



AGRONOMIC AND DROUGHT TOLERANCE EVALUATION OF DOUBLED HAPLOID RICE BREEDING LINES DERIVED FROM ANTHR CULTURE

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SUMMARY

Developing drought tolerant rainfed rice can be an alternative way to increase yield potential to achieve food security. This study aimed at selecting rainfed rice genotypes which were both high yielding and drought tolerant. Two experiments were conducted in Bogor, Indonesia in the wet season of 2016 and 2017. Two check varieties namely Ciherang and Inpari 18 and twenty eight doubled haploid lines were used for evaluating agronomic characters. The doubled haploid lines and two check varieties namely Salumpikit (drought tolerant check) and IR 20 (drought sensitive check) were used for screening at seedling stage. Both experiments were arranged using a randomized complete block design with three replications. The result revealed that there were four lines namely CG-8-18-1-2 (6.2 t/ha), CG-7-72-1-1 (4.9 t/ha), CG-8-18-1-1 (5.5 t/ha), and CG-7-72-1-6 (4.7 t/ha) had higher yield than Ciherang (3.6 t/ha) and Inpari 18 (3.3 t/ha) under optimum field conditions and were tolerant to drought. These lines showed good agronomic characters: medium plant height, high number of total tillers, medium day to harvest, and high yield. These genotypes need to be further evaluated under rainfed lowland with drought stress conditions.

Key words: Anther culture, doubled haploids, drought tolerance, rainfed rice

Key findings: These experiments were about the early evaluation to obtain advanced rainfed lowland rice lines. Doubled haploid lines were obtained from anther culture of F1 plants (crossing between upland rice variety Inpago 8 and lowland rice lines high in yield and drought tolerant). The lines showed variability in agronomic characters and drought tolerance. From these studies, we could select lines with good agronomic characters as well as drought tolerance.

INTRODUCTION

Rice is one of the staple foods consumed by more than 3 billion people in the world and is comprising of 50% to 80% of daily calorie intake (Khush, 2005). The world population is estimated to grow to 8 billion by 2030, so there must be an increase in rice production (Villa *et al.*, 2012). Climate change becomes a major problem in agriculture because it severely influences the water resources. Water deficit stress causes extensive loss to agricultural production. Drought affects approximately 23 million hectares of rainfed rice in the world (Serraj *et al.*, 2011). The problem in rainfed rice cultivation i.e. has short period of rain, low-intensity rainfall, unequal distribution of rainfall throughout the season, and variability of land biophysical-chemical condition (Serraj *et al.*, 2009). Drought is the main factor in determining productivity of rainfed rice (Fukai *et al.*, 2009).

Rainfed rice varieties that are highly adapted to dry environments and have high productivity can become solution to areas with limited water supply (Foley *et al.*, 2011). Rainfed rice varieties can be developed by using conventional or modern breeding techniques. In conventional breeding, it takes around eight to ten years from parental crossing, obtaining pure lines, and trials to variety release. On another hand, rice anther culture technique may take only one generation to produce homozygous lines, so it can increase efficiency of the selection

process as well as saving costs, time, and labor (Dewi and Purwoko, 2011, Dewi and Purwoko, 2012). Rainfed rice lines with high yielding traits and drought tolerance can be obtained by crossing a donor parent or variety with lines having high yield potential and drought tolerance (Atlin *et al.*, 2006; Bernier *et al.*, 2008; Babu, 2010). Gunarsih (2015) obtained rainfed rice lines by using anther culture from such crossing combinations. Anther culture may be performed at the first (F1) and second (F2) generation populations to obtain doubled haploid pure lines with a wide genetic diversity (Dewi and Purwoko, 2001).

One of the limitations for slow progress in breeding for drought tolerance is the lack of a standard screening method for large number of genotypes (Kamoshita *et al.*, 2008). This is mainly because of incomplete understanding of the mechanisms of drought resistance. It is important to start the drought comparative phenotypic screening of breeding material at a very early step of tested lines along with both sensitive and tolerant lines, which would allow a precise monitoring of the applied drought stress level and competitive advantage of the test material versus the promising breeding lines. Screening at a very early step will accelerate the development of tolerant lines (Boopathi *et al.*, 2013). Screening can be done at the germination stage with the help of PEG (polyethylene glycol) (Widyastuti *et al.*, 2016). Screening in seedling stage can also be performed. It takes less time, requires less area, more

replications, and unaffected by maturity period (Verulkar and Verma, 2014). Doubled haploid lines obtained from anther culture (Gunarsih 2015; Purwoko unpublished) were evaluated for agronomic character under optimum conditions in this paper. Then, screening at seedling stage was performed so the information of tolerant lines will be obtained. The objective of this study was to obtain genotypes with good agronomic characters, high yielding, and tolerant to drought.

MATERIALS AND METHODS

Plant material

Twenty eight doubled haploid lines obtained from anther culture were evaluated. The material used were F1 plants from three crosses namely upland rice variety with good agronomic character and drought tolerant (Inpago 8) and rainfed rice elite lines (candidate for new rainfed variety having good agronomic character and drought tolerance i.e. B12825E-TB-1-25, IR8770514-11-B-SKI-12, IR83140-B-11-B). The combination of the three cross breeding populations (F1s), were: 1) Code CG-7 = Inpago 8 x B12825E-TB-1-25, 2) Code CG-8 = Inpago 8 x IR8770514-11-B-SKI-12, and 3) Code CG-9 = Inpago 8 x IR83140-B-11-B (Gunarsih 2015; Purwoko, unpublished).

Twenty eight doubled haploid lines used in this study were first generation (DH 1). The list of genotypes are reported in Table 1. They were evaluated agronomically under optimum condition. Two check varieties were used (i.e. Ciherang, a mega variety of irrigated rice widely

known in Indonesia and Inpari 18, a rainfed rice variety with good agronomic characters and drought tolerance). The same doubled haploid lines were screened for drought tolerance at seedling stage. Two check varieties were used namely Salumpikit as drought tolerance check and IR20 as drought sensitive check.

Agronomic evaluation of characters

The experiment was conducted at Sawah Baru Experiment Station, Bogor, Indonesia from September 2016 to February 2017 under optimum conditions. The experiment was done in a complete randomized block design with three replications. The single factor used was the twenty eight doubled haploid lines and two check varieties. Eighteen-day old seedlings were transplanted in the first week of September 2016. Each experimental unit was a 1.5 m² plot. Three rice seedlings per hill were transplanted manually in paddy field with plant spacing of 25 cm x 25 cm. In each plot, plants were maintained with standard agronomic practices. Plots were fertilized with the doses of 90, 36, and 60 kg ha⁻¹ N, P₂O₅ and K₂O, respectively. Nitrogen was applied in split three times, i.e. 1/3 each at basal, maximum tillering, and panicle initiation stage, while the P₂O₅ and K₂O were applied as a basal application. The observation variables were plant height, total tillers per plant, days to harvest, panicle length, filled grain per panicle, unfilled grain per panicle, and grain yield per plot.

Screening at seedling stage in drought conditions

The experiment was conducted from September 2016 to December 2016 in Muara Experiment Station, Bogor, Indonesia. The experiment was arranged in complete randomized block design with three replications. The single factor used was the twenty eight doubled haploid lines and two check varieties. One seed was planted per hill with plant spacing of 10 cm x 10 cm. Watering was done until 14 days after planting and was stopped for imposition of drought stress. Scoring was conducted when IR 20 check exhibited mortality. Scoring was based on standard evaluation system (SES) IRRI (2013): (0) No symptoms, (1) Slight tip drying, (3) Tip drying extended up to ¼ length in most leaves, (5) One-fourth to 1/2 of all leaves dried, (7) More than 2/3 of all leaves fully dried, (9) All plants apparently dead. Soil sample was taken to measure soil water content. After scoring, the plants were irrigated again for ten days to measure recovery ability. The scoring was based on Standard Evaluation System (SES) IRRI (2013): (1) 90-100%, (3) 70-89%, (5) 40-69%, (7) 20-39%, (9) 0-19%. The result of scoring can determine drought tolerance ability in seedling stage.

Data analysis

Data were subjected to analysis of variance following randomized complete block design format using Statistical Tool for Agricultural Research (STAR) 2.0.1 from IRRI. The differences between treatment were tested by least significance different (LSD). The mean data of different traits were subjected to analysis of

Pearson correlation to look at the correlation among characters. Principal component analysis (PCA) was done to identify character(s) which contribute greatly to diversity (Galmoghani *et al.*, 2011). From correlation analysis and PCA, we determine important character(s) for selection index (Falconer and Mackay, 1996).

RESULTS AND DISCUSSION

Evaluation of Agronomic Characters

Analysis of variance showed that there were significant differences among lines for day to harvest, plant height, total tillers per plant, panicle length, filled grains, unfilled grains, and grain yield (Table 1). Information about agronomy characters in optimum condition is very important since it can be used as a reference to select the best lines. The selected lines are expected to inherit the good performance of their superior parent and suitable for rainfed lowland rice.

Genotypes with intermediate height were CG-8-9-1-2, CG-8-9-1-4, CG-8-93-1-1, CG-9-26-1-1, CG-9-26-1-3, and CG-9-26-1-4. Intermediate height was categorized as a good agronomic character for rainfed rice. Plants with intermediate height tend to have resistance to lodging and have efficiency in partition between grain and straw so that harvest index will be high (Peng *et al.*, 1994). Genotypes with the highest number of total tillers were CG-9-5-1-1, CG-8-115-1-1, and Ciherang (Table 1). Number of total tillers will be categorized well if they have tillers in the range 20-25 tillers (IRRI, 2013). Genotypes with good

Table 1. Means of plant height, number of total tiller per plant, day to harvest, panicle length, number of filled grains, number of unfilled grains, and grain yield.

| No. | Genotype | PH (cm) | TT | DH (days) | PL (cm) | FG | UG | GY (ton/ha) ¹ |
|-----|--------------|---------|------|-----------|---------|-------|-------|--------------------------|
| 1 | CG-7-72-1-1 | 130.8 | 16.8 | 111.3 | 30.3 | 156.0 | 61.7 | 4.9 |
| 2 | CG-7-72-1-2 | 133.8 | 15.3 | 118.0 | 30.2 | 127.1 | 102.9 | 5.3 |
| 3 | CG-7-72-1-3 | 140.1 | 15.8 | 114.3 | 29.1 | 133.2 | 74.1 | 5.0 |
| 4 | CG-7-72-1-4 | 137.2 | 15.6 | 118.0 | 30.2 | 123.4 | 88.6 | 5.1 |
| 5 | CG-7-72-1-5 | 133.1 | 14.8 | 115.0 | 31.2 | 145.0 | 45.4 | 4.8 |
| 6 | CG-7-72-1-6 | 141.9 | 14.1 | 114.3 | 29.4 | 157.3 | 94.0 | 4.7 |
| 7 | CG-7-72-1-7 | 141.5 | 13.0 | 118.0 | 29.1 | 127.3 | 51.1 | 5.1 |
| 8 | CG-8-9-1-2 | 110.4 | 19.9 | 111.7 | 29.0 | 131.1 | 62.6 | 4.4 |
| 9 | CG-8-9-1-3 | 107.8 | 15.4 | 108.7 | 27.9 | 96.7 | 99.7 | 3.8 |
| 10 | CG-8-9-1-4 | 112.7 | 14.3 | 111.7 | 27.1 | 120.9 | 71.8 | 4.3 |
| 11 | CG-8-9-1-5 | 107.8 | 19.2 | 108.0 | 28.0 | 104.4 | 92.8 | 4.5 |
| 12 | CG-8-18-1-1 | 133.6 | 16.0 | 115.0 | 26.2 | 107.0 | 36.1 | 5.5 |
| 13 | CG-8-18-1-2 | 132.0 | 16.2 | 109.3 | 25.9 | 114.4 | 23.1 | 6.2 |
| 14 | CG-8-35-1-2 | 103.1 | 19.0 | 108.3 | 27.4 | 99.5 | 58.9 | 3.7 |
| 15 | CG-8-92-1-1 | 99.4 | 18.7 | 108.3 | 25.8 | 81.4 | 57.4 | 4.2 |
| 16 | CG-8-92-1-2 | 95.0 | 18.8 | 109.0 | 23.4 | 80.1 | 41.1 | 3.4 |
| 17 | CG-8-93-1-1 | 124.9 | 14.3 | 111.3 | 25.8 | 134.3 | 42.2 | 5.2 |
| 18 | CG-8-97-1-1 | 135.8 | 14.8 | 118.0 | 28.8 | 118.2 | 73.3 | 4.4 |
| 19 | CG-8-97-1-2 | 134.6 | 14.8 | 118.0 | 29.0 | 113.6 | 94.7 | 4.0 |
| 20 | CG-8-115-1-1 | 98.2 | 21.4 | 119.0 | 26.4 | 102.0 | 96.7 | 2.9 |
| 21 | CG-9-2-1-5 | 102.4 | 15.3 | 108.7 | 24.4 | 91.2 | 87.0 | 4.5 |
| 22 | CG-9-2-1-6 | 104.8 | 16.6 | 109.7 | 23.7 | 98.7 | 69.3 | 4.4 |
| 23 | CG-9-2-1-7 | 89.4 | 19.1 | 108.0 | 23.0 | 100.9 | 56.7 | 2.9 |
| 24 | CG-9-5-1-1 | 100.1 | 22.2 | 108.3 | 24.6 | 104.4 | 66.8 | 3.5 |
| 25 | CG-9-26-1-1 | 125.4 | 15.6 | 109.7 | 25.5 | 133.0 | 32.7 | 4.9 |
| 26 | CG-9-26-1-2 | 132.2 | 19.7 | 111.3 | 26.8 | 155.9 | 48.1 | 5.5 |
| 27 | CG-9-26-1-3 | 124.8 | 15.3 | 111.7 | 25.2 | 141.7 | 38.9 | 4.6 |
| 28 | CG-9-26-1-4 | 119.2 | 17.7 | 111.7 | 25.0 | 120.8 | 43.8 | 4.5 |
| 29 | Ciherang | 106.8 | 21.0 | 109.7 | 24.5 | 89.8 | 43.5 | 3.7 |
| 30 | Inpari 18 | 90.8 | 14.6 | 109.7 | 21.7 | 56.4 | 62.1 | 3.4 |
| | Mean | 118.3 | 16.8 | 112.1 | 26.8 | 115.5 | 63.9 | 4.4 |
| | F test | ** | ** | ** | ** | ** | ** | ** |
| | CV (%) | 3.6 | 15.4 | 2.5 | 4.2 | 9.7 | 16.9 | 18.3 |
| | LSD (5%) | 13.6 | 8.3 | 9.0 | 3.6 | 75.2 | 67.9 | 2.0 |

PH: plant height, TT: number of total tiller per plant, DH: day to harvest, PL: panicle length, FG: number of filled grains, UG: number of unfilled grains, and GY: grain yield, ** significant at $P < 0.01$, * significant at $P < 0.05$

total tillers per plant consisted of 7 genotypes, including Ciherang.

Drought stress induces reduction in plant growth and development of rice (Manikavelu *et al.*, 2006). Due to the reduction in turgor pressure under stress, cell growth is severely impaired (Taiz and Zeiger, 2006). Drought affects both elongation as well as expansion growth (Shao *et al.*, 2008), and inhibits cell enlargement more than cell division (Jaleel *et al.*, 2009). Number of total tillers and plant height will be reduced under drought condition (Ashfaq *et al.*, 2012; Bunnag and Pongthai, 2013; Sokoto and Muhammad, 2014). Lines with intermediate plant height and good number of tillers are expected to have low yield reduction under drought condition.

Dewi *et al.* (2009) set criteria of days to harvest (H) of rice varieties into four classes: very early maturing ($H \leq 110$ DAS), early maturing ($110 < H \leq 115$ DAS), medium maturing ($115 < H \leq 125$ DAS), and late maturing ($125 < H \leq 150$ DAS). Based on above classification, 12 genotypes were classified as very early maturing, 11 genotypes classified as early maturing, and 7 genotypes classified as medium maturing. Based on the result, all tested lines had short days to harvest. Rice genotypes having short days to harvest and high yield is expected for rainfed lowland. Under drought in the late season, lines with short day to harvest or early flowering genotypes can escape from drought stress, therefore it is more effective in maintaining yield under terminal drought. Replacing late maturing cultivars with medium maturing cultivars that have good yield potential in rainfed lowlands provides a better chance of escaping the late season

drought (Ouk *et al.*, 2007). The tested lines with high yield and short days to harvest have potential to be developed as suitable lines for rainfed rice with the drought condition in the late season.

Genotype CG-7-72-1-1 had the highest number of filled grains (156 grains or 71.8% filled grains). Genotypes with high number of filled grains will have high grain yield. One of the ideal plant characters according to Ma *et al.* (2006) is the number of grains between 180-240 grains, with filled grain of more than 85%. Screening with a high number of filled grain character, under optimum conditions and moderate stress conditions in the reproductive phase is required to form cultivars that combine high yield potential with drought tolerance (Atlin *et al.*, 2006). Drought will increase in spikelet sterility (Raman *et al.*, 2012).

The highest productivity was achieved by line CG-8-18-1-2 (6.2 ton/ha) and significantly different from Ciherang (3.7 ton/ha) and Inpari 18 (3.4 ton/ha). High productivity was also achieved by genotype CG-8-18-1-1 (5.5 t/ha), CG-9-26-1-2 (5.5 t/ha), CG-7-72-1-2 (5.3 t/ha), and CG-8-93-1-1 (5.2 t/ha). Rice grain yield is severely reduced under drought stress (Venuprasad *et al.*, 2011; Maisura *et al.*, 2014). Information of potential yield of genotype that can be achieved under irrigation is important because it can determine the level of tolerance of the genotypes. When yield is reduced slightly by mild drought, genotypic ranking is similar between irrigated and drought conditions (Pantuwan *et al.*, 2002). Genotypes with high yield potential performed better, particularly when environmental yield level was high (Fukai *et al.*, 2009). This confirms the

importance of improving germplasm base for potential yield.

Screening on seedling stage under drought conditions

Leaf rolling is a visible sign of drought stress. Scoring based on leaf rolling can be done to obtain information about drought tolerance ability of tested genotypes. Drought treatment was given after 14 days. Tested genotypes showed symptoms of leaf rolling after 38 days of drought treatment. Sensitive check for drought namely IR 20 showed symptoms of dead for all plants (score 9) and drought tolerant check namely Salumpikit showed slight tip drying (score 1). It meant that we could evaluate drought tolerance of the tested genotypes and showed that drought treatment in the present study could distinguish tolerant and sensitive genotypes.

The results of the drought tolerance evaluation showed that CG-8-18-1-2 was categorized as tolerant or showed slight tip drying (score 1), CG-8-18-1-1, CG-8-92-1-1, CG-8-92-1-2, and CG-9-5-1-1 were categorized as mild tolerant or showed tip drying extended up to $\frac{1}{4}$ length in most leaves (score 3), ten genotypes were categorized as moderate or showed one-fourth to $\frac{1}{2}$ of all leaves dried (score 5), seven genotypes were categorized as mild sensitive or showed more than $\frac{2}{3}$ of all leaves fully dried (score 7), and six genotypes were categorized as sensitive or showed all plants dead (score 9). The result showed that tested genotypes had different levels of tolerance under drought condition (Table 2). Sensitive genotypes show strong drought symptoms by rolling and drying of the leaves as the plant

response to drought (Singh *et al.*, 1996). Leaf rolling can help in maintaining internal plant water status (Gana, 2011). If cell turgor is maintained under drought stress, it will result in delayed leaf rolling. However, increased leaf rolling under severe stress has the advantage of preventing water loss and radiation damage. Variation in leaf rolling among genotypes has a genetic basis, and QTLs associated with leaf rolling have been reported in rice (Salunkhe *et al.*, 2011).

After observation of leaf rolling, we had evaluated the recovery ability of rice plants after being re-watered showed. It was shown that lines CG-7-72-1-6, CG-8-18-1-2, CG-8-92-1-2, and CG-9-5-1-1 were categorized as tolerant or showed 90-100% recovery (score 1), CG-7-72-1-1, CG-8-9-1-4, CG-8-18-1-1, CG-8-92-1-1, and CG-9-2-1-6 were categorized as mild tolerant or showed 70-89% recovery (score 3), fifteen genotypes were categorized as moderate or showed 40-69% recovery (score 5), and one genotype was categorized as mild sensitive or showed 20-39% recovery (score 7), and three genotypes were categorized as sensitive or showed 0-19% recovery (score 9). Plants that were able to maintain the greenness of leaves will have the ability to grow. The green leaves will give the plant a chance to photosynthesize so that energy can be stored and used to stimulate root development to grow deeper. Ability to recover is also very important for the plant. After the plants experienced drought stress, its growth will slow down. When the plants are watered, the plants will grow back. Chang *et al.* (1972) reported that recovery power can be an indication of plant tolerance of plants to drought stress. Genotypes

with good recovery ability have a faster rate of growth after cessation of drought stress.

The genotypes tolerant to drought stress can be determined by its ability to maintain the greenish condition of the leaves and the recovery ability. CG-8-18-1-2 showed consistent result in maintaining

greenish condition and recovery ability. Screening on seedling stage can be effectively perform selection large and rapid selection. Friedman nonparametric analysis (Table 2) aims to change qualitative data scoring into quantitative data, so correlation analysis with other characters can be performed.

Table 2. Response of rice genotypes under drought stress conditions and recovery ability at 38 days after seedling.

| No | Genotype | Drought tolerance score | | Recovery score | |
|----|--------------|-------------------------|----------------|----------------|----------------|
| | | Mean* | Plant response | Mean* | Plant response |
| 1 | CG-7-72-1-1 | 3.7 | Moderate | 3.9 | Mild tolerant |
| 2 | CG-7-72-1-2 | 5.8 | Mild sensitive | 4.5 | Moderate |
| 3 | CG-7-72-1-3 | 4.3 | Moderate | 3.5 | Moderate |
| 4 | CG-7-72-1-4 | 7.1 | Mild sensitive | 5.3 | Moderate |
| 5 | CG-7-72-1-5 | 7.1 | Mild sensitive | 5.3 | Moderate |
| 6 | CG-7-72-1-6 | 5.2 | Mild sensitive | 2.1 | Tolerant |
| 7 | CG-7-72-1-7 | 5.1 | Moderate | 5.0 | Moderate |
| 8 | CG-8-9-1-2 | 5.2 | Mild sensitive | 4.9 | Moderate |
| 9 | CG-8-9-1-3 | 5.2 | Moderate | 2.9 | Moderate |
| 10 | CG-8-9-1-4 | 5.1 | Moderate | 3.0 | Mild tolerant |
| 11 | CG-8-9-1-5 | 6.5 | Mild sensitive | 3.9 | Moderate |
| 12 | CG-8-18-1-1 | 2.2 | Mild tolerant | 2.5 | Mild tolerant |
| 13 | CG-8-18-1-2 | 1.1 | Tolerant | 2.5 | Tolerant |
| 14 | CG-8-35-1-2 | 6.4 | Moderate | 5.5 | Moderate |
| 15 | CG-8-92-1-1 | 3.4 | Mild tolerant | 2.5 | Mild tolerant |
| 16 | CG-8-92-1-2 | 3.1 | Mild tolerant | 1.5 | Tolerant |
| 17 | CG-8-93-1-1 | 7.8 | Sensitive | 5.6 | Moderate |
| 18 | CG-8-97-1-1 | 4.9 | Moderate | 4.9 | Moderate |
| 19 | CG-8-97-1-2 | 4.9 | Moderate | 5.0 | Moderate |
| 20 | CG-8-115-1-1 | 5.4 | Moderate | 4.6 | Moderate |
| 21 | CG-9-2-1-5 | 7.1 | Sensitive | 5.0 | Moderate |
| 22 | CG-9-2-1-6 | 7.8 | Sensitive | 4.5 | Mild tolerant |
| 23 | CG-9-2-1-7 | 7.7 | Sensitive | 7.9 | Sensitive |
| 24 | CG-9-5-1-1 | 3.1 | Mild tolerant | 2.5 | Tolerant |
| 25 | CG-9-26-1-1 | 8.5 | Sensitive | 9.0 | Sensitive |
| 26 | CG-9-26-1-2 | 5.4 | Moderate | 5.3 | Moderate |
| 27 | CG-9-26-1-3 | 8.5 | Sensitive | 8.0 | Sensitive |
| 28 | CG-9-26-1-4 | 7.1 | Mild sensitive | 6.9 | Mild sensitive |
| 29 | IR 20 | 8.5 | Sensitive | 9.0 | Sensitive |
| 30 | Salumpikit | 0.8 | Tolerant | 3.1 | Tolerant |

* indicates the mean value is obtained from Friedman nonparametric statistical analysis

Correlation analysis

Table 3 shows the correlation between the characters in the agronomic character evaluation and the drought screening at seedling stage. Based on this analysis, it was expected to obtain agronomic characters correlated with drought tolerance. Productivity was significantly and positively correlated with plant height and filled grain per

panicle. This correlation indicated that increasing filled grain will be followed by grain yield. This result can be used to select genotypes with good agronomic character and high productivity. The analysis of the characters was continued with principal component analysis to be used for giving the weighted of the characters in selection index.

Table 3. Correlation analysis of tested genotypes.

| | PH | TT | PL | FG | UG | DH | GY | DRT |
|-----|----------|----------|---------|---------|--------|--------|--------|---------|
| TT | -0.663** | | | | | | | |
| PL | 0.670** | -0.383* | | | | | | |
| FG | 0.741** | -0.357 | 0.560 | | | | | |
| SG | -0.103 | 0.007 | 0.382* | -0.155 | | | | |
| DH | 0.636** | -0.388* | 0.614** | 0.355 | 0.298 | | | |
| GY | 0.766** | -0.515** | 0.361 | 0.563** | -0.367 | 0.200 | | |
| DRT | -0.089 | -0.184 | -0.091 | 0.196 | 0.103 | -0.029 | -0.140 | |
| DRR | 0.037 | -0.085 | -0.128 | 0.288 | -0.236 | 0.040 | -0.037 | 0.803** |

PH: plant height, TT: number of total tiller per plant, DH: day to harvest, PL: panicle length, FG: number of filled grains, UG: number of unfilled grains, GY: grain yield, DRT: drought tolerance, DRR: drought recovery, ** Significant at $P < 0.01$, * significant at $P < 0.05$

Drought tolerance was significantly and positively correlated with recovery ability of plant. It means genotypes with good drought tolerance will have good recovery ability. There was no correlation between agronomic characters under optimum condition with drought tolerance and recovery ability, so that we can not use this character from agronomic and drought tolerance to become direct selection character. We can perform selection gradually. First, we select genotypes with good agronomic character based on selection index. Second, we then select genotypes with good tolerance under drought condition at seedling stage.

Principal component analysis (PCA)

The PCA showed that the first three components explain about 72% of the total variance (Table 4). The purpose of breeding is to obtain high productivity genotypes, therefore the characters associated with production must be used as main criteria for selection. Characters included in the PCA are characters correlated with productivity and production component: productivity, filled grain, and plant height. The use of the characters will give more objective basis in weighted index.

The negative and positive marks on the PC value indicate a correlation of each character. PC1 only explained 34% of the total variation. Characters correlated with grain yield

were plant height and filled grain per panicle, but the PC value of each character was not significantly different. This model could not be used because we hoped that productivity had bigger PC value compared to other characters. The PC2 explained 20% of the total variation. The PC2 model could not be used because productivity did not

correlate with plant height and filled grain. PC3 explained 18% of the total variation. PC3 could be used because plant height and filled grain had correlation with production, also PC value of productivity (0.35) larger than plant height (0.08) and filled grain per panicle (0.15). The cumulative proportion of PC1 to PC3 is 72%.

Table 4. Principal component analysis of tested genotypes.

| Characters | PC1 | PC2 | PC3 |
|-----------------------------------|-------|-------|-------|
| Plant height (cm) | -0.47 | 0.05 | -0.08 |
| Number of total tillers per plant | 0.35 | -0.16 | -0.02 |
| Day to harvest (days) | -0.33 | 0.08 | 0.31 |
| Panicle length (cm) | -0.39 | -0.00 | 0.28 |
| Number of filled grains | -0.34 | 0.23 | -0.15 |
| Number of unfilled grains | 0.01 | 0.02 | 0.61 |
| Grain yield (ton/ha) | -0.37 | -0.04 | -0.35 |
| Drought tolerance | 0.08 | 0.59 | 0.02 |
| Drought recovery | 0.04 | 0.58 | 0.14 |
| Eigen values | 4.07 | 2.41 | 2.16 |
| Proportion of variance | 0.34 | 0.20 | 0.18 |
| Cumulative proportion | 0.34 | 0.54 | 0.72 |

Selection index

After correlation analysis and principal component analysis, we can use plant height, filled grain, and grain yield as weighted value for the selection index. Index selection was done by giving the weight based on PC3. Agronomic ranking of the tested lines can be obtained. Then, we perform selection for drought tolerance based on screening at seedling stage.

Selection was done based on the positive and highest index value. We can obtain 16 genotypes with good agronomic character and high yield (Table 5). We consider drought tolerance and recovery ability of these lines to obtain genotypes that are tolerant under drought condition. We

can classify these genotypes into genotypes with high productivity and tolerant to drought, genotypes with high productivity and moderately tolerant to drought, and genotypes with high productivity and sensitive to drought. The main purpose is developing high productivity and tolerance to drought stress.

Based on this grouping, we obtain genotypes with high productivity and tolerant to drought namely CG-8-18-1-2 (6.2 t/ha), CG-7-72-1-1 (4.9 t/ha), CG-8-18-1-1 (5.5 t/ha), and CG-7-72-1-6 (4.7 t/ha). Genotypes with high productivity and moderately tolerant to drought namely CG-9-26-1-2 (5.5 t/ha), CG-7-72-1-2 (5.3 t/ha), CG-8-93-1-1 (5.2 t/ha),

Table 5. Index of selection for plant height, number of filled grain per panicle, and grain yield.

| Rank | Genotype | Plant height (cm) | Number of filled grain per panicle | Grain yield (ton/ha) | Index | Drought tolerance | Drought recovery |
|------|--------------|-------------------|------------------------------------|----------------------|-------|-------------------|------------------|
| 1 | CG-8-18-1-2 | 132.0 | 114.4 | 6.2 | 0.86 | Tolerant | Tolerant |
| 2 | CG-9-26-1-2 | 132.2 | 155.9 | 5.5 | 0.80 | Moderate | Moderate |
| 3 | CG-7-72-1-2 | 133.8 | 127.1 | 5.3 | 0.54 | Mild sensitive | Moderate |
| 4 | CG-7-72-1-1 | 130.8 | 156.0 | 4.9 | 0.52 | Moderate | Mild tolerant |
| 5 | CG-8-18-1-1 | 133.6 | 107.0 | 5.5 | 0.50 | Mild tolerant | Mild tolerant |
| 6 | CG-8-93-1-1 | 124.9 | 134.3 | 5.2 | 0.49 | Sensitive | Moderate |
| 7 | CG-7-72-1-6 | 141.9 | 157.3 | 4.7 | 0.49 | Mild sensitive | Tolerant |
| 8 | CG-7-72-1-7 | 141.5 | 127.3 | 5.1 | 0.48 | Moderate | Moderate |
| 9 | CG-7-72-1-3 | 140.1 | 133.2 | 5.0 | 0.47 | Moderate | Moderate |
| 10 | CG-7-72-1-4 | 137.2 | 123.4 | 5.1 | 0.44 | Mild sensitive | Moderate |
| 11 | CG-7-72-1-5 | 133.1 | 145.0 | 4.8 | 0.41 | Mild sensitive | Moderate |
| 12 | CG-9-26-1-1 | 125.4 | 133.0 | 4.9 | 0.35 | Sensitive | Sensitive |
| 13 | CG-9-26-1-3 | 124.8 | 141.7 | 4.6 | 0.26 | Sensitive | Sensitive |
| 14 | CG-8-97-1-1 | 135.8 | 118.2 | 4.4 | 0.08 | Moderate | Moderate |
| 15 | CG-9-26-1-4 | 119.2 | 120.8 | 4.5 | 0.06 | Mild sensitive | Mild sensitive |
| 16 | CG-8-9-1-2 | 110.4 | 131.1 | 4.4 | 0.04 | Mild sensitive | Moderate |
| 17 | CG-8-9-1-4 | 112.7 | 120.9 | 4.3 | -0.06 | Moderate | Mild tolerant |
| 18 | CG-8-9-1-5 | 107.8 | 104.4 | 4.5 | -0.09 | Mild sensitive | Moderate |
| 19 | CG-8-97-1-2 | 134.6 | 113.6 | 4.0 | -0.14 | Moderate | Moderate |
| 20 | CG-9-2-1-6 | 104.8 | 98.7 | 4.4 | -0.19 | Sensitive | Mild tolerant |
| 21 | CG-9-2-1-5 | 102.4 | 91.2 | 4.5 | -0.20 | Sensitive | Moderate |
| 22 | CG-8-92-1-1 | 99.4 | 81.4 | 4.2 | -0.41 | Mild tolerant | Mild tolerant |
| 23 | CG-8-9-1-3 | 107.8 | 96.7 | 3.8 | -0.46 | Moderate | Moderate |
| 24 | CG-8-35-1-2 | 103.1 | 99.5 | 3.7 | -0.51 | Moderate | Moderate |
| 25 | Ciherang | 106.8 | 89.8 | 3.7 | -0.55 | - | - |
| 26 | CG-9-5-1-1 | 100.1 | 104.4 | 3.5 | -0.59 | Mild tolerant | Tolerant |
| 27 | CG-8-92-1-2 | 95.0 | 80.1 | 3.4 | -0.80 | Mild tolerant | Tolerant |
| 28 | CG-8-115-1-1 | 98.2 | 102.0 | 2.9 | -0.88 | Moderate | Moderate |
| 29 | CG-9-2-1-7 | 89.4 | 100.9 | 2.9 | -0.93 | Sensitive | Sensitive |
| 30 | Inpari 18 | 90.8 | 56.4 | 3.4 | -0.97 | - | - |

CG-7-72-1-7 (5.1 t/ha), CG-7-72-1-3 (5.0 t/ha), CG-7-72-1-4 (5.1 t/ha), CG-7-72-1-5 (4.8 t/ha), CG-8-97-1-1 (4.4 t/ha), and CG-8-9-1-2 (4.4 t/ha). Genotypes with high productivity and sensitive to drought namely CG-9-26-1-1 (4.9 t/ha), CG-9-26-1-3 (4.6 t/ha), and CG-9-26-1-4 (4.5 t/ha). The sensitive genotypes were suitable for irrigated or rainfed area without drought stress. They may be adapted under mild drought stress. We conducted screening for drought tolerance at seedling stage to obtain initial information on drought tolerance in some genotypes. To confirm the tolerance, the lines need to be evaluated further under rainfed lowland conditions.

CONCLUSION

There was variability among genotypes in agronomic characters and drought tolerance in doubled haploid lines evaluated. Based on our evaluation, there were four genotypes showing high productivity and drought tolerance. The lines were CG-8-18-1-2 (6.2 t/ha), CG-7-72-1-1 (4.9 t/ha), CG-8-18-1-1 (5.5 t/ha), and CG-7-72-1-6 (4.7 t/ha). The genotypes need to be evaluated further under rainfed lowland conditions.

ACKNOWLEDGEMENTS

The authors wish to thank Ministry of Research, Technology, and Higher Education, Republic of Indonesia for funding the research.

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