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THE TOLERANCE AND YIELD COMPONENTS OF RICE BREEDING LINES SELECTED UNDER LOW AND OPTIMUM NITROGEN CONDITIONS

Toleransi dan Hasil Galur Pemuliaan Padi pada Kondisi Nitrogen Rendah dan Optimum

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ABSTRACT

One of the important issues on rice breeding is to develop new rice lines suitable for nitrogen efficiency in the suboptimum environment. The study aimed to evaluate the tolerance and yield components of rice breeding lines selected under low and high nitrogen conditions. The F6 generation from two cross-combinations of Gampai/IR77674 and Progol/Asahan, were evaluated in the dry season of 2014 under N suboptimum and N optimum conditions. A total of 172 lines plus six check varieties derived from the low and high N environment selection were evaluated under low N rate (34.5 kg N ha^{-1}) and high N rate (138 kg N ha^{-1}), arranged in an augmented design. Six check varieties were relocated three times in each block. Plot size was 5.5 m^2 and plant spacing 20 cm x 20 cm. Data were collected for grain yield and major yield components. Results showed that the different status of nitrogen fertilizer affected the number of productive fillers, number of filled grains, 100-grain weight, and grain yield. Different cross combinations exhibited different results in the progeny performance. Ten lines have a higher yield also tolerant to low N condition, i.e. B14250F-6-9, B14250F-1-4, B14250F-9-9, B14250F-6-4, B14250F-5-2, B14262F-15-6, B14250F-2-6, B14262F-12-4, B14250F-5-1, and B14250F-11-4. Thus, to obtain the N tolerant lines, selection at a low N environment was more effective compared with the optimum selection environment.

[**Keywords:** Low nitrogen, optimum nitrogen, rice breeding, yield]

ABSTRAK

Salah satu masalah utama dalam pemuliaan padi adalah mengembangkan galur-galur padi efisien terhadap nitrogen di lingkungan yang kurang optimal. Penelitian bertujuan untuk mengevaluasi toleransi dan komponen hasil galur padi yang diseleksi pada kondisi nitrogen rendah dan tinggi. Generasi F6 dari dua kombinasi persilangan Gampai/IR77674 dan Progol/Asahan dievaluasi pada musim kemarau (MK) tahun 2014 dalam kondisi N sub-optimum dan N optimum. Seratus tujuh puluh dua galur ditambah enam varietas cek yang berasal dari seleksi di lingkungan N rendah dan N tinggi dievaluasi di lingkungan N rendah (34,5 kg N ha^{-1}) dan N tinggi (138 kg N ha^{-1}), menggunakan rancangan augmented*. Enam varietas cek diulang tiga kali pada setiap blok. Ukuran plot adalah 5,5 m^2 dan jarak tanam 20 cm x 20 cm. Data yang dikumpulkan meliputi hasil gabah dan komponen hasil. Hasil penelitian menunjukkan bahwa perbedaan status pupuk nitrogen memengaruhi jumlah anakan produktif, jumlah gabah isi, berat 100 gabah, dan hasil gabah. Kombinasi persilangan yang berbeda menunjukkan hasil yang berbeda. Sepuluh galur memiliki hasil yang lebih tinggi dan juga toleran terhadap kondisi N rendah, yaitu B14250F-6-9, B14250F-1-4, B14250F-9-9, B14250F-6-4, B14250F-5-2, B14262F-15-6, B14250F -2-6, B14262F-12-4, B14250F-5-1, dan B14250F-11-4. Dengan demikian, untuk mendapatkan galur padi toleran N maka seleksi pada lingkungan N rendah lebih efektif dibandingkan dengan lingkungan seleksi optimal.*

[**Kata kunci:** Hasil, pemuliaan padi, nitrogen optimum, nitrogen rendah]

INTRODUCTION

Rice grain yield is influenced by varieties, environments, and interactions between varieties and the environments. Nitrogen (N) as an essential nutrient could function as a critical factor for the selection criterion for high yield. The application of N fertilizer of 225–325 kg ha^{-1} was able to produce rice of 8.94–9.25 t ha^{-1} (Zhang et al. 2010). However, the grain yield decreased 0.2 t ha^{-1} with a low dosage of N fertilizer.

Low N conditions are common in the most rice field. Field intensively planted with rice causes the crop to suffer N deficiency if not fertilized with nitrogen (Fairhurst et al. 2007; Triadiati et al. 2012; Syakhril et al. 2014). However, fertilizer application often has a low efficiency due to volatilization (Zhong-Cheng et al.

2012), denitrification, timing and placement of fertilizer, leaching, run-off, and absorbed by harvested plants (Choudury and Kenedy 2005). Nitrogen absorbed by rice crop was only 22–30% of the applied N (Deng et al. 2012).

The use of N fertilizer worldwide increased 7-fold in the past four decades to increase rice production (Hirel et al. 2011), that it was considered too high N application for balancing the N loses (Deng et al. 2012). It is therefore considered important to identify rice genotypes efficient in using N fertilizer. On the other hand, excess of N application has a negative impact on the environment and human health (Pimentel et al. 2005; Fess et al. 2011). In certain places, farmers still use a low dose of N as little as 46–96 kg N ha^{-1} (Azwir and Ridwan 2009). In Bangladesh, farmers applied 39–175 kg N ha^{-1} because of the non-availability of resources (Hossain et al. 2005; Abrol et al. 2007). On the most intensive rice farming, Yoseftabar (2013) showed that panicle number, panicle length, panicle dry matter, and number of primary tillers increased significantly with the application of 300 kg N ha^{-1} for hybrid rice. Peng et al. (2006) suggested 60–120 kg N ha^{-1} was enough for achieving 5–9 t ha^{-1} rice yield on irrigated lands.

Metwally et al. (2010) found that the N application of 150 kg ha^{-1} resulted in the highest yield, while Kanfany et al. (2014) reported that grain yield of most rice hybrids increased with increasing N-fertilizer up to 180 kg ha^{-1}. The increase in N fertilization resulted in an increase in metabolite synthesized by rice plants. However, in contrast, Jeon (2012) reported that the N application from 70 to 150 kg ha^{-1} showed similar in yield and N efficiency of Guami variety. It might be because the variety had fewer numbers of small vascular bundles in the stem that affect nutrient translocation of rice. According to Lian et al. (2006), plants that can adapt to N deficiency have a mechanism by interrupting activities that use nutrients and energy such as photosynthate and the tricarboxylic citrid acid (TCA) cycle to survive. Nitrogen stress at the initial stage is felt by the roots but does not affect the leaf tissue.

In all cases, it is important to develop rice varieties that have N efficiency. Vinod and Heuer (2012) explained which efficient rice genotype was capable of developing physiological mechanisms with enhanced nutrient uptake via a vigorous root system, leading to the increased grain filling and grain yield under suboptimum condition.

The current research reported the N efficiency on rice genotypes. Many varieties developed by plant breeding programs almost always being done under high input conditions (Dawson et al. 2008) which cause the possibility of getting genetic diversity to low input is scarce (Ceccarelli 1996; Wissuwa et al. 2009). This approach resulted in narrow adaptive genotypes because they are only suitable for the origin of environmental conditions. Nitrogen efficient capabilities of rice germplasm are needed to identify its potential as an efficient parental in the breeding program. Information about N efficiency among local varieties was necessary to create new rice varieties that are adaptive to suboptimum N condition (Guarda et al. 2004; Toure et al. 2009; Trouche et al. 2011). Therefore, in this study, a breeding scheme that utilizes the advantages of the existing and local rice varieties, combined with the applied nitrogen, was conducted to obtain promising lines that are efficient in using the nitrogen.

Direct selection on the targeted area on the artificial environment of low N will facilitate breeders in differentiating between tolerant and sensitive genotypes. Research conducted in the suboptimum environment needs to be done to obtain lines that are adaptive to such an environment and to maintain yield (Presterl et al., 2003). The bulk selection method was suggested to be used to fix the additive genes of characters in an advanced generation that have low to moderate heritability, such as grain yield (Kumar et al. 2009). The effect of the among plant competition could be minimized by modifying the bulk method, as was commonly called modified bulk. Modified bulk referred to not to harvest inferior or off-type plants to reduce the competition effect between plants and maintain genotypes with the desired character (Acquaah 2007). By applying this method, the frequency of homozygote lines in an advanced generation is already high that it becomes easier to select lines and the progeny can be expected to be uniform. High plant population and plant density may lead to competition between individuals resulting in selection pressure to select out genotypes with poor performance. The surviving genotypes are not necessarily those to be expected the best genotype because not all characters retained from the competition is the one associated with the adaptability and yield potential.

Research using modified bulk selection on rice was carried out starting from F3 generation up to F5 on the low N environment or suboptimum N. The expected outcome would be some lines adaptive to the suboptimum N environment. This method was used on various crops such as wheat (El-Karamity et al. 2007), tomato (Salem et al. 2007), rice (Farag 2013), and soybean (Kanbar et al. 2011). To identify suitable lines for the suboptimum environment, stress tolerance index (STI) was used as a criterion for the tolerant genotype (Khodarahmpour et al. 2011). STI tends to select genotype with the high yield on both under stress and non-stress conditions (Farshadfar et al. 2013).

The study aimed to evaluate the tolerance and yield components of rice breeding lines selected under low and optimum soil nitrogen conditions.

MATERIALS AND METHODS

Plant Materials

The experiment was conducted at Muara Experimental Farm, Bogor-West Java, during the dry season of 2014. Soil analysis was shown in Table 1. A total of 172 F6 lines derived from two cross combinations of Gampai/IR77674 and Progol/Asahan were used as genetic materials for the experiment. Gampai and Progol each is a local variety, expected as the parent for low-N tolerant. Six check varieties were Ciherang, Inpari 6, Inpari 23, Inpari 33, IR77674, and Asahan. Ciherang was used as a check for high yielding variety, Inpari 6 was resistant to brown planthopper biotype 2 and 3, Inpari 23 was resistant to brown planthopper biotype 1, bacterial leaf blight strain 3, 4, 8, and Inpari 33 was resistant to brown planthopper biotype 1, 2, 3. These materials were previously selected for three generations from F3 to F5 under suboptimum N and optimum N environments.

Field Experiment

Augmented design with three replication of the six check varieties was used. Each line was planted in a plot of 5.5 m^2 as an experimental unit. Plant spacing was 20 cm x 20 cm of one 21-days-old seedling per hill. The 172 genotypes were tested on two different N levels, namely 34.5 kg N ha^{-1} as a suboptimum environment coded as N- and 138 kg N ha^{-1} as optimum environment coded as N+. Nitrogen fertilizer was applied three times (⅓ part was applied three days after planting, ⅓ part was applied at four weeks after planting, and another ⅓ part was applied at seven weeks after planting). As much as 36 kg of phosphorus (P_2O_5) and 60 kg of potassium (K_2O) were applied together at the same time with the first N application. Pest and disease control was done optimally.

Table 1. Chemical soil analysis in Muara Experimental Farm, Bogor, West Java.

Chemical component	Value	Criteria (Hardjowigeno 1987)
C-organic (%)	1.79	Low
N-total (%)	0.17	Low
C/N	11	Medium
P_2O_5 HCl 25% (ppm)	222	High
P_2O_5 Bray I (ppm)	8.8	Very Low
K (me 100^{-1} g)	0.49	Very Low
Mg (me 100^{-1} g)	2.02	Medium
Ca (me 100^{-1} g)	11.11	High
KTK (me 100^{-1} g)	15.18	Low
pH	5.4	Acid

Variables Measured and Data Analysis

At harvest time, five plant samples were taken randomly from each plot and observed for yield components including productive tiller number, panicle weight, number of filled grains per panicle, and weight of 100 filled grains. Grain yield was measured from 5 m^2 net plot. The data were analyzed following the procedure of the augmented design. Differences in the mean treatments were determined using t-test at 5% probability level. Least Significant Increase (LSI) was calculated to compare the yield of lines with a check (Saleem et al. 2013).

The tolerance indices were calculated as follows:

$$\text{Stress tolerance index (STI)} = \frac{Yp \times Ys}{\overline{Yp}^2}$$

where Ys is grain yield of each genotype under low N condition, Yp is grain yield of each genotype under optimum N condition, $\overline{Yp}$ are the mean yields of all genotypes under low N and optimum N. Determination of tolerance was calculated modified based on Sundari (2016), where the value of STI > 1 was classified as tolerant, STI between 0.5–1 was moderate, and STI < 0.5 as sensitive.

RESULTS AND DISCUSSION

The analysis of variance showed that blocks had a significant effect on the characters of productive tillers, panicle weight, and grain yield (Table 2). Differences in nitrogen treatments affected the characters of the productive tiller number, filled grain number, 100-grain weight, and yield. The interaction between block and nitrogen affected all the characters, except for 100-grains weight. The checks varieties indicated significant differences among three variables including the number of productive tillers, number of filled grains per panicle, and 100-grain weight. Genotypes indicated a significant difference between all characters. The interaction between nitrogen and genotype was significant only for panicle weight. This showed that there was a high variation of each character among lines under different nitrogen conditions. This finding was in line with Rajesh et al. (2015) and Njinju et al. (2018).

Table 2. Mean square of yield components and grain yield of rice lines under two different nitrogen conditions, Muara Experimental Farm, Bogor, West Java.

Variation	Number of productive tillers	Panicle weight	Panicle length	Number of filled grains	Weight of 100 grains	Yield
Block	35.3*	6.7*	0.9	495	0.3	$6.5.10^{7}$*
Nitrogen (N)	176.1*	0.0	4.1	4,067*	0.4*	$4.7.10^{7}$*
Block*N	101.9*	5.3*	14.2*	805*	0.0	$2.8.10^{7}$*
Lines vs check	69.8*	7.7*	47.8*	7,285*	0.6*	$5.5.10^{6}$*
Check	9.3*	1.9*	6.8	279	0.1	$2.3.10^{6}$*
Lines (check)	4.9	0.6	3.2	929*	0.1	$1.7.10^{6}$*
N*lines vs check	0.4	0.0*	0.7	197	0.0	$1.1.10^{6}$
N*check	1.8	0.2	0.6	609*	0.1	$1.0.10^{5}$
N*lines (check)	6.1*	0.6	4.2	640*	0.1	$1.7.10^{6}$*
CV (%)	17.7	14.3	6.3	11.2	10.7	17.4

*Significantly different at $P \leq 0.05$

Yield and Yield Components

The 10% best lines were selected based on grain yield at each suboptimum and optimum environments. The yield average of those 10% best lines based on the cross combination and selection environment was shown in Figure 1. The yield of lines derived from Gampai/IR77674 cross combination selected on the suboptimum environment (G/IR_N-) and the optimum environment (G/IR_N+) was not significantly different when planted under suboptimum or optimum conditions. On the other hand, the yield of lines derived from Progol/Asahan cross combination selected under suboptimum (P/A_N-) was not significantly different under two N conditions. Based on these data to obtain lines that are tolerant to suboptimum N condition, selection should be made under suboptimum N condition. This result was in line with Presterl et al. (2003) and Galais and Coque (2005) for maize that to obtain maximum genetic advance at low N-input, it was better to select genotypes under low N condition. Therefore, it appears to be quite possible to develop new varieties better adapted to the low N-input compare to the existing present varieties. As was reported by Ortiz et al. (2008), the modern varieties produced higher yield not only on the optimum but also on the suboptimum environment.

The current new varieties of rice tend to be unsuitable to the low N condition, as they were developed under optimum N condition. More tolerant variety may be derived from the progeny of cross using a local variety as a parent. Local variety generally has variable responses to different levels of nitrogen, while breed varieties have variable responses. Lines developed from the breeding program accumulated higher substance to storage organs

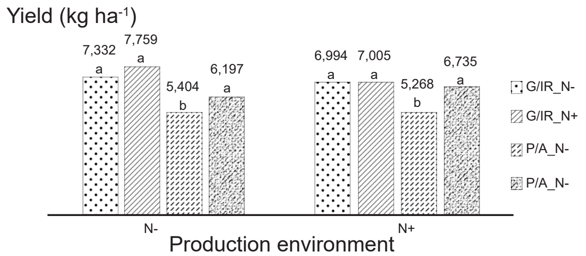

Figure 1. The yield of best 10% lines of Gampai/IR77674 (G/IR) and Progol/Asahan (P/A) derived from suboptimum (N-) and optimum (N+) selection environments and evaluated under two different N conditions.

and transmitted more assimilate from sources to its sinks. On the other hand, local varieties allocated less photosynthate to yield; hence their yields were more stable (Ahmadikhah and Mirarab 2010).

The best yield of 10% lines derived from two cross combinations selected on low and high N and tested on different N condition was shown in Table 3. The values of LSI added to yield of the checks were used to determine the significant higher yield of lines from checks. The yield of lines was significantly higher if the value was more than the yield of check added by LSI value. Data showed that there were ten lines produced a higher yield than the best check Inpari 23 under low N condition and 12 lines under optimum N condition. Most of those lines were previously selected under low N derived from the cross combination of Gampai/IR77674 which was also indicating tolerant criteria. Under the low N environment, there were 9 lines derived from the low N selection environment and 8 lines from the optimum N environment. In the

Table 3. Yield of the best 10% lines at suboptimum (N-) and optimum (N+) environments, Muara Experimental Farm, Bogor, West Java.

Production environment	Genotype	Yield (t ha^{-1})		Source		Tolerance	
		N-	N+	Selection environment	Population	Index	Criteria
N-	B14250F-6-9	9396	5656	Opt.	Gampai/IR77674	2.76	Tolerant
	B14250F-1-4	9059	3838	Subopt.	Gampai/IR77674	1.80	Tolerant
	B14250F-9-9	8434	5805	Opt.	Gampai/IR77674	2.54	Tolerant
	B14250F-6-4	8225	3421	Opt.	Gampai/IR77674	1.46	Tolerant
	B14250F-5-2	7781	3825	Subopt.	Gampai/IR77674	1.54	Tolerant
	B14262F-15-6	7641	4762	Opt.	Progol/Asahan	1.89	Tolerant
	B14250F-2-6	7029	5649	Subopt.	Gampai/IR77674	2.06	Tolerant
	B14262F-12-4	6678	3441	Opt.	Progol/Asahan	1.19	Tolerant
	B14250F-5-1	6451	6903	Opt.	Gampai/IR77674	2.31	Tolerant
	B14250F-11-4	6445	4620	Subopt.	Gampai/IR77674	1.55	Tolerant
	B14250F-3-5	6345	5139	Subopt.	Gampai/IR77674	1.69	Tolerant
	B14250F-3-7	6287	4079	Opt.	Gampai/IR77674	1.33	Tolerant
	B14262F-3-3	6216	3571	Subopt.	Progol/Asahan	1.15	Tolerant
	B14262F-11-6	6188	3579	Opt.	Progol/Asahan	1.15	Tolerant
	B14262F-9-6	6185	3427	Subopt.	Progol/Asahan	1.10	Tolerant
	B14250F-2-10	6133	6053	Subopt.	Gampai/IR77674	1.93	Tolerant
	B14250F-14-10	5981	6095	Subopt.	Gampai/IR77674	1.89	Tolerant
	Average of the best lines at N-	7087	4698				
N+	B14262F-7-1	3176	8363	Opt.	Progol/Asahan	1.38	Tolerant
	B14250F-5-3	1846	8327	Opt.	Gampai/IR77674	0.80	Moderate
	B14250F-3-10	3178	8083	Subopt.	Gampai/IR77674	1.33	Tolerant
	B14250F-1-7	3072	7467	Subopt.	Gampai/IR77674	1.19	Tolerant
	B14250F-14-3	5583	7295	Subopt.	Gampai/IR77674	2.11	Tolerant
	B14250F-15-6	2352	7148	Subopt.	Gampai/IR77674	0.87	Moderate
	B14250F-5-1	6451	6903	Opt.	Gampai/IR77674	2.31	Tolerant
	B14262F-5-2	2327	6801	Opt.	Progol/Asahan	0.82	Moderate
	B14250F-7-6	5291	6772	Subopt.	Gampai/IR77674	1.86	Tolerant
	B14250F-6-2	4534	6288	Subopt.	Gampai/IR77674	1.48	Tolerant
	B14250F-2-4	5515	6250	Opt.	Gampai/IR77674	1.79	Tolerant
	B14250F-15-4	5847	6248	Subopt.	Gampai/IR77674	1.90	Tolerant
	B14250F-5-3	5735	6145	Subopt.	Gampai/IR77674	1.83	Tolerant
	B14250F-14-10	5981	6095	Subopt.	Gampai/IR77674	1.89	Tolerant
	B14250F-12-7	5158	6075	Subopt.	Gampai/IR77674	1.63	Tolerant
	B14250F-2-10	6133	6053	Subopt.	Gampai/IR77674	1.93	Tolerant
	B14250F-5-2	4954	6016	Opt.	Gampai/IR77674	1.55	Tolerant
	Average of the best lines at N+	4537	6843				
Check+LSI*	IR77674	5428	5956			1.05	Tolerant
	Asahan	5247	5322			0.86	Moderate
	Ciherang	4754	5076			0.74	Moderate
	Inpari 6	5683	6043			1.12	Tolerant
	Inpari 23	6393	6149			1.31	Tolerant
	Inpari 33	4492	4756			0.64	Moderate

*LSI α 5% at N- is 830 and at N+ is 783.

Table 4. Yield components of two populations derived from suboptimum (N-) and optimum (N+) selection environments, evaluated at suboptimum (N-) and optimum (N+) environments, Muara Experimental Farm, Bogor, West Java.

Production environment	Selection environment	Population	NPT	WSG	NFG	PL	PW
N-	N-	Gampai/IR77674	7.8b	2.8a	138.5a	26.7b	4.1a
		Progol/Asahan	6.5b	2.9a	111.0a	26.6b	4.2a
	N+	Gampai/IR77674	12.4a	2.6a	125.0a	27.0ab	3.9a
		Progol/Asahan	11.7a	2.7a	140.6a	27.4a	4.4a
		Average	9.6	2.8	128.8	26.9	4.2
N+	N-	Gampai/IR77674	11.8a	2.6a	151.4a	27.7a	4.6a
		Progol/Asahan	12.2a	2.7a	118.7a	26.2b	4.4a
	N+	Gampai/IR77674	12.8a	2.6a	142.7a	28.5a	4.4a
		Progol/Asahan	11.4a	2.7a	137.7a	27.0b	4.6a
		Average	12.0	2.7	137.6	27.4	4.5

Numbers followed with the same letter at a column were not significantly different with t-test at $\alpha = 0.05$.

NPT = number of productive tillers, WSG = weight of 100 grains, NFG = number of filled grains, PL = panicle length, PW = panicle weight.

optimum environment, line B14262F-7-1 produced the highest grain yield of 8363 kg ha^{-1}. This line was selected under optimum N condition derived from crossing between Progol/Asahan. Among the best lines, mostly derived from Gampai/IR77674 cross than those from Progol/Asahan. Farag (2013) stated that the same selection procedure did not always effective for any populations of cross combinations. Modified bulk method on wheat, which was intended for producing high yield, was only effective on one of the three wheat populations for achieving grain yield.

There were four lines among the best 10% lines produced high yield, not only at low N environment but also at the optimum N condition. The lines were B14250F-5-2, B14250F-5-1, B14250F-2-10, and B14250F-14-10. This finding was in line with Reynolds and Borlaug (2006), who found two modern rice varieties, Farox 304 and Farox 239 that produced the best yield under high N under low N input conditions. Modern breeding programs on high input environment were able to produce rice and wheat varieties suitable for marginal production areas. Previously, Le Gouis et al. (2000) found that modern varieties of wheat performed better under low N environment.

Table 4 showed the yield components of all lines derived from Gampai/IR77674 and Progol/Asahan cross combinations. The yield component values were based on the origin of the lines, their parents, and the selection environment. In a low N environment, the selection environments affected productive tiller number and panicle length. A similar trend was found by Singh et al. (2004). Yoseftabar (2013) suggested by applying 300 kg N ha^{-1} rice crop was able to produce long panicles and higher grain numbers. According to Dhurai et al. (2014) and Dutta et al. (2013), the number of productive tillers, number of grains per panicle, and 1000-grain weight had a high heritability which may result in higher genetic gain when to be selected. Those characters were each controlled by many genes with additive gene action which is important as it directly related to grain yield (Osman et al. 2012). According to Yoseftabar (2013), panicle number, panicle length, and the number of grains per panicle were determined by N application. Similar to that result, Metwally et al. (2010) found that the application of 150 kg N ha^{-1} resulted in the highest panicle weight and the number of grains per panicle. The increasing level of N fertilizers did not lead to an increase in 1000-grain weight. Rate of N influenced rice characters such as plant height, biomass, panicle number, panicle length, grain harvest index, and grain yield. Hence, it is possible to manipulate these plant variables in favor of higher grain yield by using adequate N rate or planting N efficient genotypes (Fageria et al. 2010).

CONCLUSION

One hundred and seventy-two F6 rice lines were selected under the low and optimum N nutrient status conditions. Ten lines showed tolerance to low N fertilizer, namely B14250F-6-9, B14250F-1-4, B14250F-9-9, B14250F-6-4, B14250F-5-2, B14262F-15-6, B14250F-2-6, B14262F-12-4, B14250F-5-1, and B14250F-11-4. The different status of N fertilizer affected the number of productive fillers, number of filled grains, 100-grain weight, and grain yield. Therefore, to obtain the N tolerant lines selection at low N environmental status was more effective compared to the optimum N environmental status.

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