Selection and Evaluation of Several BC$_2$F$_2$ Rice Accession Tolerance to Submergence Stress and Backcrossing to Local Parent

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Abstract. This study aimed to conduct selection process upon the four accessions of BC$_2$F$_2$ rice genotypes which were tolerant to submergence stress during vegetative stage. The selected plants were crossed with local parents. Local parents which were used in this experiment consisted of Payak Silembuk, Siam, Pegagan, and Pelita Rampak. BC$_2$F$_2$ Accession (BC$_2$F$_2$Py.Silembuk, BC$_2$F$_2$Siam, BC$_2$F$_2$Pegagan, and BC$_2$F$_2$P.Rampak) were evaluated before and after submerging process. The data obtained was analyzed with the Least Significant Difference (LSD) formula. The results showed that BC$_2$F$_2$Py.Silembuk accession had better tolerance compared to the rest of accessions being observed. Hence, this study concluded that the accession of BC$_2$F$_2$Py.Silembuk shows the best performance among the others of BC$_2$F$_2$ accessions. The result of backcrossning to local parent, accession of BC$_2$F$_2$Py.Silembuk gave the best result.

1. Introduction

Swamp area is potential area to develop rice, but there is still several problems found so far such as high water level which is difficult to predict during rain season. This condition always makes problem in rice filed such as submergence stress which make rice filed is not optimal to produce high rice production [1]. Farmers usually overcome this condition by delaying planting rice while waiting for water is reduced to normal level [2]. One of solutions that can be applied to overcome such problem is by using the variety which is tolerant to submergence condition. Badan Litbang Pertanian has produce the variety which is tolerant to submergence condition i.e Impara 3 [3]. This variety has sub-1 gene from FR13A which has the capacity to tolerant to submergence condition for 14 days [4].

Several efforts to overcome submergence condition is producing variety that has tolerant capacity to submergence. Local variety can be maintain to serve as plasma nutfah and source of genetic variety [5]. Backcrossing is one of the plant breeding methods to produce the genotype which can have the ability to maintain submergence condition during vegetative stage [4]. This method is used to produce the varieties which have agronomic characters and better adaptation to submergence condition. Kang et al. [6] stated that the method of Backcrossing can be done to fix deficiencies on several rice varieties by introducing special gene that have capacity to maintain the bad condition during vegetatif stage of rice production. The method of crossing is used to form rice population which is required in short time. The method of backcrossing is used when the parents (recurrent) has one character which can be transfer from other variety. Backcross is only suitable for recovering the cultivars which lost one or two characters.
This research was aimed to evaluate and select BC$_2$F$_2$ which is tolerant to submergence stress during vegetative condition and backcross to local parent as continuing breeding program to get best quality.

2. Research Methodology

Randomized Completely Block Designed was used in this study which included BC$_2$F$_2$ of Siam, Pegagan, P.Rampak, Py.Silembuk. Submergence stress test was conducted after 14 days of submergence condition. Plant height and chlorophyll content were measured.

Every accession was evaluated based on tolerance capacity which included four accessions of BC$_2$F$_2$ (Siam, Pegagan, P.Rampak, Py.Silembuk). Data were analyzed by using HSD (Honestly Significant Difference) to see the difference among accessions.

Accession of BC$_2$F$_2$ was backcrossed to local parents. Backcrossing was done by crossing between local parent as female (♀) and BC$_2$F$_2$ which has submergence tolerant as male parent (♂).

Castration and emasculation were done when the plants are ready to be crossed. After crossing, all plants were covered by glacine bag until they were ready to be harvested. Harvested were conducted when the plants were ready indicated by yellow grain color. Harvested was conducted by cutting the plant and then collected based on treatments.

3. Results and Discussion

Analysis of variances from four genotypes (Table 1) to all parameters being observed such as plant height before submergence (cm), Plant Height after submergence (cm), Plant Height recovery (cm) and chlorophyll content showed that they were significant difference. On the other hand, plant height recovery and chlorophyll content showed significant difference among genotypes.

| Parameters                        | F-Value | CV (%) |
|-----------------------------------|---------|--------|
| Plant Height before submergence   | 0.36$^{**}$ | 9.80   |
| Plant Height after submergence    | 2.96$^*$  | 10.75  |
| Plant height recovery             | 5.13$^{**}$ | 9.01   |
| Chlorophyll                       | 4.21$^{**}$ | 7.72   |
| F-table 5%                        | 2.5     |        |
| 1%                                | 3.6     |        |

Note: $^{**}$ = Not significant different, $^*$ = significant different, $^{**}$ = Highly significant different.

3.1. Growth Evaluation

Each accession of BC$_2$F$_2$ after treatment with submergence condition was evaluated to see the average of variances on each observation parameter from four accessions of BC$_2$F$_2$.

3.1.1. Plant Height before Submergence

Average of plant height from four genotypes being observed were 51.32 cm to 53.25 cm (Figure 1). The genotypes that showed the highest plant height was Pelita Rampak with value of 53.25 cm and the smallest was genotype Siam which was 51.32 cm. From analysis of plant height before submergence, four accessions of BC$_2$F$_2$ Py.Silembuk, BC$_2$F$_2$ Siam, BC$_2$F$_2$ Pegagan dan BC$_2$F$_2$ P.Rampak were not significant different.
3.1.2. Plant Height After Submergence

Average plant height after submergence from 4 accessions being studied were 58.51 to 64.18 (Figure 2). Accession which showed the lowest plant height was BC2F2 Payak Selimbuk with the average of 58.51 and the highest plant height was accession of BC2F2 Siam with average number of 64.18. From HSD result, two accessions of BC2F2 Pelita rampak and BC2F2 Pegagan showed no significant different while accession of BC2F2 Siam was significant different from two accessions of BC2F2 Py.Silembuk and BC2F2 Pegagan. While accession of BC2F2 P.Rampak showed no significant different from accession BC2F2 Siam.

3.1.3. Plant Height After “Recovery”

Average of plant height after recovery from 4 accessions being observed were 71.00 to 78.02. BC2F2 which has the highest plant height was accession of BC2F2 Py Silembuk with the average of 78.02 and the lowest plant height was BC2F2 Siam with the average of 71.00. HSD result of plant height after recovery showed that three accessions of BC2F2 Siam, BC2F2 Pegagan and BC2F2 P.Rampak showed not significant different while accession of BC2F2 Py Silembuk showed significant different with BC2F2 Siam, BC2F2 Pegagan, and BC2F2 P.Rampak.
3.1.4. Chlorophyl

The average of leaves chlorphil of 4 accesions being observed were 35.59 to 38.23 (Figure 4). The chlorophyl content of BC2F2 P.Rampak was higher with the average of 38.23 and the lowest was BC2F2 Pegagan with the average of 32.59. HSD results showed that accessions of BC2F2 Pegagan and BC2F2 Siam were not significant different while populasi of BC2F2 Py.Silembuk and BC2F2 P.Rampak were significantly different.

3.2. Selected Plant

3.2.1. Accession of BC2F2 Siam

The selected plant on accessions of BC2F2 Siam have 8 genotypes which were in quadran 3,12,15,19,20,6,5,7 (Figure 5). The highest plant height was number 7 with 72 cm and number of plant that has the highest after recovery was number 5 with the plant height of 80.5.
3.2.2. \textit{BC}_2F_2 Pegagan

The highest plant height after submergence was found in number 6 which was 72.3 cm and the lowest was in number 4 which was 60.5 cm. Plant height after recovery was number 7 with the value of 75.5 cm and the lowest is number 14 which was 70.5 cm (Figure 6).
Figure 6. Accession of BC$_2$F$_2$ Pegagan on plant height after submergence for 20 days after planting, Plant height Recovery at 34 days after planting, and chlorophyll at 34 days after planting for selected plant crossing.

3.2.3. Accession BC$_2$F$_2$ Pelita Rampak

Parameter of the tallest plant height after submergence was number 2 which was 70.2 cm and the lowest was number 13 which was 65.5 cm (Figure 7). While the highest plant height after recovery was number 9 which was 86 cm and the lowest was number 5 which was 75.5 cm.
**Figure 7.** Accession of BC$_2$F$_2$ P.Rampak on plant height after submergence at 20 days after planting, Plant height Recovery at 34 days after planting, and chlorophyl at 34 days after planting to get selected plant for crossing.

### 3.2.4. Accession of BC$_2$F$_2$ Payak Silembuk

Data which showed the tallest plant height was found in plant number 6 with the value of 66.7 cm and the smallest was number 7 which was 58 cm. While the highest number for plant height after recovery was found in number 9 which was 87.5 cm and the lowest was number 7 which was 78 cm (**Figure 8**).
3.3. Backcrossing of $BC_2F_2$ to Local Parents

The successful of crossing was found in $BC_2F_2$ Py.Silembuk with number of 160 flowers which were crossed and produced 112 seeds Table 2. Approximately 48 seeds were failed. The result show that $BC_2F_2$ Siam showed the average plant height more higher compared to other accessions of $BC_2F_2$. Based on HSD test result, they were significant different on plant height after recovery. There was no significant different of Plant height after recovery on accession of $BC_2F_2$ Siam, accession of $BC_2F_2$ P.Rampak and $BC_2F_2$ Py.Silembuk.

Fukao et al. [7] stated that rice plant which has $Sub1$ gene during submergence condition might maintain the plant height. After recovery from submergence stress it showed better growth and development until harvesting. This condition occurred in accession of $BC_2F_2$ Payak Selimbuk with condition of stronger stem. Plant growed much better after submergence compared to other accessions. Ref. [7] stated that plant that has $sub$-1 gene is able to defend during submergence with the mechanism
which is called ‘quiscene’ which is indicated by slowing down the growth to save energy and carbohydrate content indicated by lower plant height during submergence.

**Table 2. The successful of breeding**

| Crossbred          | Number of flowers | Number of seeds | Number of failed seeds |
|--------------------|-------------------|-----------------|------------------------|
| Pegagan x BC2F2 Pegagan | 120               | 88              | 32                     |
| Siam x BC2F2 Siam  | 144               | 86              | 58                     |
| P.Rampak x BC2F2 P.Rampak | 124         | 65              | 59                     |
| Py.Silembuk x BC2F2 Py.Silembuk | 160     | 112             | 48                     |

Rice plant which growed in submergence condition showed that photosynthetic process was disrupted due to submergence condition. During submergence, the stomata will be closed and there will be inhibition of photosynthetic process due to the entry of sunlight for photosynthetic process was disrupted. As a result, the plant under submergence condition for long period of time will decrease the chlorophyl content [8]. In this experiment chlorophyl was measure by using SPAD 502 after treatment with submergence for several periods. The result showed that chlorophyl content of accession of BC2F2 P.Rampak and accession of BC2F2 Pegagan and BC2F2 Py.Silembuk were significant different. Singh *et al.* [9] stated that chlorophyl content after submergence will recover and the plant continued to grow. Accession of BC2F2 Py.Silembuk and BC2F2 P.Rampak showed chlorophyl content were much better compared to other BC2F2.

BC2F2 Py.Silembuk was tolerance to submergence stress compared to three other accessions of BC2F2. This result was also found by Gusmiatun [10,11 and 8]. They stated that from four accession of BC2F1, only BC2F1 Py.Silembuk showed more tolerant compared to other to submergence condition, Setter *et al.* [12] stated that the ability of rice plant to keep a live during submergence condition was correlated to the ability of plant to keep energy source during stress condition.

Selection of accession of BC2F2 was done by observing and collecting data on parameter of plant height after submergence (cm), plant height after recovery (cm), and chlorophyl content. Selection of selective plant genotipe for nect crosing was by using quadrant method which selection of two variables x and y at the same time (Figure 5), (Figure 6), (Figure 7), and (Figure 8) which showed the selected plant genotypes. Based on submergence tolerant, accession of BC2F2 Py.Silembuk was the best plant compared to others population of BC2F2. In addition, the backcrossed of this genotype to payak selimbuk as local parent showed that BC3F1 was much better compared to other genotypes (Table 2).

**4. Conclusion**

Based on plant growth accession of BC2F2Py.Silembuk was much better tolerant to submergence condition compared to other accessions. In addition accession of BC2F2Py.Silembuk showed better result of backcrossed to local parent compared to other accessions.

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