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# Parameters and Secondary Characters for Selection of Tolerance Rice Varieties under Stagnant Flooding Condition

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**ABSTRACT**

Determination of secondary characters during stagnant flooding (SF) is considered important for breeders as the selection criteria in developing SF rice tolerant varieties. Aims of this study were to find agronomical variation and to determine secondary traits that were related to SF tolerance among the rice varieties. Experiment was conducted at Indonesian Center for Rice Research in 2015. Ten rice genotypes were grown in RCBD with three replications under normal and gradual flooding conditions. Agronomic data were gathered and analysed using combined ANOVA, correlation, multiple linear regressions and genetic variability. Results showed that 92.3% variance of stress tolerance index (STI) were presented from a linear model involving weight of 1000-grains, panicle length, stem diameter, intensity of leaf green color and stem length. Stem length, intensity of leaf green color, and panicle length had broad genetic variability and high heritability these characters were important criteria when selecting the traits under flowing stress. Number of productive tillers was correlated with grain yield under SF and highly heritable, thus considered as one of determining characters for stagnant flooding tolerance. Based on STIStd, Ciherang and INPARI 30 showed more adaptive performance, while IR 42 had the least when grown under 50-60 cm stagnant water depth.

## INTRODUCTION

Water inundation in certain height for a long period, or stagnant Flooding (SF), is one of constraint for rice production in Indonesia. Under these conditions, rice plant could be submerged in the depth of at least 50 cm or even more. This conditions might be happened for several weeks or even the whole planting periods. Submerged condition in rice field is usually happened during rainy season, especially in the area with bad drainage system like those near the river bank, coastal areas (Nugraha, Vergara, Mackill, & Ismail, 2013) or in fresh water swampy (*Lebak*) area (Djamhari, 2009). During 2017, flooded devastated or affected the paddy field as much as 157,170.27 ha (PDSIP, 2017). Under SF condition, the growth of common modern rice varieties were inhibited. The suppression of growth were usually exhibited in the reduction of number of tillers and spikelet fertility and grain size, increasing of plant height, postponement heading and even collapse down (Kato, Collard, Septiningsih, & Ismail, 2014; Mallik et al., 2004; Nugraha, Vergara, Mackill, & Ismail, 2013; Singh, Mackill, & Ismail, 2011). These conditions might result in the yield lost up to 10 % or even 100 % depending duration, depth and flooding conditions. So far, a number of genotypes have been identified as stagnant flooding tolerant and most of them were land races (Vergara, Nugraha, Esguerra, Mackill, & Ismail, 2014). Up to present, no variety was officially registered as a SF tolerance in Indonesia. Since submerge condition often occurred in Indonesia, the development of rice SF tolerant varieties was considered important. In IRRI, the development of SF tolerant have been studied and the lines have been evaluated in several area of Asia and Africa during 2011 to 2012 (Mackill et al., 2010).

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Plants produce low yield because of reduction of the sink capacity such as panicle number, spikelet fertility, and grain size (Kato, Collard, Septiningsih, & Ismail, 2014; Mallik et al., 2004; Nugraha, Vergara, Mackill, & Ismail, 2013; Singh, Mackill, & Ismail, 2011).

Yield is a primary criterion of selection under SF condition (Nugraha, Vergara, Mackill, & Ismail, 2013). Tolerance mechanisms are controlled by various characters, therefore it is necessary to identify the secondary characters that strongly correlated with yield. The traits are preferably no need to destructive sample and can be observe at least before pre-flowering stage (Singh, Mackill, & Ismail, 2011). Faster shoot elongation contributed to the establishment of a larger aerial leaf area and higher light interception. These attributes might contribute to higher biomass production, and plant survival under stagnant flooding. These indicated that number of tillers, elongation ability at vegetative stage, leaf area development, and logging tolerance could be used for the determination of plant tolerance to water logging (Collard et al., 2013).

The ability of rice adaptation to long-term stagnant flooding depends on combination of morphological and physiological adaptation (Kuanar, Ray, Sethi, Chattopadhyay, & Sarkar, 2017)many different types of traditional rice varieties are being grown by the farmers. The local landraces adapted to extreme in water availability could be the sources of new gene(s. The selection of secondary characters was considered effective if they were expressed constitutively, could be observed as early as possible such as in seedlings or vegetative stages. The information regarding the secondary characters as the selection criteria would be very important for crop improvement program of the respected crop. The study was aimed to determine secondary traits correlated with stagnant flooding tolerance in rice.

## MATERIALS AND METHODS

The experiment was conducted in Experimental Station of Indonesian Center for Rice Research, Sukamandi, Subang, West Java on dry season of 2015 (April to August 2015). The research was arranged in a factorial experiment involving two factors. The first factor was rice genotypes (Table 1) and the second factors was dealt with water regimes. The experiment was constructed in nested design, in that water regimes were set up as random blocks with three replications. The size of each plot unit was 3 x 4 m.

### Plant Materials

Ten rice genotypes that were identified as tolerant, susceptible, and unknown were used as material. The genotypes used in this study, included swampy varieties, varieties with Sub1 gene, and popular irrigated varieties. (Table 1).

IRRI 119 was considered to be both of flash flooding (Nugraha, Vergara, Mackill, & Ismail, 2013) and stagnant flooding tolerant (Collard et al., 2013; Kato, Collard, Septiningsih, & Ismail, 2014; Miro & Ismail, 2013; Nugraha, Vergara, Mackill, & Ismail, 2013; Vergara, Nugraha, Esguerra, Mackill, & Ismail, 2014), while IR 42 was categorized as a stagnant flooding sensitive. INPARA 3, 4, 5, 7 were swampy tolerant, INPARI 29 and 30 were varieties with SUB1 gene (Nugraha, Hidayatun, Trisnaningsih, & Yuliani, 2017; Septiningsih et al., 2015), while Ciherang and IR 64 were popular irrigated varieties.

**Table 1.** Genetic materials used in SF study

| No | Genotype | Annotation | References |
|---|---|---|---|
| 1 | INPARA 3 | Swampy variety | |
| 2 | INPARA 7 | Swampy variety | |
| 3 | IRRI 119 | Tolerant to flash flooding and SF | (Collard et al., 2013; Kato, Collard, Septiningsih, & Ismail, 2014; Miro & Ismail, 2013; Nugraha, Vergara, Mackill, & Ismail, 2013; Vergara, Nugraha, Esguerra, Mackill, & Ismail, 2014) |
| 4 | INPARA 4 | Swampy variety (Swarna Sub1) | |
| 5 | INPARA 5 | Swampy variety (IR 64 Sub1) | |
| 6 | INPARI 30 | Irrigated variety with sub1 gene | (Nugraha, Hidayatun, Trisnaningsih, & Yuliani, 2017; Septiningsih et al., 2015) |
| 7 | IR 64 | Popular irrigated variety | |
| 8 | IR 42 | Sensitive to SF | (Vergara, Nugraha, Esguerra, Mackill, & Ismail, 2014; Yullianida, Ardie, Suwarno, & Aswidinnoor, 2015) |
| 9 | Ciherang | Popular irrigated variety | |
| 10 | INPARI 29 | Irrigated variety with sub1 gene | (Nugraha, Hidayatun, Trisnaningsih, & Yuliani, 2017) |

**Design of Field Trials**

The treatment of water regimes was divided into two conditions. First, the treatments of normal condition was managed by controlling shallow flooding at 5 cm depth during the plant growth. The second was gradual flooding which was starting at 30 days after transplanting (DAT) at 20 cm water depth. The gradual increase of 5 cm water was conducted weekly until it reached 50-60 cm in depth. After 50-60 cm had been reached, the water depth was maintained throughout the maturity. The 21 days-old seedlings were transplanted one plant per hole with the planting distance of 25 x 25 cm in a 3 x 4 m plots that were previously prepared according to the experimental set up. Procedure of Stagnant flooding Screening was conduct based on Vergara, Nugraha, Esguerra, Mackill, & Ismail (2014).

The observed agronomical and morphological traits were plant height, intensity of leaf green colour, number of productive tiller, number of filled grain per panicle, weight of 1000 grain, grain yield, stem length, stem diameter, length of leaf blade, width of leaf blade, and panicle exertion.

**Statistical Analysis**

The relative yield performance of genotypes in stagnant flooding stressed and non-stressed environment were used as an indicator to identify genotypes which tolerant to SF. Several indexes were determine on the basis of mathematical relationship between yield under stress and non-stress environments.

Stress tolerance (TOL) was proposed by Rosielle & Hamblin (1981). TOL was the differences between yield under stress (S) and non-stress (NS) environment and depicted as:

$$TOL = (Y_i)_{NS} - (Y_i)_S \qquad (1)$$

Remarks: $(Y_i)_{NS}$: Yield of each genotype under non-stress; $(Y_i)_S$: Yield of each genotype under stress

The higher values of TOL indicate susceptibility of genotype.

The mean relative performance (MRP) and relative efficiency index (REI) were calculated following Hossain, Sears, Cox, & Paulsen (1990):

$$MRP = \frac{(Y_i)_S}{Y_S} + \frac{(Y_i)_{NS}}{Y_{NS}} \qquad (2)$$

$$REI = \frac{(Y_i)_S}{Y_S} x \frac{(Y_i)_{NS}}{Y_{NS}} \qquad (3)$$

Remarks: $(Y_i)_S$: Yield of each genotype under stress; $(Y_i)_{NS}$: Yield of each genotype under non-stress; $Y_{NS}$: Overall mean yield of genotypes under non-stress

Stress tolerance index (STI) was determined based on Fernández (1992).

$$STI = \frac{((Y_i)_{NS}\ X\ (Y_i)_S)}{Y_{NS}^2} \qquad (4)$$

Remarks: $(Y_i)_{NS}$: Yield of each genotype under non-stress; $(Y_i)_S$: Yield of each genotype under stress; $Y_{NS}$: Overall mean yield of genotypes under non-stress

The STI was used to determine genotypes that showed high yield under both stress and non-stress environments. The higher STI values indicated the higher stress tolerance. A stress susceptibility index (SSI) was calculated by the reduction of yield under unfavourable compared to favourable environments (Fischer & Maurer, 1978):

$$SSI = \frac{\left(1 - \frac{(Y_i)_S}{(Y_i)_{NS}}\right)}{SI} \qquad (5)$$

$$SI = 1 - \frac{Y_S}{Y_{NS}} \qquad (6)$$

Remaks: SI: Stress Index; $(Y_i)_S$: Yield of genotype under stress; $(Y_i)_{NS}$: Yield of genotype under non-stress; $Y_{NS}$: Overall mean yield of genotypes under non-stress

The lower SSI values indicated the more tolerant to stress.

Singh, Mackill, & Ismail (2011) modified the Schenider's stress severity index (SSSI) and depicted as:

$$SSSI = \left(1 - \frac{(Y_i)_S}{(Y_i)_{NS}}\right) - \left(1 - \frac{Y_S}{Y_{NS}}\right) \qquad (7)$$

Remarks: $(Y_i)_S$: Yield of genotype under stress; $(Y_i)_{NS}$: Yield of genotype under non-stress; $Y_{NS}$: Overall mean yield of genotypes under non-stress

The SSSI values estimated the relative tolerance of a genotype relative to the population mean in grain yield reduction response due to stress. The six methods was analysed using correlation analysis to identify the relationship between methods.

To conclude based on the six indices, each method was standardized by standard deviations. In mathematics, the standardization formula is determined as:

(IndexStd)i = ((Index)i - (Overall mean)i/Std (8)

Remarks: (IndexStd)I : Standardized STI; (Index): index of SSI, SSSI, TOL; (Overall mean)i: overall mean of genotypes index; Std: standard deviation

Standardized STI was corrected (subtract) by susceptible index (standardized SSI, SSSI, and TOL) and called as STIStd. The conclusion was then based on the consistency of the corrected STI index in explaining the tolerant and susceptible genotypes.

The phenotypic variance  was estimated based on mean basis from variance estimation that given from the ANOVA. The standard deviation of variance genetic  and phenotypic  were determined based on (Visscher, Hill, & Wray, 2008). Genetic variability and phenotypic variability were determined by the ratio variance with their standard deviation. If  then the population had a broad genetic and phenotypic variabilities. The heritability was estimated by the ratio of genetic variance with phenotypic variance (Fehr, 1991).

All gathered data were analysed using combined analysis of variance across environment, correlation analysis, multiple linear regressions, genetic variability, and heritability. Analysis of variance (ANOVA) for combined environment was following Fehr (1991).

## RESULTS AND DISCUSSION

### Genotype and Water Regime Interaction on Grain Yield and Yield Components

According to the combined analysis of variance, effect of water regime on grain yield and productive tillers were significantly different. Meanwhile, the effect of genotype was significant on grain yield, number of productive tillers, filled grains, and weight of 1000 grains. However, the effects of water regime and genotype interaction were not significantly detected on grain yield and yield components. These indicated that all the tested genotype had similar responses on the of grain yield and yield components.

Stagnant flooding stress increased plant height at the average of 13 %, and among the genotypes with the value ranges of 6 – 28 % (Table 2). INPARA 4 has highest percentage of increased plant height, though the final height was still less than IRRI 119. Under the normal and stagnant flooding stress, plant height of IRRI 119 was the highest among the tested cultivars. Though considered as submerged tolerant, INPARI 29 and 30 had shorter plant height than IRRI 119. Nevertheless, the increased plant height among The submerged tolerant varieties exhibited different responses when conditioned in a stagnant flooding. Previous studies indicated that the short stature type of Swarna-Sub1 (INPARA 4) showed undesirable growth performance under stagnant flooding treatments (Khera et al., 2009; Nugraha, Vergara, Mackill, & Ismail, 2013; Singh, Mackill, & Ismail, 2011). These conditions was due to the inactive expression of sub1 gene and inhibiting stem elongation. The plants with these shorter stature were disabled to escape from flooding conditions. When the flooding extended in prolonged periods, the plants were more suffered in growth stress.

In addition to the increasing plant height, reduction on number of tillers was also a response to stagnant flooding stress (Collard et al., 2013). Stagnant flooding reduced number of productive, with average of 25 % and within the genotypes in the range of 3-46 % (Table 2). The mechanism of tolerance to water stagnation is different from that to flash flooding which is plants elongate when in contact with air (Singh, Septiningsih, Balyan, Singh, & Rai, 2017)where rice plants are completely submerged for 10-15 d during their vegetative stage, causes huge losses. Water stagnation for weeks to months also leads to substantial yield losses when large parts of rice aerial tissues are inundated. The low-yielding traditional varieties and landraces of rice adapted to these flooding conditions have been replaced by flood-sensitive high-yielding rice varieties. The ‘FR13A’ rice variety and the Submergence 1A (SUB1A. Number of tillers were negatively correlated with plant height, shoot elongation, leaf emergency, malondialdehyde (MDA) concentration in leaves and root–shoot junction, root biomass, and non-structural carbohydrate (NSC) concentration in the root–shoot junction. The results suggested existence of compensatory mechanisms between tiller growth and shoot elongation for resilience under SF. Under SF, the energy were mainly used for shoot elongation to escape flooding, thus prevented the tiller growth (Zhu, Chen, Ella, & Ismail, 2019).

The decrease of number of filled grain per panicle was moderately low (Table 3). Other genotypes even had higher number of filled grains in stagnant flooding stress than normal conditions. This was probably caused by different direction of assimilate distribution from the reduced number of productive tillers to the number of filled grains, as shown by INPARA 3, INPARI 30, and Ciherang. INPARA 4 and INPARA 5 only had slightly decrease in productive tillers number and also number of filled grains. The increasing number of panicle accounted largely for grain yield in stagnant flooding conditions (Baker, Allen Jr, & Boote, 1992; Baker, Allen, & Boote, 1990).

The average decrease of grain yield in stagnant flooding condition was 27 % compared to normal conditions and ranged from 20-41 % among the tested genotypes (Table 3). Among the tested varieties, the greater diminishing grain yield were observed at INPARI 29 and INPARA 3.

Under stagnant flooding stress, the yield reduction of IR 42 is 57 %, stem elongation is 36.7 cm, stem elongation rate is 1 cm/day and the number of tillers are 3 (Vergara, Nugraha, Esguerra, Mackill, & Ismail, 2014; Yullianida, Ardie, Suwarno, & Aswidinnoor, 2015). Meanwhile Vergara, Nugraha, Esguerra, Mackill, & Ismail (2014) reported the IR 42 yield reduction was 85 %.

**Table 2.** Plant height and number of productive tillers of the tested rice varieties under normal and stagnant flooding (SF) conditions

| Genotype | Plant height *) (cm) | | | | | Number of productive tillers *) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Normal | | SF | | SF/N (%) | Normal | | SF | | SF/N (%) | Mean |
| INPARA 3 | 114 | b | 129 | b | 113 | 15 | e | 9 | c | 59 | 12 |
| INPARA 7 | 113 | b | 125 | bc | 110 | 19 | bcd | 15 | ab | 80 | 17 |
| IRRI 119 | 129 | a | 136 | a | 106 | 17 | cde | 13 | bc | 77 | 15 |
| INPARA 4 | 91 | e | 117 | de | 128 | 19 | bcd | 19 | a | 97 | 19 |
| INPARA 5 | 101 | d | 110 | f | 109 | 17 | de | 15 | ab | 88 | 16 |
| INPARI 30 | 113 | b | 123 | bc | 109 | 23 | a | 12 | bc | 54 | 18 |
| IR 64 | 101 | d | 114 | ef | 113 | 21 | ab | 17 | ab | 79 | 19 |
| IR 42 | 106 | cd | 122 | cd | 116 | 23 | a | 16 | ab | 72 | 20 |
| Ciherang | 111 | bc | 125 | bc | 113 | 20 | abc | 15 | ab | 72 | 18 |
| INPARI 29 | 115 | b | 128 | bc | 112 | 17 | de | 13 | bc | 76 | 15 |
| Mean | 109 | | 123 | | 113 | 19 | | 14 | | 75 | |

Remarks: Values followed by different letters in the same column differ significantly under 95% of confident interval (P<0.05)

**Table 3.** Number of filled grains, weight of 1000 grains, and grain yield under normal and stagnant flooding (SF) conditions

| Genotype | Filled grains | | | | Weight of 1000 grains *) (g) | | | | | Grain yield *) (t/ha) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Normal | SF | SF/N (%) | Mean | Normal | | SF | SF/N (%) | Mean | Normal | SF | | SF/N (%) | Mean |
| INPARA 3 | 104 | 114 | 110 | 109 | 26.6 | cd | 25.8 | 97.0 | 26.2 | 6.23 | 3.86 | d | 61.96 | 5.05 |
| INPARA 7 | 94 | 89 | 94 | 91 | 28.6 | b | 27.5 | 96.3 | 28.1 | 6.83 | 5.02 | ab | 73.50 | 5.93 |
| IRRI 119 | 99 | 92 | 93 | 95 | 30.4 | a | 29.5 | 97.1 | 29.9 | 6.57 | 4.79 | abc | 72.91 | 5.68 |
| INPARA 4 | 76 | 100 | 131 | 88 | 21.2 | e | 25.1 | 118.8 | 23.1 | 6.38 | 4.99 | ab | 78.21 | 5.69 |
| INPARA 5 | 100 | 101 | 101 | 101 | 27.7 | bc | 25.1 | 90.7 | 26.4 | 5.81 | 4.43 | bcd | 76.25 | 5.12 |
| INPARI 30 | 75 | 97 | 129 | 86 | 26.7 | cd | 26.6 | 99.7 | 26.6 | 6.89 | 5.32 | ab | 77.21 | 6.11 |
| IR 64 | 91 | 78 | 86 | 84 | 26.2 | d | 26.5 | 101.2 | 26.3 | 7.42 | 5.35 | a | 72.10 | 6.39 |
| IR 42 | 75 | 72 | 96 | 74 | 21.3 | e | 23.4 | 109.5 | 22.4 | 5.10 | 4.07 | cd | 79.80 | 4.59 |
| Ciherang | 81 | 103 | 127 | 92 | 26.8 | cd | 24.9 | 92.8 | 25.9 | 6.86 | 5.49 | a | 80.03 | 6.18 |
| INPARI 29 | 94 | 90 | 95 | 92 | 26.6 | cd | 27.0 | 101.6 | 26.8 | 6.49 | 3.84 | d | 59.17 | 5.17 |
| Mean | 89 | 93 | 105 | | 26.2 | | 26 | 99.8 | | 6.46 | 4.72 | | 73.03 | |

Remarks: Values followed by different letters in the same column differ significantly under 95% of confident interval (P<0.05)

**Genotype and Water Regime Interaction on Morphological Characters**

The effects of water regime were significant on plant height, stem length, stem diameter, and length of leaf blade. The effects of genotype were significant in all observed characters. The interaction of eater regime treatment and genotypes was detected only on plant height, width of leaf blade, panicle exertion, and panicle length. These indicated that the response of genotypes on the characters were different in both treatments of water regime (normal and SF condition).

Stem elongation was one of plant adaptation mechanism to escape anaerobic respiration when submerged condition was occurred. Genotypes with the ability to elongate stem would be able to perform photosynthesis because the leaves were still positioned above the water. In the normal and stagnant flooding conditions, IRRI 119 had the longest stem but with low elongation increment (7 %). These suggested that the tolerance mechanism was not only reflected from the elongated stem, but also on plant stature at normal conditions. Stagnant flooding tolerance varieties should have a relatively higher stature than lowland varieties even in normal conditions. Genotypes with moderate stem elongation had higher survival and yield under flooding conditions. As mentioned by (Kuanar et al., 2017) many different types of traditional rice varieties are being grown by the farmers. The local landraces adapted to extreme in water availability could be the sources of new gene(s, medium elongation is important for higher plant productivity under SF. Rapid elongation underwater enhance the fitness particularly under prolonged of shallow flood (Ai Nio, Siahaan, & Peter Mantilen Ludong, 2019). Stagnation of water needed the enhancement of shoot elongation, which allowed the plant to expand their leaves out of the water and enable in contact with the atmosphere (Voesenek, Rijnders, Peeters, van de Steeg, & de Kroon, 2004). These was the possible adaptation mechanism of the submerged tolerance plants to have elongated stem during stagnant flooding conditions (Nugraha, Vergara, Mackill, & Ismail, 2013).

The average stem diameter increased 18 % from normal to stagnant flooding stress and these were observed in all tested varieties (Table 4). The increase of stem thickness in tolerant genotypes were dedicated to avoid lodging in standing water. Vergara, Nugraha, Esguerra, Mackill, & Ismail (2014) reported the increase of stem diameter might reach 10-45% during stagnant flooding than normal conditions. Visual comparison of stems between normal and stagnant flooding conditions indicated that the increase of thickness also increased hollowness, which might be important for root aeration.

The length of leaf blade increased with the average of 11.9 % (Table 5). Under normal conditions, IRRI 119 has the longest leaves among the tested varieties and the value was not significantly different with those under SF conditions. Varieties with shorter leaves under normal condition had 20 to 90 % longer leaves when exposed under stress conditions. All varieties showed narrower leaf blade in flooding condition.

**Table 4.** Length and diameter of stem under normal and stagnant flooding (SF) conditions

| Genotype | Stem length *) (cm) | | | | Stem diameter *) (dm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Normal | SF | SF/N (%) | Mean | Normal | SF | SF/N (%) | Mean |
| INPARA 3 | 85b | 101b | 119 | 93b | 5.2bc | 6.8a | 129.9 | 6abc |
| INPARA 7 | 81bc | 95c | 117 | 88c | 5.1c | 5.8b | 114.3 | 5.5de |
| IRRI 119 | 92a | 107a | 117 | 100a | 5.9a | 6.6a | 111.7 | 6.3a |
| INPARA 5 | 77cd | 89d | 116 | 83d | 5.2bc | 5.7b | 110 | 5.5de |
| INPARI 30 | 82bc | 98bc | 120 | 90bc | 5.0c | 6.3ab | 125.5 | 5.7cde |
| IR 64 | 74d | 88d | 119 | 81d | 4.8c | 5.8b | 121.2 | 5.3e |
| IR 42 | 82bc | 96c | 117 | 89c | 5.8ab | 6.5a | 112.1 | 6.1ab |
| Ciherang | 81bc | 99bc | 122 | 90bc | 5.4abc | 6.3ab | 117 | 5.8bcd |
| INPARI 29 | 82bc | 98bc | 119 | 90bc | 5.4abc | 6.6a | 122.6 | 6abc |
| Mean | 82 | 97 | 118 | | 5.3 | 6.3 | 118.1 | |

Remarks: Values followed by different letters in the same column differ significantly under 95% of confident interval (P<0.05)

**Table 5.** Length and width of leaf blade of rice under normal and stagnant flooding (SF) condition

| Genotype | Length of leaf blade *) (cm) | | | | Leaf width *) (cm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Normal | SF | SF/N (%) | Mean | Normal | SF | SF/N (%) | Mean |
| INPARA 3 | 45.1bc | 48.5abc | 107.6 | 46.8a | 1.4 | 1.4 | 100 | 1.4abc |
| INPARA 7 | 41.6cd | 43.4abc | 104.5 | 42.5b | 1.3 | 1.3 | 104 | 1.3c |
| IRRI 119 | 49.7a | 50.2a | 101.0 | 50a | 1.5 | 1.5 | 101.4 | 1.5a |
| INPARA 5 | 40.7de | 40.9c | 100.4 | 40.8bc | 1.3 | 1.3 | 104.7 | 1.3bc |
| INPARI 30 | 34.6f | 42.1bc | 121.6 | 38.3c | 1.3 | 1.4 | 104.6 | 1.3bc |
| IR 64 | 34.3f | 43.1abc | 125.5 | 38.7bc | 1.4 | 1.3 | 98.5 | 1.3bc |
| IR 42 | 36.6ef | 47.4abc | 129.6 | 42bc | 1.3 | 1.4 | 109.6 | 1.3bc |
| Ciherang | 36.1f | 44.9abc | 124.5 | 40.5bc | 1.4 | 1.4 | 100.7 | 1.4abc |
| INPARI 29 | 47.2ab | 48.8ab | 103.3 | 48a | 1.4 | 1.4 | 100.7 | 1.4ab |
| Mean | 40.7 | 45.5 | 111.9 | 43.1 | 1.3 | 1.4 | 102.6 | 1.4 |

Remarks: Values followed by different letters in the same column differ significantly under 95% of confident interval ($P<0.05$)

**Table 6.** Panicle exertion and length of panicle of rice under normal and stagnant flooding (SF) conditions

| Genotype | Panicle exertion *) (cm) | | | | Panicle length *) (cm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Normal | SF | SF/N (%) | Mean | Normal | SF | SF/N (%) | Mean |
| INPARA 3 | 3.3 | 3.5cd | 105.7 | 3.4bc | 25.8bc | 24.7bc | 96 | 25.3b |
| INPARA 7 | 2.8 | 3d | 105.7 | 2.9cd | 25.4cd | 26.4ab | 104 | 25.9b |
| IRRI 119 | 4.1 | 7.9a | 194.3 | 6.0a | 26.8ab | 27.9a | 104.1 | 27.4a |
| INPARA 5 | 2.8 | 5.6b | 198.9 | 4.2b | 25.1cd | 25.5bc | 101.6 | 25.3b |
| INPARI 30 | 3.0 | 3.8cd | 126.2 | 3.4bc | 23.7f | 25.2bc | 106.4 | 24.4c |
| IR 64 | 2.5 | 4.5bcd | 183.7 | 3.5bc | 23.8ef | 24c | 100.6 | 23.9c |
| IR 42 | 2.6 | -1.9e | -74.2 | 0.3e | 25cde | 23.9c | 95.5 | 24.5c |
| Ciherang | 3.0 | 5.2bc | 170.6 | 4.1b | 24.4def | 24.3bc | 99.7 | 24.3c |
| INPARI 29 | 1.7 | 3d | 174.7 | 2.3d | 27.8a | 28.2a | 101.4 | 28a |
| Mean | 2.9 | 3.8 | 131.739 | 3.3 | 25.3 | 25.6 | | 25.4 |

Remarks: Values followed by different letters in the same column differ significantly under 95% of confident interval ($P<0.05$)

The same result also reported by Anandan, Kumar Pradhan, Kumar Das, Behera, & Sangeetha (2015), that rapid elongation enabled the leaves to have higher photosynthesis activity to increase blade length by restoring contact between the leaves and air (El-Hendawy, Sone, Ito, & Sakagami, 2012).

Panicle exertion under normal was less varied compared stress conditions (Table 6). All genotypes showed panicle elongation, except IR 42. Panicle exertion of IR 42 was enclosed so that the panicle neck was minus. These conditions was probably due to the lower grain filling, as shown by the number of filled grain per panicle of IR 42 was low. Among the tested cultivars, the panicle length in normal and stress conditions were varied. The variances of the character which were observed in the two conditions provided an opportunity to obtain information panicle length characteristic of tolerant and susceptible genotypes. Panicle of IRRI 119 was higher than Ciherang and INPARI 30. However, based on the grain yield in drought stress, the grain yield of these genotypes were not significantly different. Although INPARI 29 had insignificant panicle length with IRRI 119, but grain yield was the lowest. These suggested that in addition to panicle length, other characters such as panicle density and number of secondary branches were also considered important for genotype selection.

**Table 7.** The index parameter of rice genotypes for stagnant flooding responses

| Genotype | STI | SSI | TOL | REI | SSSI | MRP |
|---|---|---|---|---|---|---|
| INPARA 3 | 0.58 | 1.40 | 2.37 | 0.79 | 0.11 | 1.78 |
| INPARA 7 | 0.82 | 0.95 | 1.81 | 1.12 | -0.01 | 2.12 |
| IRRI 119 | 0.76 | 0.96 | 1.79 | 1.04 | -0.01 | 2.03 |
| INPARA 4 | 0.77 | 0.79 | 1.39 | 1.05 | -0.06 | 2.05 |
| INPARA 5 | 0.62 | 0.84 | 1.38 | 0.85 | -0.04 | 1.84 |
| INPARI 30 | 0.88 | 0.84 | 1.56 | 1.21 | -0.04 | 2.20 |
| IR 64 | 0.95 | 1.02 | 2.08 | 1.30 | 0.01 | 2.28 |
| IR 42 | 0.50 | 0.73 | 1.03 | 0.68 | -0.07 | 1.65 |
| Ciherang | 0.90 | 0.71 | 1.37 | 1.24 | -0.08 | 2.22 |
| INPARI 29 | 0.60 | 1.49 | 2.64 | 0.82 | 0.13 | 1.82 |

Remarks: STI = Stress Tolerance Index; SSI = Stress Susceptibility Index; TOL = Stress Tolerance; REI = Relative Efficiency Index; SSSI = Stress Susceptibility Index; MRP = Mean Relative Performance.

**Tolerance Index of stagnant flooding stress**

Stress tolerance index (STI) was the measurement of tolerance degree of a genotype to stress. The respected analysis was used to identify genotypes that were able to produced high yield under both stress and non-stress environment. Genotype with a higher STI value was considered more tolerant. Based on the STI values, IR 64, Ciherang, INPARI 30 and INPARA 7 were considered as tolerant. While, INPARA 3 and IR 42 was categorized as sensitive genotypes.

Stress susceptibility index (SSI) was the measurement of susceptibility level of a genotype to stress. Genotypes with higher SSI values were considered more sensitive to stress (Table 7). Based on SSI, IR 42 and Ciherang was categorized as tolerant, while INPARA 3 and INPARI 29 were grouped into sensitive genotypes.

Stress tolerance (TOL) was the grain yield differences under normal and stress conditions. A genotypes was considered tolerant when it showed less differences in term of grain yield under both normal and stress conditions. Based on TOL values, IR 42 was considered tolerant, while INPARI 29 was susceptible.

According to several reports, instead of using TOL, the use of Relative Efficiency Index was also common to measure the level of adaptation of genotypes under stress conditions. Genotypes with higher REI had more tolerant capacity than those with the lower values. Based on REI, INPARI 30 and IR 64 was considered tolerant, while INPARA 3 and IR 42 was categorized as susceptible.

SSSI measured the relative stress tolerance of genotypes and was calculated from yield reduction of a genotype relative to the average grain reduction of population as a response to stress conditions. Lower value of SSSI indicated the tolerant genotypes in this case were IR 42 and Ciherang. While, INPARA 3 and INPARI 29 were identified as sensitive genotypes.

MRP indicates performance of average relative of genotype. Genotype with high value of MRP was identified as tolerant, and low values indicated sensitive. Based on MRP, IR 64, INPARI 30 and Ciherang were categorized as tolerant genotypes. While, INPARA 3 and IR 42 were identified as sensitive genotype (Table 7).

According to the six tolerance index methods, generally INPARA 3 was identified as susceptible to stagnant flooding stress. Ciherang was identified as tolerant based on 5 methods. IR 42 identified as tolerant based on 3 methods, and as susceptible based on 3 methods. Therefore, the levels of tolerance of the tested genotypes still needs deeper studies.

Coefficient of correlation among tolerance parameters indicated that STI correlated with REI and MRP. While, SSI correlated with TOL and SSSI. The significance was also showed by the consistency of tolerant genotypes that were identified using the six methods above. The genotype which was identified as tolerant by STI was also categorized as tolerant based on REI and MRP. IR 42 had a consistent determination as susceptible genotype based on STI, REI and MRP methods. Based on SSI for tolerant genotype-based, the pattern had also similar tolerance levels based on TOL and SSSI (Table 8).

**Table 8.** Correlation coefficient among stress index of rice

| | SSI | TOL | REI | SSSI | MRP |
|---|---|---|---|---|---|
| **STI** | -0.3347 | -0.0433 | 0.9999$^{**}$ | -0.3330 | 0.9990$^{**}$ |
| **SSI** | | 0.9532$^{**}$ | -0.3377 | 0.9993$^{**}$ | -0.3170 |
| **TOL** | | | -0.0468 | 0.9533$^{**}$ | -0.0251 |
| **REI** | | | | -0.3360 | 0.9989$^{**}$ |
| **SSSI** | | | | | -0.3159 |

STI was useful in identifying genotype which had high yield under normal and stress conditions. TOL and SSI proposed for identifying genotype that performed well under stress environment. MRP and REI were respectively the sum and product of two ratios, i.e. (a) genotype control mean/overall control mean and (b) genotype stress mean/overall stress mean. The index values were increase if (a) or (b) was higher. If (b) was high, the genotype entered the set of top performers though the performance under normal condition is not the top-most and vice versa. So, MRP and REI entered not very effective in distinctively discriminating genotypes that performed well under both normal and stress conditions (Raman et al., 2012).

Based on correlation analysis, stress tolerant index could be grouped into two. First was tolerance index group involving STI, REI, and MRP; and the second was sensitive index group involving SSI, TOL, and SSSI. Level of tolerance based on the six indices was determined using standardizes of index. For first group, STI was selected to be standardized with three sensitive index. Standardized STI was corrected with standardized index of sensitive group so that resulted in a new standardized index, namely STI (Table 6).

The STI showed that STIStd-ssi had insignificant differences with STIStd-sssi (Table 9). These because both formula were come from the same formula derivation. Three STIStd were consistent to identify tolerant and sensitive genotypes. High index values indicated tolerant genotype, and vice versa, the lowest index value referred to sensitive genotype. Based on three indexes, Ciherang and INPARI 30 were categorized as tolerant genotypes, while INPARA 3 and IR 42 were sensitive genotypes. IR 64 was consistent with high value based on STIStd-ssi and STIStd-sssi, but was moderate based on STIStd-tol. IRRI 119 which was identified as tolerant based on previous study, however in our study it referred as moderate sensitive.

**Table 9.** Standardized stress tolerance index of rice

| Genotype | STIStd-ssi | STIStd-tol | STIStd-sssi |
|---|---|---|---|
| INPARA 3 | -2.61 | -2.27 | -2.61 |
| INPARA 7 | 0.61 | 0.39 | 0.61 |
| IRRI 119 | 0.19 | 0.05 | 0.19 |
| INPARA 4 | 0.87 | 0.89 | 0.87 |
| INPARA 5 | -0.25 | -0.03 | -0.25 |
| INPARI 30 | 1.42 | 1.27 | 1.42 |
| IR 64 | 1.20 | 0.70 | 1.20 |
| IR 42 | -0.63 | -0.12 | -0.63 |
| Ciherang | 2.03 | 1.81 | 2.03 |
| INPARI 29 | -2.83 | -2.69 | -2.83 |

Remarks: STIStd-ssi, STIStd-tol, STIStd-sssi = standardized STI corrected by standardized SSI, TOL, dan SSSI, respectively.

**Modelling Stress Tolerance Index and Correlation Among Traits**

Regression analysis described the effect of one or more characters (designed as independent variables) on a single character (designed as dependent variable) by expressing the latter as a function of the former. In regression, the character of major importance, for example, grain yield, usually becomes the dependent variable and the factors of character that influence grain yield become independent variables (Gomez & Gomez, 1984).

Linear regression with tolerance index as Y and grain yield as x showed that the fitted model for determining stagnant flooding stress was = -0.371 + 0.235GY (GY = grain yield) with $R^2$ was adjusted by 87.76%. It could be shown that grain yield independently described by 87.76% tolerance variation. These indicated that the level of tolerance greatly affected the grain yield. To determine level precision between STI predictive value and STI actual, both variables were correlated and could be calculated from each genotypes. Correlation analysis between STI and predictive value of STI was significant with r = 0.9567 (Table 10). It was indicated that STI predictive was accurate to estimate actual value of STI.

Large proportion of variance contributed by grain yield to SF stress tolerance index indicated that the grain yield could independently distinguish the tolerant and sensitive genotypes.

The implication of these screening methods was that the selection of tolerant genotypes was able to be conducted only on SF, without normal conditions. The method would increase efficiency and effectiveness of screening method especially in relation with research cost.

Linear multiple regression with tolerance index as Y and morphological traits as x showed the fitted model to explain the stagnant flooding tolerance was STI = -3.17 + 0.08W1000 – 0.14PL – 0.56SD + 0.11SPAD + 0.04 SL with $R^2$ was adjusted by 0.923. For about 92.3 % of STI variances were sourced from weight of 1000 grain (W1000), panicle length (PL), stem diameter (SD), intensity of leaf green colour (SPAD), and stem length (SL). Weight of 1000 grain was observed at generative stage. To simplify the selection process, the traits that were expressed in seedlings and/or vegetative stages were preferred. When weight of 1000 grains were excluded from the model, however, the model became insignificant and $R^2$ was very low (0.1045) that was mostly constituted from stem diameter. The implication for the selection strategies were selection could not use only vegetative traits, but also need to consider generative traits.

Correlation analysis was design to measure the level of correlation among the observed traits under stagnant flooding stress. Plant height was highly correlated with morphological traits such as stem length, stem diameter, length of leaf blade, panicle length, and also weight of 1000 grain (Table 11). Stress Tolerance Index (STI) was significantly correlated with grain yield. While, grain yield was only correlated with number of tillers which was one of yield component. These trait was then considered as one important selection criterion for stagnant flooding tolerance (Ismail, Johnson, Ella, Vergara, & Baltazar, 2012; Kato, Collard, Septiningsih, & Ismail, 2014; Singh, Mackill, & Ismail, 2011), though the correlation between number of tillers and STI was considered low. Other yield components such as number of filled grains and weight of 1000 grains were correlated with grain yield. Between them, however, the correlation was also considered absent. These conditions were predictably resulted from the less varied number of filled grains and weight of 1000 grains variance of the tested genotypes to raise the expected correlation.

**Table 10.** Descriptive statistic and correlation of STI

| Variable | Min | Max | Mean | Stdv | r coefficient | p value |
|---|---|---|---|---|---|---|
| STI actual | 0.50 | 0.95 | 0.74 | 0.1543 | 0.9567 | 0.00000 |
| STI predictive | 0.81 | 1.25 | 1.05 | 0.1477 | | |

**Table 11.** Correlation among traits of rice genotypes under stagnant flooding stress (plot-basis)

| Traits | PT | FG | W1000 | GY | SPAD | SL | SD | LB | WB | PE | PL | STI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PH** | -0.347 | 0.023 | 0.356* | -0.193 | -0.070 | 0.850** | 0.584** | 0.569** | 0.107 | 0.196 | 0.516** | -0.130 |
| **PT** | | -0.330 | -0.314 | 0.368* | 0.108 | -0.469** | -0.305 | -0.304 | -0.011 | -0.262 | -0.294 | 0.178 |
| **FG** | | | -0.014 | 0.077 | -0.197 | 0.082 | 0.064 | -0.044 | 0.052 | 0.346 | -0.033 | -0.032 |
| **W1000** | | | | -0.026 | -0.211 | 0.416* | 0.263 | 0.112 | 0.315 | 0.399* | 0.569** | 0.151 |
| **GY** | | | | | -0.102 | -0.204 | -0.394* | -0.326 | 0.035 | 0.225 | -0.277 | 0.866** |
| **SPAD** | | | | | | -0.117 | -0.293 | -0.243 | -0.207 | -0.264 | 0.014 | -0.043 |
| **SL** | | | | | | | 0.578** | 0.662** | 0.348 | 0.322 | 0.535** | -0.129 |
| **SD** | | | | | | | | 0.467** | 0.214 | -0.030 | 0.183 | -0.332 |
| **LB** | | | | | | | | | 0.422* | 0.125 | 0.436* | -0.250 |
| **WB** | | | | | | | | | | 0.048 | 0.313 | 0.080 |
| **PE** | | | | | | | | | | | 0.351 | 0.291 |
| **PL** | | | | | | | | | | | | -0.099 |

Remarks: PH = plant height; PT = number of productive tiller; FG = number of filled grain; W1000 = weight of 1000 grains; GY = grain yield; SL = stem length; SD = stem diameter; LB = length of leaf blade; WB = width of leaf blade; PE = panicle exertion; PL = panicle length; STI = Stress Tolerance Index

**Table 12.** Genetic and phenotypic variability of rice traits under stagnant flooding stress in dry season of 2015

| Traits | Genetic, phenotypic variabilities and heritability under SF condition | | | | | |
|---|---|---|---|---|---|---|
| | | Criteria | | Criteria | H | Criteria |
| Plant height | 55.16 ± 25.43 | B | 59.54 ± 1.39 | B | 0.93 | H |
| Intensity of leaf green color | 2.28 ± 0.99 | B | 2.32 ± 0.01 | B | 0.98 | H |
| Number of productive tillers | 4.99 ± 3.31 | N | 7.27 ± 0.80 | B | 0.66 | H |
| Number of filled grain | 64.14 ± 69.30 | N | 171.60 ± 0.80 | B | 0.43 | M |
| Weight of 1000 grain | 0.97 ± 1.36 | N | 3.53 ± 0.60 | B | 0.34 | M |
| Grain yield | 0.30 ± 0.17 | N | 0.36 ± 0.60 | B | 0.77 | H |
| Stem length | 33.93 ± 15.92 | B | 32.08 ± 1.05 | B | 0.91 | H |
| Stem diameter | 0.09 ± 0.06 | N | 0.12 ± 1.05 | B | 0.68 | H |
| Length of leaf blade | 7.26 ± 6.05 | N | 14.14 ± 1.95 | B | 0.54 | M |
| Width of leaf blade | 0.00 ± 0.00 | N | 0.01 ± 1.95 | B | -0.86 | N |
| Panicle exertion | 7.31 ± 3.28 | B | 6.59 ± 0.12 | B | 0.95 | H |
| Panicle length | 2.27 ± 1.05 | B | 2.46 ± 0.06 | B | 0.93 | H |

Remarks:  is genotypic variance and its standard deviation;  is phenotypic variance and its standard deviation; $h^2$ is heritability; B = broad; N = narrow; H = high; M = medium; L = low

**Genetic Variability and Heritability**

Under stagnant flooding stress, all observed traits except panicle length showed broad phenotypic variability, and the genetic variability was varied among the traits (Table 12). These indicated that the variations were greatly influenced by the genetic backgrounds, but by the environment as well.

Many reports indicated that grain yield was quantitative trait which was governed by minor gene and had low heritability. In this study, the heritability of grain yield under stagnant flooding was high. Nugraha, Vergara, Mackill, & Ismail (2013) suggested that the flooding stress was obvious discriminator between tolerance and sensitive genotypes. These resulted in a consistent grain yield in a given environment and contributed to high heritability.

The study also found that variability of grain yield was narrow although the effects of genotype variance were significant (data not shown). The narrow grain yield variability was caused by the limited genetic background of the tested genotypes. Some varieties had the same parents. INPARA 5 (IR 64 SUB1), INPARI 30 (Ciherang SUB1), IR 64, Ciherang had a close genetic relationship. The selection based on grain yield under stagnant flooding stress could be conducted at early generations using bulk segregation.

Plant height, intensity of leaf green color, stem length, panicle exertion and panicle length had broad genetic variability and high heritability. It means that it would be relative easy to select the traits under flooding stress. The traits were then recommended as secondary trait for stagnant flooding tolerance. The selection could be carried out early generation, using bulk segregation or pedigree method.

## CONCLUSION AND SUGGESTION

A linear model involving weight of 1000 grain, panicle length, stem diameter, intensity of leaf green color, and stem length contributed 92.30 % of the variance of stress tolerance index. Intensity of leaf green color, panicle length and stem length had relatively broad genetic variability and high heritability under flooding stress, and therefore might be used for selection indices. Number of productive tillers was correlated with grain yield under SF and highly heritable, thus dedicated as one of determining characters for stagnant flooding tolerance. Based on STIStd, Ciherang and INPARI 30 had better growth performance under 50 – 60 cm of water depth while IR 42 showed the least. The tolerance levels of the genotypes still need deeper studies across several seasons to confirm the reliability of the findings.

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