Analysis the agronomic character and inheritance of fragrant genes in F2 progenies of Sigupai and Yinzhan

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Abstract. Sigupai is one of Aceh's local rice that highly popular because of the distinctive scent. Sigupai is still widely grown by the farmers in the southern western region of Aceh. However, this local rice has inferior production, height plant architecture, deep age and the average of production about 4 tons ha-1. The improvement of Sigupai local rice from the agronomic character and the harvest age can be done through crosses with Yinzhan varieties that carry sd-1 genes. The study aims to analyze changes in agronomic character and fragrant gene inheritance in Sigupai/Yinzhan F2 derived. Analysis of agronomic character is carried out by observing harvest age, plant height, weight of 1000 grains, grain weight per clump and yield potential per hectare. The planting material used was F2 derivative of Sigupai/Yinzhan as many as 104 individuals planted in pots. PCR analysis was conducted to analyze the presence of fragrant genes in F2 progenies of Sg/Yz. The data was analyzed with Chi-Square analysis. The results showed 104 plant genotypes analyzed by PCR, 64.42% of F2 progenies Sigupai/Yinzhan inherited the fragrant gene. The results of analysis of agronomic character showed 62 genotypes there were 95.08% of ripening aged, 59.01% had a short stem architecture and 6 genotypes (9.83%) had a potential yield of 4.04,-5.33 tons ha-1.

1. Introduction
Sigupai is one of Aceh's local rice which is still grown in the South-West of Aceh Province, Indonesia. Sigupai produces rather long rice seeds with fragrant, soft and tasty rice quality, resistant to drought, gives a distinctive fragrant scent and flavor to cooked, but has low yields with harvest life of in 5-6 months as well as height plant architecture (>150 cm) [1]. The scent in the seeds becomes one of the most valuable quality results and has high demand by the consumers [2,3].

The improvement of Sigupai rice quality into plants with short plant architecture (semidwarf) with good grain quality can be done by inserting the sd-1 gene through a series of breeding processes and molecular biology. The semi-dwarf gene (sd-1) is the most important gene for rice repair in Indonesia. Gen sd-1 is recessive which results in shorter stems, a high harvest index and improves response to the use of nitrogen fertilizers [4]. One of the introduced rice varieties that have the gene semidwarf (sd-1),
is the Yinzhan variety with the lifespan of the ripening plant (101 DAS), the architecture of the short plant (114) and the estimated of the highest production is > 8 tons [5].

Plants that have the sd-1 gene will have a shorter stem architecture than plants that do not have the sd-1 gene so it can play a role in preventing plants from lying in a state of high nitrogen fertilization and can improve plant architecture but not reduce the number of saplings, seed size and number of seeds [6]. The result of Sigupai and Yinzhan crossing, besides having the sd-1 gene also have inherited the fragrant gene inherited from the Sigupai elder. Sigupai has a fragrant *Pandanus amaryllifolius* Roxb. scent caused by the presence of fragrant genes from Sigupai elders, this is reinforced by the scent of the sigupai plant on the field [2].

The Fragrant gene is a carrier of the fragrant scent properties of rice as one of the quality properties of grains that are important in rice. The identification of fragrant germplasm and the proper development of polymorphic molecular markers are critical in the success of MAS (Marker Assisted Selection) technology. Badh2 is a molecular marker that has been developed by MAS from the scent gene [7]. Fragrant rice is famous for the unique scent characteristics, and the market price is higher than regular rice. Recently, Basmati rice is Indian and Pakistani, and Jasmine-type rice in Thailand is sold in the worldwide. Therefore, the selection and cultivation of fragrant rice is one of the important aspects in modern rice breeding programs to increase the economic value of rice [8].

Selection of agronomic character is usually held by visual; the plant architecture, seed shape, quality of results and components of results so that new superior varieties are obtained. Selection and identification used based on the molecular marker selection that can reduce the population required for each generation of selection [9]. The used of molecular marker to analyze the presence of genes in individual plants which influences the increasing precision of selection results from the breeding program developed [10].

The utilization of molecular marker is thought to save the time and be more accurate. Several superior rice varieties assembled through selection using molecular marker have been produced. The supreme varieties are evidence of the success of the utilization of molecular marker in support of the assembly of superior varieties. Molecular marker technology has made a real contribution and supports national food security. This research aims to analyze the character of agronomy and inheritance of genotype properties by analyzing the existence of sd-1 and fragrant genes by using molecular marker, that prespective plants are obtained to be continue to the next generation.

### 2. Materials and Methods

#### 2.1. Research implementation and genetical resources.

The research was conducted in experimental farm and Plant Breeding Laboratory, Faculty of Agriculture, Syiah Kuala University, Darussalam Banda Aceh, Indonesia from September 2018 to January 2019. The seeds used in this study were F$_2$ seeds from crossbreeding from introduced local varieties of Sigupai and variety Yinzhan (F$_2$ Sg/Yz). Sigupai was used as female recurrent parent. Sigupai and Yinzhan were also used as comparison for plant arsitecture and yield.

#### 2.2. Plants cultivation technique

F$_2$ seed were transplanted in soil mix with manure with ratio 2:1 after 15 days of sowing. In one pot contained 5 kg soil and manure were planted 2 seedling with 1 seedling in each planting hole. The basal fertilizer where used NPK and Urea 2.6 g (450 kg ha$^{-1}$) and 0.6 g (100 kg ha$^{-1}$) respectively one day before transplanting. Additional fertilizers were applied at 14, 28, and 42 days after transplanting (DAT) by using only Urea each 0.6 g pot$^{-1}$.

#### 2.3. DNA extraction

The DNA was extracted from the leaves of each 2 weeks-old rice plant sample using a modified protocol as described by [11]. Taken samples of rice plants about 0.1- 0.2 g of young leaves were cut and put inside the 2 ml microcentrifuge tube containing one stainless steel bead. Then the tube containing the
leaves of the sample plant were inserted in a microtube box and then stored in the freezer temperature -86 °C for 24 hours. Then the sample of the rice plant is destroyed by shaking the sample box containing the microcentrifuge tube 20-30 times until the rice leaf sample was destroyed. Then add 200-400 μl TPE buffers into each tube containing the crushed sample and the samples were incubated in water bath with a temperature of 65 °C for 20 minutes. After the incubation, then the sample were separated by centrifuge with 13,000 g for 10 minutes. Then the supernatant containing DNA as much as 100 μl was inserted in a 1.5 ml microtube.

2.4. **Molecular marker and Polimerase Chain Reaction (PCR) amplification**

The molecular marker used in this study was dominant STS (Sequence-Tagged Site) marker RM Fmbadh-E4-5 gene analysis [12]. Oligo primers for PCR amplification of the molecular markers had DNA sequence Uni F (5' TGC TGG ATG CTT TGA GTA 3') and Uni R (5' GTT TAG CAC ACC TGA AGG ACC A 3'). PCR was performed using EmeraldAmp PCR Master Mix (Takara). For the PCR reactions, the mixtures were initially denatured at 94°C for 120 second followed by 30 cycles of PCR amplification with the following parameters: a 45 s of denaturation at 94°C, a 45 s of annealing at 55 °C, and 60 seconds of primer extension at 72°C. Finally, the reaction mixture was maintained at 72°C for 8 min before completion. The amplified product was electrophoretically resolved on 1.5 % agarose gel in 0.5×TAE buffer. The Electrophoresis monitor was set up with 100 Volt PAC power for 38 minutes. DNA were visualized under UV transilluminator light and documented with a Canon G11 camera.

2.5. **Agronomic character analysis**

Agronomic performance of F$_2$ progenies for each plant in each genotype were collected from experiment field in dry season (September 2018). Analysis of plant phenotypic character was done on flowering and harvesting time. Others variables analysed including plant height at harvesting time, weight of seed per hill, and estimated potential yield ha$^{-1}$.

2.6. **Marker analysis**

The clearly resolved amplicons of STS were scored manually as homozygote for the allele for fgr gene from local rice parent and heterozygote carrying the alleles from both parents in the data sheet. Chi-square ($\chi^2$) test was performed to test the goodness of fit of the F$_2$ population for the phenotypic and marker data by comparing an observed frequency distribution with an expected one.

3. **Results and discussion**

3.1. **Analysis the presence of Badh gene in F2 progenies of Sg/Yz**

Molecular analysis the presence of Fragrant gene in F2 progenies of Sg/Yz for 104 plant genotype is shown in Figure 1.
Figure 1. Genetic analysis of Fragrant gene from Sigupai, Yinzhan, and F2 progenis Sg/Yz.

The results of the molecular analysis using the STS (Sequence-Tagged Site) marker RM Fmbadh-E4-5 contained 67 F2 progenis of the Sg/Yz Fragrant gene (64.42%). The genotypes that show the Fragrant gene are plants: #1, 3, 4, 6, 8, 10, 12, 13, 14, 16, 17, 18, 20, 21, 22, 24, 25, 29, 30, 31, 36, 38, 40, 41, 42, 48, 49, 50, 55, 58, 59, 60, 61, 62, 64, 67, 68, 69, 70, 72, 73, 74, 75, 76, 78, 79, 80, 81, 82, 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 97, 98, 99, 101, 102, and 104. This shows that more than half of the F2 progenis Sg/Yz progenies utilize the Fragrant gene as a gene inherited by Sigupai elders, as shown in the picture above, Sigupai analysis has Fragrant gene donors with comparison varieties, namely Sigipai and Yinzhan elders. Chi-square analysis of molecular analysis of the presence of the Fragrant gene F2 progenies Sg/Yz can be is shown in Table 1.

Table 1. Chi-Square analysis of the F2 Sg/Yz progenies with Sequence-Tagged Site (STS) marker RM Fmbadh-E4-5.

| Category         | Observed genotype | Expected genotype (1:3) | (O-E)^2 | (O-E)^2/E | χ²   | P          |
|------------------|-------------------|-------------------------|---------|-----------|-------|------------|
| Fragrance        | 67                | 78,00                   | 121     | 1,55      | 6,205 | 0,000      |
| Non Fragrance    | 37                | 26,00                   | 121     | 4,65      |       |            |
| Total            | 104               |                         |         |           |       |            |

*χ² = Chi-Square ; P = Chi-Square table

Table 1 of the results of Chi-square analysis shows that the relative observations of 67 plants have DNA bands, which indicates the presence of a genotype that inherits the Fragrant gene that carries fragrance properties in F2 progenies of Sg/Yz with the gene code carried is fgr. Plants that do not show DNA bands have 37 genotypes, but these plants may carry other donor genes. The results of the analysis
show that the F2 progenies of Sg/Yz carry a fully dominant gene (monohybrid) in a ratio of (3:1) which is a gene that completely covers the effect of its recessive allele so that the recessive allele does not appear on the genotype display [13].

3.2. Phenotypic performance of agronomy character in F2 progenies Sg/Yz

The performance of flowering age, harvest age and plant height at harvest of F2 progenies Sg/Yz plants as well as Sigupai and Yinzhan elders are shown in Table 2.

| Genotypes | Harvesting Time (DAS) | Plant Height (cm) | Genotypes | Harvesting Time (DAS) | Plant Height (cm) |
|-----------|----------------------|-------------------|-----------|----------------------|-------------------|
| Sigupai   | 136                  | 157               | Sg/Yz T37 | 110                  | 104               |
| Yinzhan   | 101                  | 115               | Sg/Yz T38 | 111                  | 113               |
| Sg/Yz T1  | 123                  | 108               | Sg/Yz T39 | 112                  | 116               |
| Sg/Yz T2  | 112                  | 102               | Sg/Yz T40 | 113                  | 117               |
| Sg/Yz T3  | 111                  | 107               | Sg/Yz T41 | 112                  | 105               |
| Sg/Yz T4  | 123                  | 106               | Sg/Yz T42 | 112                  | 105               |
| Sg/Yz T5  | 123                  | 108               | Sg/Yz T43 | 137                  | 110               |
| Sg/Yz T6  | 122                  | 102               | Sg/Yz T45 | 113                  | 102               |
| Sg/Yz T7  | 111                  | 107               | Sg/Yz T46 | 113                  | 106               |
| Sg/Yz T8  | 111                  | 112               | Sg/Yz T47 | 112                  | 111               |
| Sg/Yz T10 | 123                  | 106               | Sg/Yz T48 | 112                  | 113               |
| Sg/Yz T11 | 137                  | 108               | Sg/Yz T49 | 110                  | 111               |
| Sg/Yz T13 | 112                  | 104               | Sg/Yz T50 | 110                  | 106               |
| Sg/Yz T14 | 111                  | 103               | Sg/Yz T51 | 111                  | 113               |
| Sg/Yz T15 | 137                  | 114               | Sg/Yz T52 | 112                  | 108               |
| Sg/Yz T16 | 110                  | 95                | Sg/Yz T56 | 126                  | 131               |
| Sg/Yz T17 | 109                  | 100               | Sg/Yz T58 | 122                  | 159               |
| Sg/Yz T18 | 112                  | 99                | Sg/Yz T71 | 111                  | 150               |
| Sg/Yz T20 | 109                  | 102               | Sg/Yz T73 | 124                  | 175               |
| Sg/Yz T21 | 110                  | 102               | Sg/Yz T75 | 113                  | 131               |
| Sg/Yz T22 | 110                  | 103               | Sg/Yz T76 | 122                  | 124               |
| Sg/Yz T23 | 111                  | 106               | Sg/Yz T93 | 124                  | 143               |
| Sg/Yz T24 | 112                  | 97                | Sg/Yz T94 | 124                  | 142               |
| Sg/Yz T25 | 112                  | 102               | Sg/Yz T95 | 109                  | 145               |
| Sg/Yz T26 | 112                  | 92                | Sg/Yz T97 | 122                  | 123               |
| Sg/Yz T27 | 117                  | 107               | Sg/Yz T98 | 113                  | 137               |
| Sg/Yz T28 | 113                  | 105               | Sg/Yz T99 | 110                  | 118               |
| Sg/Yz T30 | 109                  | 109               | Sg/Yz T102| 123                  | 138               |
| Sg/Yz T31 | 112                  | 102               | Sg/Yz T103| 113                  | 155               |
| Sg/Yz T33 | 111                  | 108               | Sg/Yz T104| 121                  | 165               |
| Sg/Yz T34 | 112                  | 110               | Mean      | 115.36               | 114.66            |
| Sg/Yz T35 | 113                  | 100               | Min       | 109.00               | 92.00             |
| Sg/Yz T36 | 110                  | 92                | Maks      | 137.00               | 175.00            |

Table 2 shows that from 61 F2 progenies Sg/Yz, there were 58 genotypes (95.08%) of plants that followed Yinzhan's parents, as seen from the harvest age range of 110 to 125 DAP. The height of rice plants of F2 progenies Sg/Yz has different appearances of expressed and unexpressed phenotypic characters [14]. This is because the genetic composition of rice plants varies in each individual, so that their response to the environment is different, this is clearly seen in the appearance of the plant itself [15]. Chi-square analysis of harvest age at F2 progenies Sg/Yz can be is shown in Table 3.
Table 3. Chi-Square analysis harvesting time of F2 progenies Sg/Yz.

| Category     | Observed genotype | Expected genotype (3:1) | (O-E)$^2$ | (O-E)$^2$/E | $\chi^2$ | P   |
|--------------|-------------------|-------------------------|-----------|-------------|---------|-----|
| Genjah       | 58                | 45,75                   | 150,0625  | 3,28        | 13,120  | 0,000|
| Sedang       | 3                 | 15,25                   | 150,0625  | 9,84        |         |     |
| Total        | 61                |                         |           |             |         |     |

* $\chi^2$ = Chi-Square ; P = Chi-Square table

Table 4. Chi-Square analysis plant height of F2 progenies Sg/Yz.

| Category     | Observed genotype | Expected genotype (1:2:1) | (O-E)$^2$ | (O-E)$^2$/E | $\chi^2$ | P   |
|--------------|-------------------|---------------------------|-----------|-------------|---------|-----|
| Semidwaf Plant | 36                | 15,25                     | 430,56    | 28,2336     | 38,97   | 0,00 |
| Intermediate Plant | 13             | 30,50                     | 306,25    | 10,0410     |         |     |
| Tall Plant   | 12                | 15,25                     | 10,56     | 0,6926      |         |     |
| Total        | 61                |                           |           |             |         |     |

* $\chi^2$ = Chi-Square ; P = Chi-Square table

The high plant architecture causes the plant to fall easily so that the potential for yield loss. Classification of plant height at harvest based on IPBGR standards there are 3 categories, namely, low (<110 cm), medium (110-130 cm) and Height> 130 cm. The value of observations on the Chi-squared plant height at harvest can be seen in table 4, there are 12 plants in the tall category, namely with a height of >130 following Elder Sigupai, there are 13 plants that have a moderate value following the height at harvest, Elder Yinzhan and there are 36 plants in the category of plant height at harvest time. low harvest.

The ratio of plant height characters is 1 low: 2 moderate: 1 high, which means that the height character of the Sg/Yz-derived F2 rice plant is controlled by one dominant gene imperfectly. A ratio of 1:2:1 indicates a semi-dominance event. This event occurs when a dominant gene does not completely cover the effect of its recessive allele so that an intermediate trait will appear [16]. It can be seen that there is a trait between the two dominant traits, namely the moderate nature of the plant height at harvest.

The performance of 1000 grain grain weight, cluster grain weight and pe Ha production in Sigupai, Yinzhan and 61 Sg/Yz-derived F2 plants can be is shown in Table 5. Table 5 shows the weight of grain per clump directly affects the high and low potential yield of rice plants. The weight of the grain in the clump of 61 phenotypes was 99% below the weight of the grain per clump of the two parents, which was under 20 g [17]. In production per ha, it can be seen that there are 6 plants that have moderate production criteria, namely 4-6 tons per ha. Plants that have moderate production criteria are Plants #7, 21, 31, 45, 47 and 48. While other plants have production <4 tons per ha.

Production yields on F2 progenies of Sg/Yz were low due to lack of nutrient intake in field planting due to one pot planted with 2 samples so that there was a lack of available nutrients and a lack of space for plant growth. The high and low crop production was related to other variables such as the number...
of productive tillers and the weight of 1000 grains. Based on these data, it can be said that the increase in the amount of production is related to the large number of productive tillers and the high yield of grain/clump weight. The potential yield of rice plants is determined by the number of productive tillers, the number of grains per panicle, the percentage of pithy grain and the weight of 100 grains [18].

Table 5. Agronomic character analysis of F2 progenies Sg/yz for 61 plant genotype.

| Genotypes     | Weight of 1000 grains | Weight of filled grains per plant (g) | Estimated yield (t/ha) | Genotypes     | Berat 1000 Butir | Weight of filled grains per plant (g) | Estimated yield (t/ha) |
|---------------|-----------------------|--------------------------------------|------------------------|---------------|-----------------|--------------------------------------|------------------------|
| Sigupai       | 18.88                 | 20.56                                | 5.14                   | Sg/Yz T37     | 19.50           | 10.72                                | 2.68                   |
| Yinzhan       | 20.15                 | 33.85                                | 8.46                   | Sg/Yz T38     | 19.70           | 12.98                                | 3.25                   |
| Sg/Yz T1      | 18.60                 | 12.42                                | 3.11                   | Sg/Yz T39     | 19.30           | 8.40                                 | 2.10                   |
| Sg/Yz T2      | 19.50                 | 9.68                                 | 2.42                   | Sg/Yz T40     | 19.30           | 12.40                                | 3.10                   |
| Sg/Yz T3      | 19.57                 | 19.26                                | 4.82                   | Sg/Yz T41     | 18.90           | 13.40                                | 3.35                   |
| Sg/Yz T4      | 20.50                 | 13.65                                | 3.41                   | Sg/Yz T42     | 19.90           | 8.56                                 | 2.14                   |
| Sg/Yz T5      | 19.10                 | 12.58                                | 3.15                   | Sg/Yz T43     | 19.90           | 11.38                                | 2.85                   |
| Sg/Yz T6      | 19.80                 | 9.45                                 | 2.36                   | Sg/Yz T45     | 19.80           | 16.23                                | 4.06                   |
| Sg/Yz T7      | 20.10                 | 21.33                                | 5.33                   | Sg/Yz T46     | 18.70           | 11.46                                | 2.87                   |
| Sg/Yz T8      | 22.30                 | 16.82                                | 4.21                   | Sg/Yz T47     | 19.60           | 16.25                                | 4.06                   |
| Sg/Yz T10     | 16.90                 | 13.69                                | 3.42                   | Sg/Yz T48     | 19.80           | 18.00                                | 4.50                   |
| Sg/Yz T11     | 14.20                 | 11.21                                | 2.80                   | Sg/Yz T49     | 19.50           | 8.82                                 | 2.21                   |
| Sg/Yz T12     | 18.70                 | 9.44                                 | 2.36                   | Sg/Yz T50     | 20.00           | 7.45                                 | 1.86                   |
| Sg/Yz T13     | 18.50                 | 15.44                                | 3.86                   | Sg/Yz T51     | 19.30           | 8.06                                 | 2.02                   |
| Sg/Yz T14     | 19.30                 | 13.62                                | 3.41                   | Sg/Yz T52     | 20.50           | 8.45                                 | 2.11                   |
| Sg/Yz T15     | 19.70                 | 1.97                                 | 0.49                   | Sg/Yz T53     | 22.70           | 7.84                                 | 1.96                   |
| Sg/Yz T16     | 18.50                 | 12.12                                | 3.03                   | Sg/Yz T54     | 16.60           | 2.13                                 | 0.53                   |
| Sg/Yz T17     | 18.00                 | 1.62                                 | 0.41                   | Sg/Yz T55     | 21.10           | 9.35                                 | 2.34                   |
| Sg/Yz T18     | 19.70                 | 6.53                                 | 1.63                   | Sg/Yz T56     | 18.50           | 6.41                                 | 1.60                   |
| Sg/Yz T19     | 19.60                 | 18.14                                | 4.54                   | Sg/Yz T57     | 20.20           | 8.05                                 | 2.01                   |
| Sg/Yz T20     | 19.00                 | 9.60                                 | 2.40                   | Sg/Yz T58     | 21.60           | 6.41                                 | 1.60                   |
| Sg/Yz T21     | 17.90                 | 11.78                                | 2.95                   | Sg/Yz T59     | 21.20           | 10.35                                | 2.59                   |
| Sg/Yz T22     | 18.90                 | 10.79                                | 2.70                   | Sg/Yz T60     | 13.30           | 0.28                                 | 0.07                   |
| Sg/Yz T23     | 19.70                 | 11.22                                | 2.81                   | Sg/Yz T61     | 26.10           | 6.19                                 | 1.55                   |
| Sg/Yz T24     | 21.10                 | 6.73                                 | 1.68                   | Sg/Yz T62     | 16.90           | 7.47                                 | 1.87                   |
| Sg/Yz T25     | 19.80                 | 12.82                                | 3.21                   | Sg/Yz T63     | 16.60           | 9.44                                 | 2.36                   |
| Sg/Yz T26     | 19.80                 | 4.36                                 | 1.09                   | Sg/Yz T64     | 22.40           | 8.38                                 | 2.10                   |
| Sg/Yz T27     | 19.50                 | 15.52                                | 3.88                   | Sg/Yz T65     | 25.60           | 4.50                                 | 1.13                   |
| Sg/Yz T28     | 20.00                 | 16.17                                | 4.04                   | Sg/Yz T66     | 24.20           | 12.48                                | 3.12                   |
| Sg/Yz T29     | 17.10                 | 8.33                                 | 2.08                   | Sg/Yz T67     | 19.40           | 6.39                                 | 1.60                   |
| Sg/Yz T30     | 18.70                 | 11.23                                | 2.81                   | Mean           | 19.55           | 10.50                                | 2.63                   |
| Sg/Yz T31     | 19.30                 | 12.68                                | 3.17                   | Min            | 13.30           | 0.28                                 | 0.07                   |
| Sg/Yz T32     | 19.20                 | 12.16                                | 3.04                   | Maks           | 26.10           | 21.33                                | 5.33                   |

4. Conclusions
The results showed 104 plant genotypes analyzed by PCR, 64.42% of F2 progenies Sigupai/Yinzhan inherited the fragrant gene. The results of analysis of agronomic character showed 62 genotypes there were 95.08% of ripening aged, 59.01% had a short stem architecture and 6 genotypes (9.83%) had a potential yield of 4.04, -5.33 tons ha⁻¹.
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