crossing compatability

by F Kusmiyati

Crossing of Four Rice Cultivars to Produce Paddy F1 Which Has Tasty and Low Glycemic Index Genotype

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Abstract

Crossing between cultivars are often done by plant breeders in order to increase genetic variability. However, incompatibility and flower sterility are the main obstacles in crossing of rice. The aim of this research is to evaluate the crossing of four rice cultivars which has 2 different characteristics. Two characteristics are tasty and high-glycemic index owned by Sintanur and Gilirang while hard and low-glycemic index owned by Situ Patenggang and Logawa on the design of full diallel. The experiments were conducted in greenhouse of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, from September to December 2017. The parameters were crossing ability, length, width, and weight of grains. The data were tabulated and analyzed statistically using Dunnett test ($\alpha = 5\%$). The highest crossing ability is Sintanur × Situ Patenggang (58.00%). All F1 has seed length, width and weight smaller than the parent's except F_1 of Situ Patenggang × Logawa which has longer seed than parent's. Single Seed Descent selection in the next generations necessary to obtain the tasty-low glycemic index genotype.

Keywords: seed, crossing, ability, rice

Introduction

Rice is main source for around 95% of people in Indonesia (Sulistyo et al. 2016). The consumption of rice can be affected to blood glucose because of carbohydrate content in rice which has big impact of glycemic index (GI) response. A high glycemic index in the blood can cause Diabetes Mellitus (DM) (Truong et al. 2014). The GI is a measure of blood response after consuming food. Factors that influence GI are levels of fiber, amylose, amylopectin, fat, protein, starch digestibility and processing methods (Arif et al. 2013). Today, many farmers and consumen choose cultivars that have high production, pests and diseases resistant, and fluffy texture (Laborte et al. 2015). Rice flavour is associated with protein, fat, and amylose (Lee et al. 2014). The GI of rice has an opposite association with amylose content. Rice that has low GI sometimes has high amylose also (Elis et al. 2016). So, Diabetics is still difficult to get rice (rice) with low GI and low amylose. Therefore, genetic improvement of rice to obtain the character of pulen and low glycemic index should continue.

Genetic improvement can be done through several methods, such as gene transformation, mutation induction, and crosses (Hanafiah et al. 2010). However, the diversity germplasm collections of rice is the main requirement to get desired genotype, namely the crossing of various germplasm into an initial activity that plays an important role to combine the important properties of germplasm into the population (Hairmansis et al. 2015). Some rice genotypes crossed by Sabu et al. (2006) have been evaluated by Se et al. (2016) that natural crossing ability by selfing has a very low probability (0.5–6.8%) in making of varieties.

The success of artificial crossing is influenced by several factors. Internal factors which often become an obstacle in artificial crossing of rice are incompatibility and flowers sterility. Characteristic which can explain the ability is when pollens can pollinated the pistil and succes to become fruit. Beside it, flower sterility is affected by 2 factors. First, pollen that has capable of germination is fails to fertilize. Second, the embryo is dead after fertilization. External factors are influenced by environmental factors, weather or climate. Some cultivars that this research used were Sintanur (sticky texture, GI 91), Gilirang (sticky texture, GI 97), Situ Patenggang (pera, GI 53,7), and Logawa (pera, GI 49). This study aims to evaluate the crossing of four rice cultivars which has 2 different characteristics. Two characteristics are tasty and high-glycemic index owned by Sintanur and Gilirang while hard and low-glycemic index owned by Situ Patenggang and Logawa on the design of full diallel.

Materials and Methods

The experimental material comprising of 4 rice cultivars (Sintanur, Gilirang, Situ Patenggang, and Logawa). It taken from Balai Besar Penelitian Tanaman Padi and conducted in the greenhouse of the Faculty of Animal and Agriculture, Diponegoro University, Semarang, from September to December 2017. The rice seeds are selected by soaking in clean water for 24 hours in room temperature. Sintanur and Logawa were germinated in the first week then Situ Patenggang and Gilirang in the second week. The purpose of time differences was to get concurrent maturity flowering time of rice between parents.

The seeds sowned into bamboo box which has small size (20 cm × 20 cm × 12 cm) that contain soil:fertilizer (1:1). Plastic plate given under bamboo box to maintain of water measurement. Twelve seedlings each cultivar were choosen for planted in pots in day 21 after seedling. Three plants from each cultivars will be planted into pots that contain 15 kg media by comparison soil:fertilizer (2:1) then make it mud.

Some activities for the cultivation of rice plants were the management of pests, diseases, and weeds manually, and keep the water around ±5 cm above media surface. The artificial crossing were full diallels, the result is 16 combinations of crossing (Table 1).

Table 1. Full diallel crossing of four cultivars

Female (♀)	Male (♂)			
	Sintanur (A)	Gilirang (B)	Situ Patenggang (C)	Logawa (D)
Sintanur (A)	AA	AB	AC	AD
Gilirang (B)	BA	BB	BC	BD
Situ Patenggang (C)	CA	CB	CC	CD
Logawa (D)	DA	DB	DC	DD

Selection of female candidate happen when flowers have not bloomed, and expected to be bloom on the next day. The female flower on the panicle to be castrated leave up to ±100 flowers. One third of flower was cutted with corner (±30°) using scissors the emasculated by taking stamens using tweezers. Castration was done at 06.00–08.00 or 15.00–17.00 where the best flower stadia for castration is when the end of the stamens are in the middle of the flower. Flowers that have been cleaned from the stamens then closed by using white paper bag to keep from unwanted pollens. The selection of male flowers is performed on the same day as the blossoming of female flowers. Pollens from male flowers was taken and spread on selected female flowers, so the pollen falls and sticks to the pistil's head. Pollination is done at 09.00–13.00 or when lemma and palea are open.

After pollinated, the female flowers covered with white paper bags. Beside it, complete flowers of parents varieties is left to selfing (control) then closed with white paper bags to ensure there is no pollination from unwanted pollen. Then, each paper bag especially for crossing should have labels that shown date of crossing, number of crossing flower, name of parents, and breeder. Rice planted in the greenhouse until the seeds ready for harvest. Malai harvested four weeks after crossing, then dried for three days. Observation parameters included crossing ability (number of result crossing seed or crossing flower × 100%), seed length, seed width, and seed weight of four rice cultivars. Measurements of length, width and weight of seeds were done by random sampling of 10 seeds per crossing result and measured by using calipers. The data was tabulated and analyzed by standard statistic descriptive and the results were presented as a mean and standard deviation with SPSS statistics 17.0 software and continued with Dunnett test at 95% confidence level (α = 5%) to compare character between crossing combination and parents varieties.

Result and Discussion

The value of crossing ability four rice cultivars is from 3.12–58.00% (Table 2). The differences of percentage between this ability (lowest and highest) is wide around 54.88%. It because this research shown that some of them succes to become seed and the rest is unfilled rice or unsucces crossing or another condition this related to crossing combination itself. According to Taryono et al. (2006), the unfilled seeds in rice from the result of crossing will increase because of hybrid sterility effect or incompatibility. Research result of Mizuta et al. (2010) explains that genetic incompatibility happened in rice crossing especially because of reciprocity loose or duplicated genes subfunctionalisation. This study shown that one of the factors which has influenced to crossing ability is the position of parents or female/male. The combination of Logawa × Situ Patenggang crossing shows the lowest crossing ability (3.12%), where the combination of other crossing by placing Logawa as female shows higher crosssing ability (Logawa × Sintanur = 17.16%; Logawa × Gilirang = 24.72%).

Table 2. Crossing ability of four rice cultivars

Crossing Combination	Crossing Combination Percentage (%)	∑ Crossing Result (grain)
Sintanur × Gilirang	21.25	170
Sintanur × Situ Patenggang	58.00	290
Sintanur × Logawa	25.17	151
Gilirang × Sintanur	6.80	88
Gilirang × Situ Patenggang	5.95	33
Gilirang × Logawa	12.16	90
Situ Patenggang × Sintanur	20.80	272
Situ Patenggang × Gilirang	11.93	78
Situ Patenggang × Logawa	34.40	450
Logawa × Sintanur	17.16	121
Logawa × Gilirang	24.72	244
Logawa × Situ Patenggang	3.12	44
Sintanur × Sintanur	100.00	84
Gilirang × Gilirang	100.00	225
Situ Patenggang × Situ Patenggang	100.00	231
Logawa × Logawa	100.00	127

Crossing success can be seen in three days until a week after pollination which marked by fresh green flowers. If the flowers looked withered and turns into brown, it should be failed crossing. Successfully crossing flowers will form seeds with divers morphology (Figure 1). Logawa cultivars can be a good role as a male parent on Sintanur, Gilirang, and Situ Patenggang cultivars, with success percentages are 25.17, 12.16, and 34.40%. Although the combination of Sintanur × Situ Patenggang has the largest crossing ability about up to 58.00%, but the reciprocity of Situ Patenggang × Sintanur has only 20.80%. According to Matcubara et al. (2003), the differences between reciprocal crossing ability of both parents is because incompatibility crossing. So, number of seed is small caused by seed abortion. Beside it, cytoplasmic influences and nuclear-cytoplasmic interactions can be the causes of different hybrid sterility or differences seed formation in reciprocal crossing (Obebrese et al. 2011).

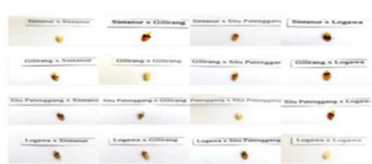

Figure 1. Full diallel seed formation of four rice cultivars

Genetic stability does not stop with the success of a crossing generation. Selection is needed to select a genotype. Selection was performed with the aim of narrowing the variability of genotype population to obtain what research want (Johannes et al. 2009). Number of seeds based on the result from crossing combination of showed around 33 to 450 seeds (Table 2). Selection method that can be used are Single Seed Descent Selection (SSD) and Bulk Selection.

Table 3. The length, width, and weight crossing seed of four rice cultivars

Crossing combination	Length (mm)	Width (mm)	Weight (g/10 grain)
Sintanur × Gilirang	0.78 ± 0.03 gpl	0.23 ± 0.02 sgp	0.16 ± 0.01 sgpl
Sintanur × Situ Patenggang	0.83 ± 0.07 g	0.23 ± 0.01 sgp	0.21 ± 0.01 sgpl
Sintanur × Logawa	0.79 ± 0.05 gl	0.21 ± 0.02 sgpl	0.16 ± 0.01 sgpl
Gilirang × Sintanur	0.78 ± 0.06 gpl	0.22 ± 0.02 sgp	0.21 ± 0.00 sgpl
Gilirang × Situ Patenggang	0.88 ± 0.02	0.22 ± 0.01 sgp	0.23 ± 0.00 sgp
Gilirang × Logawa	0.84 ± 0.10 g	0.23 ± 0.03 sgp	0.20 ± 0.01 sgpl
Situ Patenggang × Sintanur	0.87 ± 0.03 g	0.23 ± 0.04 sgp	0.20 ± 0.01 sgpl
Situ Patenggang × Gilirang	0.86 ± 0.05 g	0.22 ± 0.03 sgp	0.22 ± 0.01 sgpl
Situ Patenggang × Logawa	0.92 ± 0.06 S	0.23 ± 0.02 sgp	0.21 ± 0.01 sgpl
Logawa × Sintanur	0.80 ± 0.04 gl	0.20 ± 0.02 sgpl	0.20 ± 0.01 sgpl
Logawa × Gilirang	0.88 ± 0.04	0.22 ± 0.02 sgp	0.20 ± 0.01 sgpl
Logawa × Situ Patenggang	0.88 ± 0.03 g	0.23 ± 0.03 sgp	0.25 ± 0.00 p
Sintanur × Sintanur	0.83 ± 0.02	0.30 ± 0.01	0.26 ± 0.01
Gilirang × Gilirang	0.96 ± 0.02	0.27 ± 0.01	0.25 ± 0.01
Situ Patenggang × Situ Patenggang	0.87 ± 0.04	0.29 ± 0.01	0.28 ± 0.00
Logawa × Logawa	0.89 ± 0.01	0.25 ± 0.00	0.24 ± 0.00

The numbers followed by s (Sintanur), g (Gilirang), p (Situ Patenggang), and l (Logawa) are each significantly smaller, while the number followed by the S is larger than the Sintanur cultivar based on the Dunnett test level of $\alpha = 5\%$.

Not all seeds from crossing is able to survive after harvest because some of them were falled. The falled of young seeds can be caused of nutrition competition between seeds and number of ovule each planr. Crossing combination has a significant effect in crossing seeds (length, width and weight) (Table 3). The combination of the Situ Patenggang × Logawa has a significantly longer seed than Sintanur, while nine other crossing combinations have significantly shorter seeds than Gilirang or Situ Patenggang or Logawa (Figure 1). Although all crossing combinations have a smaller width and weight than the controls (Sintanur or Gilirang or Situ Patenggang or Logawa). This condition indicated that the crossing can cause the embryo and food reserves (endosperm) become smaller. The size of the embryo and endosperm is greatly affected to germination and growth of seedlings (Pandey et al. 1992). This information can used for study the interaction of incompatibility, morphology of seeds, and seed germination from crossing. However, the success and speed for finding the desired genotype is largely determined by the ability to separate genotypes in kind of selection (Zen 2012).

Conclusion

The highest percentage of crossing ability showed in combination Sintanur × Situ Patenggang (58.00%) and the lowest was Logawa × Situ Patenggang

(3.12%). The width and weight seeds in all crossing combination is smaller than parents cultivars (controls). Single Seed Descent (SSD) and Bulk selection method in the next generation is necessary to obtain stable genetic state with the character of pulen–low glycemic index. Based on the number of seeds of crossing, which can not be continued at the SSD selection stage is combination of Gilirang × Situ Patenggang and Logawa × Situ Patenggang.

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