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Population Structure of *Oryza alta* Swallen as a Potential Wild Rice Variety for Cultivated Rice Improvement

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ABSTRACT

Background: *O. alta* is an important wild rice harboring many elite genes. However, the wild rice belongs to the allotetraploid (CCDD) and has a complex genetic background. It is difficult to utilize its genetic resource in rice breeding. Therefore, it is necessary to systematically observe the segregation (population structure) of the offspring of its inbred population, to provide a better basis for further gene editing and selection.
Methods: Total of 371 plants from self-bred offspring of *O. alta* which were planted in the experimental field of South China Agricultural University were employed in which the main agronomic traits, of *O. alta* were investigated. Population structure of self-crossed ten generations of *O. alta* was conducted by investigating about ten agronomic traits of this wild rice.
Result: My investigation found significant differences in most of the main agronomic traits that include reduction in plant height, confirming the consistence of *O. alta* new line compared to the conventional breed. Furthermore, the experiment observed strong panicles, thicker and wider leaves in new lines as compared to typical wild and cultivated rice, approving that populating the structure of *O. alta* will surely allow the study of genomic DNA variations and pave way to DNA analysis of wild rice line using re-sequencing.

Key words: Breeding, *Oryza alta* swallen, Population, Structure.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. About half of the world's population uses rice as a staple food especially developing countries (Zhang *et al.,* 2020). Rice accounts for about 40% of the total grain output in China (Zhang *et al.,* 2018; Chaudhar *et al.,* 2018). It's important for human health as it contains bioactive compounds, including minerals and vitamins (Fukagawa and Ziska, 2019) and provide about 20% of the world's dietary energy needs (Cordero-Lara, 2020), while providing 76% of the calorific intake of the population of Southeast Asia (Bita and Gerats, 2013). Rice farmers feed and have fed more people than any other important cereal crop like wheat, barley and maize (Mohapatra and Sahu, 2021). Therefore, domestication of rice became one of the most crucial developments analogous to human civilization culture and food habit. With the continuous growth of the world's population and the continuous development of the economy (Zheng *et al.,* 2019), the requirements for the quality of human life have risen sharply (Malik, 2018) the relative arable land area has decreased (Fei *et al.,* 2021; Xie *et al.,* 2018; Li *et al.,* 2017) and the ecological environment has deteriorated (Jiang *et al.,* 2021; Zhou *et al.,* 2021) making the food scarcity prominent and attracted courtesy from all walks of life (Guo *et al.,* 2022). Furthermore, the acceleration of urbanization has caused imbalance between people and land in urban area (Zhai *et al.,* 2021; Peng *et al.,* 2023) hence demand for land increased with pressure on land resources, which led to declining crop production. Competition for land and how to use limited land resources to meet people's needs for food has become a challenge for agricultural scientific research. The increasing world population needs higher rice productivity (Yu *et al.,* 2018).

Crop improvements helps us to meet the challenge of feeding a population by breeding better varieties as faster as we can. Identification of traits contributing towards genetic diversity in each population can help in formulating effective selection criteria (Thakur and Sarma, 2023). Technologies such as genotyping, marker-assisted selection, high-throughput phenotyping, genome editing, genomic selection and de novo domestication could be galvanized by using speed breeding to enable plant breeders to keep pace with a changing environment and ever-increasing human population (Hickey *et al.,* 2019).

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The development of molecular markers combined with high throughput technologies have paved the way for achieving the desirable traits as well as induced biotic and abiotic stress tolerance in plant, which enhanced the crop breeding (Nair and Pandey, 2024). To address widespread malnutrition influencing global health, novel high-yielding rice cultivars with better nutritional quality need to be bred (Rana *et al.,* 2020). Rice is a salt-susceptible crop and, so improving the salt tolerance of rice would increase the potential of saline-alkali land and ensure food security (Qin *et al.,* 2020). Therefore, research on how to improve rice yield, quality, tolerance and resistance has become an important task for rice breeders (Peng *et al.,* 2009; Shabir *et al.,* 2017; Kumar *et al.,* 2020; Khan *et al.,* 2021) aiming to increase food production, alleviate poverty and for economic emancipation.

Cultivated rice (Oryza sativa L.) has very low genetic diversity (Qi *et al.,* 2006, Zhou *et al.,* 2022, Hour *et al.,* 2020). So far, a few wild rice landraces have been identified as a source gene transfer and utilized in rice improvement (Zhang *et al.,* 2019). The resistance of wild rice species and its gene assortment are due to the complex geographic environment and various ecological factors of wild rice; in which varieties of excellent characteristics have been formed (Nautiyal *et al.,* 2021; Shamim *et al.,* 2022). The history of using the beneficial genes of wild rice for breeding is long overdue. In 1930s, Ding Ying used Guangdong common wild rice as parents to bred “Zhongshan No. 1”, a production species with strong cold tolerance and stress resistance (Ding *et al.,* 2021, Liu *et al.,* 1998). In the 1970s, China used various ecological types of common wild rice to hybridize with cultivated rice (Guo *et al.,* 2016). At present, more than 95% of the sterile lines used in the hybrid combination in rice production are of wild type or wild type cytoplasm (Liao 2021; Xu *et al.,* 2022) with more than 20 excellent traits been identified in wild rice, mainly for disease and insect resistance, stress resistance, excellent rice quality and cytoplasmic male sterility (Sangeetha *et al.,* 2020; Huang and Liu, 2022; Long *et al.,* 2023). At the same time, wild rice is used in breeding system for strong growth advantages such as strong tillering ability (Singh *et al.,* 2022), fast growth (Varshney *et al.,* 2021), root system development (Bheemanahalli *et al.,* 2019, Panda *et al.,* 2021), strong regeneration ability (Yu *et al.,* 2021; Zhang *et al.,* 2023), functional leaf senescence resistance (Shi *et al.,* 2023), large anthers (Cao *et al.,* 2022), exposed stigma (Zheng *et al.,* 2020), long flowering time (Zsögön *et al.,* 2022) and wide compatibility (Zheng *et al.,* 2020; Peng *et al.,* 2021).

The realm of genetic diversity within rice is immense and undermining it provides the opportunity to utilize them in rice improvement programs (Jegadeeswaran *et al.,* 2024). Understanding genetic diversity of the wild species would help implementing the conservation practices measures (Gouda *et al.,* 2020; Wambugu and Henry, 2022). Zhang *et al.* (2021) further concluded that studying the population structure of wild rice will shed light to its effective conservation and utilization. Wild rice is an important part of rice germplasm resources (Liu *et al.,* 2021). Therefore, its domestication will give rise to desirable agronomic traits (He *et al.,* 2021). It has excellent characters that are not available in cultivated rice (Civan *et al.,* 2018; Gupta *et al.,* 2021). Due to its wide distribution and the complexity of the ecological environment, wild species formed strong genetic diversity during the long evolutionary process (Lakew *et al.,* 2021; Li *et al.,* 2023) hence at present, disease resistance, insect resistance, cold tolerance, heat tolerance and others have been discovered from wild rice. The natural community of wild rice has been lost in large quantities due to human inhibition (Lv *et al.,* 2018; Wang *et al.,* 2021; Yao *et al.,* 2022) and thus loss of wild rice germplasm resources worldwide is thoughtful. Protection of wild rice is of predominantly important and studies on wild rice are necessary to intensify yield, conserve and protect wild rice species. Therefore, the structure of *O.alta* must be populated to understand the main agronomic traits of this wild rice and enable the utilization and conservation of wild relatives elite genes.

The purpose of this study is to systematically observe the segregation (population structure) of *O. alta* offspring and its inbred lines to provide improved base for advanced gene editing and selection using of *O. alta* wild rice. The study is highly significant as it is only if the rice structure is populated and studied that genomic DNA variations of the wild rice line will be analyzed using re-sequencing.

MATERIALS AND METHODS

Materials

Total of 371 plants from self-bred offspring of *O. alta* which were planted in the experimental field of South China Agricultural University were used in the experiment.

Character investigation and statistical methods

The methods and standards of character investigation refer to the DUS testing guidelines for new plant varieties in the People’s Republic of China was used as a testing. The investigated traits included: (1) Plant height; (2) Panicle length; (3) Panicle number; (4) Grain length and width, the ratio of grain length to width is obtained; (5) Filled and empty panicle; (6) Seed setting rate; (7) Total grains; (8) Flag leaf length and width and so on. The obtained data were statistically analyzed by SPSS version 17.0 software and Microsoft Excel 2003.

RESULTS AND DISCUSSION

Analysis of main agronomic traits in self-crossing generation of *O. alta*

Significant differences between *O. alta* and *O. sativa* in plant height, leaf, panicle and other traits (Fig 1 to 5) were observed. As shown in Table 1, the main agronomic characters are different among plants from the self-crossing generation of *O. alta*. Plant height of *O. alta* ranged from 1.97 m to 3.03 m with an average height of 2.39 m. Among the tested plants, 46% of the plants were taller than 2.0 m in (Fig 1). The panicle number was distributed in the range of 4 to 21 and more than 74% of total plants obtained 6 to 15 panicles. The panicle length ranged between 34.5 cm to 113 cm with more than 89% ranging at 50-110 cm. The length of flag leaf of *O. alta* ranged from 31 cm to 58.7 cm and more than 82% were between 35-555 cm. The width of flag leaf ranged from 3.6 cm to 6.25 cm with more than 86% ranging between 3.6 cm to 5.5 cm. The flag leaf length/width (ratio) of *O. alta* ranged from 5.38 to 12.35 cm and more than 72% plants were between 7.5 to 10.5 cm.

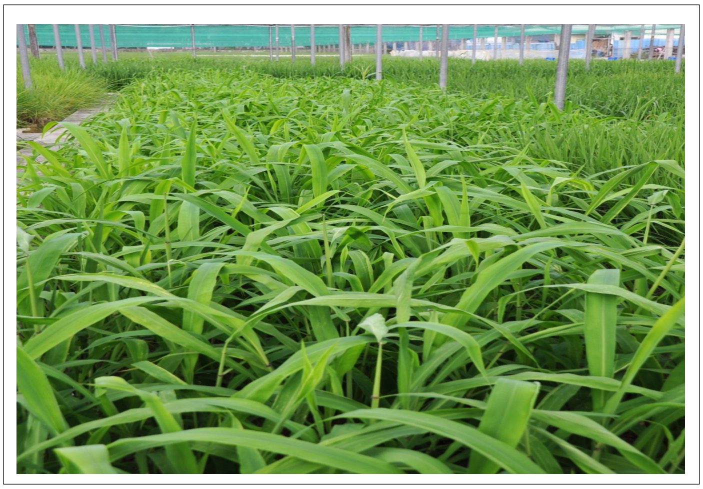

Fig 1: Plants of *O. alta* before flowering.

Fig 2: Plants of *O. alta* during flowering.

Fig 3: Plants of *O. alta* during flowering (Collecting seeds).

As presented in Table 1, the number of filled grains of *O. alta* ranged from 4 to 2017.5, while empty grains ranged from 235 to 1700. The total grains of *O. alta* ranged from 299.5 to 2497.5, with majority at the range of 500 to1800 grains. The seed setting rate ranged from 6 to 76%, with average 18.5% (Fig 5). More than 87% had their seed setting less than 20%. The grain length of *O. alta* ranged from 6.22 mm to 8.69 mm and more than 86% were at the range of 7.0 mm to 8.0 mm. The grain length/width (ratio) of *O. alta* were distributed in the range of 2.55 to 3.91 mm and more than 91% ranged 2.75 to 3.5 mm. The pollen fertility of the wild rice ranged from 0.25% to 84%, with average 27.51%.

Analysis of main agronomic traits of self-crossed 10th generation in *O. alta*

The main agronomic traits were investigated in the plants to the 10th self-bred generation of *O. alta*. The results showed that the panicle characters of *O. alta* separated after continuous self-bred for several generations, however, their characters remained consistent.

As presented in Table 1, panicle length variation ranged were 34.5~57.1 cm. The varying ranges of awn length were 14.7~51.0 mm. The variation ranges of effective panicle number were 2~9. The variation of flag leaf length was 30.0~79.8 cm. The variation of flag leaf width was 3.6~6.1 cm. The variations in filled grain number were ranged from 20 to 1947. The variation of pollen fertility was 0.3%~84.4%, the average pollen fertility is 27.5%. The variation of the seed setting rate range was 21.2%~72.3%, the average

Fig 4: Plants of *O. alta* (providing support).

Fig 5: Seeds of *O. alta.*

Table 1: The main agronomic traits of *O. alta.*

Traits	Mean ± S.E.	Minimum value	Maximum value
Plant height (m)	2.39±0.01	1.97	3.03
No. of panicle	8.64±0.25	4.00	21.00
Panicle length (cm)	67.90±0.82	34.50	113.00
Flag leaf length (cm)	41.98±0.27	31.00	58.75
Flag leaf width (cm)	4.61±0.02	3.60	6.25
Flag leaf length/width (ratio)	9.21±0.09	5.38	12.35
Penultimate leaf length (cm)	39.87±0.21	32.30	52.50
Penultimate leaf width (cm)	4.53±0.03	3.25	5.85
Penultimate leaf length/width (ratio)	8.91±0.08	7.08	13.12
Antepenultimate leaf length (cm)	46.57±0.31	34.50	58.00
Antepenultimate leaf width	4.99±0.03	3.85	6.25
Antepenultimate leaf length/width (ratio)	9.42±0.07	7.22	12.15
Filled grains	201.55±13.18	14.00	2017.50
Empty grains	768.75±17.47	235.00	1700.00
Total grains	970.30±24.03	299.50	2497.50
Seed set rate	18.50±0.40	6.00	76.00
Grain width (mm)	2.51±0.01	2.08	2.85
Grain length (mm)	7.75±0.03	6.22	8.69
Grain length/width (ratio)	3.12±0.02	2.55	3.91
Pollen fertility (%)	27.51±21.69	0.25	84.35
Grains weight per panicle (g)	2.97±0.97	1.58	4.51

seed setting rate is 52.9%. The variation of grain weight per panicle range was 1.6~4.5 g, with average 3.0 g.

Correlation analysis of main agronomic characteristics of *O. alta* population

As shown on Table 2, plant height positively correlated with grain quantity, total grains and seed setting rate with correlation coefficients variation of 0.43, 0.40 and 0.47, respectively. At the same time, plant height negatively correlated with grain length and width with coefficient variation of 0.19 and -0.20 respectively.

Consistency analysis of *O. alta* G7

After self-crossing *O. alta* to the10th generation, a line with outstanding characters was selected from its offspring and named G7.

Consistency analysis of heading date of *O. alta* G7

To further investigate G7 traits thoroughly, four lines were selected from the self-crossing off springs of *O. alta* G7 (G7-1, G7-2, G7-3, G7-4). According to the observations made within four new lines, significant differences were observed in the heading date. G7-1 took 19 days from the beginning of flowering to the end. G7-3 took 11 days, G7-4 took 5 days and G7-2 took 15 days from the beginning of flowering to the end.

Consistency analysis of panicle traits of *O. alta* G7

The effective panicle number variation coefficient of G7-1 is 0.26, G7-3 is 0.26 and the variation coefficient of G7-4 is 0.30 as shown in Table 3. The variation ranges of effective ear number of G7-1, G7-3, G7-4 and G7-2 are: 2-6, 2-6, 2-

Table 2: Correlation analysis of main agronomic characters of *O. alta* population.

Traits	Filled grain	Empty grains	Total grains	Seed setting	Grain length	Grain width
Plant height	0.42**	0.05	0.40**	0.47**	-0.19*	-0.20*
Panicle number	0.02	0.29**	0.30**	-0.06	-0.00	0.05
Panicle length	0.21**	0.06	0.23**	0.20**	-0.12	-0.07
Flag leaf	0.03	-0.11	-0.09	0.07	0.13	0.02
Flag leaf width	0.10	0.11	0.14	0.08	0.26**	0.20*
Flag leaf length/width	-0.05	-0.20*	-0.19*	-0.01	-0.13	-0.18*

Table 3: Variation parameters of effective panicle number of *O. alta* G7.

Strain	Mean	Standard deviation	Minimum	Maximum	Range	Coefficient of variation
G7-1	4.13	1.06	2.00	6.00	4.00	0.26
G7-3	3.56	0.92	2.00	6.00	4.00	0.26
G7-4	4.20	1.27	2.00	8.00	6.00	0.30
G7-2	4.62	1.65	2.00	9.00	7.00	0.36

Table 4: Variation parameters of real grain number of *O. alta* G7.

Strain	Mean	Standard deviation	Minimum	Maximum	Range	Coefficient of variation
G7-1	242.05	310.65	100.00	2047.00	1947.00	1.28
G7-3	178.00	45.56	86.00	281.00	195.00	0.26
G7-4	289.80	107.55	20.00	611.00	591.00	0.37
G7-2	198.62	86.72	30.00	375.00	345.00	0.44

Table 5: Variation parameters of empty grain number of *O. alta* G7.

Strain	Mean	Standard deviation	Minimum	Maximum	Range	Coefficient of variation
G7-1	42.79	21.03	9.00	103.00	94.00	0.49
G7-3	36.44	30.61	9.00	164.00	155.00	0.84
G7-4	67.60	89.91	9.00	390.00	381.00	1.33
G7-2	101.90	68.87	18.00	332.00	314.00	0.68

Table 6: Variation parameters of seed setting rate of *O. alta* G7.

Strain	Mean %	Standard deviation	Minimum%	Maximum%	Range%	Coefficient of Variation
G7-1	81.80	8.43	60.81	98.02	37.21	0.10
G7-3	83.42	10.12	53.01	95.54	42.54	0.12
G7-4	83.49	18.65	4.88	96.78	91.91	0.22
G7-2	65.31	22.01	12.77	93.00	80.23	0.34

8 and 2-9 respectively. The results indicated that the number of effective panicles in the offspring of *O. alta* G7 did not differentiate and the number of effective panicles in the plant line was small.

Consistency analysis of filled grain number, empty grain number and seed setting rate of *O. alta* G7

Coefficient of variation of G7-1 filled grain number is 1.28, G7-3 is 0.26, G7-4 is 0.37 and G7-2 is 0.44. G7-1, G7-3, G7-4 and G7-2 ranged from -105 real grains are 100~1947, 86~281, 20~611 and 30~375 (Table 4). The results indicated that the number of seeds in the progeny lines of *O. alta* G7 varied with low consistency and significant difference was observed between the lines.

Table 5 shows that the coefficient of variation of G7-1 empty grain number is 0.49, G7-3 is 0.84, G7-4 is 1.33 and G7-2 is 0.68. G7-1, G7-3, G7-4 and G7-2 filled grains ranged 9~103, 9~164, 9~381 and 18~332, respectively. The number of empty seeds in the G7 progeny line of *O. alta* varied with pitiable consistency and differentiation occurred in the line.

The coefficient of variation of G7-1 seed setting rate is 0.10, G7-3 is 0.12, G7-4 is 0.22 and G7-2 is 0.34. G7-1, G7-3, G7-4 and G7-2 seed setting rate raged from 60.81~98.02%, 53.01~95.54%, 4.88~96.78% and 12.77~93%, respectively (Table 6). Furthermore, seed setting rate in the offspring lines of *O. alta* G7 varied with poor consistency and differentiation occurred between lines with seed setting rates of G7-1 and G7-3 above 50%.

The result indicated reduction in plant height, confirming the consistence of *O. alta* new line compared to conventional breed. Taller plants hardly stand strong on their own especially at maturity when the seeds are set, therefore decrease in plant height of *O. alta* in this experiment is considered a milestone achievement and it will be needless to provide support to plants at maturity. Furthermore, the experiment observed strong panicles, thicker and wider leaves in new lines as compared to typical wild and cultivated rice. Thicker and wider leaves are good for plant photosynthesis. However, the seed set rate observed was very low and discouraging as well high shattering rate experienced. Therefore, further research on this wild rice will be fruitful.

CONCLUSION

The present study was conducted on 371 plants from self-bred offspring of *O. alta*. Population structure of self-crossed ten generations of *O. alta* was conducted and observations were recorded on the main agronomic traits. The results showed significant differences in most of the main agronomic traits such as reduction in plant height, strong panicles, thicker and wider leaves in new lines with low seed setting as well as high seed-shattering ability in the offspring.

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Conflict of interest

All authors declare that they have no conflict of interest.

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