Identification of gene action on rice tolerance to low temperature stress

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Abstract. The development of low temperature tolerant rice varieties is needed to increase national rice productivity. Crosses and selection are the stages of the activity of developing low temperature tolerant plants. The effectiveness of selection depends very much on the action of the genes that control the selection character. Epistatic gene action will inhibit the progress of selection on self-pollinating crops such as rice. The purpose of this study was to estimate the gene action of the character of rice growth at low temperature stresses, and predict whether the growth character was controlled by the action of additive genes or by the action of epistasis genes. The research was conducted from July 2017-March 2018 at Brastagi Experimental Farm, Karo District, altitude 1340 meters above sea level with average temperature of 19 °C. The genetic material used was 191 F2 plants from the crossing of Situbagendit x Red Sigambiri and each 10 parents. The results showed that the character of plant height, panicle exsertion, age of flowering, and production had a middle value that varied with heritability values from low to high. The action of duplicate epistasis additive genes occurs in the character of generative phase plant height and flowering age while the character of plant height in germination phase, panicle exsertion and production is governed by the action of complementary epistasis genes. The characters observed have a pattern of distribution that is continuous and controlled by many genes.

1. Introduction

Dry land utilization for rice cultivation is promising. However, up to now, only a small portion of dry land has been used for upland rice cultivation. Total upland rice area in 2014 was around 1,129 million ha. In addition, upland rice productivity is still below wetland rice. Productivity of upland rice in 2013 is 3.3 t/ha on average while wetland rice productivity is 5.3 t/ha [1]. The opportunity to increase upland rice area can be increased by utilizing dry land.

Expansion of rice planting area is began to be directed to highlands, but until now released upland rice varieties cannot adapt well in the highlands [2, 3]. In such dry land, low temperatures are the main factor limiting the development of upland rice [4, 5]. Low temperature stress can inhibit panicle excretion and increase grain emptiness [6]. In the germination phase, low temperatures can delay and reduce germination [7]. In the vegetative phase, low temperatures cause leaf yellowing, shorter plant
posture, and reduced tillering. In the reproductive phase, low temperatures cause high panicle sterility, less panicle excision, and also spikelet abortion.

Technology innovations that adaptive to low temperature stress are needed as efforts to increase upland rice production. Plant breeding approach can be performed to reach high productivity in low temperature areas that is by produce low temperature tolerant rice varieties. The efforts are include using genetic resources for varieties creation. Generally, creation of rice varieties is still use main gene sources of released varieties, local varieties, and introduced varieties that belong to indica group, so that if traced not infrequently comes from the same offspring. The narrower source of rice genetic diversity is not profitable for continuation of new superior varieties.

Cross breeding is expected to produce high recombination and genetic diversity to provide genetic material for selection. Selection success or selection efficiency to increase productivity and tolerance to low temperatures is shown by the acquisition of genetic progress from selected characters. Genetic progression is highly dependent on genetic variability and heritability [8].

Genetic diversity is very important to meet plant breeding objectives such as yield quality and quantity improvement and having wide adaptations [9]. Estimation of genetic diversity can be performed through quantitative (biometric) analysis approach to predict genetic models, number of control genes, and able to unravel genetic diversity into additive, dominant, and epistasis varieties, as well as heritability value in broad sense that compares total genetic variability with phenotypic variability [10]. Additives genetic variety is causes similarities between parents and their derivatives. Phenotypes on additive genes action is the addition of each allele without interaction with other alleles (allelic or non allelic interactions), whereas for epistasis genes action, phenotypes are determined by allele interaction from different loci [8].

Plant tolerance to low temperature stresses is not controlled by cytoplasmic genes, but by chromosomal genes found in the nucleus [11]. Heritability of low temperature stresses tolerance is very high in the early generation, so tolerance to low temperature stresses is controlled by dominant genes with additive effects [12–13] states that in lowland rice stressed by temperature, roles of dominant gene is greater than additive gene for character such as plant height, flag leaf length, harvest age, number of tillers and feather length, while for flowering age character, panicle length and grain weight, the role of additive gene is greater. Other studies have shown that genetically low temperature tolerance characters are complex and controlled by polygenic genes, but genes mechanism in controlling tolerance characters is not clear yet [14].

The study aimed to estimate gene action of upland rice growth characteristics at low temperature stresses and predict whether the growth character was controlled by additive genes action or epistasis genes.

2. Materials and methods
The experiment was carried out from July 2017 to March 2018 at Brastagi Experimental Farm, Tongkoh, Regency of Karo, located at an altitude of 1340 m above sea level and with an average temperature of 19 °C. The genetic material used is 191 plants obtained from F2 population resulting from the crossing of Situbagendit x Red Sigambiri and the two parental, each 10 plants.

Observations were made on plant height at the time of germination and generative phase, length of panicle exertion, flowering age and yield per plant. Data analysis was performed by calculating the average and conducting t-test for each character observed in each population, and followed by estimating genes action that control the characters observed. Determination of gene action and number of trait control genes is carried out based on F2 distribution analysis by looking at skewness and kurtosis value [8].

Variety and heritability components are estimated using the following equation:

\[ \text{Variance} (V) = \frac{\sum_{i=1}^{n} (X_i - \mu)^2}{n-1} \] (1)

Phenotype variance (VP) = VF2
Environment variance (VE) = \( \frac{(VP1 + VP2 + VF1)}{3} \)
Genotype variance (VG) = VF2 - VE
Wide range heritability \( (h^{2bs}) \) = \( \frac{(VG/VP)}{X} \times 100\% \).
Heritability value is categorized high when \( h^{2bs} > 50\% \), moderate when \( h^{2bs} \) is between 20-50% and low when \( h^{2bs} < 20\% \) [15].

3. Results and discussion

General condition during experiment are shown in Figure 1. Place height is 1340 m asl with rainfall of 131-327 mm/month and an average temperature of 18 °C [16]. Rice plants can grow at a temperature range of 25-33 °C [17], altitude <1000 m asl [18], and rainfall more than 200 mm for 3 consecutive months [19]. These environmental conditions are generally not in accordance with the requirements for rice growth. This condition is a condition where rice plants experience low temperature stress.

Figure 1. Temperature and rainfall data in Brastagi 2017-2018.

The finding showed that Red Sigambiri variety had higher plant height both in germination and generative phase, longer panicle exertion, longer flowering age and higher yield compared to Situbagendit variety under low temperature stress. According to Yusuf [2] and Hairmansis et al [20], Red Sigambiri is tolerant upland rice variety to low temperature. This finding is consistent with field observations that Situbagendit is not able to produce under low temperature stress (table 1). This is because Situbagendit is a plant that is sensitive to low temperatures. Low temperature in the range of 15–19°C during the reproductive stage impairs microspore development and causes the production of sterile pollen grains, resulting in poor grain filling and high spikelet sterility and reducing spikelet fertility and affecting grain quality [21].

The danger of low temperature stress is different for each variety. For tolerant variety, temperature 15 °C for 4 days in the initial phase of microspore formation will increasing grain sterility, while for susceptible cultivars, the critical temperature is at 17-19 °C. Low temperature (12 °C) for two days does not cause sterility, but if more than 6 days will causes 100% sterility [21].

Table 1. Average values of parental characters of Situbagendit (P1), Red Sigambiri (P2), and F2 population at low temperature stress.

| Characters                  | Situbagendit | Red Sigambiri | P value  | F2 population |
|-----------------------------|--------------|---------------|----------|---------------|
| Plant height (germination)  | 16.06±0.71   | 22.53±3.01    | 0.043*   | 19.43±0.42    |
| Plant height (generative phase) (cm) | 57.87±1.38   | 125.34±2.92   | 0.000**  | 91.67±1.32    |
| Panicle exertion (cm)       | 0            | 4.31±0.45     | 0.000**  | 0.52±0.07     |
| Flowering age (HST)         | 136.78±3.19  | 147.63±1.44   | 0.010**  | 137.54±1.27   |
| Yield (gr)                  | 0            | 10.99±2.42    | 0.000**  | 0.03±0.01     |

Note: * = Significant at \( \alpha 0.05 \); ** = very significant at \( \alpha 0.01 \) based on the t-test between Situbagendit and Red Sigambiri; \( \pm \) = SE value
Characters such as plant height at germination and generative phase, panicle exertion length, flowering age and yield showed significant differences between Situbagendit sensitive elders and Red Sigambiri tolerant elders, so that these characters can be used as markers for tolerant genotypes.

The observations results in F2 population indicate that F2 population has an average of plant height better than Situbagendit elders, but is still below Red Sigambiri elders. Information concerning differences in population performance and gene action for agronomic characters is very useful especially as a basis to improve genes frequency for the desired character through targeted selection activities both toward negative positive selection and both directions simultaneously. Continuous curves characteristics can be explained by the statistics of mean value, median, range, variance, standard deviation, standard error, skewness, and kurtosis. Such descriptive statistics can be used to estimate the number of genes and gene actions that control these characters in segregated populations. Skewness is a measure of curve stability from the population distribution. Kurtosis is a measure of curve thickness of population distribution. Estimation of genetic control for plant height at germination and generative phase, length of panicle exertion, flowering age and production are presented in figures 2 – 4.

**Figure 2.** Plant high distribution of germination and generative phase for F2 population at low temperatures stress.

**Figure 3.** Panicle exertion and flowering age of F2 population at low temperatures stress.
Figures 2 – 4 show that all the characters observed have a continuous distribution pattern, which means that the agronomic character of rice in low temperatures stress is controlled by many genes. All characters in F2 population as result of Situbagendit and Red Sigambiri crosses in low temperature stress do not have a normal distribution as indicated by skewness value is not equal to zero (sticking left or right). According to Roy [8] and Jayaramachandran et al. [22], distribution of quantitative characters in plants that extend left or right indicates environmental influences, genotype and environmental interactions, gene linkages, and epistasis.

F2 population distribution for flowering age characters (figure 2) shows the data is asymmetric but no extreme data is produced. F2 population produces a mean value among the two parents. Family performance is skewness value (-0.11) and kurtosis (-0.49), means that the genes action is controlled by many genes with additive genes action and there are duplicate epistasis effects.

Table 2. Skewness value, gene action, curtosis value, and graphic of F2 genotypes distribution from crossing between Situbagendit and Red Sigambiri on low temperature stresses.

| Character                  | Skewness | Genes Action                  | Kurtosis | Note                 |
|----------------------------|----------|-------------------------------|----------|----------------------|
| Plant height (germination) | 0.31     | Complementary additive + epistasis | -0.11    | controlled by many genes |
| Plant height (generative phase) (cm) | -0.46 | Duplicate additive + epistasis | 0.10     | controlled by few genes |
| Panicle Exertion (cm)      | 2.41     | Complementary additive + epistasis | 5.13     | controlled by few genes |
| Flowering age (HST)        | -0.11    | Duplicate additive + epistasis | -0.49    | controlled by many genes |
| Yield (gr)                 | 8.04     | Complementary additive + epistasis | 74.3     | controlled by few genes |

Gene’s action that cause variance on plant height, panicle exertion length, flowering age, and yield are additives and epistasis (table 2). According to Roy [8], graphs that not normally distributed is due to the involvement of non-additive genes, large environmental influences, gene linkages, and the presence of major genes in controlling variance in F2 populations. This is confirmed by Jayaramachandran [22] who stated that quantitative character of plants whose distribution patterns extend to the left or right indicates environmental influences, genotype and environmental interactions, gene linkages, or epistasis.
Table 3. Estimation values of variance components and heritability on F2 population at low temperature stresses.

| Character                     | GCV    | σ2p    | σ2e    | σ2g    | h2bs   | criteria |
|-------------------------------|--------|--------|--------|--------|--------|----------|
| Plant height (germination)    | 5.58   | 19.46  | 19.44  | 0.02   | 0.11   | low      |
| Plant height (generative phase) (cm) | 7.55 | 193.74 | 145.79 | 47.94  | 24.75  | moderate |
| Panicle Exertion (cm)         | 22.02  | 1.25   | 1.24   | 0.01   | 1.05   | low      |
| Flowering age (HST)           | 6.34   | 127.35 | 51.28  | 76.07  | 59.73  | high     |
| Yield (gr)                    | 1355   | 0.018  | 11.72  | -11.705 | 0      | low      |

Note: σ2p = Phenotype variance; σ2g = Genotype variance; σ2e = Environment variance; h2bs = Heritability value

Estimation of variance and heritability component values is performed to determine the proportion of variance caused by genetic and environmental factors [8]. The analysis results show that flowering age character has high heritability value (table 3), indicate that these characters are more controlled by genetic factors than environmental factors. Characters that have high heritability values can be considered to be selection characters [23–27]. Estimating heritability is a genetic parameter to choose an effective selection system and obtain genetic progress from a selection [28–29].

Table 4. Genotypic correlation coefficients on F2 population at low temperature stresses.

| Character                     | Plant height (germination) | Plant Height (generative phase) | Panicle Exsertion | Flowering age | Yield |
|-------------------------------|---------------------------|--------------------------------|-------------------|---------------|-------|
| Plant height (germination)    | 1                         | 0.201**                         | 0.056             | -0.162*       | -0.014|
| Plant height (generative phase) | 0.201**                  | 1                               | 0.278**           | -0.159*       | 0.110 |
| Panicle Exertion (cm)         | 0.056                     | 0.278**                         | 1                 | -0.249**      | 0.271**|
| Flowering age (HST)           | -0.162*                   | -0.159*                         | -0.249**          | 1             | 0.177*|
| Yield (gr)                    | -0.014                    | 0.110                           | 0.271**           | -0.177*       | 1     |

**. Correlation is significant at the 0.01 level (2-tailed)
*. Correlation is significant at the 0.05 level (2-tailed)

Inter character genetic correlation analysis shows that for panicle exertion and flowering age which contributed significant and positive correlation with yield.

4. Conclusion

F2 population resulted from Situbagendit and Red Sigambiri crosses in low temperature stress have a mean value between the two parents. Character of plant height at germination and vegetative phase, length of panicle exertion, flowering age and yield are controlled by the action of additive genes, with heritability values range from low to high. The duplicate action of epistasis additive genes occurs in the character of plant height at vegetative phase and flowering age while the character of plant height at germination phase, length of panicle exertion and yield is regulated by the action of complementary epistasis genes. All characters observed have a continuous distribution pattern.

5. References

[1] Kementrian Pertanian (Kementan). 2014. Agriculture statistics 2014 (in Indonesia). Pusat Data dan Sistem Informasi Pertanian. Kementrian Pertanian. Jakarta. 348p.
[2] Yusuf, A. 2014. Characteristics evaluation of gogo rice to support it’s release into North Sumatera highland as high-yielding variety (in Indonesia). Laporan Akhir BPTP Sumatera Utara. Medan.
[3] Yullianida, Hairmansis, A. Supartopo, Suwarno, 2016. Genetic resources to form root population of tolerant gogo rice in shade or highland (in Indonesia). Pros Sem Nas Masy Biodiv Indon 2-2: 175-181. DOI: 10.13057/Psnmbi/M020210.
[4] Shrestha, S., F. Asch, J. Dusserre, A. Ramanantsoainirina, and H. Brueck. 2012. Climate effects on yield components as affected by genotypic responses to variable environmental conditions in upland rice systems at different altitudes. Field Crops Res. 134:216-228.

[5] Sipaseuth, J. Basnayake, S. Fukai, T. Farrell, M. Senthonghae, Sengkeo, S. Phamixay, B. Linquist, and M. Chanphengsay. 2007. Opportunities to increasing dry season rice productivity in low temperature affected areas. Field Crops Res. 102:87-97.

[6] Cruz RP, Milach SCK, Federizzi LC. 2006. Rice cold tolerance at the reproductive stage in a controlled environment. Sci Agric 63: 255-261.

[7] Cruz RP, Sperotto RA, Cargnelutti D et al. 2013. Avoiding damage and achieving cold tolerance in rice plants. Food and energy security 2: 96-119. Doi: 10.1002/fes3.25.

[8] Roy, D. 2000. Plant Breeding. Analysis and Exploitation of Variation. New Delhi: Narosa Publishing House.

[9] Mondal MAA. 2003. Improvement pf potato (Solanum tuberosum L.) through hybridization and in vitro culture technique. PhD [Thesis]. Bangladesh: Rajshahi University, Rajshahi.

[10] Fehr WR. 1987. Principles of Cultivar Development. Vol 2. Mc Millan. New York, [USA].

[11] Shimono, H.T., Hasegawa, K. Iwama. 2001. Quantitative expression of developmental processes as a function of water temperature in rice (Oryza sativa L.) under a cool climate. J. Fac. Agric. Hokkaido Univ. 70:29-40.

[12] Shimono, H., M. Okada, E. Kanda, and I. Arakawa. 2007. Low temperature-induced sterility in rice: evidence for the effects of temperature before panicle initiation. Field Crops Res. 101:221-231.

[13] Limbongan, Y.L. 2008. Genetic analysis and superior genotype selection of rice (Oryza sativa) for adaptation in highland ecosystem (in Indonesia). Disertasi. Sekolah Pasca Sarjana, Institut Pertanian Bogor.

[14] Snape, J.W, Semokhoskii, A, Fish, Sarma, R.N, Quarrice, S.A,Galiba, G, Sutka, J. 1997. Mapping frost tolerance loci in wheat and comparative mapping with other cereals. Acta Agron. Hungar. 45: 265-270.

[15] Mangoendidjojo, W. 2003. Basics of plant breeding (in Indonesia). Penerbit Kanisius,Yogyakarta.

[16] Badan Meteorologi Klimatologi dan Geofisika (BMKG), 2018. Monthly data of temperature and rainfall 2017-2018 in Dolat Rayat sub-district. (in Indonesia). Medan : BMKG Stasiun Klimatologi Klas I Sampali.

[17] FAO. 2005. Global climate changes and rice food security. Rome (available at: http://www.fao.org/climatechange/15526-03ecb62366f779d1ed45287e698a44d2c.pdf).

[18] Acquaah, G. 2007. Principles of plant genetics and breeding. Blackwell Publishing, USA.

[19] Toha, H.M. 2012. Development of gogo rice to resolve food insecurity in marginal land area (in Indonesia). Prospek Pertanian Lahan Kering dalam Mendukung Ketahanan Pangan, hal 143-163. Badan penelitian dan Pengembangan Pertanian. Jakarta.

[20] Hairmansis A, Yullianida, Supartopo, Suwarno. 2016. Breeding of adaptable rice gogo in dry land (in Indonesia). Iptek Tanaman Pangan Vol. 11 No. 2. p95-106.

[21] Satake, T. 1969. Research on cold injury of paddy rice plants in Japan. Jpn Agric Res. 4: 5–10.

[22] Jayaramachandran, M., N. Kumaravadivel, S. Eapen, G. Kandasamy. 2010. Gene action for yield attributing characters in segregating generation (M2) of sorghum (Sorghum bicolor L.). Elec. J. Plant Breeding 1:802- 808.

[23] Rostini, N. Yuliani, E. Hermiati, N. 2006. Heritability, genetic ability, and the correlation of leaf characteristic with young fruit in 21 genotype of pineapple. Zuriat 17 (2): DOI: https://doi.org/10.24198/zuriat.v17i2.6732

[24] Wirnas D, Widodo I, Trikeosoemaningtyas, Sobir, Sopandie D. 2006. Selection of agronomic character to arrange selection index of 11 population in F6 generation of soybean (in Indonesia). Bul. Agronomi 34(1):19-24
[25] Susilaningsih, F. D. Ruswandi, N. Hermiati. 2008. Phenotypic appearance and genetic parameters of 16 cultivated gogo rice in intercropping system with peanut (3:1) in Jatinangor (in Indonesia). Zuriat 19(2). DOI: https://doi.org/10.24198/zuriat.v19i2.6658

[26] Suharsono, M. Yusuf, Paserang, A.P. 2006. Variety analysis, heritability, and estimation of advancement in selection of F2 population from crossing Slamet cultivated soybean with Nokonsawon (in Indonesia). J. Tanaman Tropika 9(2):86-93.

[27] Sihaloho, A.N., Trikoesoemaningtyas, D. Sopandie, D. Wirnas. 2015. Identification of epistasis gen action in aluminium-induced stress tolerance of soybean (in Indonesia). J. Agron. Indonesia 43:30-35.

[28] Erkul, A. A Unay, CKonak. 2010. Inheritance of Yield and Yield Components in a Bread Wheat (Triticum aestivum L.) Cross. Turkish Journal of Field Crops 15(2): 137-140.

[29] Syukur M. S. Sujiprihati, R. Yunianti, K. Nida. 2011. Estimation of variety component, heritability, and correlation to determine selection criteria for F5 population of chili (Capsicum annum L.) (in Indonesia). J. Hort. Indonesia 1 (2) :74-80. DOI: https://doi.org/10.29244/jhi.1.2.74-80