

## Feasibility of Utilization of Wild Rice (*Oryza rufipogon* Griff.) Genetic Diversity in Rice Breeding for High Yield

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**Abstract:** Common wild rice (*Oryza rufipogon* Griff.) is important to broaden the narrow genetic diversity of cultivated rice. To better understand the genetic variation, heritability and genetic correlations of agronomically important traits, 156  $F_{2:3}$  families derived from the cross between rice (*Oryza sativa*) and wide rice (*O. rufipogon* Griff.) were evaluated under field conditions. The Analysis of Variance (AOV) indicated there was highly significant genetic variation between families for all the traits tested. Transgressive segregation was observed for most traits in the  $F_{2:3}$  families. Estimates of additive genetic Coefficient of Variation (CVA) ranged from 8.03 to 33.49. Except for empty grains per panicle, estimates of the narrow sense heritability ( $h^2$ ) of the other 7 traits were moderate to high ( $h^2 = 0.42-0.90$ ). The results suggested that genetic variation for these traits was largely controlled by additive genes. Association of grain yield per plant with filled grains per plant, grain per panicle, seed setting rate and 1000-grain weight was positive and significant. Overall, there was high genetic variation which promised high rate of arising elite transgressive individuals and moderate heritability for most traits in the populations tested. It is feasible and efficient to use wild rice abundant genetic diversity to develop rice cultivars for high yield.

**Keywords:** Breeding, genetic variation, heritability, rice (*Oryza sativa* L.), wild rice (*Oryza rufipogon* Griff.)

### INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important food crops and feeds more than half the world's population. The world population will reach 8 billion by 2030 and rice production must increase by 60% from a base of 1995 to meet the demand of increasing population (Yuan, 2005). Genetic diversity is the basis of rice breeding to develop high yield and quality cultivars. The development of new modern rice varieties has depended on the continued availability of genetic diversity (Jackson and Juggan, 1993; Bellon *et al.*, 1998). However, the widespread adoption of Modern Varieties (MVs), use of fertilizer and pesticide, development of irrigation and increasingly market-oriented production have contributed to the loss of genetic diversity, known as genetic erosion (Tripp and Vander Heide, 1996; Tanksley and McCouch, 1997). Loss of genetic diversity has been one limitation for the hybrid rice production (Xu *et al.*, 2003). Maintenance of broad genetic diversity is important to explore elite genes resources to develop high yielding, good quality and multi-resistant rice cultivars and so ensures high and stable rice production.

The main source of genetic diversity of Modern Varieties (MVs) is the traditional varieties that have been grown and selected for generations (Bellon *et al.*,

1998). However, wild species are considered as a unique source of genetic variation. Common wild rice (*Oryza rufipogon* Griff.) is the ancestor of cultivated rice (*O. sativa* L.). (Second, 1982; Oka, 1988; Wang *et al.*, 1992). During the course of domestication from wild rice to cultivated rice, many genes were lost through natural and human selection, leading to the lower genetic diversity of the cultivated rice (Sun *et al.*, 2001; Caicedo *et al.*, 2007; Zhu *et al.*, 2007). It is feasible to explore and utilize the gene resources in wild rice to solve the problem faced in modern rice production. The most successful examples are the use of genes from *Oryza nivara* to breed rice varieties with long-lasting resistance to grassy stunt virus (Khush *et al.*, 1977; Plucknett *et al.*, 1987) and the use of wild abortive cytoplasmic male sterility from *O. spontanea* to hybrid rice (Li and Zhu, 1988). Despite these successes, some progress has been made in molecular breeding of wild rice germplasm resources for the improvement of quantitatively inherited traits, such as yield (Xiao *et al.*, 1998). Xiao *et al.* (1998) identified two QTLs that could increase yield by 17 and 18% from *O. rufipogon*. By analysis of the genotype of the BC<sub>4</sub> generation derived from *O. rufipogon*, Li *et al.* (2002) identified two QTLs, which could increase yield by 25.9 and 23.3%, respectively. However, agriculturally desirable alleles are present in low

frequency and linked with unfavorable genes, so utilization of those QTLs identified from wild rice by molecular analysis might result in transfer of undesirable traits (Moncada *et al.*, 2001; Septiningsih *et al.*, 2003; Xiao *et al.*, 1998; Sabu *et al.*, 2009). Hence it is important to understand the genetic variation, heritability and correlations of agronomically important traits in the lines derived from cross between wide rice and cultivated rice before any further utilization of wide rice germplasm in breeding program.

Common wild rice (*Oryza rufipogon* Griff.) DP3 is a perennial form, originated from Guangxi, where wild rice in China is most diverse (Vaughan *et al.*, 2008). It grows to a height of approximately 143 cm and shows extremely high tillering ability, with high resistance to Bacterial Leaf Streak (BLS) (He *et al.*, 2012). *Oryza sativa* ssp. *indica* cv. 93-11 is an elite cultivar in china and is the paternal cultivar of a "super" hybrid rice Liang-You-Pei-Jiu (LYP9), which increase yield by 20 to 30% yield per hectare over the other rice crops in cultivation (Li *et al.*, 2011). The objectives of the present research were:

- To estimate the genetic variation of the springs derived from the cross between *Oryza sativa* and *O. rufipogon*
- Evaluate the heritability which is important for traits under selection
- Analyze the correlations between traits to know whether selection for one trait will have an effect on another

## MATERIALS AND METHODS

**Plant materials:** A cross was made between wild rice (*Oryza rufipogon* Griff.) DP3 and elite cultivar 93-11 to produce F<sub>1</sub> hybrid seed in 2009. In the early crop season of 2010, one F<sub>1</sub> seed was planted and self crossed to produce a F<sub>2</sub> population. In the late crop season of 2010, the F<sub>2</sub> population was planted and a random sample of 156 F<sub>2</sub> individuals was taken to harvest the seed of F<sub>2:3</sub> families. Field experiment was conducted at Nanning region, Guangxi province, China. All F<sub>2:3</sub> families, parents DP3 and 93-11 were planted in 2011, using completely randomized trial (Kearsey and Pooni, 1996). Five plants of each F<sub>2:3</sub> families were collected as sample and panicle length, panicles per plant, filled grains per plant, empty grains per plant and grains per panicle, seed setting rate, 1000-Grain Weight, g (GW) and grain yield per plant were evaluated.

**Analysis of Variance (ANOVA):** One way Analysis of Variance (one-way ANOVA) for all traits was

Table 1: Analysis of variance on individual plants of F<sub>2:3</sub> families

| Source           | df      | Mean square     | Expected mean squares      |
|------------------|---------|-----------------|----------------------------|
| Between families | n-1     | MS <sub>B</sub> | $\sigma_w^2 + r\sigma_B^2$ |
| Within families  | n (r-1) | MS <sub>W</sub> | $\sigma_w^2$               |

n & r: Number of F<sub>2:3</sub> families and individual plants per family, respectively

performed to test differences between F<sub>2:3</sub> families using the model given by Kearsey and Pooni (1996) (Table 1).

**Narrow-sense heritability:** As advised by Kearsey and Pooni (1996), four parameters, namely VA (Additive genetic Variance), VD (Dominant genetic Variance), VEC (Common Environmental Variance of families) and VE (Environmental Variance within families) should be used to estimate the genetic and environmental variance of the F<sub>2:3</sub> populations. However, there are only two statistics (Table 1),  $\sigma_w^2$  and  $\sigma_B^2$ , so it is necessary to ignore two parameters VD and VEC (Kearsey and Pooni, 1996). In ANOVA of F<sub>2:3</sub> generation (Table 1), the Mean Squares variance between (MS<sub>B</sub>) and the within (MS<sub>W</sub>) families had expectations  $\sigma_w^2 + r\sigma_B^2$ ,  $\sigma_w^2$ , respectively. Using MSB and MSW, the expectations  $\sigma_w^2$  and  $\sigma_B^2$  were calculated as  $\sigma_w^2 = MS_W$ ,  $\sigma_B^2 = (MS_B - MS_W) / r$ , then the parameters VA and VE were evaluated as  $VA = \sigma_B^2$ ,  $VE = \sigma_w^2 - \frac{1}{2}VA$ , finally, the narrow-sense heritability of the trait tested was estimated as follow:  $h^2 = VA / (VA + VE)$  (Kearsey and Pooni, 1996). Number of families and number of individual plants per family are shown by r and n, respectively.

**Correlations and genetic variation analysis:** Correlation between yield and yield related traits was calculated at two probability levels (p<0.05 and p<0.01) in Microsoft Excel. Coefficient of Additive genetic Variation (CVA) was computed by using formula:  $CVA = 100 \sqrt{V_A} / \bar{X}$  (Houle, 1992). VA had been calculated above and  $\bar{X}$  was the mean of the trait test.

## RESULTS AND DISCUSSION

### Results:

**Assessment of yield and its related traits:** There were highly significant differences for yield and all yield related traits between families (Table 2). The panicle length of DP3 and 93-11 was 21.20 and 23.20 cm, respectively (Table 2). In F<sub>2:3</sub> families, the panicle length ranged from 20.0 to 29.7 cm, with an average of 23.63 cm (Table 2). The panicle length of about 62% families ranged from 20.0 to 24.0 cm and about 8% ranged from 28.0 to 30.0 cm with extremely transgressive showing (Fig. 1a).

Table 2: Statistics, analysis of variance, Additive genetic Variation (CVA) and narrow-sense heritability ( $h^2$ ) of yield and yield related traits

| Traits | Parent  |        | F <sub>2,3</sub> families |      |        | Mean squares    |                 | F value | CVA   | h <sup>2</sup> |
|--------|---------|--------|---------------------------|------|--------|-----------------|-----------------|---------|-------|----------------|
|        | DP3     | 93-11  | Min.                      | Max. | Mean   | MS <sub>B</sub> | MS <sub>w</sub> |         |       |                |
| PL     | 21.20   | 23.20  | 20.0                      | 29.7 | 23.63  | 13.65           | 2.84            | 4.81**  | 8.03  | 0.77           |
| PPL    | 30.00   | 7.00   | 5                         | 21   | 12.21  | 40.03           | 13.08           | 3.06**  | 28.36 | 0.62           |
| FGP    | 1308.60 | 964.44 | 318                       | 1830 | 898.69 | 344702.20       | 72939.62        | 4.73**  | 33.49 | 0.77           |
| EGP    | 603.90  | 180.63 | 64                        | 1068 | 257.46 | 39826.92        | 29711.79        | 1.34**  | 22.55 | 0.11           |
| GP     | 63.75   | 163.58 | 30                        | 231  | 106.82 | 4046.37         | 1557.67         | 2.60**  | 26.96 | 0.42           |
| SSR    | 68.42   | 84.23  | 22                        | 89   | 77.03  | 0.03            | 0.01            | 3.00**  | 10.80 | 0.42           |
| GW     | 19.20   | 29.10  | 12.6                      | 34.1 | 21.57  | 27.94           | 4.70            | 5.94**  | 12.91 | 0.90           |
| PY     | 25.10   | 28.05  | 6.2                       | 36.6 | 19.31  | 145.65          | 48.87           | 2.98**  | 29.41 | 0.50           |

PL: Panicle Length, cm; PPL: Panicles Per Plant; FGP: Filled Grains per Plant; EGP: Empty Grains per Plant; GP: Grains per Panicle; SSR: Seed Setting Rate, percent; GW: 1000-Grain Weight, g; PY: grain yield per plant, g; \*: Significant at  $p < 0.05$ ; \*\*: Significant at  $p < 0.01$

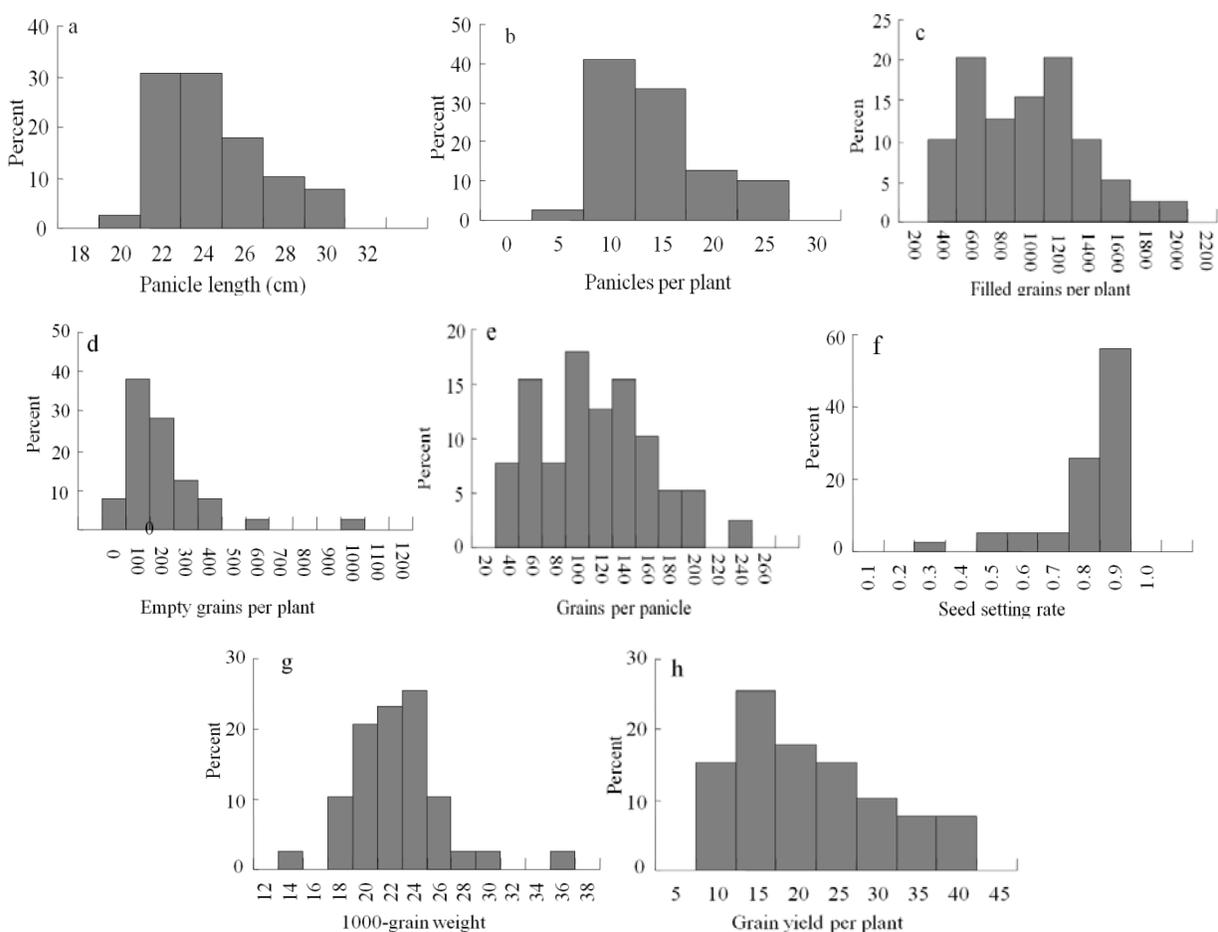


Fig. 1: Frequency distribution of agronomic traits in F<sub>2,3</sub> families derived from the cross between *Oryza sativa* (93-11) and *O. rufipogon* (DP3), (a) panicle length, (b) panicles per plant, (c) filled grains per plant, (d) empty grains per plant, (e) grains per panicle, (f) seed setting rate, (g) 1000-grain weight, (h) grain yield per plant

A large gap existed between DP3 (30.00) and 93-11 (7.00) for panicles per plant. The panicles per plant of about 74% families ranged from 5 to 21 (Fig. 1b), with an average of 12.21 (Table 2). No transgressive individual was observed in the F<sub>2,3</sub> families for panicles per plant.

The filled grains per plant of DP3 were 1308.60 and that of 93-11 were 964.44 (Table 2). In the F<sub>2,3</sub>

families, filled grains per plant ranged from 318 to 1830 in large amplitude with an average of 898.69 (Table 2). From the frequency distribution, about 10% families got this index ranging from 1400 to 2200 with extremely transgressive exhibition (Fig. 1c).

Empty grains per plant did not show a continuous distribution (Fig. 1d). The grains per panicle of DP3 (63.75) was much smaller than that of 93-11 (163.58)

(Table 2). In the  $F_{2:3}$  families, the grains per panicle ranged from 30 to 231, with an average of 106.82 (Table 2). For grains per panicle, about 8% families ranged from 180 to 240 with extremely transgressive showing too (Fig. 1e).

The frequency distribution of seed setting rate was not continuous and about 82% individuals ranged from 0.7 to 0.9 (Fig. 1f). The 1000-grain weight of DP3 (19.20 g) was much smaller than that of 93-11 (29.1 g) (Table 2). In the  $F_{2:3}$  families, 1000-grain weight ranged from 12.6 to 34.1 g, with an average of 21.57 g. The frequency distribution of 1000-grain weight was not continuous too and about 3% families ranged from 18.0 to 26.0 g with transgressive exhibition (Fig. 1g).

The grain yield per plant of DP3 was 25.10 g and that of 93-11 was 28.05 g (Table 2). In the  $F_{2:3}$  families, grain yield per plant ranged from 6.2 to 36.6 g in large amplitude with an average of 19.31 g (Table 2). The frequency distribution of grain yield per plant demonstrated almost normal distribution and about 15% families ranged from 30.0 to 40.0 g with transgressive showing (Fig. 1h).

**CVA and narrow-sense heritability:** The Coefficient of Additive genetic Variation (CVA) of yield and yield related traits are shown in Table 2. Filled grains per plant got the highest CVA of 33.49. Panicles per plant and grain yield per plant had similar CVA of 28.36 and 29.41, respectively. The CVA of empty grains per plant and grains per panicle was 22.55 and 26.96, respectively. Seed setting rate, 1000-grain weight and panicle length had low CVA of 10.80, 12.91 and 8.03, respectively.

As shown in Table 2, 1000-grain weight had the highest narrow-sense heritability ( $h^2$ ) of 0.90. Panicle length and filled grains per plant had the same  $h^2$  of 0.77. Panicles per plant had moderate  $h^2$  of 0.62. The  $h^2$  of grain yield per plant was 0.50. Grains per panicle and seed setting rate had the same low  $h^2$  of 0.42. Empty grains per panicle had the lowest  $h^2$  of 0.11.

**Correlations:** Association of grain yield per plant with FGP, GP, SSR and GW was positive and significant (Table 3). SSR was significantly and positively associated with FGP and EGP (Table 3). Association of Grains per Panicle (GP) with PPL and FGP was also positive and significant (Table 3). There were no significant correlations among all other traits measured (Table 3).

**Discussion:**

**Transgressive segregation:** *O. rufipogon* is abundant in genetic diversity and some beneficial alleles for

Table 3: Correlation coefficient between traits in  $F_{2:3}$  families

| Traits | PL | PPL    | FGP   | EGP    | GP       | SSR      | GW     | PY      |
|--------|----|--------|-------|--------|----------|----------|--------|---------|
| PL     | 1  | -0.148 | 0.167 | -0.051 | 0.144    | 0.165    | 0.132  | 0.195   |
| PPL    |    | 1      | 0.300 | -0.088 | -0.633** | 0.173    | -0.094 | 0.205   |
| FGP    |    |        | 1     | 0.019  | 0.353*   | 0.503**  | -0.054 | 0.920** |
| EGP    |    |        |       | 1      | -0.157   | -0.804** | -0.091 | -0.027  |
| GP     |    |        |       |        | 1        | -0.157   | -0.005 | 0.354*  |
| SSR    |    |        |       |        |          | 1        | 0.067  | 0.508** |
| GW     |    |        |       |        |          |          | 1      | 0.316*  |
| PY     |    |        |       |        |          |          |        | 1       |

PL: Panicle Length, cm; PPL: Panicles Per Plant; FGP: Filled Grains per Plant; EGP: Empty Grains per Plant; GP: Grains per Panicle; SSR: Seed Setting Rate, percent; GW: 1000-Grain Weight, g; PY: grain yield per plant, g; \*: Significant at  $p > 0.05$ ; \*\*: Significant at  $p < 0.01$

diverse quantitative traits including grain size, grain weight, grain yield, grain quality, cold tolerance, aluminum tolerance and flowering time have been found in *O. rufipogon* (Kovach and McCouch, 2008). Therefore, crosses between high-yielding/low-diversity elite cultivars and low-yielding/high-diversity wild accessions often give rise to superior offspring, with wild alleles conferring increased performance in the context of the elite cultivar genetic background. So, wild germplasm has been considered as a resource for capturing positive transgressive segregation (Xiao *et al.*, 1998; Septiningsih *et al.*, 2003; Kovach and McCouch, 2008; Sabu *et al.*, 2009). In the present study, for yield, superior offspring had been found in the  $F_{2:3}$  families with grain yield per plant of 36.6 g. Transgressive segregation were observed for most traits tested. High rate of transgressive segregation mean that it is feasible to breeding elite high yield rice cultivars from the offspring derived from the cross between *Oryza sativa* and *O. rufipogon*.

**Genetic diversity:** Genetic diversity is very important for transgressive breeding and the efficiency of transgressive breeding is usually positively associated with the genetic distance between the parents. In the present study, high genetic variation (29.41) was observed in the  $F_{2:3}$  families for grain yield per plant. Genetic variation of the present study is similar with that of offspring derived from the cross between *Oryza sativa* and *O. rufipogon* in previous researches (Jing *et al.*, 2011; Sudhakar *et al.*, 2012) and much higher than that of offspring of *Oryza sativa* or derived from the cross between cultivated rice in early reports (Ravindra Babu *et al.*, 2012; Sangam *et al.*, 2011; Priyanka *et al.*, 2010; Padmaja *et al.*, 2008). More genetic diversity between two parents could produce higher genetic variation in their offspring and higher genetic variation mean higher rate of transgressive segregation arising in the offspring populations.

**Heritability in offspring derived from *O. rufipogon*:** In breeding practice, the breeder should concern about

heritability, especially narrow-sense heritability, which is an important parameter to estimate the genetic advancement of relative traits under selection. By now, few jobs have been conducted to illustrate the narrow-sense heritability of yield and its relative traits in the offspring population derived from the cross between *Oryza sativa* and *O. rufipogon*. In the present research, except for empty grains per panicle, estimates of the narrow sense heritability ( $h^2$ ) of the other 7 traits were moderate to high ( $h^2 = 0.42-0.90$ ). The results suggested that genetic variation for these traits was largely controlled by additive gene. It is effective to select these traits in breeding practices.

**Correlations between traits tested:** As Table 3 showed, grain yield per plant had the highest correlation coefficient with filled grains per plant. So in high yield breeding of *O. rufipogon* utilization, among all yield related traits, filled grains per plant should be the most important traits to be considered. Grain yield per plant has the second large correlation coefficient with seed setting rate. The other traits, grains per panicle and 1000-grain weight were positively associated with grain yield per plant.

### CONCLUSION

High genet variation was observed in the offsprings derived from the cross between *Oryza sativa* and *O. rufipogon* and great genetic variation promises high rate of arising transgressive segregation. It is efficient to make selection for yield and most yield related traits. In high yield breeding of *O. rufipogon* utilization, filled grains per plant should be the most important traits to be considered. It is efficient and feasible to utilize *O. rufipogon* abundant genetic diversity to develop rice cultivars for high yield.

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