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High yielding capabilities and genetic variation in crossing of sheath blight disease resistant rice line

Yuba Raj Gaihre, Akihiro Nose*

Saga University, Faculty of Agriculture, 1 Honjo-Machi, Saga City 840-8502, Japan

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ABSTRACT

Rice breeding has achieved significant progress toward enhancement effects of genetic improvement for the yield but the development of high yielding cultivar with fungal disease resistance is still an important step needed to fulfill the future demand of food for growing population. Rice line 32R is a well-documented source of durable and broad spectrum resistance to sheath blight disease. The objective of this study was to determine the genetic component of yielding capabilities of rice line 32R and quantifying physio-morphological characteristics related to high yielding capacity. Characteristics of sink and source were studied in relation to grain yield and disease resistant. This study revealed that 12.5 metric ton per hectare yielding capacity in F_1 progeny was attributed because of improved performance culm length, panicle length, number of tiller, tillering angle, RuBisCo content in leaf, nonstructural carbohydrate (NSC), dry matter accumulation (DMA), leaf area and number of filled grain per panicle. Especially, multiple regressions showed that number of filled grain per panicle, panicle length and leaf area had contributed 83.0, 28.4 and 29.9% of its effort to the grain yield, respectively in F_1 progeny.

1. Introduction

Rapid population growth demands more food production, while agricultural land is gradually reducing with urbanization (Timsina and Connor, 2001). Rice (*Oryza sativa* L.) is one of the most important food crops of the world. Demand for rice is increasing every year. Yield losses caused by diseases represent a major threat to fulfill the future rice demand. Sheath blight is a rice fungal disease which can cause yield loss up to 50% (Marchetti and Bollich, 1991). The epidemic area of sheath blight disease in Japan is increasing every year because of global warming (Iizumi and Yokozawa, 2008). Nowadays, sheath blight disease is mainly controlled by the use of harmful fungicides (Gorth and Bond, 2007). This method of control is not compatible with sustainable crop management. Rice sheath blight disease is caused by the fungal pathogen *Rhizoctonia solani* Kuhn. The pathogen can survive both in soil and water. Moreover, it produces a phytotoxin (RS toxin), which can reproduce most of the symptoms of the disease (Vidhyasekaran et al., 1997). Environmental factors, in particular temperature and humidity, affect both infection and development of the fungus (Han et al., 2003). Due to the semi-saprophytic nature and low specificity of the pathogenicity mechanisms in *R. solani*, the fungus infects a large number of plant species such as maize, tomato, cabbage, Chinese cabbage and so on (Zeng et al., 2011). Despite extensive efforts to identify sources of resistance in rice germ-plasms, major genes which provide complete resistances to the fungus have not been identified (Wasano, 1988; Jia et al., 2012).

However, large variations in susceptibility to sheath blight disease among rice cultivars have been observed under field conditions (Wasano and Hirota, 1986). There are some rice lines such as Tetep, Tadukan, Teqing, Saza, Marsi, Tauli, Brimful, Jasmine 85, ZYQ8, Minghui 63, LSBR-5 and LSBR-33 in which a high degree of quantitative resistance is available against this pathogen under field conditions (Khush, 1977; Wasano, 1988; Groth and Nowick, 1992; Li et al., 1995; Pan et al., 1999). Among them Tetep offers excellent protection against the pathogen under the field condition and this variety shows fewer and smaller lesions, suggesting the presence of physiological mechanisms of resistance (Wasano et al., 1983; Groth and Nowick, 1992). Wasano et al. (1985) developed a short-culm elite line, 32R, from partial resistant variety Tetep (Indica variety), crossing with CN_4-4-2 (Japonica variety). The rice line CN_4-4-2 was obtained by crossing Chugoku 45 with Nipponbare. The rice line 32R was continuously screened for sheath blight disease resistance over 15 years, along with another sheath blight susceptible rice line 29S, which is genetically related to 32R. Study on different aspects of sheath blight pathogen including disease symptoms in field, metabolic pathways and proteomics analysis after *R. solani* infection showed that 32R has strong resistance capacity against sheath blight disease pathogen (Wasano et al., 1985; Wasano and Hirota, 1986; Danson, 2000; Miyagi et al.,

* Corresponding author. Tel.: +81 952 28 8737.
E-mail addresses: nosea@cc.saga-u.ac.jp, yubarajgaihre@gmail.com (A. Nose).

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2006; Mutuku and Nose, 2010; Gaihre et al., 2011; Mutuku and Nose, 2012). Furthermore, the study of temperature effect showed that 32R has strong growing capacity under different temperature regimes (Kiet and Nose, 2011). These all facts indicate that 32R has high degree of quantitative resistance and also has important agronomic traits for the development of sustainable rice. However, the yielding capacity of 32R has not been studied and attempt of improving yielding capacity of this important rice line was not done. So, to develop high yielding sheath blight resistance rice line by improving the yielding capacity of 32R, it is crossed with one of the high yielding earlier parent cultivar Nipponbare.

Screening for combinations of physio-morphological characteristics and resistance for sheath blight using markers derived by QTL analysis is a potentially interesting approach for the development of high yielding cultivar with high degree of disease resistance. It is apparent that information of morphological and physiological aspects of rice is also key feature to plan a resourceful breeding program (Peng et al., 2008). Sink and source functions, and their relationships including nonstructural carbohydrates (NSC), RuBisCo content, leaf area, panicle length and number of filled grain per panicle are fundamental physio-morphological basis of biomass production and yield in rice. Thus, the development of plant architecture suitable for disease resistance and high yielding capacity by the utilization of information of morphological and physiological traits is required for developing sustainable high yielding crop varieties. Yield traits are quantitatively inherited and affected by different biotic and abiotic factors (Kreye et al., 2009). Hence it is also necessary to detect QTLs associated with yield along with disease resistance using reliable populations in order to understand their genetic bases well and for that selection of best parent is very important. Study of QTL analysis has been found QTLs of sheath blight disease resistance in chromosome 1, 3, 5, 7, 8 and 9 on rice line 32R (Gaihre et al., 2011).

The objective of the present study was to identify the yielding capacity of sheath blight resistance line and to quantify the different physio-morphological traits contributing to the high grain yield. For the evaluation of yield capacity, plant characteristics and its combining effort for photosynthesis, growth, and grain production based on knowledge of plant and crop physio-morphology is used. This work is the process of constructing reliable genetic resources by utilizing the sheath blight disease resistance characteristics of 32R and yield oriented characteristics of Nipponbare for the marker assisted breeding.

2. Materials and methods

2.1. Plant materials

The development of F_1 progeny was done in 2007 and 2008. Parent lines used for the experiments were Nipponbare (Japonica variety) as a male parent and 2F18-7-32 (32R) as a female parent. The later cultivar was developed from a crossing Tetep (Indica variety) as the female parent and CN_4-4-2 (Japonica variety) the male parent. CN_4-4-2 resulted from a crossing Chugoku 45 with Nipponbare resulted in the development of CN_4-4-2 (Wasano et al., 1985). F_1 rice hybrid progeny was obtained by artificial emasculation and pollination at the flowering stage of 32R with Nipponbare by the modified method of rice crossing (Sarkarung, 1991).

2.2. Plant cultivation

The experiment was conducted at the agronomy field of Saga University, Saga, Japan (33° 16′ N and 130° 18′ E) during April 2007 to October 2009 in a heavy clay soil. Seeds of parent cultivars and F_1 were treated with a systemic insecticide and fungicide. 0.1% of Sumichion, an insecticide (Yashima Chemicals Industry Co., Ltd) and 0.5% Tekurido C, a fungicide (Kumiai Chemicals Industry Co., Ltd) for 24 h and then washed by tap water and incubated at 28 °C for 48 h for germination. Pre-germinated seeds were sown on seedling trays. A common procedure was followed in rising of seedling in bed. Seedlings of 30 days old plant were transplanted in the well-puddled experimental plots as single plant per hill with a spacing of 30 cm × 25 cm. Nitrogen, phosphorus and potash were applied at 50, 33 and 33 kg/ha respectively just before transplanting.

2.3. Measurement of physio-morphological properties

The physio-morphological parameters were measured at the physiological maturity stage taking 20 plants from each cultivar. Based on the identification of effective improvement of sheath blight disease resistance and yielding capacity in F_1 progeny in 2008, to identify the relation of sheath blight disease resistance, yielding capacity and physio-morphological traits, the physio-morphological parameter and sheath blight disease resistance of each plant was measured in 2009 by increasing the measurement of physiological parameter then in 2008.

Fresh weight of total biomass was measured at mature stage and the dry weight of each sample was also determined after oven drying at 80 °C to constant weight. Harvesting index was estimated by using dry weight of total plant to the total grain yield. Data were analyzed on single plant basis. Tiller and leaf angles are important traits associated with the morphology of ideal plant type. Specially, erect leaves and relatively small tillering angle, allowing a high leaf area are desired characteristics for rice breeding. Leaf area, leaf sheath area and lesion area were measured by using LIA 32 scanner software (Yamamoto, 2004). Second leaf was selected for the comparative study of leaf angle and leaf area as it is reasonable for sheath blight resistance breeding to select the erect second leaf (Han et al., 2003). It is almost exposed to sun and it is less affected by the grain weight during grain filling, also it is similar to third leaf. Leaf angle was measured from the ventral part of the leaf to vertical stem. Productive tiller of plant were counted to determine the total number of tiller in each plant. Tillering angle of tiller was measured from the vertical line from where the tiller attached to the soil surface. The length, width and thickness of seed determine the grain quality and grain shape. The length, width and thickness of seed were measured with bran by using Absolute Digimetric Caliper (Mitutoyo, Japan). First heading date of each cultivar was determined when any one plant of the cultivar have shown ear emergence starting from the day of showing. Culm length was measured from soil surface to panicle base in centimeters. Panicle length was measured from panicle base to tip in centimeters. The 1000 grains weight of each cultivar was measured after harvesting. Harvest index (HI) was calculated for each cross as

$$\text{HI}\ \ (\%) = \left(\frac{\text{dry weight of grain yield}}{\text{dry weight of whole plant}}\right) \times 100.$$

2.4. Total nonstructural carbohydrate measurement

The leaf sheaths were dried at 80 °C for 3 days. Nonstructural carbohydrate (NSC) accumulated in the leaf sheath was measured according to the method of Tsukaguchi et al. (1996) with some modifications: milled samples (0.5 g) were added into 30 ml of water and autoclaved at 120 °C for 20 min to extract NSC. After cooling, 1.5 mg *a*-amylase (A0521, Sigma–Aldrich) and 0.5 mg amyloglucosidase (A9228, Sigma–Aldrich) was added in 20 ml buffer made by 88.7 mM KH_2PO_4 and 11.1 mM Na_2HPO_4–$12H_2O$ and incubated at 40 °C for 24 h to disbranch NSC into monosaccharides. After filtrating incubated samples, residuals were dried at 80 °C for 24 h and