1 **Genotypic Diversity and Environmental Stability of Starch Physicochemical**
2 **Properties in the USDA Rice Mini-Core Collection**

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ABSTRACT

The USDA rice mini-core collection consists of 217 accessions representative of a world-wide germplasm bank. We investigated its genotypic diversity in starch physicochemical properties and the effects of genotype, environment and GxE interaction in this study. High genotypic diversity was found in all 18 measured starch quality traits in the mini-core rice in two location-years in China. Genotype, environment and GxE effects on these traits were analysed using 115 common accessions successfully produced in both environments. Thermal properties (T_o , T_p and T_c) were very stable whereas most other traits differed significantly between environments. However, when these accessions were divided into five subgroups based on amylose content, environment was found to have differential effects. GxE interaction also played a significant role in determining the starch traits. These findings will provide guidance for selection from the diverse genotypes in the USDA mini-core collection for cultivation and for developing cultivars with desired cooking and eating quality.

Keywords: Rice starch; Physicochemical properties; Genotypic diversity; Environmental effects; Apparent amylose content; USDA rice mini-core collection

Abbreviations: AAC, apparent amylose content; ADH, adhesiveness; BD, breakdown; COH, cohesiveness; CPV, cold paste viscosity; CS, consistency; DSC, differential scanning calorimetry; GT, gelatinization temperature; HD, hardness; HPV, hot paste viscosity; Ptime, peak time; PT, pasting temperature; PV, peak viscosity; RVA, Rapid Visco-Analyzer; R%, percentage of retrogradation; SB, setback; T_o , onset temperature;

T_c , conclusion temperature; T_p , peak temperature; ΔH_g , enthalpy of gelatinization;
 ΔH_r , enthalpy of retrogradation; $\Delta T_{1/2}$, width at half peak height.

1. Introduction

Accounting for nearly 90% of grain dry weight, starch is the major component of rice grain, and rice eating and cooking qualities are thus largely determined by starch physicochemical properties (Chikuba, Watanabe, Sugimoto, Manabe, Sakai, Taniguchi, 1985). With improved living standards, consumers demand enhanced eating and cooking quality, which has hence become the focus of many rice breeding programmes across the world. Furthermore, apart from serving as the staple food for about half of the world's population, rice starch has many other applications in the food and non-food industries, *e.g.* as a stabilizing, thickening or filling agent. Rice consumers with different cultural preferences require cultivars of varied eating and cooking qualities, meanwhile, diverse rice applications require rice with specific starch quality. Therefore, a wide diversity in rice starch quality is essential to meet these diverse demands.

Starch is composed of amylose (mostly linear with few branches) and highly branched amylopectin. Many factors contribute to variation in starch quality, including amylose/amylopectin ratio, the shape and size of starch granules and fine structure of amylopectin (Lin, Singh, Chang & Chang, 2011; Yu, Ma, Menager & Sun, 2012). Several indicators of starch physicochemical quality are established and routinely used in evaluation of rice eating and cooking qualities, *i.e.*, apparent amylose content (AAC), gel consistency (GC), textural properties, Rapid Visco-Analyser (RVA) pasting viscosity, thermal and retrogradation properties.

79 According to Wang et al. (2007), rice eating and cooking quality parameters can be
80 divided into two trait groups. AAC, GC and most pasting viscosity are in one group
81 determining eating quality; and the other group includes alkali spreading value,
82 pasting temperature and pasting time which characterize the cooking process. Yang et
83 al. (2014) also reported that AAC-related traits include most pasting viscosity
84 parameters, and GT-related traits include pasting temperature. AAC has long been the
85 key indicator of rice eating and cooking qualities. Generally, high AAC leads to a hard,
86 fluffy and separate texture, whereas low AAC is usually associated with a tender,
87 cohesive and glossy texture. Gelatinization temperature (GT) reflects the ease or
88 difficulty to cook rice, rice with a high GT needs more energy and time in cooking.
89 GT has also been classified as low, intermediate and high (Juliano & Villareal, 1993).
90 High-AAC rice usually has intermediate or low GT, low-AAC rice usually has high or
91 low GT, but other combinations are rare, *i.e.* high-AAC with high GT, low-AAC with
92 intermediate GT (Juliano et al., 1993). Screening rice starch physicochemical
93 properties in a diverse germplasm collection can help to discover such rare AAC-GT
94 combinations.

95 Starch biosynthesis in rice endosperm occurs through a concerted network of four
96 groups of enzymes: ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS),
97 starch branching enzymes (SBE) and starch debranching enzymes (DBE) (Pandey et
98 al., 2012). Among them, the insoluble starch synthase, granule bound starch synthase I
99 (GBSSI) encoded by *Waxy* gene is the primary enzyme responsible for amylose
100 production in rice endosperm (He et al., 2009; Wang et al., 1995), while the *SSIIa* was
101 identified as the major gene for GT and thermal properties (He et al., 2009; Lapitan,
102 Redona, Abe & Brar, 2009; Wang et al., 2007). The *Waxy* and *SSIIa* genes were also
103 reported to be responsible for variations in gel texture and pasting viscosity (Lapitan

et al., 2009; Wang et al., 2007).

Starch physicochemical properties are not entirely determined by genes, however, as various environmental factors, including air temperature, atmospheric carbon dioxide, light, water, and soil nutrients, affect starch structure and functionality (Patindol, Siebenmorgen & Wang, 2015). By comparing the starch physicochemical properties of rice grown in different locations, significant differences were found in all measured parameters (Bao, Kong, Xie & Xu, 2004b; Cameron, Wang & Moldenhauer, 2007; Xu, Xie, Kong & Bao, 2004). Bao et al. (2004b) reported that cool paste viscosity (CPV), setback viscosity (SB), breakdown viscosity (BD) and peak time (Ptime) of viscosity parameters were mainly affected by genotype while peak viscosity (PV) and hot paste viscosity (HPV) were mainly affected by environmental factors. Tong et al. (2014) reported genotype was the main factor in determining PV, HPV, CPV, BD, SB, CS and PT of viscosity parameters. However, both of these previous studies covered only a small number of rice samples. The effects of genotype $\times$ environment interaction on rice starch physicochemical properties have hence not been adequately studied.

The USDA rice mini-core collection contains 217 accessions belonging to six ancestry groups originated from 76 countries of 15 geographic regions (Agrama et al., 2009; Bryant et al., 2013; Li et al., 2010). The mini-core represents a miniature of the whole USDA rice gene bank which contains more than 18,000 rice genotypes collected from all over the world. Its population structure and genetic diversity have been investigated using molecular markers and phenotypic traits (Li et al., 2010). However, starch physicochemical properties in the rice mini-core collection remains unknown. A comprehensive study of this germplasm resource is necessary in order to find rice accessions with desirable starch physicochemical properties for use in

breeding programmes. The diverse mini-core accessions also allow comparison of rice starch physicochemical properties among geographic regions and ancestry groups, which has not been covered by previous reports due to their limited number of samples. Furthermore, the rice mini-core collection can provide diverse genotypes, ideal for studying the effects of genotype, environment and GxE interaction on starch quality traits. In this study, we investigated genotypic diversity in starch physicochemical properties in the rice mini-core collection, and the effects of genotype, environment and their interaction on these properties.

2. Materials and methods

2.1. Plant materials

The seeds of 217 accessions in USDA mini-core collection were provided by U.S. Department of Agriculture - Agricultural Research Service (USDA-ARS). All of the accessions were grown in two environments, in Hainan Island (18° N) from December 2013 to April 2014, and in Hangzhou (30° N) from June to September 2014. However, not all of the accessions produced enough seeds for measurements of starch quality parameters.

For measurement of starch physicochemical properties, sufficient quantity of grains was obtained from 163 of the harvested accessions in Hainan Island (this set of samples was denoted as HN-163) and from 155 of the harvested accessions in Hangzhou (denoted as HZ-155). In total, we obtained enough seeds for 195 genotypes from the two plantings for analysing starch physicochemical properties. For studying $G \times E$ interaction, only 115 of the accessions that were successfully produced and

common to both plantings were used in the data analysis.

The harvested seeds were stored at 4°C before milling to white rice with a Satake rice machine (Satake, Hiroshima, Japan) and then ground to flour using a Cyclone sample mill (UDY, Fort Collins, CO, USA) with a 100-mesh sieve.

2.2. *Apparent amylose content (AAC)*

AAC was analysed using the method of Perez and Juliano (Perez & Juliano, 1978).

2.3. *Pasting viscosity*

Rice pasting properties were measured with a Rapid Visco Analyzer (RVA, model 3-D, Newport Scientific, Warriewood, Australia) equipped with Thermocline for Windows software (version 1.2). Rice flour (3 g, 12% moisture basis) was mixed with 25 mL of 0.5 mM AgNO₃ solution in a RVA sample can. A programmed heating and cooling cycle was used: the samples were held at 50°C for 1 min, heated to 95°C in 3.8 min, held at 95°C for 2.5 min, then cooled down to 50°C in 3.8 min and held at 50°C for 1.4 min. The peak viscosity (PV), hot paste viscosity (HPV), cold paste viscosity (CPV) and their derivative parameters breakdown (BD=PV – HPV), consistency (CS=CPV – HPV) and setback (SB=CPV – PV), plus pasting temperature (PT) and peak time (Ptime) were recorded. Viscosities were determined in Rapid Visco Units (RVU).

2.4. *Textural properties*

The sample cans containing rice starch gels formed in RVA analysis were sealed by ParafilmTM and kept at 4°C for 24 h. Textural properties were determined by a TA-XT2i Texture Analyzer (Stable Micro Systems, Surrey, England) equipped with Texture Expert software (version 1.2) in a two-cycle programme. The gel samples were pressed twice by a flat-ended cylinder probe with a 5 mm diameter for 10 mm distance at the speed of 1.0 mm/s. Hardness (HD, g) adhesiveness (ADH, g.s) and cohesiveness (COH) were calculated with the Texture Expert software (version 1.2).

2.5. Thermal and retrogradation properties

The thermal and retrogradation properties were analysed using a DSC 2920 thermal analyser (TA Instruments, Newcastle, DE, USA) with DSC standard and dual sample cells. Rice flour (2 mg, dry basis) was weighed into an aluminium pan and mixed with 6µl DW. The pan was hermetically sealed and heated from 30°C to 110°C at a rate of 10°C /min after equilibration at room temperature for 2 h; a sealed empty pan was used as a reference sample. Onset (T_o), peak (T_p), conclusion (T_c) temperatures, width at half peak height ($\Delta T_{1/2}$) and enthalpy of gelatinization (ΔH_g) were determined by the Universal Analysis Programme, Version 1.9D (TA Instruments). The scanned sample pans were kept at 4°C for 7 days, then retrogradation properties were measured by rescanning from 25°C to 110°C at a rate of 10°C /min. The enthalpy (ΔH_r) of retrograded starch was determined and the percentage of retrogradation (R%) was calculated as $\Delta H_r / \Delta H_g * 100\%$.

2.6. Statistical analysis

All 18 parameters of starch quality traits were measured in duplicate, and the data analysis was performed with SAS for Windows version 8 (SAS Institute Inc., Cary, NC, USA). The Student-Newman-Keuls test at $P < 0.05$ was conducted for comparison of mean values of accessions from different regional and ancestry groups. For the 115 rice accessions common to both environments, the Duncan's multiple range test at $P < 0.05$ was conducted. Analysis of variance was performed to determine genotypic and environmental variation among the parameters.

3. Results

3.1. Genotypic diversity of starch physicochemical properties

3.1.1 Apparent amylose content (AAC)

Wide diversity in AAC was observed both in HN-163 and HZ-155 (Table 1). For HN-163, AAC ranged from 1.1% (KECHENGNUO NO. 4, IND) to 29.4% (81B/25, IND) and averaged 20.6%; For HZ-155, the mean value was 20.3% with a similar wide range from 0.7% (Khao Luang, Admix) to 29.4% (AMANE, IND). In both environments, the majority of the accessions showed intermediate and high AAC (Fig. 1). In HN-163 group, 53 accessions (32.5%) fell into the intermediate AAC class and 60 accessions (36.8%) into the high AAC class; In HZ-155 group, 54 accessions (34.8%) and 46 accessions (29.7%) had intermediate and high AAC, respectively.

3.1.2. Textural properties

High genotypic diversity for textural properties was found in HN-163 as well as in HZ-155 (Table 1). In HN-163, HD ranged from 6.4 g (WIR 911, TEJ) to 89.7 g (B805D-MR-16-8-3, IND) and averaged 44 g; ADH ranged from -113.4 g.s (ECIA76-S89-1, IND) to -7.8 g.s (Nahng Sawn,) and averaged -62.7 g.s; and COH ranged from 0.499 (Quinimpol, TRJ) to 0.690 (WIR 911, TEJ) and averaged 0.566. In HZ-155, HD ranged from 6.2 g (C 5560, Admix) to 75.9 g (TOg 7025, *O. glaberrima*) and averaged 39.4 g; ADH averaged -56.5 g.s and ranged from -111.3 g.s (TOg 7135, *O. glaberrima*) to -6.2 g.s (KhaoPhoi, Admix); and COH averaged 0.568 and ranged from 0.491 (WAB462-10-3-1, TRJ) to 0.694 (C 5560, Admix).

3.1.3. Paste viscosity parameters

Variation among the rice genotypes in paste viscosity parameters was also very high in both HN-163 and HZ-155 (Table 1). Comparing the two groups, the HN-163 accessions had higher mean values of PV, HPV, CPV, CS, BD but lower SB, Ptime and PT. In Hainan, “IR 58614-B-B-8-2” (IND cultivar) showed the highest CPV (525.4 RVU), CS (284.6 RVU) and SB (133.2 RVU), while “Tobura” (Admix, 176.0 RVU), “C 5560” (Admix, 35.3 RVU) and “4484” (IND, -148.6 RVU) showed the lowest CPV, CS and SB, respectively. However, the highest BD was also observed in “4484” (253.3 RVU). “IARI 6626” (AUS) had the highest PV (453.2 RVU) and “Shui Ya Jien” (IND) had the highest HPV (286.5 RVU). “DJ 24” (AUS, 212.0 RVU), “CNTLR80076-44-1-1-1” (IND, 109.4 RVU) and “Romeno” (TEJ, 64.9 RVU) showed the lowest PV, HPV and BD, respectively.

In Hangzhou locality, “YOU-I B” (IND) showed the highest CPV (493.0 RVU), CS

(274.7 RVU) and SB (170 RVU); “Khao Phoi” (Admix) showed the lowest CPV (160.5 RVU) and CS (38.1 RVU); and Toga (IND) showed the lowest SB (-119.3 RVU). “IARI 6626” (AUS) which had the highest PV in the Hainan environment also showed the highest PV (424.3 RVU) and BD (233.5 RVU) in Hangzhou. “Acheh” had the highest HPV (253.2 RVU), and “ASWINA 330” (AUS, 184.4 RVU), “PATNAI 6” (AUS, 97.4 RVU) and “Egyptian Wild Type” (TEJ, 49.6 RVU) showed the lowest PV, HPV and BD, respectively. The extreme values for most pasting viscosity parameters were obtained in the AUS and IND cultivars.

3.1.4. Thermal and retrogradation properties

Gelatinization temperature (T_p) ranged from 66.7 (Bombon, TEJ) to 81.0°C (Quinimpol, TRJ) in HN-163 and ranged from 66.1 (RP2151-173-1-8, IND) to 82.3°C (CHUNJIANGZAO NO. 1, TEJ) in HZ-155 (Table 1). Most of the accessions belonged to the high GT class, 121 accessions (74.2%) in HN-163 and 120 accessions (77.4%) in HZ-155 having a GT higher than 74°C.

Retrogradation percentage ranged from 0.1% to 55% (JP5, Admix) in HN-163 and from 0.3% to 30.7% (TJ, IND) in HZ-155. It should be noted, however, most of the accessions had $R\% < 10\%$, with a mean value of 9.2% in HN-163 and 8.9% in HZ-155.

3.2. Diversity of starch physicochemical properties in different ancestry groups

According to Bryant et al. (2013), the rice accessions in this study can be divided into six ancestry groups. Among them, IND group had the most accessions, and AUS,

TRJ and TEJ had similar numbers of accessions. The IND rice showed the broadest AAC range both in HN-163 (1.1%-29.4%) and in HZ-155 (2.7%-29.4%) (Supplementary Table 1). The AUS accessions had the highest mean value of AAC in both HN-163 (24.9%) and HZ-155 (25.1%, excluding *O. glaberrima* accessions). The mean AAC of the AUS and IND accessions were greater than the overall mean AAC of the mini-core, whereas the mean values of TRJ and ARO were similar to that of the mini-core. Meanwhile, TEJ and admix accessions had significant lower mean AAC than that of the mini-core (Table 1, Supplementary Table 1). IND rice had higher mean AAC than TEJ and TRJ rice in both environments, and the differences between IND and TEJ, TRJ and TEJ were significant.

The highest mean values of viscosity parameters were found in TRJ, TEJ or IND subpopulations in both environments. For example, the IND group had the highest CS and CPV, and the TRJ group had the largest mean BD in both location-years. In Hainan, the TEJ group had the highest mean PV and HPV, and in Hangzhou TRJ and IND had the highest mean PV and HPV.

The pattern of differences in textural properties among these ancestry groups was quite consistent with that in AAC. High mean AAC was usually associated with high mean HD but low means of ADH and COH.

In both environments, TRJ rice had the highest mean GT and TEJ had the lowest GT. The broadest range of GT was observed in the TEJ group in HN-163, and this group had the second widest GT range in HZ-155. The ARO accessions had the narrowest range but the highest mean R%, whereas TEJ had the lowest mean R%, in both environments.

3.3. Diversity of starch physicochemical properties in different regions

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305 The rice accessions in the mini-core have been grouped into 15 regional
306 subpopulations including the group with unknown origin (Agrama et al., 2009). The
307 difference in starch physicochemical properties among all these subpopulations
308 (excluding the Unknown group) is given in Supplementary Table 2. South Pacific,
309 North Pacific, China, Subcontinent and Southeast Asia regional groups all had a wide
310 range of AAC, whereas North America, Central America, South America, Africa, and
311 Mideast all had a narrow AAC range. The Oceania group had the narrowest AAC
312 range although it did not have the fewest number of accessions. The Mideast,
313 Subcontinent and Africa groups all had high mean AAC, whereas those accessions
314 native to Eastern Europe and Western Europe had the lowest mean AAC. However, in
315 both Hainan and Hangzhou environments, the Western Europe group had the highest
316 mean PV, HPV and PT of viscosity parameters, and its mean CPV was also quite high,
317 comparable to the highest China group. The North America group which showed
318 relatively low mean AAC had the highest mean BD in both location-years. In HN-163,
319 the Mideast and Subcontinent group which showed the highest AAC also showed
320 higher CS, SB and PT than many other groups; In HZ-155, Central Asia group had the
321 highest CS and PT, Mideast and Western Europe had the highest SB and Ptime,
322 respectively. Interestingly, for CS and SB parameters, the regional groups that had
323 high mean values usually also had high mean AAC. For other parameters (PV, HPV,
324 CPV, BD, Ptime, and PT), the regional group with the highest mean values was
325 usually associated with low mean AAC.

326 The differences in GT between different regional groups were less significant,
327 compared with the differences in other parameters. But in both location-years, the
328 Western Europe group had the lowest T_o , T_p , and T_c . The Mideast, Western Europe

and Eastern Europe had similar and high $\Delta T_{1/2}$. The Central America group had the highest R%.

3.4. Plot of AAC-GT combination

The plot of AAC-GT combination for each accession in both environments and in different ancestry and regional groups was visualized in Fig. 1 and Fig. 2. The results showed that all accessions in the two environments could be placed into four major groups. Taking AAC 20% as a threshold, both low AAC and high AAC rice accessions could be divided into low GT and high GT subgroups. Furthermore, the mean GT of low AAC/high GT group is higher than that of high AAC/high GT group, and the GT of low AAC/low GT group is higher than that of high AAC/low GT group.

Among the six ancestry groups, TEJ and IND had all four types of AAC-GT combinations, whereas other ancestry groups consisted of mainly high AAC/high GT type of rice. Among the 15 regional groups, South pacific, China and Subcontinent had more diverse AAC-GT combinations compared to all other groups.

3.5. Comparison of starch physicochemical properties of 115 accessions from both Hainan and Hangzhou environments

3.5.1. Starch traits deviation between environments

For the subset of 115 accessions that were successfully produced in both Hainan and Hangzhou, the ranges and mean values of AAC, textural properties and most thermal and retrogradation properties were similar between the two environments

(Supplementary Table 3). In contrast, all RVA viscosity parameters, except for CS, showed significant differences in the means between the two location-years.

The 115 common accessions harvested in both location-years were divided into five subgroups according to their AAC measured in Hainan: waxy (< 5%), very low (5-12%), low (13-20%), intermediate (21-25%) and high (> 25%). The deviation of 18 starch traits between environments was visualized in Fig. 3.

Although the overall mean of AAC in 115 accessions did not differ significantly between environments, under scrutiny, different patterns of deviations were noted in the five AAC subgroups. In general, compared to the Hainan (HN) environment, most of rice accessions with waxy (< 5%), very low (5-12%) or low (13-20%) AAC were elevated in AAC in Hangzhou (HZ), with only few accessions showing a slight drop. However, the other two subgroups showed different patterns of deviation from the former three subgroups: about one third of rice genotypes with intermediate (21-25%) AAC had lower AAC in HZ, and nearly all rice genotypes with high (> 25%) AAC had lower AAC in HZ.

On the other hand, rice of all five subgroups shared the same pattern of deviation for nearly all the RVA parameters, except for CS. Compared to HN, nearly all 115 accessions had lower PV, HPV, CPV and BD while having higher SB, Ptime and PT. However, the pattern of CS deviation was complicated and very much like that of AAC: most rice with high AAC dropped substantially while other rice showed slight increase in CS. Furthermore, the HD and ADH of gel texture properties also followed this trend in deviation.

In contrast, the 115 rice accessions only showed negligible deviation in T_o , T_p and T_c between environments. For $\Delta T_{1/2}$, ΔHg and $R\%$, the deviation between environments seemed to be random.

3.5.2. Analysis of variance for rice starch physicochemical properties

Analysis of variance (ANOVA) for 18 parameters of starch quality was performed using the 115 common accessions harvested from both environments. The results indicated that genotypic variances were significant for all parameters (Table 2). AAC was mainly affected by genotypic effect which accounts for 92.9% of the total variation. Genotype caused 64.1% and 62.5% of the total variance for HD and ADH, respectively, suggesting that these two textural properties are largely affected by genotype. Except for T_c and ΔHg , all thermal and retrogradation parameters were mainly affected by genotype. In contrast, CS was the only viscosity parameter that was mainly affected by genotype, accounting for 83.5% of the total variance.

ANOVA indicated that environment variance was also significant at $P < 0.001$ for nearly all parameters. Environmental variance accounted for 92.9%, 79.9%, 64.4%, 95.1%, 92.5%, 73.7% and 73.4% of the total variance in PV, HPV, CPV, SB, BD, Ptime and PT respectively, suggesting that these viscosity parameters are greatly affected by environmental factors. Environmental effects were also large on T_c and ΔHg , accounting for 55.4% and 71.9% of the total variation respectively.

GxE interaction accounted for a small proportion of the total variation in all traits, but the effect was still significant ($P < 0.001$). For instance, it was responsible for 19.2% of the total variance in $\Delta T_{1/2}$.

3.5.3. Analysis of variance for five AAC subgroups

We divided all the 115 rice accessions into waxy (< 5%), very low (5-12%), low

(13-20%), intermediate (21-25%) and high (> 25%) subgroups according to their AAC measured in Hainan Island. Analysis of variance for starch traits was then performed in the five subgroups. The results were very informative (Table 3). Genotype, environment and $G \times E$ effects were all highly significant ($P < 0.0001$) on the 18 starch parameters in each of the five subgroups. However, the weight of these three effects varied significantly among subgroups for many traits. For example, the mean square values in Table 3 suggested that, for rice in very low (5-12%) and high AAC (> 25%) groups, environment was the source causing greatest variance for AAC. In contrast, it was genotype which was mainly responsible for variance in the low AAC (13-20%) group. The results revealed that even for the same trait, environment exerted different effect on rice of different subgroups, and some groups were more likely affected by environment. For example, as mean square value suggested, for CS, rice with high AAC was much more affected by environment while rice in other four AAC classes were less affected.

4. Discussion

Many studies have attempted to find high genetic diversity in starch physicochemical properties using large rice populations (Asante et al., 2013; Bao, Corke & Sun, 2004a; Bao, Shen, Sun & Corke, 2006; Bao, Sun & Corke, 2007; Caffagni et al., 2013; Zhao et al., 2013), but the effort has not always been successful. The 217 accessions in the USDA rice mini-core collection have diverse ancestry and geographical origins (Agrama et al., 2009; Bryant et al., 2013; Li et al., 2010), adequately captured most of genetic diversity in the whole USDA rice gene bank. Our

investigation of the genotypic diversity in starch physicochemical properties in the rice mini-core collection provides the information needed for breeders to find the most suitable parental germplasm for improvement of eating and cooking qualities of rice.

Previous studies have revealed that starch physicochemical properties can vary dramatically among rice genotypes. Juliano et al. (1981) reported five AAC types: waxy (0-2%), very low (5-12%), low (12-20%), intermediate (20-25%) and high (25-33%). Among the rice genotypes in the mini-core, there exists a wide range of AAC (0.7% - 29.4%) covering all five AAC types, with the majority belonging to the intermediate or high AAC type. The ranges of PV, HPV and CPV observed in the mini-core are much wider than all the previous reports, such as by Asante et al. (2013) for 65 African accessions and 23 US accessions; and Bao et al. (2006) for 516 accessions. Only the ranges of gelatinization temperature (66.7 - 81°C in HN-163; and 66.1 - 82.3°C in HZ-155) are mostly comparable to the previous reports. For instance, Bao et al. (2004a, 2007) observed GT ranging from 66.9 to 79.0 °C in 56 waxy rice accessions and from 63.2 to 79.8°C in 236 nonwaxy rice accessions. Our findings indicate that the USDA rice mini-core collection is a highly valuable germplasm resource for finding novel combinations of AAC and other parameters of starch quality.

The rice accessions in the mini-core were divided into six ancestry groups (Bryant et al., 2013) and 15 regional groups (Agrama et al., 2009). Among the six ancestry groups, it was found that the AUS and IND rice had higher mean AAC than other groups but less diversity. The AUS group showed the least diversity among all groups although it had the highest mean AAC. All AUS accessions belonged to intermediate or high AAC class with one exception. This finding was consistent with Tong et al.

(2014), showing that AUS rice with a high mean AAC but less diversity. Compared to TRJ, both TEJ and IND are more diverse in AAC type but lower in mean AAC. Amylose biosynthesis in rice is mainly controlled by *Waxy* gene, and five *Waxy* gene haplotypes can distinguish rice from the five AAC classes (Teng et al., 2013). Therefore, the difference in AAC are likely due to the difference in *Waxy* haplotype constitution in these ancestry groups. Among the 15 regional groups, the South Pacific, China and Subcontinent groups have the greatest diversity in terms of AAC, GT types and AAC-GT combinations. This could be partially attributed to the larger numbers of accessions in these groups. However, the South America and Southeast Asia groups have similar large numbers of accessions but have a smaller range of AAC, GT and less diverse AAC-GT combinations.

The plotting of AAC and GT showed that both AAC and GT in the mini-core could each be divided into two major classes, resulting in four classes of AAC-GT combinations. Using 20% as a boundary, both low-AAC and high-AAC rice accessions could be divided into low-GT and high-GT groups. Furthermore, the GT of low-AAC/high-GT group is higher than that of high-AAC/high-GT group; and the GT of low-AAC/low-GT group is higher than that of high-AAC/low-GT group. These findings agreed with previous reports by Bao et al. (2007) and Yang et al. (2014). Concerning different ancestry, TEJ and IND were the only two groups that had rice accessions with all four AAC-GT combinations. In contrast, nearly all AUS accessions were in high-AAC/high-GT class. Juliano et al. (1993) have observed that it is difficult to find rice having high-AAC/high-GT and low-AAC/intermediate-GT combinations. However, screening of large collections of rice germplasm can reveal the rare AAC-GT combinations (Bao et al., 2006; Bao et al., 2007; Juliano, Perez, & Resurreccion, 2009). Since the USDA mini-core collection consists of diverse

genotypes, we were able to find the rare low-AAC/intermediate-GT and high-AAC/high-GT rice accessions. This precious germplasm resource can provide materials for further studies on AAC-GT interaction and underlying genes in starch biosynthesis.

Analysis of variance (ANOVA) was used in previous studies to dissect the genotype, environment and GxE interaction effect on starch traits (e.g., Bao et al., 2004; Xu et al., 2004; Tong et al., 2014). Bao et al. (2004) reported PV and HPV were mainly affected by environment but CPV, SB, BD and PT were mainly affected by genotype. However, Tong et al. (2014) reported that all RVA parameters were mainly affected by genotypic factors. By the way, it can be pointed out that Tong et al. (2014) used the high correlations of the measurements from the same genotypes between two years to infer that environmental effect was small on these starch traits. This inference might not be sound because high correlation between years should be expected if all the trait values increased or decreased to the same extent. As shown in this study (Fig. 3), the correlation between two environments was also very high in PV, HPV, CPV, etc., but environmental effect on these traits was high ($P < 0.001$) based on ANOVA (Table 2). Overall, high correlation in starch parameter measurements between years should not be interpreted as indicating a small environmental effect.

The contrasting findings about environmental effect on RVA parameters indicate more studies are necessary before any firm conclusions can be made. Different results from previous studies could arise from different experimental designs. The eight rice genotypes used in Bao et al. (2004b) have large differences in amylose content, and hence their genotypic differences in CPV, BD, SB and Ptime parameters would be large. Tong et al. (2014) used 20 rice genotypes but planted them at the same location in the same season in two years, hence the environmental effects on the measurements

must be small compared to other studies conducted in multiple locations in different seasons/years. More importantly, as our data revealed, ANOVA could lead to totally different results when using rice samples with different AAC types (Table 3), which means different sampling strategy could lead to different results from different studies. It is should be stressed that our data suggested that even for the same trait, environment exerted different effect on rice belonging to different groups (grouped by AAC classes). For example, environment only caused great variation in CS in rice with high AAC (> 25%), in contrast, there was no significant change in CS of rice with other AAC types. According to previous reports, rice in different AAC classes may have different *Waxy* gene alleles (Teng et al. 2013; Chen, Bergman, Pinson & Fjellstrom 2008a), and Chen et al. (2008b) once reported that environment exerted different effect on AAC of rice with different *Waxy* haplotypes. For future work, the rice accessions of mini-core should be genotyped for *Waxy* gene, and a systematic analysis composing *Waxy* alleles and starch traits variation between environments should be conducted.

In conclusion, the present study revealed a wide variation in starch quality traits in the USDA rice mini-core collection. Some previous studies have attempted to mine genes of interest using the mini-core collection, for example, to find and map QTLs for grain yield and protein concentration (Bryant et al., 2013; Li et al., 2011). As revealed in this study, high diversity in starch quality traits in the rice mini-core collection indicates this germplasm resource is highly suitable for association mapping between starch physicochemical properties and starch biosynthesis related genes in order to develop new rice cultivars with improved starch quality via marker-assisted breeding programmes. The ANOVA analysis of 18 parameters of starch physicochemical properties indicated that for rice with different AAC types,

environment has different effects on their starch traits. This is likely caused by differential regulation of gene expression at the *Waxy* locus in rice groups with different *Waxy* alleles. The information should provide valuable guidance for rice cultivation, that environments significantly affected rice starch traits, and growers should select suitable environments in order to produce rice grain with desired quality.

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Figure captions

Fig. 1. Plot of AAC-GT combination for rice accessions in HN-163, HZ-155, and six different ancestry groups. TRJ: tropical *japonica*, TEJ: temperate *japonica*, IND: *indica*, AUS: *aus*, ARO: aromatic, and Admix: accessions having admixed ancestry. Square: accessions harvested from Hainan; Diamond: accessions from Hangzhou.

Fig. 2. Plot of AAC-GT combination for rice accessions in different regional groups. Square: accessions harvested from Hainan; Diamond: accessions from Hangzhou.

Fig. 3. Starch trait deviation between Hainan (HN) and Hangzhou (HZ) environments. The rice accessions were divided into five subgroups according to their AAC measured in HN: subgroup I (< 5%), II (5-12%), III (13-20%), IV (21-25%), V (> 25%).

References

- Agrama, H. A., Yan, W. G., Lee, F., Fjellstrom, R., Chen, M. H., Jia, M., et al. (2009). Genetic assessment of a mini-core subset developed from the USDA rice genebank. *Crop Science*, 49, 1336–1346.
- Asante, M. D., Offei, S. K., Gracen, V., Adu-Dapaah, H., Danquah, EY., Bryant, R., et al. (2013). Starch physicochemical properties of rice accessions and their association with molecular markers. *Starch – Stärke*, 65, 1022–1028.
- Bao, J., Corke, H., & Sun, M. (2004a). Genetic diversity in the physicochemical properties of waxy rice (*Oryza sativa* L) starch. *Journal of the Science of Food and Agriculture*, 84, 1299–1306.
- Bao, J., Kong, X., Xie, J., & Xu, L. (2004b) Analysis of genotypic and environmental effects on rice starch. 1. Apparent amylose content, pasting viscosity, and gel texture. *Journal of Agricultural and Food Chemistry*, 52, 6010–6016.
- Bao, J., Shen, S., Sun, M., & Corke, H. (2006). Analysis of genotypic diversity in the starch physicochemical properties of nonwaxy rice: apparent amylose content, pasting viscosity and gel texture. *Starch – Stärke*, 58, 259–267.
- Bao, J., Sun, M., & Corke, H. (2007). Analysis of genotypic diversity in starch thermal and retrogradation properties in nonwaxy rice. *Carbohydrate Polymers*, 67, 174–181.
- Bryant, R. J., Jackson, A. K., Yeater, K. M., Yan, W. G., McClung, A. M., & Fjellstrom, R. G. (2013) Genetic variation and association mapping of protein concentration in brown rice using a diverse rice germplasm collection. *Cereal Chemistry*, 90, 445–452.
- Caffagni, A., Albertazzi, G., Gavina, G., Ravaglia, S., Gianinetti, A., Pecchioni, N., et

585 al. (2013). Characterization of an Italian rice germplasm collection with genetic
 586 markers useful for breeding to improve eating and cooking quality. *Euphytica*, 194,
 587 383–399.

588 Cameron, D. K., Wang, Y. J., & Moldenhauer, K. A. (2007). Comparison of starch
 589 physicochemical properties from medium-grain rice cultivars grown in California and
 590 Arkansas. *Starch – Stärke*, 59, 600–608.

591 Chen, M. H., Bergman, C., Pinson, S., & Fjellstrom, R. (2008a) Waxy gene
 592 haplotypes: Association with pasting properties in an international rice germplasm
 593 collection. *Journal of Cereal Science*, 48, 781–788.

594 Chen, M. H., Bergman, C., Pinson, S., & Fjellstrom, R. (2008b) Waxy gene
 595 haplotypes: Association with apparent amylose content and the effect by the
 596 environment in an international rice germplasm collection. *Journal of Cereal Science*,
 597 47, 536–545.

598 Chikuba, S., Watanabe, S., Sugimoto, T., Manabe, N., Sakai, F., & Taniguchi, Y.
 599 (1985). Relation between palatability evaluations of cooked rice and physicochemical
 600 properties of rice. Part 2. Establishment of palatability estimations formula of rice by
 601 multiple regression analysis. *Journal of the Japanese Society for Starch Science*, 32,
 602 51–60.

603 He, P., Li, S. G., Qian, Q., Ma, Y. Q., Li, J. Z., Wang, W. M., et al. (2009). Genetic
 604 analysis of rice grain quality. *Theoretical and Applied Genetics*, 98, 502–508.

605 Juliano, B. O., Perez, C. M., Blakeney, A. B., Castillo, D. T., Kongseree, N., Laignelet,
 606 B., et al. (1981). International cooperative testing on the amylose content of milled
 607 rice. *Starch – Stärke*, 33, 157–162.

608 Juliano, B. O., Perez, C. M., & Resurreccion, A. P. (2009). Apparent amylose content
609 and gelatinization temperature types of Philippine rice accessions in the IRRI gene
610 bank. *Philippine Agricultural Scientist*, 92, 106–109.

611 Juliano, B. O., & Villareal, C. P. (1993). *Grain Quality Evaluation of World Rices*
612 (page 7). International Rice Research Institute, Manila, Philippines.

613 Kong, X., Zhu, P., Sui, Z., & Bao, J. (2015). Physicochemical properties of starches
614 from diverse rice cultivars varying in apparent amylose and gelatinization temperature
615 combinations. *Food Chemistry*, 172, 433–440.

616 Lapitan, V. C., Redona E. D., Abe, T., & Brar, D. S. (2009). Mapping of quantitative
617 trait loci using a double-haploid population from the cross of *indica* and *japonica*
618 cultivars of rice. *Crop Science*, 49, 1620–1628

619 Li, X., Yan, W., Agrama, H., Hu, B., Jia, L., Jia, M., et al. (2010). Genotypic and
620 phenotypic characterization of genetic differentiation and diversity in the USDA rice
621 mini-core collection. *Genetica*, 138, 1221–1230.

622 Li, X., Yan, W., Agrama, H., Jia, L., Shen, X., Jackson, A., et al. (2011). Mapping
623 QTLs for improving grain yield using the USDA rice mini-core collection. *Planta*,
624 234, 347–361.

625 Lin, J. H., Singh, H., Chang, Y. T., & Chang, Y. O. (2011). Factor analysis of the
626 functional properties of the flours from mutant genotypes. *Food Chemistry*, 126,
627 1108–1114.

628 Pandey, M. K., Rani, N. S., Madhav, M. S., Sundaram, R. M., Varaprasad, G. S.,
629 Sivaranjani, A. K., et al. (2012). Different isoforms of starch-synthesizing enzymes
630 controlling amylose and amylopectin content in rice (*Oryza sativa* L.). *Biotechnology*

631 *Advances*, 30(6), 1697–1706.

632 Patindol, J. A., Siebenmorgen, T. J., & Wang, Y. J. (2015). Impact of environmental
 633 factors in rice starch structure: A review. *Starch – Stärke*, 67, 42–54.

634 Perez, C. M., & Juliano, B. O. (1978). Modification of the simplified amylose test for
 635 milled rice. *Starch – Stärke*, 30, 424–426.

636 Teng B., Zhang Y., Wu J., Cong X., Wang R., Han Y., et al. (2013). Association
 637 between allelic variation at the *Waxy* locus and starch physicochemical properties
 638 using single-segment substitution lines in rice (*Oryza sativa* L.). *Starch – Stärke*, 65,
 639 1–9.

640 Tong, C., Chen, Y., Tang, F., Xu, F., Huang, Y., Chen, H., et al. (2014). Genetic
 641 diversity of amylose content and RVA pasting parameters in 20 rice accessions grown
 642 in Hainan, China. *Food Chemistry*, 161, 239–245.

643 Wang, L., Liu, W., Xu, Y., He, Y., Luo, L., Xing, Y., et al. (2007). Genetic basis of 17
 644 traits and viscosity parameters characterizing the eating and cooking quality of rice
 645 grain. *Theoretical and Applied Genetics*, 115, 463–476.

646 Xu L., Xie J., Kong X., & Bao J. (2004). Analysis of genotypic and environmental
 647 effects on rice starch. 2. Thermal and retrogradation properties. *Journal of*
 648 *Agricultural and Food Chemistry*, 52, 6017–6022.

649 Yang, F., Chen, Y., Tong, C., Huang, Y., Xu, F., Li, K., et al. (2014). Association
 650 mapping of starch physicochemical properties with starch synthesis-related gene
 651 markers in nonwaxy rice (*Oryza sativa* L.). *Molecular Breeding*, 34, 1747–1763.

652 Yu, S., Ma, Y., Menager L., & Sun, D. (2012). Physicochemical properties of starch
 653 and flour from different rice cultivars. *Food and Bioprocess Technology*, 5, 626–637.

654 Zhao, W. G., Chung, J. W., Kwon, S. W., Lee, J. H., Ma, K. H., & Park, Y. J. (2013).
655 Association analysis of physicochemical traits on eating quality in rice (*Oryza sativa*
656 L.). *Euphytica*, 191, 9–21.

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Table 1

Means and ranges of starch physicochemical properties in rice harvested from Hainan (HN-163) and Hangzhou (HZ-155) environments.

Parameters	HN-163			HZ-155		
	Mean $\pm$ SD	Min	Max	Mean $\pm$ SD	Min	Max
AAC (%)	20.6 $\pm$ 7.3	1.1	29.4	20.3 $\pm$ 6.6	0.7	29.4
HD (g)	44.0 $\pm$ 20.9	6.4	89.7	39.4 $\pm$ 18.2	6.2	75.9
ADH (g.s)	-62.7 $\pm$ 29.5	-113.4	-7.8	-56.5 $\pm$ 26.7	-111.3	-6.2
COH	0.566 $\pm$ 0.03	0.499	0.69	0.568 $\pm$ 0.03	0.491	0.694
PV (RVU)	314.3 $\pm$ 55.6	212	453.2	285.9 $\pm$ 50.3	184.4	424.3
HPV (RVU)	179.7 $\pm$ 34.8	109.4	286.5	169.9 $\pm$ 33.5	97.4	253.2
CS (RVU)	161.0 $\pm$ 44.9	35.3	284.6	156.6 $\pm$ 41.0	38.1	274.7
BD (RVU)	134.5 $\pm$ 37.1	64.9	253.3	116 $\pm$ 34.3	49.6	233.5
CPV (RVU)	341.6 $\pm$ 63.7	176	525.4	326.5 $\pm$ 58.2	160.5	493
SB (RVU)	27.3 $\pm$ 66.3	-148.6	133.2	40.6 $\pm$ 62.6	-119.3	169.9
PT (°C)	75.9 $\pm$ 2.8	67.6	84.1	76.6 $\pm$ 2.4	68.9	82.4
Ptime (min)	5.6 $\pm$ 0.5	3.7	6.4	5.8 $\pm$ 0.5	3.6	6.5
To (°C)	70.2 $\pm$ 3.8	59.2	76.6	70.4 $\pm$ 3.8	59.1	78.1
Tp (°C)	75.3 $\pm$ 3.1	66.7	81	75.7 $\pm$ 3.0	66.1	82.3
Tc (°C)	80.4 $\pm$ 2.9	71.8	87.7	81.2 $\pm$ 2.5	71.3	86.8
$\Delta T_{1/2}$ (°C)	6.8 $\pm$ 1.5	4.2	12.7	6.9 $\pm$ 1.4	3.9	12.4
ΔH_g (J/g)	8.5 $\pm$ 2.8	1.4	17.9	9.4 $\pm$ 2.2	1.8	15.5
R%	9.2 $\pm$ 7.4	0.1	55	8.9 $\pm$ 6.2	0.3	30.7

Table 2

Mean square values from analysis of variance for starch physicochemical property parameters of 115 rice accessions successfully grown in both Hainan and Hangzhou environments.

Source	df	AAC	HD	ADH	COH	PV	HPV	CPV	CS	SB	BD	Ptime	PT	T _o	T _p	T _c	ΔT _{1/2}	ΔHg	R%
Genotype	114	196.0 ^{***}	1395.9 ^{***}	2929.1 ^{***}	0.001 ^{***}	10681.2 ^{***}	4217.8 ^{***}	14429.4 ^{***}	7107.7 ^{***}	7186.1 ^{***}	4822.7 ^{***}	0.914 ^{***}	21.8 ^{***}	46.4 ^{***}	31.8 ^{***}	22.8 ^{***}	5.7 ^{***}	15.8 ^{***}	130.3 ^{***}
Year	1	10.1 ^{***}	701.4 ^{***}	1629.9 ^{***}	0	148943.2 ^{***}	17758.5 ^{***}	27400.3 ^{***}	1047.2 ^{***}	215216.0 ^{***}	63801.8 ^{***}	2.634 ^{***}	66.0 ^{***}	3.5 ^{***}	5.9 ^{***}	31.7 ^{***}	0.6 ^{***}	62.3 ^{***}	5.1 ^{***}
G*E	114	4.9 ^{***}	79.1 ^{***}	125.9 ^{***}	0.001 ^{***}	627.8 ^{***}	241.6 ^{***}	688.5 ^{***}	353.6 ^{***}	3960.7 ^{***}	350.1 ^{***}	0.025 ^{***}	2.1 ^{***}	1.6 ^{***}	0.9 ^{***}	2.7 ^{***}	1.5 ^{***}	8.5 ^{***}	23.4 ^{***}

^{***} indicate significance at $P < 0.001$ level.

669 **Table 3**

670 Mean square values from analysis of variance for starch physicochemical property
 671 parameters of rice accessions in different subgroups.

Parameter	Subgroup ¹	No. of accessions	Mean Square ²		
			G	E	G x E
AAC	I	8	6.6	6.5	9.0
	II	11	15.7	30.3	3.9
	III	17	33.8	20.7	2.7
	IV	40	11.0	10.6	4.2
	V	39	3.8	24.6	4.3
HD	I	8	2.6	2.8	2.5
	II	11	68.9	1.5	3.1
	III	17	303.4	22.7	33.2
	IV	38	409.8	165.4	113.6
	V	38	231.5	1434.3	90.7
ADH	I	8	19.0	4.8	5.9
	II	11	174.3	0.1	3.5
	III	17	346.0	2.2	52.2
	IV	38	948.7	447.2	206.9
	V	38	491.9	2561.9	124.9
COH	I	8	0.0058	0.0006	0.0114
	II	11	0.0021	0.0049	0.0004
	III	17	0.0009	0.0030	0.0010
	IV	38	0.0009	0.0020	0.0003
	V	38	0.0004	0.0014	0.0003
PV	I	7	2219.5	6745.1	410.4
	II	10	4896.3	28015.0	1815.9
	III	16	4977.5	19222.7	854.4
	IV	38	7095.6	25607.9	410.6
	V	38	7932.0	58240.9	488.3
HPV	I	7	545.3	1670.1	118.5
	II	10	1040.9	5712.1	476.2
	III	16	2491.7	2612.7	154.4
	IV	38	4082.9	3143.6	182.7
	V	38	5680.6	4311.1	284.8
CPV	I	7	940.1	1065.8	926.3
	II	10	2169.5	6453.7	910.2
	III	16	2447.4	1135.6	483.2
	IV	38	11395.4	4643.0	601.0
	V	38	12850.5	13228.5	699.6
BD	I	7	765.2	1702.5	332.5
	II	10	5526.1	8426.9	609.7
	III	16	2965.7	7661.7	602.8
	IV	38	3359.7	10807.0	269.2
	V	38	1047.2	30860.7	221.2
SB	I	7	973.4	2448.5	1091.5
	II	10	5740.3	7576.3	910.1
	III	16	8892.1	11014.1	663.0
	IV	38	9546.6	8442.8	595.3
	V	38	2588.7	15955.8	367.0

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 673 Cont. on next page.

Parameter	Subgroup ¹	No. of accessions	Mean Square ²		
			G	E	G x E
CS	I	7	189.1	67.6	494.3
	II	10	627.9	22.6	297.1
	III	16	2395.9	303.3	207.0
	IV	38	3220.3	145.7	384.4
	V	38	2126.7	2436.0	305.9
PT	I	7	70.1	2.8	0.6
	II	10	50.4	1.8	2.0
	III	16	31.7	13.0	1.3
	IV	38	6.8	20.0	3.0
	V	38	5.4	27.1	2.0
Ptime	I	7	0.53	0.01	0.05
	II	10	0.87	0.24	0.02
	III	16	0.14	0.32	0.02
	IV	38	0.11	0.65	0.02
	V	38	0.12	1.56	0.01
T _o	I	8	79.8	4.4	2.5
	II	11	100.2	3.9	10.4
	III	17	63.0	4.4	1.8
	IV	39	33.6	2.2	2.0
	V	39	20.8	2.8	0.8
T _p	I	8	66.8	1.4	1.2
	II	11	58.6	3.8	0.5
	III	17	43.3	1.2	1.5
	IV	39	26.9	1.4	1.0
	V	39	16.9	0.5	0.6
T _c	I	8	59.8	4.0	6.7
	II	11	30.0	13.4	2.4
	III	17	27.8	1.6	2.9
	IV	39	18.5	6.1	2.9
	V	39	16.3	12.0	2.0
$\Delta T_{1/2}$	I	8	4.5	3.0	2.1
	II	11	9.4	0.2	0.6
	III	17	4.5	2.4	1.5
	IV	39	6.1	1.6	1.9
	V	39	1.7	4.0	1.1
ΔHg	I	8	11.8	8.3	5.8
	II	11	19.9	22.1	8.1
	III	17	10.7	0.4	11.0
	IV	39	12.7	51.2	7.4
	V	39	19.9	31.1	8.6
R%	I	8	15.6	2.7	5.0
	II	9	59.4	20.6	10.9
	III	16	38.7	37.0	13.8
	IV	36	172.7	24.3	41.1
	V	38	84.6	5.7	15.9

¹The 115 accessions were divided into five subgroups according to their AAC measured in HN: I (< 5%), II (5-12%), III (13-20%), IV (21-25%), and V (> 25%).

²All mean square values were significant at $P < 0.0001$ level.

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