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Growth Evaluation of Selfcrossed Progenies BC_1F_2 Experiencing Submerged and Non-submerged at BC_1F_1 and its Parents

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Abstract: *A non-tidal swamp can increase rice cultivation and production, but its use is still dependent on natural conditions, making plants vulnerable to submergence and drought stress. Developing dual-tolerant varieties is essential for rice cultivation in non-tidal swamps. Inpara 8 carries the SUB1 gene, providing tolerance to submergence stress, while Inpago 5 is tolerant to drought stress. This study aimed to evaluate the growth of BC1F2 progeny from a cross between Inpara 8 and Inpago 5 under submerged stress, along with their parents (Inpago 5 and Inpara 8). The research was conducted in the greenhouse of the Agriculture Faculty at Sriwijaya University at coordinates 3°13'11.1"S 104°38'49.4"E from July 2021 to January 2022. Inpago 5 exhibited the highest average plant height (145.81 cm), the most tillers (4.73), the most productive tillers (3.87), the highest total grain per clump (720.87 grains), the heaviest clump grain weight (15.86 g), and the lowest percentage of empty grains (33.47%). The donor parent Inpara 8 had the highest average total grain per panicle (192.08) and the highest dry weight (20.69 g). BC1F2 Submerged progeny showed the highest average 1000-grain weight (25.26 g) and the fastest harvest age (114.50 DAP). The BC1F2 self-cross progeny subjected to submergence stress displayed characteristics similar to its parents, with no significant differences in observed parameters.*

Keywords: *Dual tolerance, Inpago 5, Inpara 8*

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1. INTRODUCTION

Oryza sativa L. or Rice is an important carbohydrate food source in Indonesia because there almost the majority of the population in Indonesia (98.35% of 275.77 million people) consume rice as a daily carbohydrate source [1] This number absolutely can increase every year but according to BPS Indonesia (2023) rice production for food consumption in 2023 is estimated to be around 30.90 million tonnes and this figure is down 2.05% from the previous year, due to the area of rice harvested land. which also decreased by 255.79 thousand hectares. This phenomenon must be addressed immediately so that food needs in Indonesia can be fulfilled. The effort that can be made is by utilizing sub-optimal land, the non-tidal swamp land for rice cultivation.

Swamp land in Indonesia is divided into two, non-tidal swamp land and tidal swamp land. Indonesia has 25.20 million hectares of non-tidal swamp land [2]. Non-tidal swamp land is sub-optimal land that potential for agricultural development [3] However in its utilization in agriculture, non-tidal swamp still depends on natural conditions, so the

utilization of non-tidal swamp land is still small compared to tidal swamp land [4].

The water source in non-tidal swamps comes from rainfall, so the height of the water level is influenced by the rainfall in that area [5] In non-tidal swamp land rice cultivation is differentiated by season. The first-season plant is prone to harvest failure due to drought and in the next season is prone to submerged stress [6]. The development of rice varieties that have dual-tolerant (tolerant when the rice submerged during the vegetative phase[7] and drought tolerant during the generative phase [8]–[10]) to those abiotic stress can be the solution to the problem [11].

Rice plants, especially the Inpara 8 variety have genes that help them to tolerate submergence, SUB1, or Submergence1 genes on chromosome 9 [12], [13]. This SUB1 gene helps plants to suppress their metabolism so the growth is restricted by inhibiting the production of ethylene and reducing the work of gibbereline [14] The tolerance trait carried by the SUB1 genes can be inherited [15], [16].

Rice cultivation in non-tidal swamp land prone to submergence during the vegetative phase and drought during the generative phase [6] the SUB1 gene that can tolerate submergence stress in Inpara 8 can be inserted into Inpago 5 variety which has tolerance to drought stress [17].

A cross and selection has been carried out between Inpago 5 as the recipent parent and SUB1 gene donor parent Inpara 8 to produce the selected BC_1F_1 accession which has been given submerged and non-submerged treatment and then carried out self crossing to obtain the selected BC_1F_2. This reasearch aims to evaluate the growth of BC_1F_2, Inpago 5 recipient Parents and Inpara 8 donor parents.

2. MATERIALS AND METHODS

The study was carried out in Juli 2021 – January 2022 at the greenhouse of the Agriculture Faculty at coordinates 3°13'11.1"S 104°38'49.4"E, Sriwijaya University

Soil materials.

Non-tidal swamp soil was taken from non-tidal swampland in the observation area of the Agriculture Faculty, Universitas Sriwijaya, Indralaya. The soil was put into pots, 5 Liters for each pot, and added the limestone. The media was placed in a flooded condition.

Plant materials.

BC_1F_2 from submerged and non-submerged stress, Inpago 5 and Inpara 8 seeds were used in this study. BC_1F_2 seeds were progenies from self-crossing BC_1F_1 from the recipient parent Inpago 5 and the donor parent Inpara 8 [18] Inpara 8 has the SUB1 gene which makes it tolerant to submerged stress [12], [13]

Seedling.

The seeds were soaked for 24 hours and germinated on wet paper in a tray and kept moist. Germinated seed sown in the trays filled soil for 14 days

Pot Experiment.

14-day-old seedlings were transplanted into the soil-filled pots and orderly arranged in greenhouse. There were 25 pots/treatment and 15 pots/ parents. Seedlings were fertilized regularly with urea (1,5 g/pots), KCl (0,75 g/pots), and SP36 (0,75 g/pots) at 7, 23, and 42 days after transplanting (DAT)

Harvest.

The harvest time showed when the panicles already droop, drying, and yellowing leaves and grains. Usually harvest in ±115 days after transplanting.

Growth Evaluation.

The evaluation plants will be observed for growth with the following parameters: plant height, the total number of tillers,

number of productive tillers (panicle), days to flowering, days to harvesting, number of total grains per panicles, number of total grains per plant, percentage of sterile spikelets, biomass dry weight, grain weight per plant, weight of 1000 grain.

Data Analysis.

The statistical comparison among treatments was conducted using the analysis of variance (ANOVA). If the F count > F-table at 5 % the test level means the treatment has a real effect. Furthermore, so the difference between varieties, further tests are using the least significant different test at 5% test level.

3. RESULTS AND DISCUSSION

The result of the analysis of variance (ANOVA) on all parameters is represented in Table 1. It outlined that the growth of height and number of tillers BC_1F_2 Submerged, BC_1F_2 Non-submerged, Inpara 8, and Inpago 5 showed the same growth and did not show significant differences, in the same growth environment, the four groups of progeny populations showed indications of genetics similarity with their parents[18].

Productive tillers in the population of BC_1F_2 submerged, BC_1F_2 non-submerged, Inpara 8 and Inpago 5 showed significant differences, while at days of flowering, day of harvesting, the number of grains per panicle, the number of grains per plant, percentage of sterile spikelets, biomass dryweight, grain weight per plant, weight of 1000 grains in the populations of BC_1F_2 submerged, BC_1F_2 non-submerged, Inpara 8 and Inpago 5 showed no significant differences.

Plant Height.

For submergence-tolerant plants, height is a crucial parameter affecting productivity and survival after stress conditions [19]. Table 2 shows that plant height did not significantly differ at 14 and 28 DAT. Similarly, no significant differences were observed at 42, 56, and 70 DAT. According to the Rice Standard Evaluation System [20], rice plant height is categorized as follows: (1) Semi-dwarf (less than 110 cm for lowland rice and less than 90 cm for upland rice), (2) Intermediate (110-130 cm for lowland rice and 90-125 cm for upland rice), and (3) Tall (more than 130 cm for lowland rice and more than 125 cm for upland rice).

Based on Figure 1a. BC_1F_2 Submerged got an average plant height was 97.53 cm, higher than Inpara 8 (97.30 cm) and lower than BC_1F_2 non-submerged (98.64 cm). Inpago 5 has the highest average plant height compared to the other populations which is 103.79 cm, so the BC_1F_2 submerged, BC_1F_2 non-submerged, Inpara 8, and Inpago 5 can be grouped into semidwarf of lowland rice and intermediate lowland rice. The result on Tabel 5. Showed the plant height of BC_1F_2 Submeged was correlated to days of flowering ($r = 0.999$) as the previous study [21]showed plant height was correlated to day after flowering.

Number of tiller and Productive tiller.

On the Table 2. The number of tillers in BC_1F_2 submerged, BC_1F_2 non-submerged, Inpara 8 and Inpago 5 at the initial observation showed no significant difference, at 26 and 42 DAT populations BC_1F_2 submerged were significantly different from Inpago 5 and at observation 56 dan 70 DAT the development of the number of tillers all populations showed not significantly difference. This phenomenon happened because rice ended its vegetative phase marked by slowing the growth of the number of tillers due to competition in nutrient absorption for preparing the generative phase[22]

Table 1. F Values and coefficient of variance (%) of growth parameters of BC_1F_2 submerged, BC_1F_2 non-submerged, Inpara 8 and Inpago 5

Parameters	F Value		Coefficient of Variance (%)
	Treatment	Group	
Plant Height			
14 DAT	0.47^{ns}	0.74^{ns}	8.90
28 DAT	0.42^{ns}	0.64^{ns}	3.91
42 DAT	0.65^{ns}	0.51^{ns}	1.00
56 DAT	0.07^{ns}	1.16^{ns}	1.53
70 DAT	1.19^{ns}	1.52^{ns}	7.51
The Number of Tillers			
14 DAT	033^{ns}	2.33^{ns}	2.23
28 DAT	5.06^{*}	0.01^{ns}	4.76
42 DAT	3.13^{ns}	0.001^{ns}	3.95
56 DAT	1.68^{ns}	0.42^{ns}	4.86
70 DAT	2.29^{ns}	0.75^{ns}	8.77
Productive Tillers	4.77^{*}	1.74^{ns}	7.50
Days to Flowering	1.51^{ns}	0.98^{ns}	7.50
Days to Harvesting	1.11^{ns}	0.97^{ns}	7.77
Total Spikelets per Panicle	3.01^{ns}	3.37^{ns}	13.54
Total Spikelets per Plant	4.10^{ns}	2.42^{ns}	9.89
Percentage of Sterile Spikelets	0.90^{ns}	1.14^{ns}	10.58
Biomass Dry Weight	1.46^{ns}	0.64^{ns}	8.68
Grains Weight per Plant	2.73^{ns}	2.87^{ns}	9.98
Weight of 1000 Grains	1.25^{ns}	1.50^{ns}	1.47
F Tabel 0.05	4.76	5.14	

Note: values within a row followed by the same letters were not significantly different based on the LSD at P≤0.05; ns = not significant at ≤0.05; * and ** = significant ≤0.05; DAT = Days After Transplanting

Tabel 2. Plant Height Average (cm) dan jumlah anakan total pada populasi BC_1F_2 Submerged, BC_1F_2 Non-Submerged, Inpara 8 dan Inpago 5

Population	14 DAT	28 DAT	42 DAT	56 DAT	70 DAT
	Plant Height (cm)				
BC_1F_2 S	41.50±2.78a	69.02±5.63a	106.13±4.30abc	126.73±11.01a	144.27±8.97a
BC_1F_2 NS	43.80±0.4a	72.22±0.47a	105.75±0.97ab	125.61±1.43a	140.60±4.24a
Inpago 5	56.16±31.8a	79.27±20.92a	108.21±5.54bc	124.50±10.47a	145.81±16.30a
Inpara 8	44.54±2.82a	72.42±3.08a	103.35±3.71a	127.30±4.02a	138.90±4.97a
LSD 0.05	**33.09**	**22.89**	**8.52**	**15.45**	**78.0**
	The Number of Tillers				
BC_1F_2 S	1.07±0,11a	1.33±0.41a	1.93±0.41abc	3.13±0.75a	4.47±1.22a
BC_1F_2 NS	1.13±±0.11a	1.93±0.30abc	2.02±0.36ab	2.80±0.69a	3.30±1.27a
Inpago 5	1.17±0.11a	2.47±0.30bc	2.47±0.11b	3.93±0.41a	4.73±0.30a
Inpara 8	1.13±0.11a	1.80±0.2ab	1.80±0.00a	3.20±0.40a	3.53±0.64a
LSD 0.05	**0.18**	**0.67**	**0.61**	**1.19**	**2.46**

Note: values within a row followed by the same letters were not significantly different based on the LSD at P ≤0,05; DAT = Days after transplanting; S = Submerged; NS = Non-submerged

In the Figure 1b. The number of tillers Inpago 5 showed the highest average of 2.95, followed by BC_1F_2 Non-submerged (2.54), BC_1F_2 Submerged (2.44) and Inpara

8 showed the lowest average number of tiller was 2.29. The number of tillers is influenced by the environmental conditions [23] such as the application of nitrogen fertilizer that can increase the amount of cytokinin in the tiller book so it can also increase the process of forming new tillers [22], [24]

The Table 3. The highest productive tillers were shown in Inpago 5 with the average was 3.87 tillers. Productive tillers Inpago 5 and Inpara 8 showed no significant difference while in BC_1F_2 Non-submerged showed a significant difference with Inpago 5. The number of productive tillers is influenced by the number of tillers per plant, the more tillers the higher chance that the plant to produce productive tillers [25] Table 6. BC1F2 Non-submerged the number of tillers was positively correlated with productive tillers (r = 0.999) so the more tillers produced, they can increase the number of productive tillers.

Days to Flowering and Days to Harvesting.

The results showed that BC1F2 Submerged, BC1F2 Non-submerged, Inpara 8, and Inpago 5 did not differ significantly in flowering and harvesting times (Table 3). Inpago 5 flowered at 92 days after sowing (DAS) and had the shortest grain filling period until harvest. Inpara 8 flowered at 90 DAS. BC1F2 Submerged flowered at 84 DAS, with a grain filling period of 34 days until harvest at 118 DAS, which is one day longer than BC1F2 Non-submerged. BC1F2 Non-submerged flowered at 83 DAS but had a grain filling period 3 days shorter than BC1F2 Submerged.

Table 6. BC_1F_2 Non-submerged days to flowering showed a positive correlation with grain weight per plant (r =1) similar to a previous study by Renawake et al (2014) flowering at the age of 80-90 days will produce a positive correlation to yield.

Table 3. Agronomy parameters of the vegetative and generative stage of BC_1F_2 Submerged, BC_1F_2 Non-submerged, Inpara 8, and Inpago 5

Population	PL	DF	DH	TSP	TSC
BC_1F_2 S	2.93±1.22a	84.07±3.75a	118.33±1.15a	126.67±40.12a	401.27±289.61ab
BC_1F_2 NS	2.30±1.33a	83.00±4.80a	114.50±0.70a	123.60±13.85a	330.20±22.62a
Inpago 5	3.87±0.46a	92.80±2.88a	116.33±0.57a	191.01±38.42a	720.87±96.38bc
Inpara 8	3.80±0.2a	90.00±0.52a	115.66±0.57a	192.08±98.86a	650.73±282.80abc
LSD 0.05	**1.62**	**45.5**	**62.3**	**100.21**	**371.33**

Note: values within a row followed by the same letters were not significantly different based on the LSD at P ≤0,05; S = Submerged; NS = Non-submerged; PL = Number of productive tillers; DF = Day to flowering; DH = Day to harvesting; TSP = Number of total spikelets per panicle; TSC = Number of total spikelets per plant

Table 4. Agronomy parameters of vegetative and generative stage of BC_1F_2 Submerged, BC_1F_2Non-submerged, Inpara 8, and Inpago 5

Population	PS	BD	WGP	WP
BC_1F_2 S	39.40±3.89a	16.71±4.40a	9.66±6.92a	25.26±0.97a
BC_1F_2 NS	44.10±31.70a	16.62±9.62a	6.65±5.79a	23.67±13.74a
Inpago 5	33.47±2.96a	17.58±2.16a	15.86±2.12bc	23.68±1.85a
Inpara 8	49.80±6.68a	20.69±1.79a	15.59±6.85ab	23.54±1.25a
LSD 0.05	**30.31**	**10.08**	**8.95**	**12.38**

Note: values within a row followed by the same letters were not significantly different based on the LSD at P ≤0,05; S = Submerged; NS = Non-submerged; PS = Percentage of sterile spikelets (%); BD =Biomass dry weight (g); WG = Weight of 1000 grains (g); WGP = Weight grains per Plant (g)

Total Spikelets per Penicles and Total Spikelets per Plant

Total spikelets per panicles BC_1F_2 Non-submerged showed a positive correlation with total spikelets per plant (r = 0.999) (Table 6.). Total spikelets per plant BC1F2 Submerged had a positive correlation with grain weight per plant (r =1), Inpago 5 (r =1), and Inpara 8 (r = 0.997). Total spikelets per panicle BC_1F_2 Submerged showed a positive correlation with total tillers (r =0,998). Good ability to produce tillers is a profitable capability to have a plant with a high production yield because the number of tillers is related to the number of panicles per plant, the panicles themselves will produce grains. [26], [27]

Percentage of sterile spikelets.

Inpara 8 showed the highest percentage of sterile spikelets was 49,80% and Inpago 5 showed the lowest percentage was 33,47%. The percentage of spikelets BC_1F_2 Submerged and BC_1F_2 Non-submerged showed no significant difference with its parents were 39,40% and 44,10% (Table 3). The high percentage of the sterile spikelets was due to the phase toward generative many plants were attacked by pests and disease. the brown planthopper and stem rot causing the developing spikelets to become sterile and empty. Just like a previous study by Nabilah (2022) pest attacks can reduse the percentage of grains. Table 8. Showed the percentage of sterile spikelets Inpara 8 has a negative correlation with a weight of 1000 grains (r=-0,999), the higher the percentage of sterile spikelets the lower the weight of 1000 grains.

Grains Weight per Plant, Weight 1000 Grains, Dry Weight.

In Table 4. Inpago 5 showed significant differences with the other three populations with 15.86 g which was also the highest yield of grain weight, followed by Inpara 8 (15.59 g), BC_1F_2 submerged (9.66 g), and BC_1F_2 non-submerged had the lowest grain weight of 6.65 g. Grain production is determined by the coordination of the distribution of the sink and source of photosynthetic results and the increase in production when the photosynthetic results are transferred more to the panicle and fill the grain than to other parts of the plant body [28], [29]. In Table 6. Grains weight per plant BC_1F_2 non-submerged showed a positive correlation to the weight of 1000 grains (r = 1). Table 4 showed that BC_1F_2 submerged had the highest weight of 1000 grains with 25.26 g and showed no significant difference with the population of BC_1F_2 Non-submerged (23.67 g), Inpago 5 (23.68 g), and Inpara 8 showed the lowest weight of 1000 grains

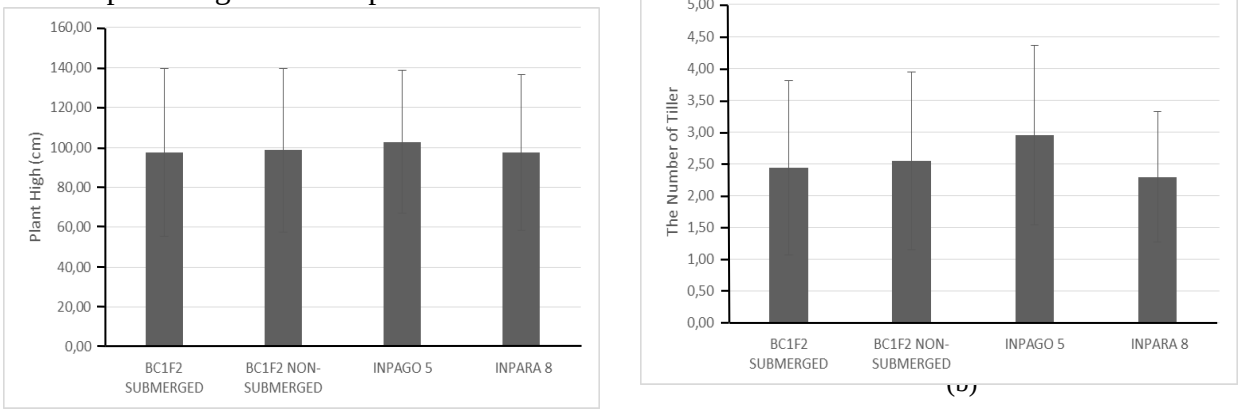

Figure 1. Average of Plant High (a) and The Number of Tiller (b) of BC_1F_2 Submerged, BC_1F_2 Non-submerged, Inpara 8, and Inpago 5

was 23.54 g. Dry weight was shown to be the highest in the population of Inpara 8 with 20.69 g and the lowest in BC_1F_2 non-submerged 16.71 g. Table 6 showed BC1F2 non-submerged has a positive correlation to the weight of (r =1) and the weight of 1000 grains of grain (r =1). The dry weight of plants showed indicators of plant growth and development, the photosynthate will form more plant organs to increase the production of plant dry matter [30]

Table 5. Comparison of correlation analysis result among growth parameters of BC_1F_2 Submerged

	PH	*NT*	*PL*	*DF*	*DH*	*TSP*	*TSC*	*PS*	*BD*	*WGP*	*WG*
PH	1										
NT	0,917	1									
PL	0,703	0,929	1								
DF	0,999*	0,936	0,738	1							
DH	-0,997*	-0,945	-0,756	-1*	1						
TSP	0,889	0,998*	0,950	0,912	-0,922	1					
`TSC	0,850	0,990	0,972	0,876	-0,888	0,997	1				
PS	-0,960	-0,992	-0,874	-0,973	0,979	-0,982	-0,964	1			
DB	0,989	0,966	0,801	0,995	-0,997*	0,948	0,919	-0,991	1		
WGP	0,849	0,989	0,972	0,875	-0,888	0,997	1**	-0,963	0,918	1	
WG	-0,672	-0,320	0,055	-0,633	0,612	-0,260	-0,181	0,437	-0,554	-0,180	1

Table 6. Comparison of correlation analysis result among growth parameters of BC_1F_2 Non-submerged

	PH	*NT*	*PL*	*DF*	*DH*	*TSP*	*TSC*	*PS*	*BD*	*WGP*	*WG*
PH	1										
NT	0,997*	1									
PL	0,994	0,999*	1								
DF	0,994	1*	1**	1							
DH	1*	0,999*	0,997	0,997	1						
TSP	0,985	0,995	0,998*	0,998	0,990	1					
TSC	0,993	0,999*	1**	1**	0,996	0,999*	1				
PS	0,780	0,826	0,845	0,843	0,798	0,877	0,850	1			
DB	0,999*	0,993	0,988	0,988	0,997*	0,976	0,986	0,752	1		
WGP	0,997*	0,989	0,983	0,984	0,995	0,970	0,981	0,733	1*	1	
WG	0,998*	0,989	0,984	0,984	0,995	0,971	0,982	0,735	1*	1**	1

Table 7. Comparison of correlation analysis result among growth parameters of Inpara 8

	PH	*NT*	*PL*	*DF*	*DH*	*TSP*	*TSC*	*PS*	*BD*	*WGP*	*WG*
PH	1										
NT	-0,700	1									
PL	-0,997	0,756	1								
DF	-0,661	0,999*	0,721	1							
DH	0,569	0,189	-0,500	0,240	1						
TSP	0,374	-0,924	-0,449	-0,943	-0,550	1					
TSC	-0,403	-0,372	0,326	-0,420	-0,982	0,698	1				
PS	0,925	-0,919	-0,953	-0,897	0,214	0,699	-0,025	1			
DB	-0,998*	0,747	1**	0,711	-0,512	-0,437	0,339	-0,949	1		
WGP	-0,404	-0,371	0,328	-0,419	-0,982	0,697	1**	-0,026	0,340	1	
WG	0,616	-0,994	-0,679	-0,998*	-0,297	0,961	0,473	0,869	-0,669	0,471	1

Table 8. Comparison of correlation analysis result among growth parameters of Inpago 5

	PH	*NT*	*PL*	*DF*	*DH*	*TSP*	*TSC*	*PS*	*BD*	*WGP*	*WG*
PH	1										
NT	0,725	1									
PL	-0,793	-0,156	1								
DF	0,550	-0,176	-0,945	1							
DH	0,609	0,988	0,000	-0,327	1						
TSP	-0,683	-0,998	0,097	0,234	-0,995	1					
TSC	-0,784	-0,996	0,245	0,086	-0,970	0,989	1				
PS	-0,025	0,670	0,629	-0,849	0,778	-0,713	-0,600	1			
DB	0,896	0,955	-0,440	0,122	0,898	-0,936	-0,978	0,421	1		
WGP	-0,733	-1**	0,167	0,165	-0,986	0,997	0,997*	-0,662	-0,959	1	
WG	0,059	-0,645	-0,655	0,866	-0,756	0,689	0,573	-0,999*	-0,390	0,636	1

Note: (Table 5, Table 6, Table 7, Table 8) PH = Plant height; NT = Number of total tillers; PL = Number of productive tillers; DF = Day to flowering; DH = Day to harvesting; TSP = Number of total spikelets per panicle; TSC = Number of total spikelets per plant; PS = Percentage of sterile spikelets (%); BD= Biomass dry weight (g); WG = Weight of 1000 grains (g); WGP = Weight grains per plant (g); * = Correlation is significant at the 0.05 level; **= Correlation is significant at the 0.01 level

4. CONCLUSION

The results showed that the Inpago 5 recipient parent had the highest average plant height, number of tillers, productive tillers, and grain weight per plant, along with the lowest number of total spikelets per plant and percentage of sterile spikelets. The donor parent Inpara 8 had the highest number of total spikelets per panicle and dry biomass weight. BC1F2 submerged progeny had the highest average weight of 1000 grains and the fastest time to harvest. The self-crossed BC1F2 submerged and non-submerged progeny had characteristics similar to their parents, with no significant differences in the observed parameters.

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