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Quantification of Chalky Area, Amylose and Amylopectin in Single Rice Grain

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Abstract

Rice grains with chalkiness are considered inferior in cooking quality. Chalky grains contain air spaces in the accumulated starch granules and may vary in amylose content from translucent grains of the same genotype. Further, chalky area percentage (CAP) varies among the genotypes. Hence, this study assessed CAP, amylose and amylopectin contents in individual chalky and translucent grains of seven rice varieties cultivated in wet and dry seasons. Besides, other cooking quality parameters, kernel length after cooking (KLAC), alkali spreading value (ASV), volume expansion ratio (VER) and pasting profiles were assessed in translucent and chalky grains separately. CAP widely varied (5-75%) in the individual grains of varieties. Grains with CAP >75 were more in dry season indicating the influence of environment on chalkiness. In chalky and translucent grains, variation in KLAC and VER were highly significant while variation in ASV was non-significant. Amylose and amylopectin exhibited inverse and direct relationships with CAP, respectively. Compared with translucent grains, the four measured and three derived viscosities showed significant variations in the chalky grains. The cool paste or final viscosity values were lower in the chalky grains. Variation in the cooking quality behaviour of chalky grains is genotype dependent. However, genotypes categorized as VOC may not affect the overall pasting properties or cooking quality of the rice genotype.

Keywords: Rice grain quality, Chalkiness, Amylose, Amylopectin, Chalky area, Single grain

Introduction

Chalkiness is one of the crucial parameters of appearance quality of rice grain. Rice cultivars with more than 20% chalky kernels are in general unacceptable in the world markets. Generally, chalkiness is identified by the presence of partial or complete opaque area inside the endosperm and is influenced by genetic and environment factors (Juliano, 1979). The extent of induced chalkiness varies among the varieties (Yamakawa *et al*., 2007) and the same variety may contain both chalky and translucent grains.

Mutant male sterile [ms-h (t)] gene (Koh *et al.,* 1999), lack of starch-branching enzyme IIb (BEIIb) gene (Nishi *et al.,* 2001), 1-aminocylopropane-1-carboxylic acid (ACC) contents and variation in the activities of the key enzymes involved in starch biosynthesis during grain filling (Jin *et al.,* 2010) are the genetic factors responsible for chalkiness. Whereas environmental factors like sulphur deficiency (Shaeffer *et al.,* 1987), infection by rice blast and sheath blight (Candole *et al.,* 2000), elevated carbon dioxide (Yang *et al.,* 2007), increase in temperature during grain filling period and particularly night time temperatures (Lanning *et al.,* 2011; Tu *et al.,* 2023; Jaldhani *et al.,* 2021), resulting in deterioration of grain quality traits including amylose content, gel consistency and head rice recovery under high-temperature stress. Similarly, rise in irrigation water temperature (Nisha *et al.,* 2025) increases chalkiness.

Chalky grain is filled with loosely packed, round and large compound starch granules while translucent grain contains tightly packed, polyhedral and small single starch granules (Yamakawa *et al.,* 1980, Singh *et al.,* 2006, Fujita *et al.,* 2007).

These reports imply that the accumulation of starch in chalky grain can vary from the translucent grain either in quality, quantity or both. Since starch is the dominant component of the grain change in its quality or quantity can affect the overall grain quality. Though starch contains two linear polysaccharides, amylose (unbranched) and amylopectin (branched), the former was more studied in relation to chalkiness. Considering the variation in chalky size in the cultivars and the possibility of independent inheritance of chalky kernel and chalky area (Zhang *et al.,* 2009), it is essential to reveal the relation between chalky area and starch components in individual grains.

Translucently chalky area in individual grains can be measured manually using a magnifying glass. Though expensive, digital imaging technologies (Yoshioka *et al.,* 2007, Armstrong *et al.,* 2019) can minimize analysis time and human errors as chalky grains transmit less amount of light than translucent grains. They are useful for chalkiness quantification at single grain level in terms of length-width ratio, percentage of grains with chalkiness, area of chalky endosperm and degree of endosperm chalkiness (Wan *et al.,* 2005).

Pasting properties were studied in rice among genotypes (Singh *et al.,* 2006, Champagna *et al.,* 1999, Bao *et al.,* 2002, Bao *et al.,* 1999, Sun *et al.,* 2011, Liu *et al.,* 2009, Devi *et al.,* 2009), lines of Koshihikari × Kasalath mapping population varying in percentage of grains with chalkiness (PGWC) (Zhang *et al.,* 2012), mixture of rice having 0 to 100% chalky grains (Chun *et al.,* 2009), gene edited (Liu *et al.,* 2022), rice cultivated at varying seasonal temperatures (Tu *et al.,* 2023).

Based on this background, rice grain of seven genotypes cultivated in both wet and dry seasons were screened for chalkiness, cooking quality parameters and pasting properties to explore for any inter-relationship between chalkiness and the quality parameters as well as pasting properties.

Materials and Methods

Seven rice varieties, Aditya, Dhanarasi, Jaya, Nidhi, Suraksha, Swarnadhan and Tulasi, were cultivated during wet season 2015 and dry season 2016 in the experimental fields of ICAR-IIRR, Hyderabad, following standard package of practices. The harvested grain of each sample was collected and dried (14% moisture content) under ambient conditions. The grain was stored at room temperature for three months and then processed as below.

Sample Processing

Each paddy sample was dehusked using Satake TH4 (No. 1012077) and the brown rice was polished using Satake TM05C (No. 554456) miller.

Quality analysis

The milled grain was manually separated into translucent (T) and chalky (C) grain using a trans-illuminator (Armstrong *et al.,* 2019). Each chalky grain was placed on trans-illumination box keeping the germ side of the grain on the left bottom and its image was recorded along with a needle of known length (placed above grain) using Nikon D80 camera at 60.0 mm focal length with 0.016 min exposure time. Later, each image was processed separately with i*mageJ* software. Scale for each image was adjusted using the known dimensions of the needle, image was converted to 8-bit, chalky area of the grain was selected by adjusting the threshold value and the selected area was measured using analyse particle tool of the software. Similarly, total area of the grain (same image) was also determined which was considered 100% while calculating the chalky area percentage (CAP) in each grain. Chalky area was estimated in 93 individual chalky grains of the above varieties cultivated in both the seasons.

CAP in single grain = (Chalky area of the grain/Total area of the grain) × 100.

Chalky (having highest CAP) and translucent grains of each sample were separately subjected to alkaline spreading value (ASV) (Little *et al.,* 1958), kernel length after cooking (KLAC) (Juliano, 1980), amylose (AC) and amylopectin (AP) estimation using non-interference equations (Lander *et al.,* 1991, Sanjeeva Rao *et al.,* 2024).

Chalky (having various values of CAP) and translucent grains of each sample were separately powdered (100 mesh) manually in mortar and pestle. Powder (5g) of each sample was separately subjected to rice viscosity analysis (Jayakody *et al.,* 2007) using Physica Smart Starch analyser having MCR 52 and Rheoplus software (Anton Paar).

Statistical analysis

Statistical analysis was performed in R software (version 4.4.2) using *Agricolae* package for ANOVA, ggplot2 and reshape2 packages for regression analysis and performance analytics package for correlation analysis.

Results and Discussion

ANOVA analysis indicates that the variation in chalky area % (CAP) in individual grains was significant (P 0.5) among the genotypes and highly significant (P 0.001) with the grain type **(Table 1).** Variation in individual grain weight was significant for grain type and highly significant among the genotypes as well as Genotype × Season interaction. Variation in amylose (AC) was significant among the genotypes, significant between genotype × grain type (G:T) and highly significant between genotype × season (G:S) interaction. Variation in amylopectin content (AP) was significant among the genotypes, grain type and season. While it was highly significant for G:S and significant for G:T:S.

In dry season, the mean CAP was least in Aditya (40.0%) ranging from 14.0 to 76.1% and highest in Dhanarasi (73.7%) ranging from 55.2 to 91.3% **(Table 2).** Whereas in wet season, CAP was least in Tulasi (30.7%) ranging from 7.9 to 63.2% and highest in Dhanarasi (57.1) ranging from 12.4 to 86.7%. Except Aditya and Suraksha, grains possessing CAP above 75% were more in dry season where temperature during grain filling period is usually higher than the wet season. Variation in chalky size in cultivars was observed and opined that there is possibility of independent inheritance of chalky kernel and chalky area (Zhang *et al.,* 2009). The images of rice grain obtained with Cano Scan 9950FV digital image scanner were processed and classified the rice grains based on the position and proportion of chalky area into white-based, white-back, white-back cum based, white-belly, white-core and milky-white rice (Yoshika *et al.,* 2007).

Although variation was observed in individual grain weight of chalky and translucent grains, the mean individual grain weights were statistically same for four (Dhanarasi, Jaya, Suraksha and Swarnadhan) genotypes. In chalky grains, grain weight decreased with the increase in CAP **(Figure 1)** in all the genotypes in both the seasons. The regression (R^2) was significant with Swarnadhan in both the seasons. While R^2 was significant for Jaya in dry season only.

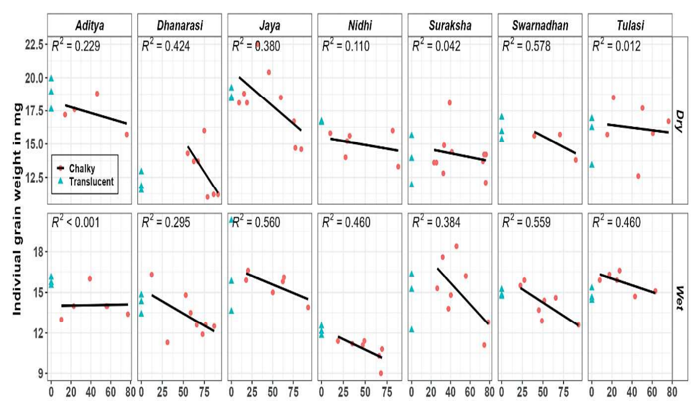


Figure 1: Influence of chalky area % on individual grain weight of rice cultivated in dry and wet seasons. Normal grains in green were not included in regression line

Amylose, Amylopectin and CAP

Considering the variation in chalky area of individual grains within a variety, starch components (AC and AP) were estimated (Sanjeeva Rao *et al.,* 2024) in individual grains in this study to reveal the influence of chalky area on starch components. Both AC and AP varied in chalky grains in both the seasons **(Table 2).** The mean AC in chalky grains was similar in both the seasons in three genotypes (Jaya, Nidhi and Tulasi), marginally higher in wet season over dry season in two genotypes (Dhanarasi and Swarnadhan) and vice-versa in remaining two genotypes (Aditya and Suraksha). The mean AC was statistically similar in both translucent and chalky grains, except Aditya in wet season and Swarnadhan in dry season. The mean AP in chalky grains was similar in both the seasons in two genotypes (Suraksha and Tulasi), higher in wet season than dry season in three genotypes (Aditya, Jaya and Nidhi) and vice-versa in two genotypes (Dhanarasi and Swarnadhan). The mean AP of translucent grains was statistically dissimilar to that of the mean AP in chalky grains of in two genotypes each in wet (Aditya and Swarnadhan) and dry (Jaya and Swarnadhan) seasons.

Although the mean AC and AP in chalky grains were similar to translucent grains in both the seasons, AC decreased (except Tulasi) and AP increased with the increase in CAP (**Figures 2** and **3**). This indicates that the chalky grains with more CAP contains lesser AC and higher AP in comparison with the translucent grains.

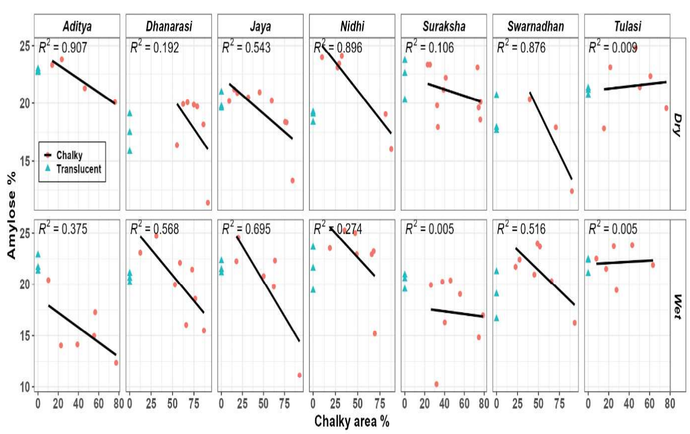


Figure 2: Effect of chalky area % on amylose content of rice grain cultivated in dry and wet seasons. Normal grains in green were not included in regression line

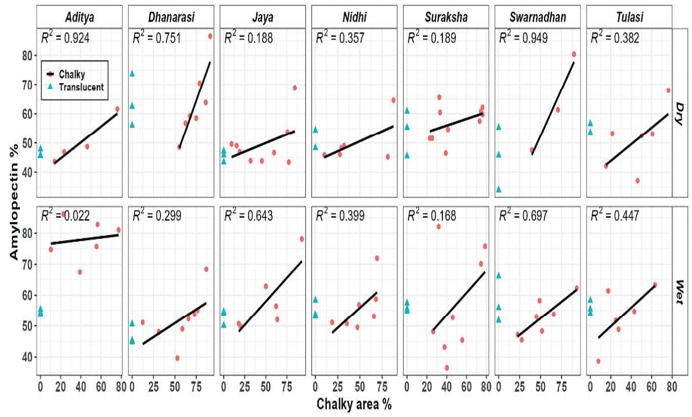


Figure 3: Effect of chalky area % on amylopectin content of rice grain cultivated in dry and wet seasons. Normal grains in green were not included in regression line

In CAP vs AC graph (**Figure 2**), in dry season, the regression value (R^2) was highly significant (> 0.7) in three (Aditya, Nidhi and Swarnadhan) genotypes, significant (> 0.5 and < 0.7) in Jaya and non-significant (< 0.5) in the remaining three (Dhanarasi, Suraksha and Tulasi) genotypes. While in wet season, the R^2 was significant in three (Dhanarasi, Jaya and Swarnadhan) genotypes and non-significant in the remaining four (Aditya, Nidhi, Suraksha and Tulasi) genotypes. This indicates that the chalky grains of Suraksha and Tulasi were more stable in AC in both seasons.

In CAP vs AP graph **(Figure 3)**, in dry season, the R^2 value was highly significant in three (Aditya, Dhanarasi and Swarnadhan) genotypes and non-significant in the remaining four (Jaya, Nidhi, Suraksha and Tulasi). While in wet season, almost highly significant in Swarnadhan, significant in Jaya and non-significant in the remaining five (Aditya, Dhanarasi, Nidhi, Suraksha and Tulasi) genotypes. This indicates that the chalky grains of Nidhi, Suraksha and Tulasi were more stable in AP in both the seasons.

Marginal decrease in AC was observed in chalky grains with insignificant correlation (Zhang *et al.,* 2009, Chun *et al.,* 2009, Zhou *et al.,* 2009). The response of amylose content to elevated temperature appears to be genotype dependent. In heat-tolerant QTL introgressed restorers of KMR-3R, Jaldhani *et al.* (2021) observed both increases and decreases in amylose content under high-temperature stress, indicating differential stability of starch composition among genotypes. Significant low AC (1.7% in Nipponbare to 4.8% in Hatsuboshi) was reported in chalky grains of five varieties ripened at high temperature (Yamakawa *et al.,* 2007). Varied trend in AC was noticed among the six chalky mutants in comparison to the wild Nipponbare wherein AC increased in 2nd, 3rd and 4th mutants, decreased in 1st and 6th mutants and was similar to wild in 5th mutant (Jin *et al.,* 2010).

Thus, it is evident that AC in the chalky grain can vary from translucent grain and the extent of variation depends on CAP of the grain. However, this variation could not appear in earlier reports where amylose content was estimated from the powder obtained from bulk seeds of chalky and translucent grains. Further, means of amylose and amylopectin contents were calculated separately using the values obtained from individual grains for each variety **(Table 2)** to compare the present results with the reported observations. Either negligible or marginal variations were observed in the means of amylose content of both chalky and translucent grains. Amylose content was more in translucent grains of Suraksha in both the seasons as well as Aditya in wet season while it was more in chalky grains of Nidhi in both the seasons. Genetic studies involving bulk seed analysis observed high amylose as incompletely dominant to low whereas single grain analysis revealed high amylose as completely dominant to intermediate or low amylose (Kumar *et al.,* 1987) and thus, emphasized the importance of phenotypic analysis at single grain.

Regarding amylopectin in chalky grains, although quantity was not mentioned, less number of small chains and more number of long chains (Yamakawa *et al.,* 2007) and vice-versa (Chun *et al.,* 2009, Patishdol *et al.,* 2003) were observed.

High temperature stress induced synthesis of α-amylase mRNA followed by the degradation of starch after the middle stage of grain filling leads to reduced starch content (Yamakawa *et al.,* 2007, Kawakatsu *et al.,* 2009). High-temperature stress is also known to increase grain chalkiness and reduce head rice recovery. Evaluation of heat-tolerant KMR-3R introgression lines under late-sown conditions revealed substantial reductions in head rice recovery, largely attributed to increased chalky grain formation (Jaldhani *et al.,* 2021). In contrast, this study reports increased starch content with the increase in CAP since the extent of increase in amylopectin was more than the degree of decrease in amylose (Liu *et al.,* 2010).

KLAC, VER and ASV in chalky and translucent grains

Even translucent rice grain becomes opaque on cooking and it is difficult to distinguish whether a cooked grain is from chalky grain or translucent grain. Hence, kernel length after cooking (KLAC), alkali spreading value (ASV) and volume expansion ratio (VER) were analysed in milled rice in chalky (highest CAP) and translucent grains of each genotype. ANOVA indicates that the variation in KLAC and VER were highly significant or significant among the genotypes, season, grain type and their interactions **(Table 3).**

Marginal difference (<1 mm) was observed in the KLAC values of translucent and chalky grains of the varieties cultivated in wet season **(Table 4).** Whereas this difference was >1 mm in Suraksha, Swarnadhan and Tulasi cultivated in dry season. In both the seasons, KLAC of translucent grain was marginally higher than chalky grain of Aditya and Dhanrasi. Whereas KLAC of chalky grain was higher in the case of Suraksha and minor variation was noted for Nidhi. In Swarnadhan and Tulasi, KLAC of translucent grain was marginally higher in wet season while KLAC of chalky grain was >1 mm in dry season.

In wet season, VER values were similar in both chalky and translucent grains of Dhanarasi and Tulasi. The values of VER were more in the chalky grains of

Aditya and Jaya and vice-versa in Nidhi, Suraksha and Swarnadhan. In dry season, the VER values were higher in the translucent grains of Aditya, Dhanarasi, Nidhi, Swarnadhan and Tulasi. Whereas the VER values were higher in the chalky grains of Jaya and Suraksha.

ASV is inversely proportional to GT (IRRI, 1976). The requirements and protocol for the conduction of ASV experiment is simple compared to the determination of GT. Difference in ASV between translucent and chalky grains (highest CAP) was either absent or negligible for varieties in both the seasons except for Tulasi cultivated in wet season **(Table 1)**. Similarly, insignificant correlation was reported between GT and chalkiness (Zhang *et al.,* 2009) which indicates that GT is unaffected by chalkiness. The 107 glutinous (low amylose) rice noted wide ASV from 2 to 7 (Srinivas *et al.,* 2023) which indicates that decrease in AC due to chalkiness **(Figure 3)** may not necessarily change the ASV.

During cooking, starch granules of rice grain absorb water and swell where their size is manifold higher than the original size of the starch granules and finally solubilise. This irreversible change is gelatinization and this temperature is designated as gelatinization temperature (GT). In addition, separately cooked chalky grains of all genotypes showed undulated texture and were disintegrated with less force whereas the cooked translucent grain had uniform texture and was stable when similar force was applied. Non-uniform distribution of starch granules interspersed by air spaces in chalky grain (Yamakawa *et al.,* 2007, Singh *et al.,* 2006, Fujita *et al.,* 2007) can be related to the undulated texture of cooked chalky grain.

Pasting behaviour of chalky and translucent grain powders

The pasting curve of rice starch popularly known as rapid visco analyser (RVA) profile includes hot-hold-cool hold protocol (Bao *et al.,* 1999, Sun *et al.,* 2011). The paste profile sequentially passes through pasting temperature (PT) as well as pasting viscosity (PV), peak viscosity (PKV), through viscosity or hot paste viscosity (HPV) and final viscosity or cool paste viscosity (CPV). While breakdown viscosity (BDV=PKV-HPV), setback viscosity (SBV=CPV-PKV) and setback from trough or consistency viscosity (CTV=CPV-HPV) are derived from the above measured viscosities.

ANOVA indicates that variation in PV, PKV, CPV, BDV, SBV and CTV were highly significant with change in genotype (G), season (S), grain type (T) and their interactions **(Table 5)**. Variation in HPV was also highly significant in all parameters except T (non-significant). Variation in PT was highly significant for G, S and G:S while significant for GT, S:T and G:S:T. These results emphasize that chalky grains (with all types of CAP) can behave differently during pasting properties in comparison with translucent grains. The pasting viscosities significantly varied among the genotypes (Bao *et al.,* 2002).

The mean values of the above parameters are presented in **(Table 6)** for each genotype in both the seasons in translucent and chalky grains. In wet season, in translucent grains, PT ranged from 70.3 °C (Tulasi) to 77.3 °C (Swarnadhan), PV from 2054 (Dhanarasi) to 6828 (Aditya), PKV from 20160 (Tulasi) to 30610 (Dhanarasi), HPV from 1385 (Dhanarasi) to 3584 (Nidhi), CPV from 1021 (Dhanarasi) to 8506 (Swarnadhan), BDV from 16900 (Swarnadhan) to 29230 (Dhanarasi), SBV from 12760 (Swarnadhan) to 29590 (Dhanarasi) and CTV from -364 (Dhanarasi) to 5033 (Swarnadhan). In chalky grains, 70.9°C (Nidhi) to 78.8°C (Swarnadhan), PV from 428 (Dhanarasi) to 3627 (Swarnadhan), PKV from 22980 (Nidhi) to 32260 (Aditya), HPV from 457 (Aditya) to 3003 (Swarnadhan), CPV from 730 (Aditya) to 15030 (Suraksha), BDV from 21950 (Nidhi) to 31800 (Aditya), SBV from 14180 (Suraksha) to 31530 (Aditya) and CTV from -160 (Nidhi) to 14500 (Suraksha).

In dry season, in translucent grains, PT ranged from 65.3°C (Jaya and Dhanarasi) to 73.6°C (Nidhi), PV from 150 (Dhanarasi) to 4777 (Nidhi), PKV from 21430 (Nidhi) to 30360 (Tulasi), HPV from 621 (Jaya) to 9278 (Dhanarasi), CPV from 496 (Jaya) to 14110 (Dhanarasi), BDV from 15870 (Nidhi) to 27600 (Jaya), SBV from 12920 (Dhanarasi) to 29370 (Tulasi) and CTV from -1928 (Tulasi) to 4830 (Dhanarasi). In chalky grains, PT ranged from 65.6 °C (Jaya) to 74.7 °C (Dhanarasi), PV from 316 (Dhanarasi) to 5447 (Tulasi), PKV from 20680 (Tulasi) to 32190 (Jaya), HPV from 399 (Jaya) to 14960 (Suraksha), CPV from 488 (Tulasi) to 24420 (Suraksha), BDV from 9825 (Dhanarasi) to 31790 (Jaya), SBV from 2420 (Suraksha) to 31560 (Jaya) and CTV from 74 (Tulasi) to 5908 (Aditya).

Further, PT values were statistically similar for each genotype for both translucent and chalky grains in both the seasons. Most of the PKV values were higher in chalky grain powders. While most of the values of PV, HPV and CPV differed statistically from translucent to chalky grains and were higher in translucent grains. The per cent change of chalky over translucent grain powders **(Figure 4)** both the seasons indicates that chalky grain behaves differently during cooking. The positive value indicates that the respective parameter in chalky grain powder was more than the value noted for translucent grain and vice-versa for negative values. The % change in PT was less than ±5% except Dhanarasi in dry season. Among the viscosities, the % change was least for BDV and highest for CTV as well as CPV. Among the genotypes, highest values of % change in chalky grain were noted for Suraksha (CTV & CPV) and Dhanarasi (CPV).

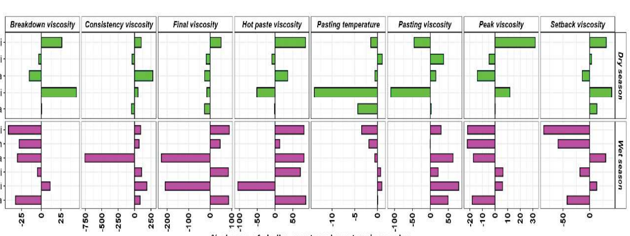


Figure 4: The % change of chalky grain powders over translucent grain powders in pasting profile of rice grain cultivated in dry and wet seasons

In multiple correlation analysis was performed separately for translucent **(Figure 5)** and chalky **(Figure 6)** grains. In translucent grains, KLAC noted significant negative correlation (P 0.1) with CTV only. VER noted significant negative association (P 0.05) with PKV, BDV and SBV. AC noted significant positive correlation (P 0.05) with PV and significant negative correlation (P 0.05) with HPV. AP noted significant positive correlation (P 0.01) with HPV and CPV. The genotypes used in this study contain similar AC and AP contents, however, minor variation in AP content could be responsible for the variation in the pasting properties. In addition, among the measured (PKV, PV, HPV and CPV) viscosities, PKV noted significant negative association (P 0.5) with CPV. While HPV noted highly significant positive association (P 0.001) with CPV.

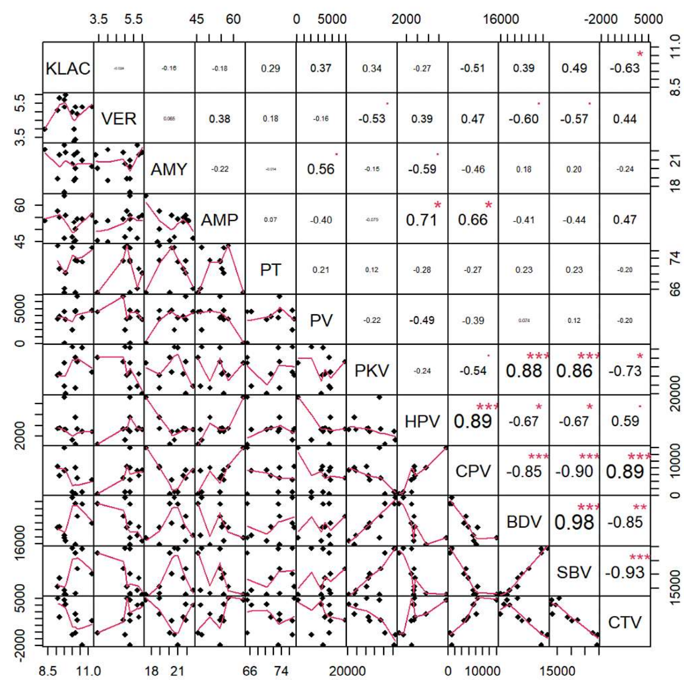


Figure 5: Multiple correlation among cooking quality and pasting parameters in translucent grains.

KLAC (kernel length after cooking), VER (volume expansion ratio), AC (AMY) (amylose %), AP (AMP) (amylopectin %), PT (pasting temperature), PV (pasting viscosity), PKV (peak viscosity), HPV (hot plate viscosity), CPV (cool paste viscosity), BDV (breakdown viscosity), SBV (setback viscosity) and CTV (consistency viscosity) Symbols *, **, * and dot represent p-values 0, 0.001, 0.01, 0.1 and 0.05 respectively**

In multiple correlation analysis of chalky grains, KLAC noted significant positive association (P 0.01) with

CTV and this relationship was opposite to translucent grains. Similarly, VER noted significant positive association (P 0.01) with PKV only. As AC decreased and AP increased with the increase in CAP **(Figures 2** and **3)** in chalky grains in these genotypes, AC noted significant negative association with AP (P 0.01). Although both AC (decreased) and AP (increased) varied among the chalky grains with the increase in CAP, AC alone noted significant negative association (P 0.05) with PKV and it indicates that PKV was more affected by change in AC content. Further, among the measured viscosity parameters, PV noted significant negative association (P 0.01) with HPV and CPV. HPV noted highly significant positive association (P 0.001) with CPV.

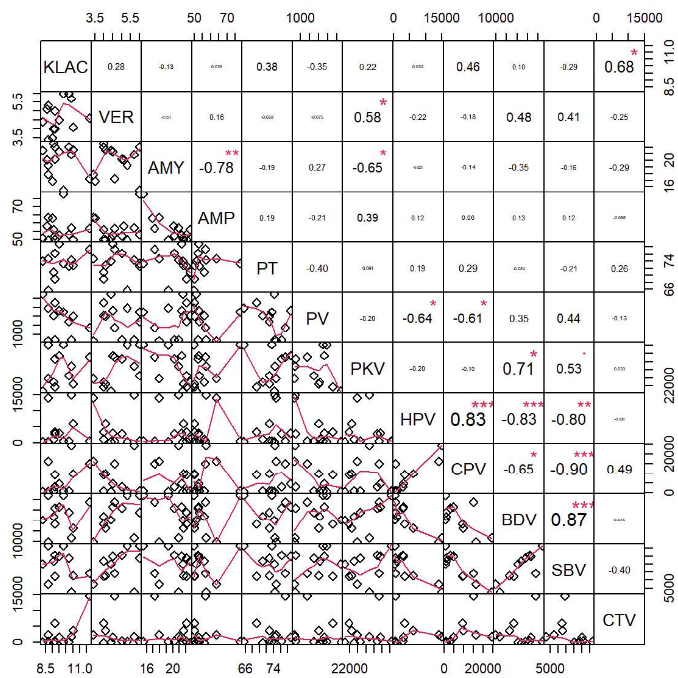


Figure 6: Multiple correlation among cooking quality and pasting parameters in chalky grains.

KLAC (kernel length after cooking), VER (volume expansion ratio), AC (AMY) (amylose %), AP (AMP) (amylopectin %), PT (pasting temperature), PV (pasting viscosity), PKV (peak viscosity), HPV (hot plate viscosity), CPV (cool paste viscosity), BDV (breakdown viscosity), SBV (setback viscosity) and CTV (consistency viscosity). Symbols *, **, * and dot represent p-values 0, 0.001, 0.01, 0.1 and 0.05 respectively**

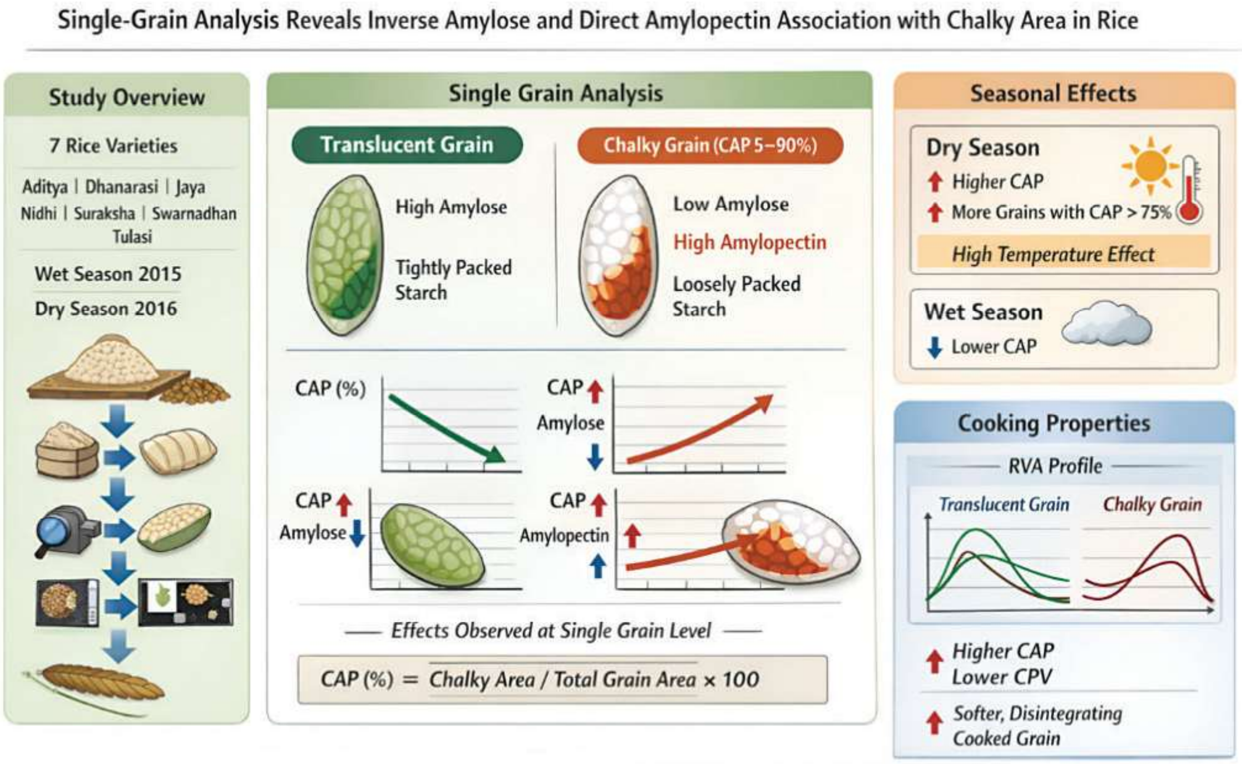
Rice grain with high BDV, PKV and low CTV, CPV, SBV, PT and peak viscosity time were believed to possess soft texture and good eating quality (Liu *et al.,* 2009). The pasting profile properties varied in rice grains cultivated at lower and higher seasonal temperatures (Tu *et al.,* 2023).

Amylose noted significant negative correlation with PV, HPV, CPV and BDV and the correlation is genotype dependent (Bao *et al.,* 2002). Removal of 1st intron of Wx gene increased AC in the rice grain, however, significant variation was not observed in pasting profiles (Liu *et al.,* 2022). The percentage of grains with chalkiness (PGWC) noted negative association with PKV and BDV in Koshihikari x Kasalath mapping population (Zheng *et al.,* 2012). Variation in pasting profiles were significant from translucent to chalky grains, however, the variation of pasting profiles was non-significant from 5 to 100% increase in the proportion of chalky grain (Chun *et al.,* 2009).

The grain of rice genotypes with similar starch content and composition can vary in pasting properties (Champagna *et al.,* 1999, Bao *et al.,* 2002). The onset of gelatinization and increase in initial viscosity is PT. The maximum viscosity reached during heating is PKV which represents un-gelatinized starch. The swelling of starch granules and pasting is promoted by AP and inhibited by AC (Singh *et al.,* 2006). A higher PKV indicates a greater proportion of un-gelatinized starch and vice versa (Devi *et al.,* 2009). HPV represents the viscosity noted at the end of 5 min of heating at 95°C where breaking of starch granules was noted (Singh *et al.,* 2006). CPV represents the viscosity noted at the end of cooling the gel to 50°C (Bao *et al.,* 1999, Sun *et al.,* 2011). Among the various measured and derived pasting properties, CPV or final viscosity is more relevant to the rice consumer.

Compared with translucent grains, CPV values were lower in chalky grains. Hence, chalky grains with higher CAP can accumulate lesser AC and more AP with lower CPV. Such cooked grains can behave like low amylose or waxy rice genotypes and can disintegrate with lesser force.

The grain quality of chalkiness is measured manually in a milled rice sample that contains both translucent and chalky grains. Further, chalkiness is expressed as absent (A), very occasionally chalky (VOC), occasionally chalky (OC) and present (P) if the number of chalky grains are absent, lesser than 10%, from 11 to 20% and more than 20% in the total sample (Arvind Kumar *et al.,* 2021).



In conclusion, grains with chalky area percentage (CAP) >75 were more in dry season indicating the influence of environment on chalkiness. Variation in KLAC and VER values was observed in translucent and chalky grains. However, ASV test could not differentiate chalky from translucent grains. Further, amylose content decreased while amylopectin content increased with the increase in CAP. Compared with translucent grains, the four measured and three derived viscosities showed significant variations in the chalky grains. The cool paste or final viscosity values were lower in the chalky grains. The chalky grains with higher CAP can accumulate lesser amylose and more amylopectin and such grains exhibited lower cool paste viscosity. Variation in the cooking quality behaviour of chalky grains is genotype dependent. However, genotypes categorized as VOC may not affect the overall pasting properties or cooking quality of the rice genotype.

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