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Effect of Heat Stress on Grain Quality of different Rice Varieties

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ABSTRACT: Rice grain quality is sensitive to heat stress during grain filling. To analyse the effect, twelve different varieties of rice were grown in field and then transferred to pots at their early tillering stage. One set of each genotype was grown under control conditions while another set was shifted to 2-3°C higher temperature in a glasshouse (maintained between 9 AM to 3 PM with diurnal variation). There was a negative impact of high temperature on grain chalkiness and amylose content. Rheological properties, including peak viscosity, breakdown viscosity, final viscosity, and setback viscosity showed significant differences between the control and heat stress. Scanning electron microscopic examination showed that the arrangement of starch granules in rice grains during grain filling stage was affected by high temperature. Developing heat-tolerant rice varieties with improved grain filling, stable starch synthesis and resistance to high temperatures, along with implementing enhanced cultivation practices, can have great potential for sustainable and high-quality rice production.

Keyword: Grain chalkiness, Paste viscosity, High temperature, Rice.

INTRODUCTION

Rice is one of the most important staple food crops for more than half of the world's population, so ensuring its grain quality is paramount. Global warming due to changing climate has already shown recurrent heat stress in many parts of the world, severely affecting primary crop production worldwide. Rice is highly affected by the high temperature during grain filling. Although considerable research has been conducted where heat stress is the primary source of concern, and to cope with these changes, identifying and developing varieties that can perform better under harsh climates is one of the major targets. An increase in temperature, spatiotemporal changes, and unpredictable seasons have negatively impacted the quantity and quality of food grain production. It is projected that the mean global temperature will surpass the recommended limit of 1.5°C due to the current rate of global warming, with an increase that is more than twice the limit (IPCC 2013; IPCC 2022). (Lyman *et al.*, 2013) studies show that an increase in average temperature by 1°C during the rice growing season can reduce paddy yield by 6.2%. Their study also reported that the total milled rice yield decreased by 7.1% to 8.0%, head rice yield by 9.0% to 13.8%, and total milling revenue by 8.1% to 11.0%. These results demonstrate that rising temperatures could significantly impact rice production and revenue. Rice is a critical food crop for many people worldwide, but the impact of high-temperature stress on its yield could be significant. Without implementing adaptation measures, it is estimated that the grain yield of Rice could decrease by as much as 41% by the end of the 21st century (Ceccarelli *et al.*, 2010). Grain appearance is an essential criterion in determining the quality of Rice, consumer preference, and price. Chalkiness affects the quality of the grains with increased hardness, brittleness, and decreased pliability, reducing shelf life and making them less suitable for consumption. Endosperm chalkiness is a varietal characteristic that negatively affects the appearance and milling properties, texture, and palatability of cooked Rice (Chen *et al.*, 2012). Chalkiness of the endosperm is caused when the starch granules do not grow enough, and the spaces remain between the granules, which results in the loose arrangement of starch granules, thus reflecting light and making the grain appear chalky. Scanning electron microscopy studies suggest that these starch granules consist of starch glucan, principally amylopectin and amylose, arranged into three-dimensional, semi-crystalline structures (Lisle *et al.*, 2000; Singh *et al.*, 2003; Tashiro & Wardlaw, 1991) Amylose forms a single helical structure and is packed in an amorphous region (Buleon *et al.*,1998). It is reported that granules packed by orderly arranged amylopectin branches, with clusters formed by double helices of amylopectin, result in closed packaging of starch granules showing high crystallinity and uniformity. In contrast, loosely packed starch granules show low crystallinity and non-uniformity (Chun *et al.*, 2009). Since rice quality has become one of the most critical factors for consumer preference, rice taste analyser and texture analyser has been widely used for comprehensive analysis of the palatability of Rice RVA is widely used to evaluate the taste and quality of Rice because it requires a small sample size, is simple to use, and have high repeatability (Tong *et al.,* 2014). Rice's main components are starch and protein. Starch consists mainly of amylose and amylopectin and accounts for around 80-85% of chemical constituents, showing its physiochemical properties, such as gel strength and pasting properties (Singh *et al*., 2000; Martin *et al*., 2002; Butardo & Sreenivasulu 2016), despite 6-8% protein content. There have been many studies where researchers have explored the correlation between RVA and taste value (Zhang *et al*., 2008). Majorly the japonica rice cultivars are used for the preparation of essential food materials in Asian countries. Manufacturers give preference for rice flour instead of starch for preparing processed food. However, the pasting properties and swelling of starch granules are not only affected by starch but also by lipids, protein, and mineral compounds (Singh *et al*., 2000). To better understand the viscosity properties of rice starch, RVA works as an estimation method for physical indexing and precursor for cooking and processing qualities (Martin *et al*., 2002; Jun *et al*., 2004; Bao *et al*., 2006; Bryant *et al*., 2012).

Rapid Visco Analyser (RVA) studies provide an accurate method to measure the viscosity parameters. RVA uses a rotational viscometer to measure fluid flow properties under shear stress. The viscosity is measured by determining the time it takes for a starch suspension in water to become viscous. The RVA does this by measuring the resistance to the flow of a small probe inserted into the suspension. The results of an RVA study can be used to profile different types of Rice and compare varieties. Quality estimation of rice starch properties setback and breakdown values play a notable role in cooking rather than peak viscosity. Since it has been found that grain chalkiness affects quality production in Rice, thus it is a principal target for rice crop improvement. Hence, in this study, we investigated the differences in quality between controlled environmental conditions (referred to as heat stress) and normal environmental conditions (referred to as control) and analysed the impact of heat stress on rice grains in various rice varieties.

MATERIAL AND METHOD

A. Plant Material

Twelve different rice varieties cultivated across India were analyzed, including Pusa Sugandh 5(PS 5), Pusa Sugandh 2 (PS2), Pusa Basmati 1509 (PB1509), Pusa Basmati 1401 (PB1401), Nagina 22 (N 22), Jaya, IR36, Pusa 677 (P677), MTU 1010, IR 64, Pusa 44 (P44) and Vandana.

B. Experimental design, location, and agro-management

The field experiment was conducted during the kharif season 2021 at the Indian Agricultural Research Institute, New Delhi. After 30 days in the nursery, the seedlings were transplanted in the experimental field with a plot size of 20 m^2 per genotype. The planting was done in a randomized block design in two replications. Plants were shifted to pots with the same field soil at tillering stage (prior to the booting stage) and left for seven days to acclimatize (plants were selected from the centre of the plots). At the booting to heading stage, plants were shifted into a glass house chamber, maintaining a diurnal temperature variation in the range of 36°C to 38 °C. The experiment was conducted with recommended weed, pest management, and agronomic inputs.

C. Evaluation of chalkiness cooking quality parameters

For phenotyping, ten random plants were selected and tagged. On the 30^{th} day after anthesis, the panicles were collected and left for complete drying at room temperature to a moisture content of 14 percent. The upper two- third portion of the panicle was cut out to remove any biases, and seeds were collected and milled using palm husker and polished for further studies. The phenotyping for grain chalkiness was done by visual inspection of each genotype in three replicates using 100 seeds for each replication. The paddy and kernel length and width were taken using a laboratory vernier's caliper. All the data were recorded as per the standard evaluation system for rice (Ikehashi & Khush 1979). Cooking was done by taking ten intact milled grains in test tubes and measuring the length before cooking length (BCL). After soaking in distilled water for 30 min, the grains were transferred into 50 ml test tubes. Ten milliliters of distilled water was poured into each test tube, placed in the stand, kept in a boiling water bath for ten minutes, simmered for five minutes (after the power shut off), and allowed to cool down. The cooked kernels were carefully removed by pouring the cooked grains on adsorbent paper. The length and width of the cooked kernels were carefully measured manually using graph paper, and further length-width ratio is calculated (Golam & Prodhan 2013). The Amylose content (AC) was measured by the method of (Perez & Juliano 1978) with minor modifications.

D. Rapid Visco analyser **(***RVA) profiling*

Rice flour pasting properties were studied using an RVA (MCR 52 Anton Paar). Flour (3 g, 12% moisture) was mixed with 16 ml of double-deionized water in the RVA aluminium sample can. The RVA was run using a program suggested by (Tong *et al*., 2014) with slight modifications; a heating and cooling cycle was set as (1) holding at 50°C for 1 min, (2) heating at 95° C for 3.8 min, (3) holding at 95° C for 2.5 min, (4) cooling at 50° C for 3.8 min, (5) holding at 50° C for 1.4 min. The RVA paddle speed was 960 rpm for the first 10 s, after which the speed was reduced to 160 rpm. The peak (PV), hot paste (HPV), cool paste (CPV) viscosities and their derivative parameters breakdown (BD =PV–HPV), setback (SB = PV–PV), and consistency (CS = CPV–HPV) were recorded. Statistical analysis of the physicochemical parameters was done at least in duplicate. Data analyses was performed using SPAR software available at ICAR-IASRI, New Delhi.

E. Scanning electron microscopy (SEM)

Selection of grains for SEM was made after phenotyping. Since PB1401 showed very little chalkiness as compared to other Basmati varieties under field condition, we selected Pusa Basmati 1401 grain for the SEM studies under control and heat stress conditions. Five full grains were selected for each treatment and examined by scanning electron microscopy. Grains were transversally cut in half with a razor blade, the half-grains were transferred to double adhesive carbon tape attached to aluminium specimen stubs, and the specimen's cut surface was coated with gold using an ion sputter coater. The specimens were observed and photographed with a scanning electron microscope (Tescan Vega3).

RESULTS AND DISCUSSION

In this study, we focused on the effect of heat stress on some locally adapted cultivars and mega varieties of rice, including basmati and non-basmati types. High temperature causes increased chalkiness and thus affects the grain quality of rice. Our results show that the least affected rice variety was Aus cultivar N 22, where 18% of chalkiness was reported at high temperature (38 °C) stress (Table 1). Among the medium slender genotypes, the most widely affected cultivar was MTU 1010, cultivated widely in Southern India. High-yielding long-grain rice genotypes, widely cultivated in northern India, were highly susceptible to elevated temperature, showed up to 80% grain chalkiness, and mostly depicted white belly and white core. Basmati variety PB 1509 showed around 30% grain chalkiness under control conditions, which was increased to 80% under heat stress, while PB1401 showed 40% chalkiness under heat stress conditions. The main disadvantage of chalky grains is that it causes breakage during milling and directly lead to post-harvest losses.

Other high-yielding varieties, namely P 677, P 44, IR 36, Vandana, and Jaya, show moderate chalkiness under heat stress. Aromatic long-grain varieties PS 2 and PS 5, were also influenced by high-temperature stress. The kernel length/breadth ratio showed no significant difference between heat stress and control conditions. The cooked kernel elongation ratio did not differ significantly among varieties between the control and stress groups. Table 2 represents the descriptive statistics among control and stress groups for kernel L/B ratio and kernel elongation ratio, respectively. Endosperm chalkiness in rice is mainly caused by decreased assimilate supply and increased grain filling rate under high temperatures (Tsukaguchi *et al*., 2008, Terashima *et al*., 2001). Rice with high chalkiness tends to have reduced milling yield and lower head rice recovery, leading to a lower market value for the produce. Chalkiness in the rice grain alters its physical properties, such as hardness and translucency, making it more difficult to mill. Chalkiness causes rice grain breakage during milling, resulting in a lower milling yield. The percent of head rice recovered during milling can also decrease, as chalky grains tend to break apart more easily than non-chalky grains. Here, we observed a significant increase in chalkiness in some rice genotypes, such as MTU 1010 and PB1509, in the 80-90% range under heat stress conditions. Some short grain varieties like N 22 responded better than any other genotype and reported less chalkiness and no breakage during hulling and milling. Generally, long-grain rice is more prone to breakage during milling. If chalkiness persists, it loses its hardness and transparency, thus resulting in lower market value, directly affecting the farmer's profit.

Generally, HT reduces grain size, weight, and yield due to decreased accumulation of dry matter in the grain. There was a decrease in grain amylose content under heat stress for each genotype. Under control, the highest amylose content (26%) was recorded for PS 5, followed by PS 2, PB 1509, and PB 1401 among the long-grain type genotypes. In contrast, medium grain cultivar N 22 showed around 22% amylose, followed by Jaya, IR 36, P 677, MTU 1010, IR 64, P 44, and the least amount for Vandana. All these varieties showed moderate amylose content as per the standard evaluation system of rice. At higher temperatures, there was a decrease in amylose content leading to deterioration in grain quality. The most affected variety was the mega variety MTU 1010 which showed an average decrease in amylose content of around 14% at high temperature. While other genotypes were affected in the range of 2.5%-8% reduction in amylose content under heat stress compared to control (Fig. 1).

Table 1: Percentage of chalky grains in twelve different rice varieties under control (35 °C) and heat stress (38 °C) conditions during grain filling.

Sr. No.	Variety	Percent Chalkiness ± SEM	
		(control)	(stress)
1.	N 22	3±2	18±2.7
2.	MTU 1010	30±2.0	90±3.0
3.	Pusa Sugandha 2	17±1.0	37±3.5
4.	Vandana	15±1.0	28±2.0
5.	PUSA 677	15±2.0	38±2.3
6.	Pusa Sugandha 5	6±1.50	20±5.0
7.	Jaya	11±2.1	30±1.5
8.	Pusa 44	14±2.0	20±4.0
9.	IR 64	12±2.0	30±5.0
10.	PB 1509	30±5.4	80±2.0
11.	IR 36	8±1.0	20±3.0
12.	PB1401	3±2.0	40±2.0

Table 2: Descriptive statistics of kernel L/B and elongation ratio under control and stress condition.

Descriptive Statistics	Descriptive Statistics	Kernel length/breadth ratio		Cooked kernel elongation ratio	
		Control	Stress	Control	Stress
Mean	Mean	3.38	3.45	1.76	1.66
Standard Error	Standard Error	0.22	0.23	0.11	0.10
Median	Median	3.19	3.21	1.67	1.63
Standard Deviation	Standard Deviation	0.78	0.80	0.39	0.35
Variance	Variance	0.61	0.64	0.15	0.12
Minimum	Minimum	2.44	2.54	1.25	1.1
Maximum	Maximum	4.68	4.89	2.3	2.1

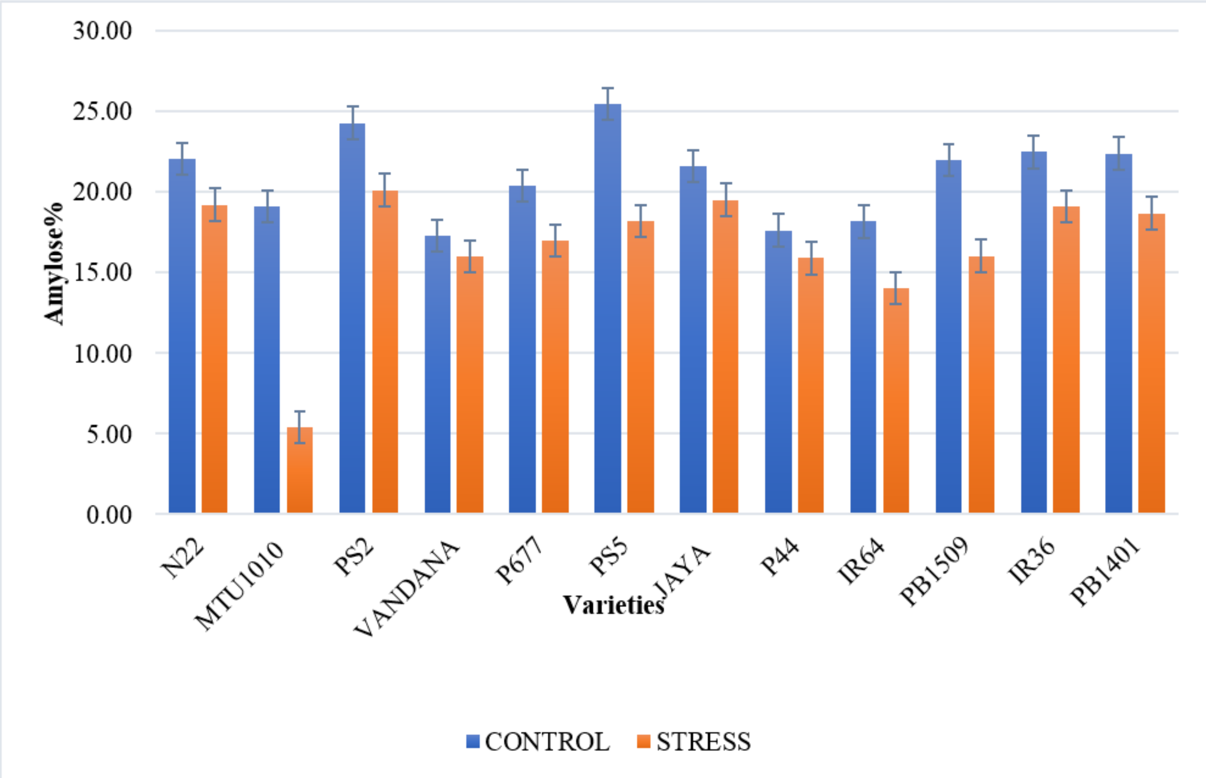

Fig. 1. Grain amylose content of 12 rice varieties under control (35°C) and heat stress (38°C). The error bars represent standard error of means.

High temperature can affect the ratio of amylose to amylopectin in the grain. It affects the biochemical and physiological processes that determine the starch composition in the grain. It causes an increase in respiration rates in the grain, which can cause a reduction in the amount of carbon available for starch synthesis. This, in turn, can lead to a reduction in amylose and amylopectin content in the grain. Studies have shown that high temperatures can affect the enzymes involved in starch synthesis and degradation. For example, the activity of ADP-glucose pyrophosphorylase, which is involved in the initial step of starch synthesis, can be reduced at high temperatures, leading to a lower rate of starch synthesis, and the activity of enzymes involved in starch degradation, such as alpha-amylase, can increase, leading to a reduction in starch accumulation (Geigenberger *et al*., 1998). Under high-temperature stress, there can be a reduction in the proportion of amylose in the grain, leading to a higher proportion of amylopectin because the enzymes involved in amylose synthesis are more sensitive to high temperatures than those involved in amylopectin synthesis (Umemoto *et al*., 1995 and Jiang *et al*., 2003). Ai *et al*. (2022) reported that high amylose cultivar showed a higher negative effect under High-temperature stress. Ahmed *et al*. (2015) studies show the negative impact of high temperatures on basmati rice amylose content. Our findings show a decrease in amylose content under heat stress. The p-value of 0.0036 shows a significant difference between the stress and control genotypes. The amylose content varied between varieties, but heat stress significantly impacts amylose content during the grain filling period in rice. A gradual decrease in amylose content ranged from 2.5% to 14%. Our studies report the reduction in amylose content under high-temperature diurnal variation among twelve rice genotypes, influencing the functional properties of starch viscosity and affecting rice eating quality.

Rapid Visco-analyzer (RVA) profiling was done for all the control and treated genotypes. The paired T-test showed a significant difference between control and heat stress conditions between peak viscosity, pasting temperature, holding strength, breakdown viscosity, final viscosity, and setback values. At high temperatures, there was a significant decrease in setback value from the peak in all the genotypes. Pasting temperature, varied significantly and slightly increased in Vandana, PS 2, PB 1509 and Jaya under heat stress condition. While, the holding strength, breakdown viscosity, and final viscosity was also decreased under heat stress for each genotype. The final viscosity decides the taste value of rice. Table 3, shows the RVA values recorded under control and heat stress for this study's selected genotypes. The composition of starch granules directly affects the viscosity properties and taste deterioration. The gelatinization properties of rice starch are commonly evaluated using RVA. The extent of swelling of starch granules is reflected by peak viscosity, while the temperature at which the starch paste begins to rise is known as the pasting temperature (Kesarwani *et al*., 2015). Breakdown assesses the ease of disintegration of swollen starch granules (AACC, 2000). Setback indicates the tendency of starch pastes to retrograde, an index of starch retrogradation (Kuo *et al*., 2001). Good cooking quality is indicated by low setback so that rice does not retrograde to become hard upon cooling (Raina *et al*., 2007). We found significant differences in peak viscosity (P = 0.003), breakdown (p-value = 0.003071), final viscosity (p-value = 2.507e-09), and setback from trough (P = 3.367e-10) between control and stress, which is highly significant. Our results concur with the findings of (Nakamura *et al*., 2021), where the pasting properties of chalky rice grains were found to be approximately 0.9 times lower compared to whole rice grains under high temperatures, and further, their findings align with the results reported by (Chun *et al*., 2009; Okuda *et al*., 2010 and Nakamura *et al*., 2012). Scanning electron microscopy (SEM) studies showed that starch granules lose their closed compact structure and appear like loosely packed swollen granules under heat stress. Observations for PB 1401, a classical long grain basmati rice variety suggests that under control condition starch deposition was organized as tightly packed granules that exhibit high crystallinity and uniformity. Conversely, under heat stress the arrangement was of loosely packed starch granules with low crystallinity and lack of uniformity (Fig. 2).

Table 3: Comparison of viscosity properties of different varieties of rice under control and heat stress conditions.

Varieties (G)	Treatments (T)	Pasting temperature (PT) (°C)	Peak viscosity (PV) (mPa.s)	Holding strength (HS) (mPa.s)	Breakdown (B) (mPa.s)	Setback from peak (SBP) (mPa.s)	Setback from trough (SBT) (mPa.s)	Final viscosity (FV) (mPa.s)
IR 36	CONROL	65.59	1180	362.45	819.5	-5341	6161	6523
	STRESS	63.99	158.8	130.9	279.1	-4316.5	4344.5	4475.5
N 22	CONTROL	72.64	647.25	154.35	492.9	-4725.5	5218	5372.5
	STRESS	69.69	267.55	143.2	124.4	-5006	5130	5273
PS 5	CONTROL	69.25	294.9	270.015	171.9	-5181.5	5353.5	5423.5
	STRESS	70.93	241.2	161.35	132.85	-3762	3895	4056.5
IR 64	CONTROL	70.25	597.55	173.7	423.8	-4743	5167	5340.5
	STRESS	69.68	338.75	136.7	202.05	-4202.5	4404.5	4541.5
VANDANA	CONTROL	68.49	881.1	265.7	615.5	-3319.5	3944.5	4210
	STRESS	73.52	732.9	249.95	482.95	-76	4821.5	5072
PS 2	CONTROL	69.35	422.7	201.25	221.8	-4903	5124.5	5325.5
	STRESS	72.35	454.25	194.95	259.25	-3859	4118.5	4313.5
PUSA 44	CONTROL	69.33	1404	491.9	898.6	-5982.5	6894.5	7386
	STRESS	69.83	243.8	155	188.78	-5441.5	5530.5	5685.5
PUSA 677	CONTROL	67.77	767	221.6	545.25	-6038	6583	6804.5
	STRESS	63.87	330.2	160.8	169.35	-4294	4463.5	4624.5
PB 1509	CONTROL	67.45	324.5	172.5	152	-3864	4462	4634
	STRESS	72.54	225.5	144.95	80.555	-3726	3807	3951.5
JAYA	CONTROL	67.99	2680	1195.5	888.25	-4912	6557.5	7592
	STRESS	74.07	379.1	167.15	212.05	-4910	5122	5289
MTU 1010	CONTROL	68.17	1935	637.7	1297.5	-2134.5	3432.5	4989.5
	STRESS	63.50	1635	585.8	1049	-3348	4397	4063
PB 1401	CONTROL	70.51	1290	440.85	848.85	-6024.5	6873.5	7314
	STRESS	63.44	510.8	243.9	266.85	-5330	5596.5	5840.5

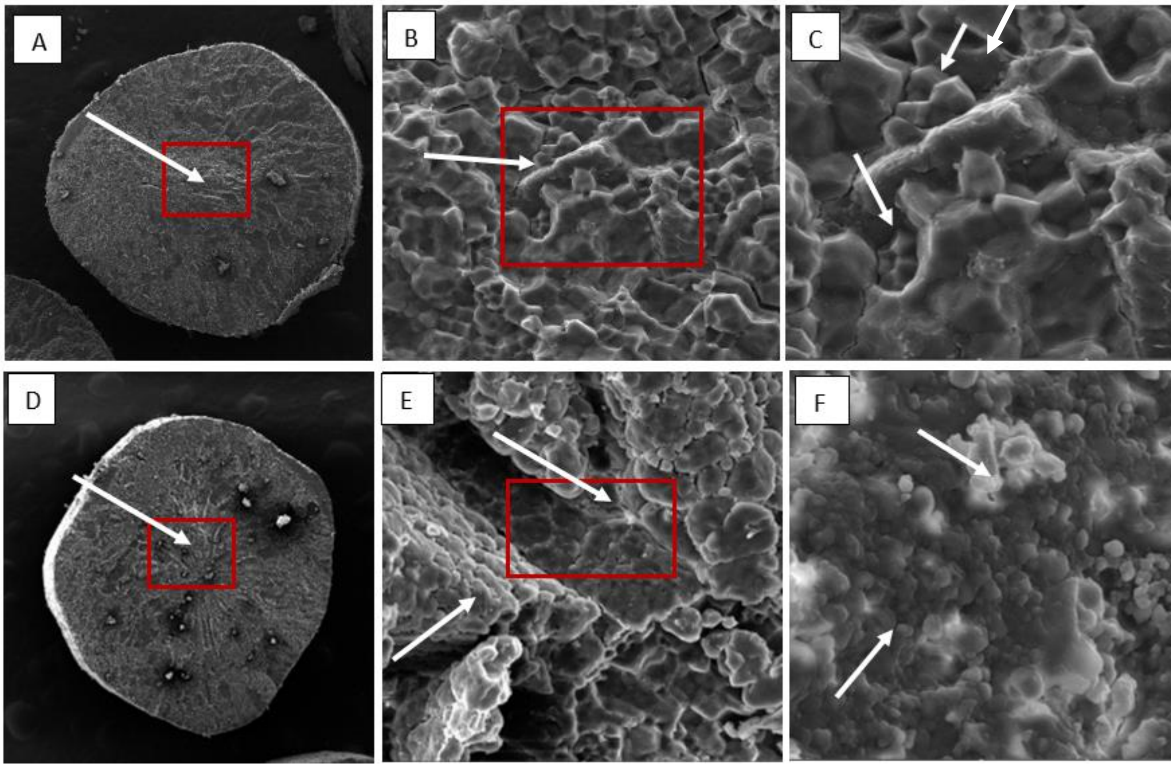

Fig. 2. SEM images of mature endosperm of rice variety PB 1401 under control are A, B and C. B represents the compact structure of starch granules shown in higher resolution of granules compactly arranged. The white arrow in A, B represents the position from where the image is being captured in high resolution. Arrow in B and C (2.00kx and 4.00kx image resolution respectively) reparents the compact arrangement of starch granules under controlled condition. D, E and F are Sem images of PB1401 under heat stress condition shows the loose packaging of starch granules, F shown in higher resolution of granules are loosely packed and arranged. The white arrow in D and E represents the position from where the image is being captured in high resolution. Arrows in E and F (2.00kx and 4.00kx magnified image resolution respectively) represents the loose arrangement of starch granules under stress condition. Red rectangular box in A, B, D, E represents the position from where image is being captured and further magnified image is captured.

CONCLUSION AND PROSPECTS

In conclusion, high temperature during grain filling in rice showed negative impact on starch accumulation and its composition. Development of rice varieties that are more tolerant to high temperatures during grain filling and stable starch synthesis and packing is essential to overcome these negative effects. This study enlightens the adverse effect of high temperature on rice grain chalkiness and starch qualities, thus exploring the problem at gene level and developing heat tolerant genotypes using molecular breeding approaches is required.

Conflict of interest. None.

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Author contribution. PS did the field and lab experiments and manuscript writing, AMS guided and edited the manuscript, SGR helped in RVA profiling, AKS in experiment designing, NKS in overall guidance, experimental design and final editing of the manuscript.

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