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Research Article

Study on physicochemical properties of rice landraces for amylose, gel consistency and gelatinization temperature

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Abstract
Rice grain quality depends on physical, biochemical, cooking quality and organoleptic characters. Grain quality analysis in rice involves a physicochemical and sensory tests. In this study, morphologically different 60 rice landraces of Tamil Nadu along with ADT (R) 45 as a check variety were used for physicochemical analysis based on the amylose content (AC), gel consistency (GC) and gelatinization temperature (GT). Highly significant variation ($p<0.05$) was observed among the 60 landraces for all the characters. Most of the landraces registered low amylose content. Nine landraces exhibited high and 18 landraces exhibited intermediate AC. In the case of a gel consistency, most of the landraces exhibited soft GC, while four landraces *viz.,* Athur kichadi, Kavuni nel, Uppu mulagai and Valan registered medium gel. With respect to GT, all the landraces exhibited high to intermediate GT except Kattu ponni, Kavuni nel and Sugandni samba which were identified with low GT. The check variety exhibited intermediate amylose, soft gel and high gelatinization temperature. The landraces Chennellu, Kattu vanipam, Kichali samba, Kuttala samba, Pome, Revathi, Samba masanam, Saysree, Sembalai, Sivappu Kavuni, Soora kuruvai, Thailand Kavuni, Thanga samba and Thirupathisaram were identified as promising landraces based on intermediate amylose, soft gel consistency and intermediate gelatinization temperature. High genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) coupled with high heritability and high genetic advance as a percentage of mean were recorded in all three characters indicating additive gene action. This indicates that there was a low environmental influence on the expression of these characters and hence one can practice selection. The results of this study provide vital information on amylose content gel consistency and gelatinization temperature of various rice landraces which can be utilized in a breeding programme directed towards improving the cooking and eating quality. Landraces with high amylose content can be further studied for identifying landraces with high resistant starch and low Glycaemic Index.

Key words: Amylose content, Gel consistency, Gelatinization temperature, landraces, rice

INTRODUCTION

Rice (*Oryza sativa.* L.) is a staple food crop in South and South East Asia and forms the major carbohydrate source for humans. Grain yield enhancement became a prime objective for a plant breeder for several decades but demand for good quality rice was also increased since the late 90s onwards as the living standard of the people was gradually improved (Rathi *et al.,* 2010). Therefore, the production of quality rice became a major objective in rice breeding programmes to meet consumer preference and market demand (Anang *et al.,* 2011). Rice varieties having good milling parameters, appearance, cooking and eating parameters are considered as superior grain quality rice variety which increases the total economic value of rice (Custodio *et al.,* 2019). Rice is an important food for most of the people, giving around 80 per cent in every day energy intake (Palanisamy and Usha, 2020). Rice consumers in Thailand, Malaysia and the Philippines prefer long and slender grains, while consumers in Indonesia and Bangladesh prefer grains that are medium in length and slender. Whereas in South India fine or short slender grain varieties with intermediate amylose and alkali spreading value, soft gel consistency and high-volume expansion of cooked rice are preferred and in North-Western Indian states *viz.,* Punjab and Haryana, prefer extra-long grains.

Many locally cultivated landraces, local varieties, indigenous lines play a pivotal role in food nutritional and health security besides place a role in providing a genetic source for resistance to diseases, pests and tolerance to abiotic stress *viz.,* drought, salinity and flood conditions (Hassan *et al*., 2020). Many landraces are adapted to a specific region and harbour genes for physical properties include the grain shape, degree of milling, grain appearance, milling recovery, kernel shape and quality traits including GC, alkali digestion, GT, AC and fragrance (Sattari *et al.,* 2015).

Many landraces are not being taken as white rice due to their unsatisfiable palatability properties. These eating qualities are majorly controlled by the quantity and quality of amylose and amylopectin. The amylose content shows varied diversification *viz.,* waxy (0-2%), very low (3-9%), low (10−19%), intermediate (20−25%), and high (>25%) (Kumar and Khush, 1986). Most of the Indian consumers prefer intermediate amylose due to their good palatability. So, the development of variety with intermediate amylose is a common goal for plant breeders. Gel consistency measures the cold paste viscosity of cooked milled rice flour an index used in distinguishing cooked rice texture of high amylose landraces. Gel consistency varies from soft to hard (Cagampang *et al.,* 1973). The Association of starch polymers in the aqueous phase determines weak and rigid gels. Rice with soft gel consistency is preferred more by the consumers than hard gels. Gelatinization temperature, is another important physical property of starch, which decides the cooking time of rice wherein at least 90 per cent of the starch granules swell irreversibly in hot water. The gelatinization temperature of rice varieties may be classified as low (55 to 69°C), intermediate (70 to 74°C), and high (>74°C). An estimate of the gelatinization temperature is indexed by the alkali digestibility test (Little *et al.,* 1958). The degree of spreading the value of individual milled rice kernels in a weak alkali solution (1.7% KOH) is very closely correlated with gelatinization temperature. Keeping these points in view, the present research was conducted to evaluate the available rice landraces for their physicochemical properties and to identify the desirable landraces for a breeding programme.

MATERIALS AND METHODS

The experimental material comprised of 60 traditional rice landraces collected from the Plant Breeding and Genetics unit, TRRI, Aduthurai and a high yielding variety ADT (R) 45 as a check (**Table 1**). These landraces were raised during September 2019 at Tamil Nadu Rice Research Institute (TRRI), Aduthurai, Thanjavur. The area lies 20 meters above mean sea level with 11.0140° N, 79.4811° E longitudes. Twenty-five days old seedlings were transplanted into the main field in randomized block design in two replications with a spacing of 20 x 20 cm. Landraces were grown under irrigated conditions and standard crop production practices were followed. All the samples were harvested at the physiological maturity stage and moisture content was maintained to 12-14 per cent by using a hot air oven (Stericox-STXLO45) and kept at room temperature for four months then used for grain quality parameter estimation. All these landraces along with check variety ADT (R) 45 were subjected to cooking and eating quality analysis in two biological replicates.

Grain quality analysis was done at Quality lab, Plant Breeding and Genetics unit, TRRI, Aduthurai. The amylose content of each landrace was estimated by following the method of Juliano (1972). 100 mg rice flour was taken into a clean and dried100 ml volumetric flask and added 1ml ethanol (95%) with 9 ml of 1 N sodium hydroxide (NaOH). The samples were kept on a boiling water bath (89^{0}C) followed by cooling for 10 minutes at room temperature. The final volume was made up to 100 ml by adding distilled water. Thereafter, a 5 ml aliquot was pipetted out from 100 ml stock solution into another 100 ml volumetric flask and1ml of 1 N acetic acid and 2 ml of freshly prepared iodine solution was added. Solutions were shaken properly and kept in a dark place for 20 minutes and determine the absorbance at 620 nm using a UV-Vis spectrophotometer (Jasco, Cambridge, UK). Amylose content was quantified from a standard curve generated from absorbance values of known standard rice variety as a check (ADT (R) 45).

The properly dried paddy was subjected to hulling and milling by using Zaccaria's Rice Miller (Mod. PAZ-1/DTA). The polished rice of each landrace and the check variety was powdered using pestle and mortar followed by sieved with 1 mm sieve. For the estimation of a gel consistency, in a long (1.8 x 15 cm) clean and dried test tube, 100 mg of fine rice powder was taken followed by adding of 0.2 ml of ethanol containing 0.25% thymol blue and 2ml 0.2N Sodium hydroxide in each test tube and mix the contents properly. All the samples were kept in the hot water bath for 8 minutes then cooled for 5 minutes. All

Table 1. List of rice landraces used in the study

S. No.	Landraces	S. No.	Landraces
1	Adukan	32	Norungan
2	Altera	33	Ottadam
3	Anai komban	34	Palkichadi
4	Athira	35	Pome
5	Athur kichadi	36	Poongar
6	Bhavani	37	Rajalakshmi
7	Burma Kavuni	38	Rajamannar
8	Chennellu	39	Rasakadam
9	Chinkini Kar	40	Rathasaali
10	Chithrai kar	41	Revathi
11	Garudan samba	42	Salem samba
12	Kaivara samba	43	Samba masanam
13	Kalanamak	44	Sanga samba
14	Kandasali	45	Saysree
15	Karuppu Kavuni	46	Sembalai
16	Karuthakar	47	Sivappu Kavuni
17	Kattai kar	48	Sivappu malli
18	Kattu ponni	49	Soora kuruvai
19	Kattu vannipam	50	Sowttara samba
20	Kavuni nel	51	Sugandni samba
21	Kichali samba	52	Thailand Kavuni
22	Kudavarghai	53	Thanga samba
23	Kuttala Samba	54	Thengai poo samba
24	Lalmati	55	Thirupathisaram
25	Manda maranellu	56	Thodipaliyan
26	Manjal ponni	57	Uppu mulagai
27	Manvilayan	58	Vaalan
28	Mappillai samba	59	Varppu kudachan
29	Melaki	60	Vasura mundan
30	Mysore malli	Check	ADT 45
31	Nammahuar		

the samples were vortexed again and kept in an ice water bath (Clifton-NE2D-4/22) for 20 minutes. Later on, all the tubes were taken out and laid horizontally on laminated graph paper for one hour to take the measurement. The landraces were grouped based on the length of the gel as hard (length of gel :40 mm), medium (length of gel :41-60 mm), and soft (length of gel : 61 mm) (Graham, 2002).

Gelatinization temperature was measured by the extent of Alkali Spreading Value (ASV) of milled rice (Singh *et al*., 2000). Six whole grains were taken in petri plates and 10 ml of 1.7 % potassium hydroxide solution (KOH) was added and kept for 23 hours at 30°C (Singh *et al*., 2000). They were evenly placed inside the petri plates so that each kernel does not disturb each other during expansion. After 23 hours, scores (1, 2 - high, 3-intermediate to high, 4, 5-intermediate, 6, 7-low) (Singh *et al.,* 2000) were given based on the visual observation. Rice with low GT disintegrates completely, the ones with intermediate GT were slow in disintegration while those with high GT were unaffected.

Analysis of variance (ANOVA) with post-hoc analysis and variability studies was carried out to check the significant difference, amount of variation and nature of traits present in the landraces for AC, GC and GT. Variability analysis was estimated as Burton (1952) for calculation of GCV and PCV. Broad sense heritability (h^2) was calculated as per the method suggested by Hanson *et al.* (1956). Similarly, the method suggested by Johnson *et al.* (1955) was used for the calculation of genetic advance (GA). PCV, GCV, heritability and genetic advance were analyse dusing TNAUSTAT-Statistical package (v1.1) (Manivannan, 2014).

RESULTS AND DISCUSSION

Amylose content is one of the major quality traits used for selection in a breeding programme for cooking and eating quality (Sarawgi *et al.,* 2000). Among the 60 rice landraces no landraces registered for waxy type amylose (0-2%). Very low amylose content (3-9%) was observed in two landraces *viz.,* Uppu mulagai and Barma Kavuni. Thirty-one landraces exhibited low amylose

Table 2. Physicochemical properties of rice landraces used in this study

S. No.	Landraces	Amylose		Gel consistency		Gelatinization temperature	
		Amylose (%)	Category	Gel length (mm)	Type	Score	Type
1	Aanai komban	18.56	Low	92	Soft gel	4	Intermediate
2	Adukan	15.50	Low	110	Soft gel	5	Intermediate
3	Altera	28.10	High	90	Soft gel	5	Intermediate
4	Athira	26.94	High	68	Soft gel	3	Intermediate to high
5	Athur Kichadi	22.48	Intermediate	46	Medium gel	1	High
6	Burma Kavuni	9.59	Very low	82	Soft gel	3	High or intermediate
7	Bhavani	17.53	Low	73	Soft gel	4	Intermediate
8	Chennellu	20.65	Intermediate	71	Soft gel	5	Intermediate
9	Chinkini Kar	19.45	Low	90	Soft gel	5	Intermediate
10	Chitrai Kar	10.74	Low	83	Soft gel	5	Intermediate
11	Garudan Samba	10.25	Low	63	Soft gel	5	Intermediate
12	Kaivara samba	14.05	Low	71	Soft gel	4	Intermediate
13	Kalanamak	15.70	Low	90	Soft gel	3	High or intermediate
14	Kandasali	17.79	Low	97	Soft gel	3	High or intermediate
15	Karuppu Kavuni	24.63	Intermediate	111	Soft gel	5	Intermediate
16	Karuthakar	15.04	Low	96	Soft gel	5	Intermediate
17	Kattai kar	19.50	Low	105	Soft gel	4	Intermediate
18	Kattu ponni	22.48	Intermediate	110	Soft gel	6	Low
19	Kattu vanipam	24.63	Intermediate	111	Soft gel	5	Intermediate
20	Kavuni Nel	27.60	High	58	Medium gel	7	Low
21	Kichali samba	21.65	Intermediate	107	Soft gel	5	Intermediate
22	Kudavarghai	19.24	Low	79	Soft gel	5	Intermediate
23	Kuttala samba	22.00	Intermediate	102	Soft gel	5	Intermediate
24	Lalmati	19.65	Low	130	Soft gel	4	Intermediate
25	Manda maranellu	16.74	Low	106	Soft gel	4	Intermediate
26	Manjal ponni	16.92	Low	109	Soft gel	4	Intermediate
27	Manvilayan	19.85	Low	82	Soft gel	4	Intermediate
28	Mappillai samba	28.93	High	95	Soft gel	5	Intermediate
29	Melaki	16.03	Low	117	Soft gel	5	Intermediate
30	Mysore malli	17.53	Low	105	Soft gel	3	High or intermediate
31	Nammahuar	28.76	High	107	Soft gel	5	Intermediate
32	Norugan	18.96	Low	89	Soft gel	5	Intermediate
33	Ottadai	10.25	Low	70	Soft gel	3	High or intermediate
34	Palkichadi	31.30	High	87	Soft gel	5	Intermediate
35	Pome	22.35	Intermediate	88	Soft gel	4	Intermediate
36	Poongar	26.61	High	85	Soft gel	4	Intermediate
37	Rajalakshmi	27.93	High	71	Soft gel	5	Intermediate
38	Rajamannar	17.63	Low	82	Soft gel	4	Intermediate
39	Rasakadam	15.70	Low	75	Soft gel	4	Intermediate
40	Rathasaali	25.79	High	72	Soft gel	4	Intermediate
41	Revathi	21.00	Intermediate	65	Soft gel	4	Intermediate
42	Salam samba	16.40	Low	76	Soft gel	4	Intermediate
43	Samba masanam	20.83	Intermediate	91	Soft gel	4	Intermediate
44	Sanga samba	18.00	Low	68	Soft gel	4	Intermediate
45	Saysree	21.52	Intermediate	125	Soft gel	4	Intermediate
46	Sembalai	20.00	Intermediate	110	Soft gel	4	Intermediate
47	Sivappu Kavuni	21.37	Intermediate	105	Soft gel	4	Intermediate
48	Sivappu malli	16.50	Low	79	Soft gel	4	Intermediate
49	Soora kuruvai	24.25	Intermediate	125	Soft gel	4	Intermediate
50	Sowttara samba	10.25	Low	93	Soft gel	4	Intermediate
51	Sugandni samba	17.52	Low	73	Soft gel	7	Low
52	Thailand Kavuni	21.16	Intermediate	104	Soft gel	4	Intermediate
53	Thanga samba	22.15	Intermediate	86	Soft gel	4	Intermediate
54	Thengai poo samba	17.28	Low	82	Soft gel	4	Intermediate
55	Thirupathisaram	20.00	Intermediate	70	Soft gel	4	Intermediate
56	Thodipaliyan	18.50	Low	78	Soft gel	4	Intermediate
57	Uppu mulagai	4.63	Very low	54	Medium gel	4	Intermediate
58	Valan	15.50	Low	47	Medium gel	4	Intermediate
59	Varppu kudachan	19.50	Low	66	Soft gel	4	Intermediate
60	Vasara mundan	21.65	Intermediate	70	Soft gel	3	High or intermediate
Check	ADT 45	24.21	Intermediate	66.50	Soft gel	3	High or intermediate

(10-19 %), 18 landraces along with the check variety ADT (R) 45 recorded intermediate amylose (20-25%) and nine landraces registered high amylose content (>25%) (**Table 2**). Most consumers prefer rice with intermediate AC since cooked rice will be soft, flaky with good palatability (Cuevas *et al.,* 2018). Amylose content variation in all levels (low, intermediate and high) in rice landraces was reported by Sahu *et al.* (2017), Taratima *et al.* (2019) and Pokharel *et al*. (2020). This could be due to the variation in genetic level. In contrast, most of the genotypes were registered intermediate AC in the work of Palanisamy and Usha (2020) which indicates narrow variation in genotypes and cooked rice was soft in texture.

Gel consistency analysis was developed as an indirect method to screen the cooked rice for its hardness (Juliano, 1985). Based on gel consistency values, these landraces were classified into soft, intermediate and hard gel consistency (Graham, 2002). Landraces with soft gel consistency had a gel length that is more than 61 mm. GC values ranged from 46.00 mm in Athur kichadi to 130.00 mm in Lalmati. Among the 60 rice landraces, 56 landraces along with check variety exhibited soft gel consistency (>60 mm) and four landraces registered medium gel consistency (41-60 mm) (**Table 2 & Fig. 1**). The majority of the landraces in this study registered soft gel consistency, this could be due to the low level of amylose content since these traits were related to each other (Jan and Subhash, 2020). Rice with soft gel consistency cooks more tenderly and remains soft even after cooling (Pokharel *et al.,* 2020). Similar results of soft GC in most of the genotypes were reported by Bharath *et al.* (2018) and found that rice remains soft after cooking was due to soft GC.

Gelatinization temperature is one of the important characters which decide the time taken to cook the rice. GT can be estimated indirectly by analysing alkali digestion in weak potassium hydroxide solution (Juliano, 1972). In this study 60 landraces were subjected to alkali spreading estimation and found that Athur kichadi showed high GT, seven landraces exhibited high to intermediate GC, three landraces exhibited low GT, remaining 49 landraces registered intermediate gelatinization temperature and high-intermediate GT was exhibited by check variety (**Table 2**). The rice varieties with intermediate GT are preferred all over the world, as the high GT rice becomes excessively soft when overcooked elongate less (Singh *et al.,* 2020). Similar work on gelatinization temperature was carried out by (Hasan *et al.,* 2020) and found that most of the genotypes exhibited intermediate GT. The wide diversification of the physicochemical results might be due to the wide genetic background of the rice landraces under study.

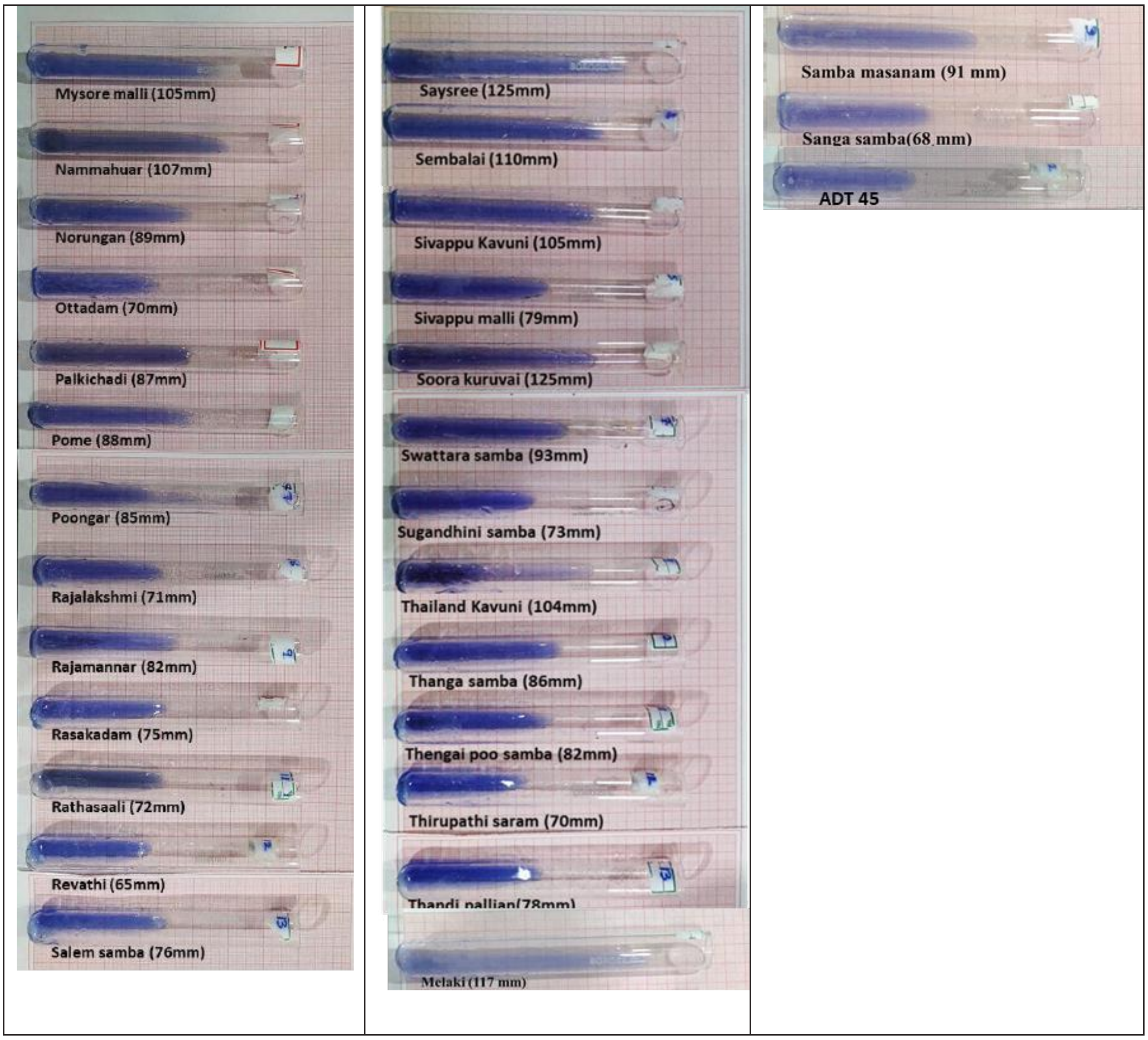

Fig. 1. Gel consistency of 60 rice landraces

The ANOVA among 60 landraces indicated highly significant differences ($p<0.01$) (based on F value and mean sum of square) with respect to all the three characters studied (**Table 3**). The magnitude of variation between landraces was reflected by high values of mean and range for traits studied (Jan and Subhash, 2020). Among three characters high mean and range was observed in GC followed by AC and GT (**Table 4**), which indicated that the landraces under study were genetically diverse. Post-hoc analysis was carried out, to follow up the significant difference observed in ANOVA and to check the difference between characters. In post-hoc analysis, a high significant difference ($p<0.01$) was observed between AC-GT followed by GC-GT and AC-GC (**Table 3**) suggesting that all three characters are differs from each other.

In this study, high GCV and PCV was observed in AC (26.27%, 26.41%), GC (22.19%, 22.13%) and GT (22.17%, 22.37%) (**Table 4**) and the difference between GCV and PCV was very minimum. Narrow differences between the GCV and PCV implies the lesser influence of the environment on these traits and a consequently greater role of genetic factors on the expression of traits. Hence, these characters can be relied upon and simple selection can be practised for further improvement. Similar observations were also noted earlier by Bharath *et al.* (2018) and Sharma *et al.* (2020) for AC, GC and GT in rice. In contrary to the present study, moderate PCV and GCV was reported by Savitha and Kumari (2016) for amylose content which indicates the fair level of scope for phenotypic selection.

Estimates of phenotypic and genotypic coefficients of variation alone are not sufficient to enough assess the heritable variation. For a more reliable conclusion, estimates of high heritability and high genetic gain should be considered together (Johnson *et al.,* 1955). In this study high heritability coupled with high genetic advance as percentage of mean were recorded in AC (98.94%,

Table 3. Analysis of variance with post-hoc test for physicochemical properties

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	234764.41	2	117382.20**	871.828	2.2926E-92	3.047012
Within Groups	23831.12	177	134.64			
Total	258595.53	179				

** Significance at 1 per cent level; Bonferroni corrected alpha - 0.0167

Post-hoc test

Characters	Mean	Variance			P(T<=t)
Amylose (AC)	19.550	27.889	**AC**	GC	5.9301E-51
Gel consistency (GC)	87.366	375.151		GT	1.0433E-43
Gelatinization temperature (GT)	4.267	0.877	**GC**	GT	1.0844E-61

Table 4. Variability analysis in landraces for physicochemical properties

Traits	Mean	Range	PV (δ^2p)	GV (δ^2g)	PCV (%)	GCV (%)	h^2 (%)	GA	GAM (%)
Amylose	19.46	4.63-31.30	26.42	26.14	26.41	26.27	98.94	10.48	53.83
Gel consistency	87.33	46-130	375.65	375.50	22.33	22.19	99.96	39.91	45.70
Gelatinisation temperature	4.28	1-7	0.92	0.90	22.37	22.17	98.23	1.94	45.26

PV - phenotypic variance; GV –genotypic variance; PCV - phenotypic coefficient of variation; GCV - genotypic coefficient of variation; h^2 - broad sense heritability; GA –genetic advance; GAM –genetic advance as percentage of mean

53.83%), GC (99.96%, 45.70%) and GT (98.23%, 45.26%) (**Table 4**). This indicates that these traits were controlled by additive gene action and hence one can practice selection for improvement. The heritable fraction of the variation provides the base to the plant breeder for selection based on phenotypic performance and broad sense heritability gives an idea about the portion of observed variability attributable to genetic differences (Sahu *et al*., 2017). Heritability estimation helps the plant breeder in selecting better genotypes, hence prior knowledge about the heritability of the traits is necessary in plant breeding for selection (Singh *et al.,* 2018). Therefore, the estimates of heritability and genetic advance would help to formulate a sound breeding programme. High heritability may not always be associated with high genetic advances. Since high heritability does not always indicate a high genetic gain, heritability is recommended to be considered in association with genetic advance to predict the effect of selecting superior crops varieties. Characters with high heritability coupled with high genetic advance reported for better selection than those with high heritability and low genetic advance (Jan and Subhash, 2020). High heritability coupled with high genetic advance as per cent of mean was also reported by Sharma *et al.* (2020) for amylose content, gel consistency and gelatinization temperature.

The results obtained from the study suggested that physicochemical characterization based on AC, GC and GT exhibited considerable variation among the landraces. Most of the rice landraces under study exhibited low amylose content, soft gel consistency and intermediate gelatinization temperature and these traits are important in deciding the cooking quality and palatability of rice. Based on intermediate amylose, soft gel consistency and intermediate gelatinization temperature.,Chennellu, Kattu vanipam, Kichali samba,Kuttala samba, Pome, Revathi, Samba masanam, Saysree, Sembalai, Sivappu Kavuni, Soora kuruvai, Thailand Kavuni, Thanga samba and Thirupathisaram were identified as promising landraces. The variation in these parameters can be exploited by the rice breeders through a hybridization programme to improve the existing varieties and to develop varieties with desirable characters, for their better acceptance by farmers and consumers.

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