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Amylose Content and Grain Length of New Rice Transgressive Variants Derived from a Cross Between *O. rufipogon* and Malaysian Rice Cultivar MR219

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***Abstract*— Amylose content is one of the important grain quality properties of rice. A total of 10 new rice genotypes (BC_2F_7 generation) derived from a cross between *O. rufipogon* Griff. accession IRGC105491 and *O. sativa* subspecies *indica* cv. MR219 with high yield were evaluated for amylose content in three environments in Peninsular Malaysia. One of the parents, a popular high yielding Malaysian rice cultivar MR219 was used as a check. Based on the average amylose content across the environments, the genotype G13 showed significantly ($p < 0.05$) different amylose content (23.88 %) in comparison to other genotypes. Two genotypes G13 (25.7%) and G15 (25.6%) were higher than MR219 (25.1%) in terms of amylose content in Sungai Besar environment. There was a positive (r^2=0.018) but no significant correlation between amylose content and grain length.**

Keywords— ***Oryza rufipogon*; *Oryza sativa*;Transgressive variants; Amylose content.**

I. INTRODUCTION

Rice is one of the most important staple leading food crop of more than one-half of the world population with approximately 95% of production in Asia [3]. In general it is marketed as milled grains as opposed to wheat and corn which are processed to yield flour. In Malaysia, rice is the most important food crop and is cultivated in about 660 thousand ha of arable land in the country [9]. Rice production in Malaysia has witnessed an impressive rise in the recent past. Rice quality is a main concern for all people involved in producing, processing and consuming rice, since it influences the nutritional and commercial value of grains. The most important quality components encompass appearance, milling quality, cooking and nutritional quality. Amylose content (AC) is being considered to be one of the most important traits related to the cooking quality of rice [10]. The starch is the major component of rice endosperm and consists of amylose and amylopectin. The amylose is a relatively less- branched α (1,4) – linked glucose polymer [21]. The enzyme granule bound starch synthase l (GBSSl) is required for amylose synthesis, and several alleles are encoded by the *Wx* locus [55], [56]. AC in endosperm is closely correlated with cooking and eating quality of rice and is mainly governed by genetic effects and environmental conditions [29]. AC milled rice is considered as one of the most important quality parameters for predicting rice eating and processing behaviour [17],[25],[34]-[35]. Rice grain length and shape are important to consumers because they determine the physical appearance and affect the cooking quality of the grain. Seed size or weight is important in the evolution of cereal crops because humans tend to select for large seed size during the early domestication process, as evidenced by the fact that most cultivated species have larger seeds than their wild relatives [40]-[41]. Over the last decade, wild species in rice have been successfully utilized for introgression of diverse traits such as cytoplasmic male sterility (cms) [25], [11], abiotic and biotic stress [14],[1], yield and its components [37], [32], and grain quality [28], [2], into modern cultivars. Conventional and molecular breeding have demonstrated that the introgression of *O. rufipogon* gene could add new traits or improve the existing agronomic traits of *O. sativa* such as grain quality [28], [2]. Three wild rice species, *Oryza rufipogon*, *O. longistaminata* and *O. glaberrima*, have been widely used in rice breeding

[7], [33], [38]. *Oryza rufipogon*, known as the ancestor of Asian cultivated rice (*O. sativa*), has been proven to be a valuable gene pool for rice genetic improvement and thus plays a critical role in rice breeding. The aim of this study was to evaluate the relation between high yielding advanced breeding lines for amylose content and grain length in three different environments for cultivar development.

II. Materials and Methods

The wild parent *O. rufipogon* and the high yielding cultivar MR219 were crossed to develop transgressive variants with high yield and nutritional value [26]. The variants used in the present study were selected based on the field performance and pericarp colour in BC_2F_5 and BC_2F_6 generations (Table 1, Fig.1). Paddy-rice samples were obtained from Bumbong Lima, Gurun Kedah and Sungai Besar in Malaysia.

TABLE I

List of the evaluated transgressive variants

Designation	Code
R2-10-18-2-B-B	G4
R6-2-31-2-B-B	G7
R7-6-38-2-B-B	G8
R7-7-39-4-B-B	G9
R14-9-69-2-B-B	G13
R14-9-69-4-B-B	G15
R14-9-69-5-B-B	G16
R17-1-83-3-B-B	G19
R26-2-108-1-B-B	G25
R26-6-113-1-B-B	G26

Fig.1 Grain samples of two parents MR219, *O. rufipogon* and genotype G7

A. Amylose content

Samples of rough rice were hulled with a dehusker machine (Motion Smith Co., Singapore), milled using a milling machine (Satake Co., Japan) and passed through a 500um sieve screen to obtain rice powder. Moisture was determined by drying at 130 oC to constant mass. Amylose content was determined based on ISO method (1&2) [12]-[13]. Briefly rice samples were defat by refluxing with methanol for 6 hours in a soxtec extraction unit. After defatting, the samples were left for two days in the same room to allow evaporation of residual methanol. About 9 ml of sodium hydroxide solution (1 mol/L) and 1 ml ether were added to 0.1 gram of defatted samples. The mixture heated in a boiling water bath for 10 minutes. Samples were cooled down and transferred to a 100 ml volumetric flask. The volume was adjusted with distilled water and mixed. The absorbance was measured using a FIA star (FOSS Co., Sweden) in triplicate.

B. Length

The length of milled rice was measured by randomly picking fifty whole grains and measured with the help of a digital micrometer (Steinmeyer, Germany).

C. Data Analysis

Analysis of variance (ANOVA) was performed for amylose content and grain length using the Generalised Linear Model (GLM) procedure assuming a random effects model and type III sum of squares from SAS version 9.1.3 (SAS Institute Inc. 2003). Duncan's Multiple Range Test ($\alpha = 0.05$) was determined for mean separation using PROC MEANS from SAS version 9.1.3 (SAS Institute Inc. 2003).

III. Results and discussion

Besides AC, grain size is also a highly important quality trait in rice. Although the preference for rice grain characteristics varies with consumer groups, long and slender grain is generally preferred for *Indica* rice by the majority of consumers in the USA and most Asian countries [43]-[44]. There was a positive but not significant correlation between grain size and AC (r^2=0.018) in this study. The relationships between the cooking quality and the appearance quality of rice has been well documented, with some studies having shown a negative correlation between AC and appearance quality trait [47], [43], [50], [45], while other studies have reported positive correlations between these pairwise traits in rice [44]-[46], [48]-[49]. It has been shown that rice grain shape is simultaneously controlled by triploid endosperm genes, cytoplasmic genes, embryo and maternal plant genes [53]-[54] and their genotype × environment interaction effects [51]-[52]. AC is one of the significant starch properties that influence the cooking and eating characteristics of rice grains. The cooking properties of rice have close relation to AC of rice starch [23]. Rice varieties on the basis of their AC are classified into waxy (0 to 2%), very low (3 to 9%), low (10 to 19%), intermediate (20 to 25%), and high greater than 25% [15]. AC of rice starches generally ranges from 15-35%. Higher AC of rice reveals higher volume expansion ratio and higher degree of flakiness. If AC is high in grains than rice becomes dry, less tender, and turn hard upon cooling. As compared to, low amylose contents of rice will cook moist and sticky. Juliano [16] studied pasting properties of rice in South Asia. He found that the milled rice samples of five countries had protein content ranging from 5.74 to 10.98% and AC from 2.9 to 31.8%. Analysis of variance showed highly significant differences ($p < 0.05$) for AC among rice genotypes in three different environments investigated in this study. The distribution of all genotypes could be categorized into two amylose groups: 9 were in the group having more than 20% amylose (intermediate), while one sample (G25) was less than 20% amylose (low) (Fig. 2). Since non-sticky rice is generally preferred in Malaysia, varieties with high yield and intermediate AC have special significance in local breeding efforts. Genetic studies showed that the nonwaxy trait is dominant over the waxy trait [20]. Among non-waxy parents, high amylose is completely dominant over low or intermediate amylose, and intermediate is dominant over low [19]. It is obvious from the results that effect of rice samples and environments was found to be significant for AC. AC can vary as much as 6% depending upon environmental condition [8]. The average AC was found to be higher in G13 followed by G15 but lower than MR219 and the lowest AC was observed in G25. The average grain length of G15 was 7.2 mm and for G13 it was 7.1 (Fig. 3), however there was no significant correlation between AC and grain length. In Sungai Besar the genotypes G13 and G15 showed higher AC than MR219, however MR219 was similar to G9. In all the three environments amylose content was in the range of 18-25 percent however the results were not same for all genotypes in different environments. The results of this study are in conformity with the earlier findings of Dipti [8] and Bultosa [6] who reported the AC in different rice varieties range from 18.60 to 28.0% and 20.0 to 25.8%, respectively. AC in long grain rice varieties can range from 23.0 to 26.0% [57]. Panlasigui [24] found 26.0 to 27.0% AC in white rice of different rice varieties. In the present study the average of AC for the three different environments was intermediate (except one genotype) which co-relates to extreme elongation during cooking and soft texture of cooked rice as reported by Juliano and Pascual [18]. The intermediate amylose varieties are generally most preferred. This finding supports the data previously reported in a study where the percentage of AC for rice varieties from Goa ranged from 14-25% [5]. It is seen that when one of the parents has high amylose, the progenies also have high amylose. However in the progenies with low AC all the parents had intermediate AC. In the case of progenies with intermediate AC at least one of the parents had intermediate AC thus showing the complexity of inheritance of AC [27]. Previous studies [3], [4], [30], [36], showed that the rice grain quality traits such as AC, and PC were easily influenced by different environmental factors such as field location, temperature, and solar radiation.

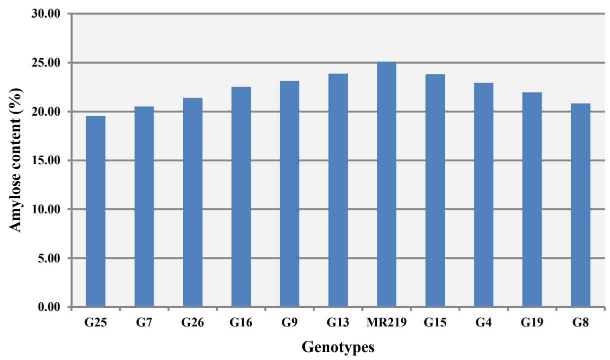

Fig.2 Amylose content (%) of the evaluated genotypes including MR219

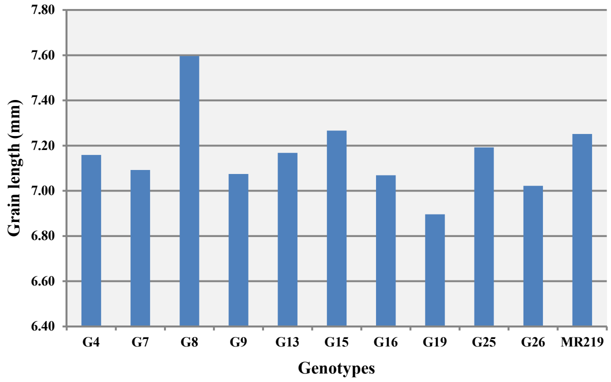

Fig.3 Grain length genotypes including MR219

IV. Conclusion

All the genotypes showed intermediate AC (except G25) with long grain; traits preferred for cooking properties.

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