073 ns | 0.79041 | 7.70865 |
| Within groups | 24.42947 | 4 | 6.10737 | | | |
| Total | 24.92253 | 5 | | | | |

ns – not statistically significant at 5% level of significance.

**Appendix Table 2.** ANOVA on the effect of leveling practice on weed management.

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between groups | 0.09375 | 1 | 0.09375 | 0.01829 ns | 0.898947 | 7.708647 |
| Within groups | 20.5 | 4 | 5.125 | | | |
| Total | 20.59375 | 5 | | | | |

ns – not statistically significant at 5% level of significance.

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# EVALUATION OF ORGANIC NITROGEN SOURCES TO FURTHER INCREASE YIELD IN AN ORGANIC-BASED NUTRIENT MANAGEMENT IN IRRIGATED PADDY SOILS

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## Abstract

Basic studies show that there is no standard nitrogen requirement for rice crop under organic-based nutrient management (OBNM) as application depends on the sources of organic materials. Thus, technology packages were observed in five rice cropping seasons in two phases to determine the most suitable alternative nitrogen source in attaining the optimum yield in an organic-based farming system. Rice straw compost with chicken manure and rice straw compost alone were previously studied and compared as basal fertilizer to provide the initial nutrient needed by the rice crop. Results showed that no organic source was enough to give the required nitrogen up to booting stage. Urea, green manure (wild sunflower), organic foliar fertilizer (OFF), and inorganic foliar fertilizer (IFF) were used as top-dress fertilizer treatments. In 2012 dry season, urea (7.4 t $ha^{-1}$) is significantly different from other top-dress but in 2012 wet season, green manure (5.8 t $ha^{-1}$) is not significantly different from urea (5.9 t $ha^{-1}$). Green manure recorded the highest grain yield (7.3 t $ha^{-1}$ and 5.6 t $ha^{-1}$) among the topdress in 2013 for both dry and wet season while OFF and IFF had comparable yield. Thus, green manure showed potential as an alternative nitrogen source in an organic-based nutrient management in the paddy.

**Keywords:** *Chicken Manure, Foliar Fertilizer, Green Manure, Leaf Color Chart, Organic, Wild Sunflower.*

**Introduction**

Organic agriculture had grown popular, yet its current status is still far from its full potential because of the meager formal support to its supply chain including input supply, production, research and development on seeds, and nutrient and pest management (Maghirang, 2011). The need for more research from the scientific community in improving the agricultural technologies was also highlighted in papers discussing the sustainability of organic agriculture. However, research and development is just one of the holistic approaches that can contribute to the success of organic agriculture. This research aimed to package an optimized organic-based nutrient management with farm wastes as nutrient sources.

In the previous 10 year-trial of this research, the mineralization of different organic materials was done in fully-flooded soils; hence, the basis of the proper application time of basal organic fertilizer (Javier and Tabien, 2003). The dynamics of nutrient availability was also monitored, and showed that nitrogen (N) decreased at the maximum tillering or at early panicle initiation – the most critical growth of rice plants that directly dictates the grain yield. Thus, nitrogen topdressing is needed to boost yield. From the same organic fertilizer trial, results showed that organic material with lower C/N ratio (wild sunflower) released nitrogen faster than those with higher C/N ratio (rice straw and commercial organic fertilizers). Nitrogen releases were observed 1-2 days after soil incorporation of green manure. From this result, another study was established to determine the best possible organic topdress fertilizer for the organically-basal fertilized plots. Results showed that green manure (*Azolla, Sesbania, Aeschynomene, Indigo*, mungbean) and urea had similar potential for yield increase.

The leaf color chart (LCC) was developed for effective nitrogen management for rice. This tool is already packaged in the PalayCheck® system being disseminated by Philippine Rice Research Institute (PhilRice) and in the Site-Specific Nutrient Management (SSNM) by International Rice Research Institute. Essentially, LCC offers substantial opportunities for farmers to estimate plant nitrogen demand in real time for more efficient fertilizer use and higher rice yield. The existing LCC was developed under irrigated rice ecosystem and was calibrated as part of a judicious use of chemical fertilizers.

However, use of organic fertilizer is not included on managing nutrients. Simple trial using LCC and chlorophyll meter (SPAD) instead of the chemical analyses (Javier and Grospe, 2009; 2010) showed that nitrogen declined in plants applied with inorganic nutrient sources. Thus using LCC as basis of green manure (GM) application days after transplanting is a challenge.

In 2008 wet season (WS) and 2009 dry season (DS), more inconsistencies in critical LCC readings were observed when organic was included in the nutrient management than when growth-based inorganic N was applied (Javier and Grospe, 2009; 2010). When organic fertilizer was basally applied, LCC cannot predict the real time N demand of topdressing necessary for the succeeding growth stages particularly during panicle production, spikelet formation, and grain filling. Apparently, the decomposition time and nutrient releases by the basally applied rice straw and/or with chicken manure affected the real time N demand of the rice plants. Therefore, there is a perceived need to test the efficiency of LCC in the organic-based nutrient management for irrigated rice soils in terms of yield.

In the previous studies that excluded LCC use, rice plants applied with sunflower and chicken manure as topdress in addition to rice straw, produced higher yield than crop not given with additional fertilizer. However, yield was still lower when inorganic N fertilizer was applied (Javier and Grospe, 2009). Alongside these results, it is relevant to seek answer on whether commercially advocated organic foliar supplements be considered as an alternative organic-N topdress sources in a purely organic rice crop production. As such, this study aimed to assess several options of organic sources of nutrients as top-dress nitrogen fertilizers as compared with the inorganic sources and evaluate the efficiency of LCC-based OBNM (organic-based nutrient management) in irrigated system.

## Materials and Methods

The study was conducted in five seasons from 2011 to 2013 WS at PhilRice-Central Experimental Station, Maligaya, Science City of Muñoz, Nueva Ecija, Philippines in two phases. The first phase was established in 2011-2012 WS, in which treatments were set in factorial split plot design with four replications. The main plot contained rice straw compost (RSC) alone and rice straw with chicken manure (RSCM), both applied at 14 days before transplanting (DBT). The subplot treatments were topdress fertilizer options: urea, freshly chopped *Tithonia diversifolia* or wild sunflower (WSF) as representative of green manure, organic fertilizer spray (OFF), and inorganic foliar fertilizer (IFF). Fertilizer application was dictated by two LCC readings set at critical readings 3 and 4. At the 4$^{th}$ and 5$^{th}$ cropping season (2013 dry and wet season) of the experiment, treatments were simplified, in which all treatment plots was applied with only RSCM as basal fertilizer at 14 DBT. As in the first trials, application of topdress fertilizer was based on LCC readings (critical readings of 3 and 4) taken every 7 days from 14 days after transplanting (DAT) until the early flowering stage.

The quantity of the topdress green manure (wild sunflower) was based on the 23 kg N ha$^{-1}$ for wet season and 35 kg N ha$^{-1}$ for dry season applied until early flowering as recommended in PalayCheck® System particularly Key Check 5: nutrient management (inorganic-based) (Table 1). The volume by weight was based on the analyzed N content of the material plus the moisture content as this was applied as fresh. For the inorganic and organic foliar spray, the recommended (as registered at the FPA) rate and dosage of application was followed.

In the first phase, double rate was used for the LCC 4 treatments during dry season to reach the 4 reading. However, input needed for the green manure to achieve the reading was insufficient leading to high input cost; thus, recommended rate was reduced to 23 kg N ha$^{-1}$ during the second phase. This was also based on the Key Check 5 (inorganic-based nutrient management) of the PalayCheck System®.

Organic inputs (rice straw, chicken manure, and wild sunflower) were acid digested and analyzed using Kjeldahl Method for N analysis and Vanado-molybdate method for P and K.

Plant responses were analyzed using R-Cropstat analytical software. Means were computed at 0.05 level of probability using LSD. Different topdress fertilizer management in the 2013 DS and WS was compared with RSCM as basal organic-based fertilizer. To validate the previous results, grain yield data from the RSCM plots with its corresponding topdress fertilizer application was re-run in similar statistical analysis as the second set of trial.

## Results and Discussion

### *Organic Basal Fertilizer*

Rice straw compost (RSC) alone and rice straw compost with chicken manure (RSCM) showed significant grain yield only at the third cropping season; whereby, RSCM (5.6 t ha$^{-1}$) was higher than RSC (5.5 t ha$^{-1}$). RSCM contributed higher grain yield than RSC alone for two consecutive seasons. RSCM had 3% yield advantage in 2012 DS and 1.8% in 2013 WS due to additional nutrient from chicken manure (Table 2). Based on chemical analysis, chicken manure (CM) has 2% N, 1.65% P, and 1.45% K, while RSC alone has

**Table 1**. Total top-dress fertilizer applied based on LCC reading.

| | | 1st phase | | | | | | 2nd phase | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Wet Season 2011 | | Dry Season 2012* | | Wet Season 2012 | | Dry Season 2013 | | Wet Season 2013 | |
| Treatments | Fertilizer Grade (% N-P-K) | Total volume applied $ha^{-1}$ | Estimated N kg applied $ha^{-1}$ | Total volume applied $ha^{-1}$ | Estimated N kg applied $ha^{-1}$ | Total Volume applied $ha^{-1}$ | Estimated N kg applied $ha^{-1}$ | Total volume applied $ha^{-1}$ | Estimated N kg applied $ha^{-1}$ | Total volume applied $ha^{-1}$ | Estimated N kg applied $ha^{-1}$ |
| LCC3-Urea | | 1 bag | 23.00 | 5.25 bags | 120.80 | 3 bags | 69.00 | 3 bags | 69.00 | 3 bags | 69.00 |
| LCC4-Urea | 46-0-0 | 4 bags | 92.00 | 12.50bags | 287.50 | 4.50 bags | 103.50 | 10.5 bags | 241.50 | 7 bags | 161.00 |
| LCC3-WSF | | 3 t | 95.10 | 27.38 t | 867.90 | 9 t | 285.30 | 18 t | 570.60 | 12 t | 380.40 |
| LCC4-WSF | 3.8-0.2-2.3 | 12 t | 380.40 | 44.84 t | 1421.14 | 18 t | 570.60 | 21 t | 665.70 | 21 t | 665.70 |
| LCC3-OFF | | 1 bottle | 0.01 | 17 bottles | 0.17 | 3 bottles | 0.03 | 7 bottles | 0.07 | 6 bottles | 0.06 |
| LCC4-OFF | | 4 bottles | 0.04 | 18 bottles | 0.18 | 6 bottles | 0.06 | 8 bottles | 0.08 | 8 bottles | 0.08 |
| LCC3-IFF | | 1 pack | 0.19 | 18 packs | 3.42 | 3 packs | 0.57 | 6 packs | 1.09 | 6 packs | 1.09 |
| LCC4-IFF | 19-19-19 | 4 packs | 0.76 | 19 packs | 3.61 | 6 packs | 1.14 | 7 packs | 1.28 | 7 packs | 1.28 |

**Table 2.** Average grain yield (t ha$^{-1}$) of rice plants applied with RSC and RSCM as basal fertilizers and with the different topdress fertilizers based on LCC reading. 2011 WS, 2012 DS, and 2012 WS. Maligaya soil series. PhilRice Experimental Station, Science City of Muñoz, Nueva Ecija.

| | Grain Yield (t ha$^{-1}$) | | |
|---|---|---|---|
| | Wet Season | Dry Season | Wet Season |
| **Treatment** | **2011** | **2012** | **2012** |
| **Main Plot** | | | |
| RSC | 3.7 | 6.1 | 5.5 |
| RSCM | 3.6 | 6.3 | 5.6 |
| | ns | ns | * |
| **Sub Plots** | | | |
| LCC3 Urea | 3.64 abc | 7.75 a | 5.85 a |
| LCC3 WSF | 3.62 abc | 6.69 b | 5.59 ab |
| LCC3 OFF | 3.30 bc | 5.17 c | 5.22 b |
| LCC3 IFF | 3.45 bc | 5.37 c | 5.11 b |
| LCC4 Urea | 4.12 a | 6.98 ab | 6.03 a |
| LCC4 WSF | 3.89 ab | 7.16 ab | 6.06 b |
| LCC4 OFF | 3.70 abc | 5.16 c | 5.17 b |
| LCC4 IFF | 3.25 c | 5.35 c | 5.09 b |
| | ** | *** | *** |
| **MP x SP** | ns | ns | |

Means of the same letter are not significantly (ns) different at 5% level of significance using the CropStat software program. LCC 3= critical reading of 3 by the leaf color chart, LCC 4 = critical reading of 4 by the leaf color chart. WSF = wild sunflower, OFF= organic foliar fertilizer, IFF = inorganic foliar fertilizer

0.75% N, 0.28% P, and 0.12% K. Same results were found by IRRI as published in Rice Knowledge Bank. IRRI researchers gathered that manure consisting of 1.5-3% N, 1.15-2.25% P, and 1-1.4% K had higher essential nutrient than the compost alone (0.5-2.0 %N, 0.44-0.88% P, and 0.4-1.5% K). Applying organic fertilizer increases phosphorus mobilization and soil microbial activity that improves crop root system and nutrition (Ibrahim et al., 2008).

With the preliminary results, RSCM was used as the basal fertilizer during the 2nd phase to provide the initial nutrients needed by the rice crop and to further focus on the topdress nutrient management for a high-yielding rice varieties.

### *Topdress Fertilizer*

The grain yield attained from LCC 3 and LCC 4 were comparable regardless of the basal and top-dress fertilizer treatments used (Table 2). But generally, LCC 4 produced higher average grain yield than LCC 3 due to the frequent application of topdress fertilizer, which increased the amount of fertilizer applied (Table 1). Before harvesting, 100% lodging was observed in LCC 4 from grain filling until maturity when urea was incorporated; consequently, decreasing yield. In Japan, rice yield decreased up to 50% due to lodging (Rajkumara, 2008). Lodging may occur due to absence of potassium resulting in poor root

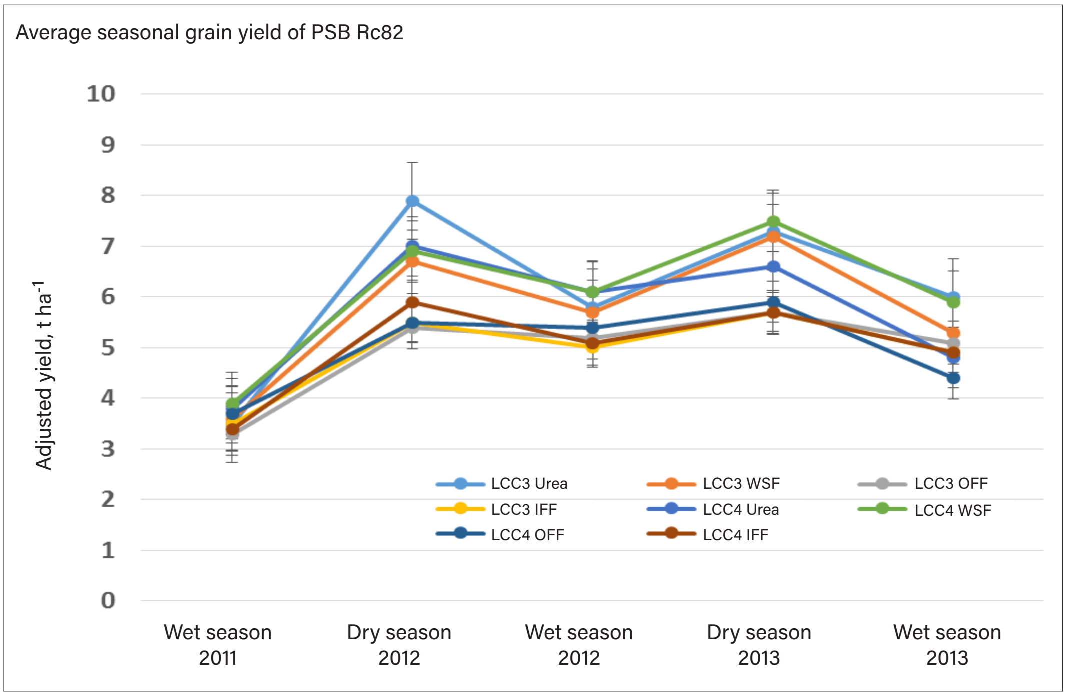


**Figure 1.** Average grain yield (t ha$^{-1}$) of rice plants applied with RSCM as basal fertilizers and with the different topdress fertilizers based on LCC reading. 2011 WS-2013 WS. Maligaya soil series. PhilRice Experimental Station, Science City of Muñoz, Nueva Ecija.

growth and reduced plant vigor (Bhiah et al., 2010); and excessive nitrogen application that reduced stem diameter and stem wall width, which also affects the strength of stem base and its anchorage system (Hobbs et al.1998; Rajkumara, 2008). These explain why lodging occurred in LCC 4-urea treatment.

Topdress fertilizers showed significant differences during the 1st phase of the experiment especially in 2012 DS, in which LCC 3-urea, LCC 4-urea, and LCC 4-WSF produced comparable yield, but higher than LCC 3-WSF. In the 2012 WS, however, yield was not significantly different from either urea or WSF based on either LCC 3 or LCC 4 (Table 2). Given the equivalent nitrogen rate as the urea, WSF applied at 23 kg N $ha^{-1}$ in the WS and 35 kg for the DS almost doubled the total N applied per hectare when LCC 4 was used as the basis. The frequency of organic-based N supplement by the LCC 4 was also doubled for the other treatment or the foliar fertilizers. However, grain yield was not much of significant different from LCC 3 and LCC 4 as basis for topdressing and between the use of WSF and urea. On the other hand, the use of urea and WSF gave generally higher yield (average of 5.6 t $ha^{-1}$) than foliar supplements (4.6 t $ha^{-1}$), whether inorganic or organic source.

Different topdress fertilizer management in the 2013 DS and WS was compared with RSCM as the basal organic-based fertilizer. To validate the previous results, grain yield data from the RSCM plots with its corresponding topdress fertilizer application were re-run in similar statistical analysis as the second set of trial. The general yield was observed to have increased only during the wet seasons while yield in the two dry seasons was sustained (Figure 1). Consistently, urea gave the highest yield among the topdress fertilizers in all trial seasons. WSF, given the same nitrogen rate per hectare in every LCC critical readings, showed potential as an organic-based topdress N fertilizer (Table 3). Taking the averages of grain yield, it showed that the yield was comparable between the two LCC readings as basis of topdressing fertilizer (average 5.42 t $ha^{-1}$). The same was observed between organic and inorganic foliar (average 4.96 t $ha^{-1}$) and between urea and WSF (average 5.88 t $ha^{-1}$).

Despite application of organic-based basal fertilizers or supplement, there has been no significant differences between the use of LCC 3 and LCC 4. Urea application based on LCC 3 was not consistently better than LCC 4. Considering the volume and number of times of application of either WSF or urea to get high yield and the projected cost of input relative to the attained yield in an organic-based nutrient management, the use of LCC3-based OBNM is a better option.

Similarly, as there had been no significant differences in yield between urea and WSF, and between OFF and IFF, the choice of the fertilizer to apply will depend on farmers' budget. Generally, the results showed that it is still better to use the solid/granular fertilizers than the spray/foliar fertilizers. As observed, the solid/granular fertilizers gave an average of 920 kg $ha^{-1}$ higher grain yield than organic/inorganic foliar fertilizers (Figure 2). This is regardless of LCC readings as basis of topdressing application time.

Results show that inorganic fertilizers produced higher yield than organic fertilizers but do not usually produce micronutrients, which can be provided by manures and other organic sources (IRRI, 2014). These micronutrients are important in chemical processes and plant activities such as photosynthesis, respiration, chlorophyll formation, and enzymatic reactions. They are also needed in nitrogen fixation, root growth, growth hormone production, seed development, and processes within the plant involving nitrogen

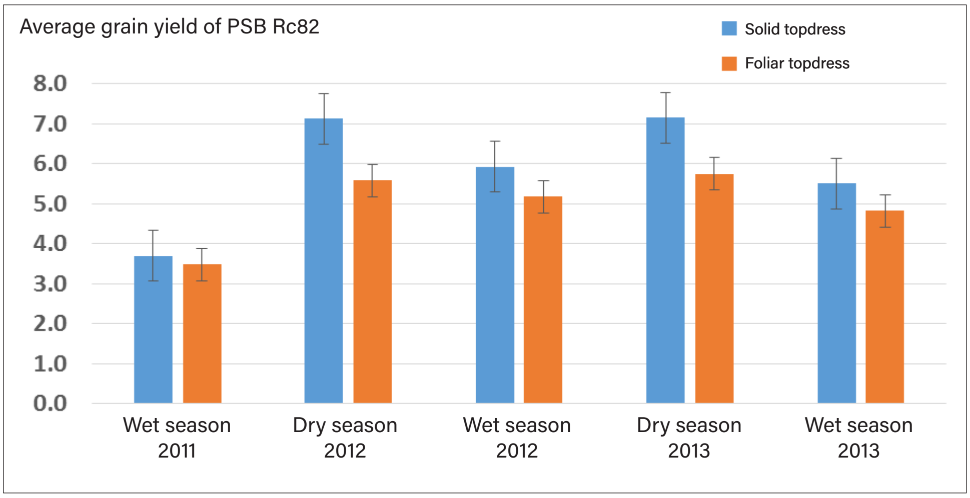


**Figure 2.** Average yield of PSB Rc82 as affected by the application of solid topdress fertilizers and foliar topdress supplement to basally-applied plants with rice straw+chicken manure. WS 2011-2013.

**Table 3.** Average grain yield (t $ha^{-1}$) of rice plants applied with RSCM as basal fertilizers and with the different topdress fertilizers based on LCC reading. 2011 WS-2013 DS. Maligaya soil series. PhilRice Experimental Station, Science City of Muñoz, Nueva Ecija.

| Treatments | Grain Yield (t $ha^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Wet Season 2011 | Dry Season 2012 | Wet Season 2012 | Dry Season 2013 | Wet Season 2013 |
| LCC3 Urea | 3.5 cd | 7.9 a | 5.8 ab | 7.3 ab | 6.0 a |
| LCC3 WSF | 3.6 bc | 6.7 bc | 5.7 abc | 7.2 b | 5.3 bc |
| LCC3 OFF | 3.3 d | 5.4 d | 5.2 cd | 5.7 d | 5.1 c |
| LCC3 IFF | 3.5 cd | 5.5 d | 5.0 d | 5.7 d | 4.9 cd |
| LCC4 Urea | 3.8 ab | 7.0 b | 6.1 a | 6.6 bc | 4.8 cd |
| LCC4 WSF | 3.9 a | 6.9 b | 6.1 a | 7.5 a | 5.9 b |
| LCC4 OFF | 3.7 abc | 5.5 d | 5.4 bcd | 5.9 cd | 4.4 d |
| LCC4 IFF | 3.4 cd | 5.9 cd | 5.1 d | 5.7 d | 4.9 cd |
| SE | 0.009 | 0.314 | 0.186 | 0.258 | 0.02 |
| cv (%) | 5.0 | 9.9 | 6.7 | 8.0 | 7.8 |

Means with similar letter are not significantly different at 5% level using the CropStat statistical software program. LCC 3= critical reading of 3 by the leaf color chart, LCC 4 = critical reading of 4 by the leaf color chart. WSF = wild sunflower, OFF= organic foliar fertilizer, IFF = inorganic foliar fertilizer

(University of Hawaii, 2015). However, inorganic chemical fertilizer is essential for crop nutrition to maximize productivity. Hence, the use of organic manures and composted organic materials along with chemical fertilizers may be effective to further increase crop yield (Ibrahim et al., 2008). Applying green manure may improve economic viability of rice production while reducing environmental impacts of agriculture. However, such approaches are complex because they depend on interactions among green manure, environment, and management (Cherr et al., 2006). *Tithonia diversifolia* (wild sunflower, WSF) is a high-quality organic source in terms of nutrient release and supplying capacity (Nziguheba, et al., 1998; Olabode et al., 2007). Considering though the habitat of wild sunflower, the Azolla spp, an aquatic plant, can be a better option as it can grow simultaneously with rice.

## Summary and Conclusion

This study used rice straw as the main organic-based basal fertilizers to develop holistic organic-based management. Rice straw is the main farm waste in a rice-rice cropping system. Because of its high carbon:nitrogen ration, there is a need to hasten its decomposition through inoculant. Chicken manure was also used in this study.

The use of urea, wild sunflower, organic foliar, and inorganic foliar as topdress fertilizers based on LCC readings of 3 and 4 were assessed to determine if they can help attain higher yield. Results showed that applying nitrogen as topdress fertilizer based on LCC readings did not produce significant differences in increasing yield of PSB Rc82, a high yielding variety under an organic-based basal nutrient management. Apparently, the synchronization between the net N mineralization and plant N uptake can explain the ineffectiveness of using the LCC in an organic-based nutrient management for rice. The laboratory result of Korsaeth and others (2002) showed that the incorporation of a mixture of N-rich white clover and N-poor barley straw created a transient accumulation of clover derived inorganic N during the first period. Thereafter, microbial N demand during the straw C utilization resulted in net immobilization of most of the clover derived inorganic N.

Among the topdress fertilizers, urea produced the highest yield. Considering the volume and number of times of WSF application and the projected input cost in an organic-based nutrient management, the use of LCC can be further studied. Topdressing of green manure can be better based on critical growth stages of the rice plants.

Generally, results showed that it is still better to use the solid/granular fertilizers than spray/foliar fertilizers either in organic or inorganic origin. As observed, the solid/granular fertilizers gave an average of 920 kg $ha^{-1}$ higher grain yield than use of organic/inorganic foliar fertilizers.

For pure and to-be-certified organic rice production management, WSF has potential to be used as an alternative topdress nutrient source for rice in an organic based nutrient management based on LCC 4 as critical value in determining the application time. However, with the issue on the synchrony of N release and N uptake from the organic N source, the use of LCC 4 may require more and frequent topdress application, thus higher input cost should be considered.

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