Vietnam Journal of Agricultural Sciences

ISSN 2588-1299

VJAS 2022; 5(2): 1435-1444
https://doi.org/10.31817/vjas.2022.5.2.01

Genetic Divergence of Cultivated Rice Varieties in North Vietnam for Grain Quality Traits Using D^2 Cluster Analysis

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Abstract

The study was carried out with twenty-four genotypes of rice during the Spring season, 2020 in Hanoi, Vietnam in order to exploit the attributes and divergence of quality properties from existing rice cultivars in North Vietnam. The rice genotypes were characterized based on ten quality parameters of milling, physical appearance and physiochemical characters using Mahalanobis D^2 statistic. D^2 analysis allocated the 24 genotypes into eight clusters, of which cluster I was the largest with 12 genotypes. The clustering showed the random distribution of genotypes into different clusters indicating that the origin and release duration were not related to the genetic diversity of grain quality. Among different traits, amylose content, length/width ratio and gel consistency were identified as topmost contributors in total divergence. Cluster I possessed a maximum intra-cluster value of 296.34 and the maximum inter-cluster distance was recorded between cluster IV and VI (2247.39) succeeded by cluster I and VI (2245.94) designing the members presented in these clusters can be exploited as potential donors in a breeding program to result good physicochemical quality traits in rice.

Keywords

Rice, genetic divergence, quality, cluster, Mahalanobis

Introduction

Rice grain quality is the same importance as yield because rice is mainly consumed in the form of whole cooked grains (Asante, 2017) and not as processed products. Furthermore, rice quality defines the market value and consumer acceptance of rice varieties (Cuevas *et al.*, 2016). Grain quality encompasses the physical biochemical and physiological properties of rice grains (Sattari *et al.*, 2015). The physical properties include the grain shape, degree of milling, grain appearance, milling recovery, kernel shape. The physicochemical properties comprise of gel consistency, alkali digestion, amylose content (Sattari *et al.*, 2015; Cruz & Khush, 2000; Rohilla *et al.*, 2000). Different rice genotypes have diverse physicochemical and

Received: October 21, 2021
Accepted: May 13, 2022

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physical properties, which influence the grain quality properties.

Rice is one of the main crops in Vietnam, contributing more than 90% of total cereal production (Bettman *et al*., 2014). The average daily consumption of rice per capita in the country is more than 300g (Nguyen *et al*., 2019). Annually, North Vietnam contributes 31.8% and 30.4 % of the nationwide cultivation area and paddy production of rice, respectively (General Statistics Office of Vietnam, 2021). Recently, numerous newly improved rice varieties have been developed by Vietnamese scientists and rice breeders, however, it has been estimated that still approximately 50-70% of the rice varieties being grown in the southern part were originated from IRRI, whereas the improved varieties imported from China have been planted in the northern part (Le *et al*., 2010; Thuy *et al*., 2020). Along with living standards improved, there is a universally growing concern of enhancement of quality in rice as attractive grain character and good cooking quality will accelerate the acceptance and spread of new variety. Nowadays, the grain quality of rice has emerged as the most important element for rice production since it is directly linked with its market value, and thus affects grower profits (Gibson & Kim, 2013; Keyser *et al*., 2013; Cuevas *et al*., 2016).

Knowing the relationship between physicochemical properties and end-use qualities will help breeders to select rice genotypes with desirable characteristics. According to Brown *et al*. (2014), screening to select proper materials is a must for launching any breeding program. In the Central and the Southern parts of Vietnam, some released rice varieties and developed lines were collected and evaluated with various aspects of milling quality, physical properties and cooking quality to identify good grain (Nguyen Thanh Tuong *et al*., 2005; Nguyen *et al*., 2018; Tran *et al*., 2012). Genetic divergence of cultivated or improved rice varieties regarding in North regarding grain quality has not been reported, however, some studies were published but with upland or local germplasms only (Chu *et al*., 2011; Hoai *et al*., 2008; Nguyen *et al*., 2010; Nguyen *et al*., 2012).

The knowledge of diversity and genetic distance among groups of genotypes helps to identify parental lines for decision-making on hybridization programs. Clustering analysis has been widely used to investigate the diversity of germplasm pools in rice (Binodh *et al*., 2010; Mohanty *et al*., 2010; Subudhi *et al*., 2021), wheat (Ali *et al*., 2017), soybean (Jain *et al*., 2016), common bean (Al-Ballat & Al-Araby, 2019), tomato (Ullah *et al*., 2015) and chili (Yatung *et al*., 2014). This study was therefore designed to evaluate the physical and physiochemical quality characteristics of inbred varieties with diverse origins and released in different periods in North Vietnam to determine the level of genetic divergence through cluster analysis.

Materials and Methods

Plant materials and experimental design

The experimental materials consisted of 24 indica inbred varieties which were categorized in three groups based on their release years and origins presented in **Table 1**.

Rice quality assessment

Seeds were collected from each plot in each replication by manual harvesting at the maturity stage and dried to 14% of moisture content. Samples of 150g from each plot were used for analyzing ten quality characters *viz*. brown rice percentage (%), milling percentage (%), head rice recovery (%), chalkiness (score), grain length (mm), grain width (mm), length/width ratio, gel consistency (mm), alkali digestion (score) and amylose content (%). The analysis was carried out in the rice quality lab of Food Crop Research Institutes in 2020. Standard evaluation system described by Cruz & Khush (2000) was applied for milling quality and physical quality measurement. Amylose content was measured by procedures described by TCVN5716: 2008, gel consistency was estimated according to TCVN8369: 2010, whereas alkali digestion was determined following the protocol of TCVN 5715: 1993.

Rice with soft to medium gel consistency, intermediate amylose content and intermediate

Table 1. List of rice varieties (genotypes) used in the experiment

No	Variety	Year of release	Origin or pedigree	No	Variety	Year of release	Origin or pedigree
1	CR203	1984	IR8423-132-622	13	AC5	2005	C70//CR203/10TGMS
2	13/2	1988	IR17494-32-3-1-1-3	14	BC15	2008	Spontaneous mutant of 13/2
3	DT10	1990	Gramma and chemical mutation of C4-63	15	RVT	2011	Introduced from Taiwan
4	C70	1994	C671177/Milyang23	16	Thien uu 8	2011	Developed by private company
5	Bac thom 7	1998	Originated from China	17	VTNA2	2011	Originated from China
6	Ai 32	1999	Originated from China	18	Bac thinh	2015	MS4/HT1
7	Khang dan 18	1999	Originated from China	19	TBR225	2015	K2/TBR27
8	Kim Cuong90	1999	Originated from China	20	TBR45	2015	Developed by private company
9	Q5	1999	Originated from China	21	Ha Phat 3	2017	Originated from China
10	Xi23	1999	Introduced from IRRI	22	QP5	2017	Gamma mutation of ST19
11	P6	2000	IR2588/Xuan so 2	23	VTNA6	2018	BM9962/TBR18
12	HT1	2004	Originated from China	24	Dai thom 8	2019	BVN/OM4900

gelatinization temperature are generally favored by most consumers (Rohilla *et al*., 2000; Pang *et al*., 2016). These important attributes related to cooking quality were evaluated following scales:

Regarding amylose content, rice is classified into five groups, *viz* waxy (0-2%), very low (3-9%), low (10-19%), intermediate (20-25%), and high (> 25%) (Juliano *et al*., 1981; Cruz & Khush, 2000). Rice with a higher amylose content always has a harder, less sticky texture after cooking than rice with a lower amylose content (Lu *et al*., 2009; Jang *et al*., 2016) so that rice grains with an amylose content of 16-20% are the most popular type in markets and meet the demand of eating and cooking quality from customers (Song *et al*., 2019).

The classification of gel consistency was followed by the method reported previously by Graham (2002):

(i) Very shelly rice with hard gel consistency (gel length: 40mm or less)

(ii) Shelly rice with medium gel consistency (gel length: 41-60mm)

(iii) Soft rice with soft gel consistency (gel length: above 60mm)

For alkali digestion, the seven-point scale used in assigning different values based on kernel spreading (International Rice Research Institute, 1996) (**Table 2**):

Table 2. Numerical scale for scoring gelatinization temperature (Graham, 2002)

Score	Spreading	Alkali digestion	Gelatinization temperature
1	Kernel not affected	Low	High
2	Kernel swollen	Low	High
3	Kernel swollen, collar complete or narrow	Low or intermediate	High-intermediate
4	Kernel swollen, collar complete and wide	Intermediate	Intermediate
5	Kernel split or segregated, collar complete and wide	Intermediate	Intermediate
6	Kernel dispersed, merging with collar	High	Low
7	Kernel completely dispersed and intermingled	High	Low

Data analysis

The mean data of each character were assembled from all genotypes in each replication were subjected to divergence analysis using Mahalanobis D^2 statistics extended by Rao (1952). On the basis of the magnitude of the D^2 value, clustering of genotypes was implemented according to Tocher's method (quoted by Rao, 1952). Analyses of variance (ANOVA) were conducted for all grain quality traits using STAR version 2.0.1 together with pairwise comparison of each quality parameter among the clusters using Tukeys's Honest Significant Difference (HSD) Test at the 0.05 probability level. Relative importance of variables based on the squared generalized Mahalanobis distance as outlined by Singh (1981). The cluster analysis and the graphs were performed by using "Biotools" package in RStudio.

Results and Discussion

The 24 rice genotypes were assembled in eight clusters based on relative magnitude D^2 estimate (**Table 2**). Among the different clusters, cluster I contained 12 genotypes followed by cluster II and cluster III accommodated 3 genotypes each, cluster IV contained 2 genotypes namely TBR225 and VTNA6. Furthermore, clusters V, VI, VII, and VIII were mono-genotypic clusters because of having one genotype each in a cluster (**Table 3**).

Considering varietal origin in **Table 1**, genotypes originated from China were the most popular in this study, succeeded by domestically developed genotypes (varieties). Genotypes with different origins were distributed in all clusters in which 3 or more genotypes above were determined. Similarly, in relation to release duration, cluster I contained 12 genotypes released after 1998, excepting the Bac thom 7, which also belong in this group by virtue of its low amylose content and grain width traits. Cluster II, III, V, VI, and VIII consisted of the genotypes which have been released two decades or more (before 2000) whereas cluster IV and VII included only genotypes released from 2015 to 2018 (**Table 1**). Especially, cluster II, III, V and VIII consisted of CR203, DT10, Khang dan 18, etc. which have been cultivated in Vietnam for over two decades and were envisaged as the history of rice breeding in North Vietnam (Khanh *et al.*, 2021). Cluster IV included VTNA6 and TBR225 which were newly released, high-yielding and good quality genotypes that are grown widely in North Vietnam (Doan Tri Tue, 2016; Tran Manh Bao *et al.*, 2016). That information also showed release duration and origin (pedigree) of the genotypes played a minor role in clustering for physic and physiochemical grain quality.

The average intra and inter-cluster D^2 values and average genetic distance within and between clusters for quality traits are presented in **Table 4**. The maximum intra cluster divergence was in

Table 3. Cluster composition of 24 rice genotypes

Cluster	Number of genotypes	Name of the genotypes
I	12	QP5, RVT, Thien uu 8, P6, HT1, VTNA2, Bac Thinh, BC15, TBR45, Dai thom 8, AC5, Bac thom 7
II	3	Ai32, Khang dan 18, C70
III	3	13/2, Kim Cuong 90, Q5
IV	2	TBR225, VTNA6
V	1	CR203
VI	1	DT10
VII	1	Ha Phat 3
VIII	1	Xi23

cluster I followed by cluster II and cluster III. The intra cluster divergence was zero in cluster V, VI, VII and VIII since there was only 1 genotype in each of them. The highest intra-cluster distance (296.34) was identified in cluster I comprised of 12 genotypes: so-called QP5, RVT, Thien uu 8, P6, HT1, VTNA2, Bac Thinh, BC15, TBR45, Dai thom 8, AC5, and Bac thom 7 which was indicative of wide genetic divergence among the constituent genotypes. Genotypes within a cluster with a high degree of divergence and different origins would produce more desirable breeding materials for achieving the uppermost genetic advance related to yield and adaptability *per se* provided that there is adequate complementation.

The inter-cluster distance analysis revealed the highest inter-cluster distance between the cluster IV - VI (2247.39), followed by cluster I - VI (2245.94), cluster II - IV (2184.05) and cluster IV - V (2180.61) indicating high divergence of genotypes included in these two cluster pairs (**Table 4**). According to Rahaman *et al*. (1997) and Szamosi *et al*. (2010), the crossing between highly divergent genotypes would construct a wide spectrum of trait variability supporting further selection and improvement. Therefore, the varieties developed from the genotypes within the traits of compatibility of these clusters in a crossing program may produce high magnitude of desirable transgressive segregants. The lowest inter-cluster distance was between the clusters I and IV (438.20) indicating that genotypes included in these two clusters were closely related. The results also revealed that cluster IV had high inter-cluster distance with most of the clusters.

Selection within such clusters might be implemented based on the maximum mean value for the desirable characters (Binodh *et al*., 2010).

For cluster mean analysis, average cluster mean of more than 6.60mm grain length was found in cluster IV, VI, VII whereas only cluster VII has been recorded for grain length/width ratio (grain shape) at >3.0. Brown rice percentage of clusters I (81.28%) and cluster VIII (78.80%) were determined as lowest however, those clusters had the highest head rice recovery: 56.58% and 54.63% respectively. Highest desirable value for head rice recovery (56.58%), chalkiness (0.44), long slender grain (L/W ratio ≥3) were observed in cluster I, while cluster VII included only one genotype of Ha Phat 3 that had the same level as those parameters excepting grain length was significantly higher (7.20mm) (**Table 5**). Generally, the hulls form 20% to 22% of the rough rice, although variation of 16% to 28% has been recorded (Juliano & Gonzales,

Table 4. Average intra-cluster (diagonal and bold) and inter-cluster (off-diagonal) D^2 values for eight clusters derived from 24 rice genotypes

Cluster	I	II	III	IV	V	VI	VII	VIII
I	**296.34**	1758.63	1659.46	438.20	1933.65	2245.94	561.65	749.65
II		**265.58**	540.52	2184.05	453.31	900.62	1821.20	711.06
III			**254.36**	1715.17	477.10	448.38	1856.68	927.74
IV				**165.53**	2180.61	2247.39	561.89	1196.63
V					**0.00**	540.16	1754.04	961.49
VI						**0.00**	1827.07	1060.36
VII							**0.00**	601.05
VIII								**0.00**

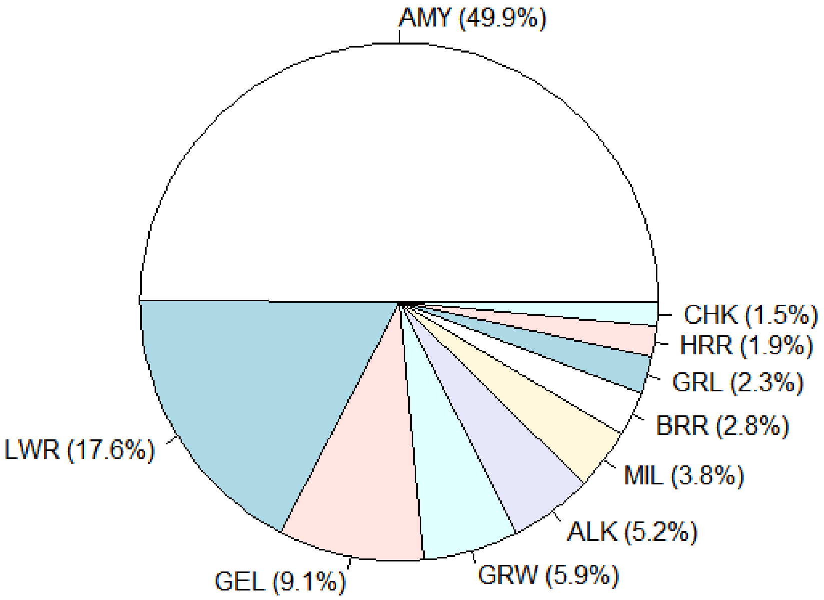
1989). Bran and embryos constitute another 8% to 10%. Thus, from a given sample of rough rice, about 70% milled rice is obtained (Graham, 2002). The proportion of whole grains is known as head rice recovery and is expressed as the percentage of rough rice. Thus, the rough rice sample is 100g, 70g of milled rice is obtained. Some studies concluded that the highest milling percentage of modern rice varieties can be up to 73% (Biswas *et al*., 1992; Sun & Siebenmorgen, 1993). The head-rice recovery may vary from as low as 25% to as high as 65% (Khush *et al*., 1979). Findings from Rao *et al*., (2017) showed that rice grains with an amylose content of 14-18%, soft gel consistency, low gelatinization temperature, long slender and head rice recovery >45%, chalkiness <10% are the most popular needs in markets and meet the demand of eating and cooking quality from Vietnamese customers. Comparing with the above market requirements, amylose content is an important quality parameter which normally is preferred from 14-18% by consumers was found in cluster I, IV and VII. Cluster I showed the most favorable value of gel consistency (53.72mm) and alkali digestion (3.82). Cluster IV and VII showed significantly different values of alkali digestion: 3.03 and 4.00 respectively but those values are still in the preferred range.

The results in **Table 5** show that the average cluster mean values for most of the quality parameters were found promising in clusters I, IV and VII. Among the above clusters, higher cluster distance was observed between clusters I and VI; between cluster IV, cluster IV and cluster II indicating that it could be possible to use the promising genotypes with high mean values for different traits from divergent clusters in hybridization program depending upon the objective of the breeding program to derive superior transgressive segregants.

Contribution of different quality characters to the genetic divergence among rice genotypes under this study is presented in **Figure 1**. The amylose content (49.9%), length/width ratio (17.6%) and gel consistency (9.1%) were among the major contributing factors towards genetic diversity among 24 rice genotypes. The amylose content and length/width ratio on genetic divergence in rice have been reported earlier (Binodh *et al*., 2010) which agreed with the results of the present study. According to Anacleto *et al*. (2015), amylose content is widely explored to capture the diversity of rice quality whereas Tomlins *et al*. (2007) found grain size and shape is an important visual parameter of rice that influences consumers' preference thereby making it one of the grain quality raits being

Table 5. Cluster means of physicochemical characters in 24 genotypes

Character	Clusters							
	I	II	III	IV	V	VI	VII	VIII
Brown rice percentage (%)	81.29	82.20	82.43	82.20	82.20	82.73	82.30	78.80
Milling rice percentage (%)	68.39	69.63	64.87	69.10	69.30	62.97	69.40	65.10
Head rice recovery (%)	56.58	50.97	48.73	49.30	47.10	47.90	51.50	54.60
Chalkiness (score)	0.44	2.77	5.47	3.30	6.30	5.00	0.70	2.30
Grain length (mm)	6.48	5.87	5.90	6.70	6.20	6.97	7.20	6.30
Grain width (mm)	2.20	2.50	2.73	2.40	2.50	2.79	2.30	2.50
Length/width ratio	2.98	2.40	2.20	2.85	2.50	2.50	3.00	2.50
Gel consistency (mm)	53.73	32.77	40.20	77.85	33.00	35.33	63.30	41.70
Alkali digestion (score)	3.83	5.83	3.13	3.05	2.00	2.67	4.00	4.70
Amylose content (%)	15.72	27.17	26.87	16.15	27.60	28.20	16.80	21.30



Note: AMY: amylose content; LWR: length/width ratio; GEL: gel consistency; GRW: grain width; ALK: alkali digestion; MIL: milling rice percentage; BRR: brown rice percentage; GRL: grain length; HRR: head rice recovery; CHK: chalkiness.

Figure 1. Relative contribution of ten quality characters to D^2 values in 24 genotypes

considered in varietal development and improvement. The grain width (5.9%), alkali digestion (5.2%), milling rice percentage (3.8%), brown rice percentage (2.8%) and grain length (2.3%) were moderate contributing traits towards genetic divergence. The least contribution was observed in head rice recovery (1.9%) and chalkiness (1.5%) towards the total genetic divergence. Hence, the parameters like amylose content, length/width ratio, gel consistency can be utilized as key factors of selection in the segregating generations.

Vietnam possesses a vast diversity of native and traditional rice varieties due to its geographical situation, latitudinal range and diversity of ecosystems (Fukuoka *et al*., 2003). This diversity along with the divergence of cultivated varieties constitutes a largely untapped and highly valuable genetic resource for local and international breeding programs (Higgins *et al*., 2021). Nguyen Thanh Tuong *et al*. (2005) evaluated 55 rice varieties grown coastal of Ben Tre, Long An, Tien Giang, and Tra Vinh provinces (in South Vietnam) found that the amylose content was the most divergent trait. In the Central of Vietnam, Tran *et al*. (2012) reported that rice cultivars display a wide range of variations in amylose content. Those findings were similar to the results of this study. Other researchers used the molecular marker-based PCR analysis and multivariate analysis was done by PCA and cluster analysis with of 350 diverse accessions in Mekong delta found large diversity and variable in the gene pool also suggested breeder should have priority for exploring the genetic variation of the amylose content (Nguyen *et al*., 2018).

Conclusions

The inter-cluster distances were generally larger than the intra cluster distance in this study showing wider diversity was present among genotypes of distant groups. The highest intra-cluster distance was (296.34) in cluster I which contains 12 genotypes released in different duration, and the maximum inter-cluster distance was between clusters IV and VI. The divergence of quality characteristics among 24 rice genotypes did not show a clear relationship to release duration.

Regarding cluster mean, almost desirable quality values were identified in cluster I such as amylose content, head rice recovery, chalkiness, gel consistency and alkali digestion. The three of the most important factors contributing to genetic divergence in this study were amylose content (49.9%), length/width ratio (17.6%) and gel consistency (9.1%) among ten studied traits. Overall, the results indicated that the priority should be used for the members of cluster I and cluster IV comprising of diverse genotypes with different release duration for high-quality breeding programs.

Acknowledgments

The author is highly thankful for the technical support given by Ms. Nguyen Thi Lan Anh (Food Crop Research Institutes) in laboratory work and Dr. Nguyen Thi Thu Ha, Center of Agricultural Research and Ecological Studies (Vietnam National University of Agriculture) for critical comments to improve the manuscript.

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