Characterization of the multiple crossing tropical wheat “magic population” using SSR marker associated with physiological characters

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Abstract. Heat stress is a challenge in the development of wheat in tropical environments. Characterization of heat tolerant wheat lines based on physiological characters is still very rarely carried out in tropics, therefore early detection using heat stress linked markers related to physiological characters is very necessary in order to produce adaptive wheat varieties in tropics. The study aims to characterize wheat lines from the "magic population" crossing based on SSR markers related to physiological characters and identified by field observations. The research used 23 genotypes for SSR marker characterization and continued to field experiment using 10 selected genotypes with Guri-5 Agritan and Guri-6 Agritan varieties as check. The experiment was arranged in randomized block design with 3 replications.

The results showed polymorphism on Xbarc84 and Xgwm285 markers at the genotypes number 203, 145 and 184. Xbarc84 markers could show a relation to flowering time on genotype 203 with longer flowering time and harvest time. Xgwm285 marker show relation to HTI at genotypes no.145 with no band was formed, and the genotypes show weakness on growing in the field and did not show any relation on genotypes no.184. Correlation analysis between characters showed that SRF, SL and NSp characters could be used as secondary characters for heat tolerance wheat selection.

1. Introduction
One of the main objectives of wheat breeding is to determine specific genome areas that control the character of plant growth both agronomic and physiology [1]. Many studies use specific populations to analyze and find QTL that is responsible for the expression of component yield genes. The QTL detection approach with association analysis shows higher yield resolution than QTL mapping [2]. Detecting stable QTLs and character associated with markers can accelerate the selection process in order to produce high yielding varieties [3].

High temperature stress is a challenge in developing wheat in tropical environments such as Indonesia and influencing the stages of plant development [4]. High humidity is a major trigger for increasing temperatures in tropical environments. Decreased yield of wheat is due to gene expression for yield characters and yield components are inhibited by inappropriate temperatures. Heat stress that exceeds the tolerance threshold is a cause of decreased crop production [5]. The effect of heat stress on wheat influences the metabolic at each stage of the life cycle of wheat plants which causes a decrease in yield [6], Therefore early detection of the yield character using high temperature link to markers is necessary in order to produce adaptive varieties in tropical environments [7].

SSR markers are widely used in detecting heat tolerance in wheat. Characteristics related to heat temperature are yield character (plot yield, 1000 seed weight, seed filling time and effective number of tillers per plant), morphological characters (early ground cover, stay green, Epicuticular wax, leaf
rolling and biomass), physiological characters (canopy temperature, photosynthesis level, chlorophyll content, stomata conductance, stem reserve and membrane thermo stability) [8]. For this reason, the use of SSR markers regarding these characters will be very helpful in screening wheat plants that are adaptive to the tropical environment. The results of previous studies show that the SSR XGWM, XBARC and XGDM markers are mostly constructed in order to detect quantitative temperature tolerance traits in wheat [9]. Variation of alleles from these markers can be used as a reference to find out how the interaction of these alleles on wheat agronomic growth. The results of the markers analysis can provide information regarding population diversity with allelic variations in population to make it more efficient in germplasm collection. These markers can also be used to find specific genes that are tolerant of heat in tropical environments. Molecular characterization will also help in determining the pattern of inheritance of a species, gene flow inherited within or between populations of the same or related species.

The use of SSR markers related to physiological characteristics such as flowering time, anthesis time, temperature tolerance index and thermo stability membrane are very helpful in detecting high temperature tolerance traits in wheat. Most of these characters are striking and affect the filling of seeds in wheat and not much is done on tropical wheat in Indonesia. The importance of this research is to characterize and to know the effectiveness of the use of SSR markers related to the nature of flowering physiology and temperature resistance which are closely related to the characters expressed in the field.

2. Materials and Methods

The research was carried out in the greenhouse of the Indonesia Cereals Research Institute (ICeRi), Maros, South Sulawesi and the bio-molecular laboratory of the Forestry Faculty, Hasanuddin University (UNHAS), Makassar, South Sulawesi on February until August 2018. The genotype tested consisted of 19 wheat genotypes results of crossing magic population and 2 varieties as check (Guri 5 and Guri 6). Molecular characterization was carried out through DNA isolation of 21 wheat genotypes taken from fresh leaves 10 DAP in greenhouse. DNA isolation followed procedure developed by George et al [10]. DNA quality was calculated by agarose gel electrophoresis with comparator markers, whereas DNA quantity was measured using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific, USA). Amplification using 10 SSR primers related to physiological characters (Table 1), mixed ingredient for band amplification using Hotstar PCR mix. PCR reactions were carried out in a 15µl volume containing 1 × PCR buffer, dNTPs (dATP, dCTP, dGTP, and dTTP), primers 0.5 µl (F and R), 50 ng DNA 1 µl. The PCR program used in this study was 3 minutes at 94ºC for initial denaturation, then 35 cycles consisted of 30 seconds at 94ºC for denaturation, 30 seconds at annealing temperature, 120 seconds for primer extension, 120 seconds for final extension. DNA staining was done using Gelred.

Field research was carried out in Muneng, East Java at altitude of 50 m asl. The research took place from May to September 2018. The research was carried out in 3 replications using randomized block design (RBD), plot size 1.5 m x 5 m. The genetic material used was 28 lines of the "Magic Population" crossing and 2 varieties as check (Guri-5 Agritan and Guri - 6 Agritan). Planting is done on each genotype/plot and planted 6 rows along 5 m with spacing between rows about 25 cm.

Plant maintenance includes irrigation, cleaning of weeds, fertilizing, controlling pests and diseases. The first fertilization is done at plant 10-15 DAP with a dose of 150 Urea kg ha⁻¹, 200 SP36 kg ha⁻¹ and 100 KCl kg ha⁻¹, the second fertilization is carried out at 30-35 DAP with a dose of Urea 150 kg ha⁻¹. Before planting the seeds were given 85% of carbaryl insecticide treatment and at the time of planting the hole was given carbofuran with a dose of 17 kg ha⁻¹.

Observation variables include flowering time (FT) (days), harvesting time (HT) (days), plant height (PH) (cm), number of spike / meters (NSM), spike length (PL) (cm), number of seeds / spike (NSS), seed weight (g) / spike (SWS), number of spikelet (NSp),Seed filling rate (SFR) number of florets (NF), number of hollow florets (NHF), water content (WC), weight of 1000 seeds (W1000S) (g) , and yield (Y) (t / ha). Data were analyzed by using Variance F test. The average characters those showed the real / very real effect on the F test followed by the smallest significant difference test (LSD) at 5% level compared to the check varieties. All data were analyzed using the STAR.
Table 1. Primer, Chromosome, sequence, annealing temperature, motif, base size and characteristics

| No | Primer      | Chromosome | Sequence                                                                 | Annealing Temperature | Contain motif | SSR Size (bp) | Character          |
|----|-------------|------------|-------------------------------------------------------------------------|------------------------|---------------|---------------|--------------------|
| 1  | Xbarc 13    | 2B         | 5' GCA GGA ACA ACC ACG CCA TCT TAC 3', 5' GCG TCG CAA TTT GAA GAA AAT CAT C 3' | 52                     | (TTC)5+3+2    | 142           | flowering time     |
| 2  | Xbarc84     | 3B         | 5' GCG ATA ACC GTT GGG AAG ACA TCT G 3', 5' GGT GCA ACT AGA ACG TAC TTC CAG TC3' | 58                     | (ATG)8        | 110           | flowering time     |
| 3  | Xbarc217    | 4D         | 5' GCG TTG TGT TGA AGG CTG AGC ATC CCA 3', 5' GCG GAG TAG CTA AAC GGC GGT GGA GGA AAC 3' | 52                     | (CT)12        | 95            | flowering time     |
| 4  | Xbarc197    | 3A         | 5' CGC ATG GTC AGT TTT CTT TTA AGC CT 3', 5' GCG CTC TCC TTC ATT TAT GGT TTG TTG 3' | 50 (45 s)              | (TAA)15       | 176           | Heat tolerance index|
| 5  | Xgwm285     | 3B         | 5' ATG ACC CTT CTG CCA AAC AC 3', 5' ATC GAC CGG GAT CTA GCC 3' | 60 (1 m)               | (GA)27        | 227 (Altar)   | Heat tolerance index|
| 6  | Xbarc1044   | 3A         | 5' GCG TAT GTA TGT CTA TTT TCC TAT C 3', 5' CCC AAT TTT GCT AAG TGC TCT ACT 3' | 55                     | (TATC)9       | 130           | days to heading    |
| 7  | Xgwm666.2   | 3A         | 5' GCA CCC ACA TCT TCG ACC 3', 5' TGC TGC TGG TCT CGC 3' | 60 (1 m)               | (CA)13        | 114           | days to heading    |
| 8  | Xgwm635     | 7A         | 5' TTC CTC ACT GTA AGG GCG TT 3', 5' CAG CCT TAG CCT TGG CG 3' | 60 (1 m)               | (CA)10 (GA)14 | 109 and 99 (Opata M85) | days to heading |
|    |             |            |                                                                         |                        |               | 93 (Altar 84/Ae) |                    |
| 9  | Xgdm125     | 4D         | 5' GCA GGC GTG TTA CTC CAA GT 3', 5' CCG AGG TGG ATA GGA GGA AA 3' | 60                     | (CA)29        | 222.24        | days to anthesis   |
| 10 | Xgwm156     | 3B         | 5' CCA ACC GTG CTA TTA GTC ATT C 3', 5' CAA TGC AGG CCC TCC TAA C 3' | 60 (1 m)               | (GT)14        | 300 (Opata M85) | membrane thermostabi lity |
|    |             |            |                                                                         |                        |               | 279 (Altar 84/Ae) |                    |
3. Results and discussion
The analysis results used 10 SSR markers linked to physiological characters, only on Xbarc84 and Xgwm285 primers showed polymorphism, whereas the others markers did not show any polymorphism. The Xbarc84 primer on genotypes No. 203 showed the polymorphism of the band with the addition of a smaller sized band (<100 bp), while the primer Xgwm285 showed the polymorphism at genotypes no. 145 band is was not formed and genotypes no. 184 band formed with a larger size (300 bp).

The polymorphism of the Xbarc84 primer at genotypes No. 203 indicated the sequence of the Genotypes genome with the same SSR motif but having a smaller base size (<100 bp). These different base sizes can be identified to find more diversity in wheat populations and confirmed by field observations. Field observations on genotypes No. 203 showed the flowering time longer than the others. Xbarc84 is a primer related to the character of flowering time. The results of wheat research in tropical environments showed that heat stress followed by high humidity accelerates the flowering time in wheat [11]. Flowering character is strongly influenced by heat stress followed by long drought [12]. Stress flowering in wheat plants in a tropical environment is very important because it can affect the time of seed filling. Finding genes responsible for flowering time in tropical environments can help in assembling more adaptive wheat in tropical environments [13]. Knowing the location of genes by using mapped markers can speed up isolation of genes and study their functions and transform them.

Polymorphism in primary Xgwm285 with base size 222-227 bp which is related to the character of Heat tolerance index (HTI) is not formed band on genotypes no.145, this indicated that there was no locus on the genotypes due to mutations in the site where the primer will be annealed, it caused the locus cannot be
read and cloned by the primer. On genotypes no. 184 band polymorphisms formed with a larger base size (300 bp), this result has similarity with OHP12a1-1-9, but they could not explain it in detail [14]. This indicated the insertion or addition of base sequences of ± 100 bp at the Xgwm285 locus in the Genotypes genome no.184. For the case in plant no.145, the plant cannot grow well and die before reaching the flowering stage, this may be due to the inability of the plant to withstand the heat stress that occurs in the greenhouse, whereas in the case of plant no. 184 shows no difference in growth with other plants. This shows that the absence of bands formed on the xgwm285 marker is an indication that the plant is sensitive to high temperatures or has low index tolerance to heat (HTI). The polymorphism of the two genotypes will be confirmed based on characters linked to HTI. There are a lot of characters can interpret HTI in wheat [15]. Analyzing HTI requires observations in two environments (stress and without stress), but in this study we did not experience the plant in these two conditions, only at stress condition.

We used association analysis to find the relation between the marker and the field test. Association analysis between marker analysis and field observations are necessary in order to find which markers has relation to the desired character, but the analysis of character associations with markers requires a comprehensive planting in several locations and several seasons to see the consistency of the band with the character of observation, this activity is difficult if the population is too large and if genetic material is eliminated in the next selection process. The use of multi-locations results data on genotypes that carry allelic diversity can be a solution for tracking alleles that are stable and useful for heat tolerance wheat in tropical environments.

The field studies result to the production of 23 lines of advanced wheat F6 "Magic Population" generation, only 12 lines can be observed to get seed production. Based on the results of variance analysis shows that the characters of flowering time (FT) (days), harvesting time (HT) (days), plant height (PH) (cm), number of spike / meters (NSM), spike length (PL) (cm), number of seeds / spike (NSS), seed weight (g) / spike (SWS), number of spikelet (NSp), Seed filling rate (SFR), weight of 1000 seeds (W1000S) (g), and yield (Y) (t / ha), different significantly compared to checks (Tables 2 and 3).

### Table 2. Agronomic Characters of 12 Tropical Wheat lines on the Lowlands, in Muneng, East Java in 2018

| Genotypes | FT  | HT  | SRF | PH   | W1000S | NSpM |
|-----------|-----|-----|-----|------|--------|------|
|           | - days - | - days - | - days - | - cm - | - g -  |
| CBF6-119  | 51  | 77  | 26.00 | 46.31 | 31.12  | 162.00 |
| CBF6-192  | 55  | 83  | 28.00 | 46.53 | 33.19  | 123.50 |
| CBF6-184  | 55  | 82  | 27.00 | 45.25 | 32.88  | 173.00 |
| CBF6-159  | 55  | 82  | 27.33 | 46.33 | 32.85  | 170.00 |
| CBF6-203  | 75  | 105 | 30.00 | 49.57 | 13.64  | 154.25 |
| CBF6-154  | 54  | 81  | 27.00 | 45.66 | 32.39  | 156.50 |
| CBF6-147  | 53  | 77  | 24.00 | 45.11 | 31.92  | 175.00 |
| CBF6-171  | 55  | 82  | 27.00 | 47.49 | 33.13  | 93.50  |
| CBF6-115  | 52  | 79  | 26.67 | 47.35 | 31.50  | 200.00 |
| CBF6-158  | 55  | 82  | 27.00 | 47.12 | 32.27  | 143.50 |
| GURI-5    | 50  | 75  | 24.67 | 50.83 | 30.37  | 128.00 |
| GURI-6    | 48  | 71  | 23.33 | 58.65 | 28.81  | 274.00 |
| **Average** | 54.72 | 81.22 | 26.50 | 48.18 | 30.34  | 162.77 |

| Genotypes | FT  | HT  | SRF | PH   | W1000S | NSpM |
|-----------|-----|-----|-----|------|--------|------|
| **CV**    | 3.96 | 3.88 | 5.31 | 4.88 | 5.32  | 10.25 |
| **LSD**<sub>0.05</sub> | 3.66 | 5.33 | 2.38 | 3.98 | 2.73  | 28.25 |

**Notes:** FT : Flowering time, HT : harvesting time, PH : Plant height, W1000S : Weight of 1000 seeds, NSM : Number of spike / meters
Table 3. Character of yield component of 12 Tropical Wheat lines on the Lowlands, in Muneng, East Java in 2018

| Genotypes  | WSM g  | SL cm | NSM g  | WSS g  | NSp  | Y t/ha |
|------------|--------|-------|--------|--------|------|--------|
| CBF6-119   | 131.83 | 7.30  | 26.35  | 0.81   | 15.75| 1.40   |
| CBF6-192   | 114.10 | 7.43  | 27.40  | 0.92   | 16.20| 1.11   |
| CBF6-184   | 131.62 | 7.55  | 23.90  | 0.77   | 15.85| 1.44   |
| CBF6-159   | 143.78 | 7.31  | 24.60  | 0.84   | 15.73| 1.20   |
| CBF6-203   | 20.80  | 9.43  | 16.58  | 0.14   | 18.48| 0.06   |
| CBF6-154   | 123.15 | 7.16  | 24.00  | 0.79   | 15.20| 1.38   |
| CBF6-147   | 154.18 | 7.19  | 27.05  | 0.88   | 16.40| 1.68   |
| CBF6-171   | 84.07  | 7.70  | 27.38  | 0.91   | 17.45| 0.80   |
| CBF6-115   | 163.48 | 7.49  | 25.55  | 0.83   | 15.40| 1.38   |
| CBF6-158   | 127.94 | 8.03  | 27.10  | 0.89   | 16.65| 0.93   |
| GURI-5     | 111.89 | 8.28  | 29.05  | 0.88   | 16.85| 1.13   |
| GURI-6     | 201.06 | 8.11  | 25.85  | 0.73   | 15.05| 1.04   |
| Average    | 125.66 | 7.75  | 25.40  | 0.78   | 16.25| 1.13   |
| Genotypes  | **     | **    | **     | **     | **   | **     |
| CV         | 10.34  | 3.21  | 5.82   | 6.90   | 4.38 | 16.66  |
| LSD0.05    | 22.01  | 0.42  | 2.50   | 0.09   | 1.20 | 0.31   |

Notes : WSM : Weight of seed/meter, SL : Spike length, NSM : Number of seed/meter, WSM: Weight of seed/spike, NSp : Number of spikelet, Y : Yield

Correlation analysis can be used to select the secondary character for heat tolerance wheat selection. The results of correlation analysis between agronomic characters in Muneng, East Java showed that the flowering characters were highly correlated and were positively related to FT, SRF, SL and NSp characters and were highly correlated and negatively correlated to the characters of W1000S, WSS, NSSp, WSM and Y, while for the characters harvest time has a positive and significant correlation only on SRF, SL and NSp characters (Table 4). These three characters greatly affect the yield of wheat which will affect the character of seed filling rate (SFR). The importance of knowing the magnitude of the correlation value is to find out how much the influence of the character has and whether it correlates positively or negatively, which will influence the secondary character selection to determine the important character of heat tolerant wheat.
Table 4. Correlation of agronomic characters of Tropical Wheat on the Lowlands, in Muneng, East Java 2018

|       | HT   | SFR  | PH   | W1000S | NSpM | WSM  | SL   | NSM  | WSS  | NSp  | Y   |
|-------|------|------|------|--------|------|------|------|------|------|------|-----|
| coef  | 0.98 | 0.70 | -0.11| -0.73  | -0.26| -0.79| 0.63 | -0.76| -0.79| 0.62 | -0.68|
| p-value| 0.00*| 0.00*| 0.54  | 0.00*  | 0.13 | 0.00*| 0.00*| 0.00*| 0.00*| 0.00*|
| SFR   | coef | 0.82 | -0.15| -0.67  | -0.32| -0.80| 0.57 | -0.72| -0.74| 0.61 | -0.67|
| p-value| 0.00*| 0.39 | 0.00* | 0.05*  | 0.00*| 0.00*| 0.00*| 0.00*| 0.00*| 0.00*|
| PH    | coef | -0.24| -0.30 | -0.46 | -0.65| 0.27 | -0.44| -0.38| 0.43 | 0.51 |
| p-value| 0.15 | 0.07 | 0.01* | 0.00*  | 0.12 | 0.01*| 0.02*| 0.01*| 0.00*|
| W1000S| coef | -0.09| 0.60  | -0.78 | 0.76 | 0.93 | -0.52| 0.74 |
| p-value| 0.60 | 0.00*| 0.00* | 0.00*  | 0.00*| 0.00*| 0.00*| 0.00*|
| NSpM  | coef | 0.70 | -0.01 | -0.20 | -0.18| -0.59| 0.26 |
| p-value| 0.00*| 0.97 | 0.24  | 0.29   | 0.00*| 0.13 |
| WSM   | coef | -0.56| 0.49  | 0.58   | -0.78| 0.73 |
| p-value| 0.00*| 0.00*| 0.00*  | 0.00*  | 0.00*|
| SL    | coef | -0.46| -0.71 | 0.68   | 0.79 |
| p-value| 0.01*| 0.00*| 0.00*  | 0.00*  | 0.00*|
| NSM   | coef | 0.92 | -0.16 | 0.52   |
| p-value| 0.00*| 0.35 | 0.00*  |
| WSS   | coef | -0.08| 0.80  |
| p-value| 0.02*| 0.00*|
| NSp   | coef | -0.69|       |
| p-value| 0.00*|       |

4. Conclusion
The results showed polymorphism on Xbarc84 and Xgwm285 markers at the genotypes number 203, 145 and 184. Xbarc84 markers could show a relation to flowering time on genotype 203 with longer flowering time and harvest time around 25 and 27 days longer than check. Xgwm285 marker show relation to HTI at genotypes no.145 with no band was formed, and the genotypes show weakness on growing in the field and did not show any relation on genotypes no.184. Correlation analysis between characters showed that SRF, SL and NSp characters could be used as secondary characters for heat tolerance wheat selection.

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