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Adverse effect of increase in minimum temperature during early grain filling period on grain growth and quality in *indica* rice (*Oryza sativa*) cultivars

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ABSTRACT

Increasing global temperatures have a deterimental effect on rice quality besides leading to yield penalty. Past data shows that minimum temperature has increased more than maximum temperature in India as well as other parts of the world. The effect of this increase in mean minimum temperature on grain growth and quality of *indica* rice (*Oryza sativa* L.) cultivars is obscure. Our study submits the evaluation of grain quality of early, mid-early and medium duration *indica* rice cultivars to moderate increase in mean minimum temperature from anthesis to maturity in two seasons (*kharif* 2014 and 2015) . Early duration susceptible cultivars, Vandana and Parijat were significantly affected during the early phase of grain filling resulting in 4.6 and 6.4% decline in test weight and 12-18% in high density grains respectively, when the mean minimum temperatures were ≥ 25°C. Slower grain growth rate under elevated mean minimum temperature accounted for decrease in dry matter accumulation in these cultivars. In general, percentage chalkiness was less in all the *indica* cultivars. Head rice recovery was not affected but amylose content reduced in all cultivars with a significant effect on Bakal and Sahbhagi Dhan when minimum temperatures increased by more than 23°C. Rice cooking temperature determined by alkali spreading value was not stable across the environment in early duration cultivars, Vandana and Parijat. Increase in mean minimum temperature elicited the vulnerability of early duration *indica* cultivars, by adversely affecting quality traits like test weight, high density grains and gelatinization temperature.

Key words: Grain growth, Minimum temperature, Quality traits, Rice

Rice (*Oryza sativa* L.) is a staple cereal diet for a large part of the world's population and accounts for 30-75% of the daily calorie intake in Asia (Krishnan *et al.* 2011). The market value and consumer acceptance of rice is decided by its various quality traits (Lapitan *et al.* 2007). Grain and cooking quality is strongly influenced by genetic and environmental factors (Shi *et al.* 1997) and high temperature during grain growth and filling can cause quality variation in rice (Cooper *et al.* 2008) due to its effect on cellular and developmental processes (Barnabas *et al.* 2008). Globally, the mean surface temperature has increased by 0.85˚C from 1800 to 2012 and is further projected to increase by 1.3-3.7°C by the end of 2100 (IPCC 2013). Rice production is predicted to be threatened widely in the subtropical and tropical rice producing areas with this temperature increase (IPCC 2013). Perusal of the temperature data reveals that a diurnal asymmetry occurs with more rapid increase in the night-time than day-time temperatures (Davy *et al.* 2016). This has been attributed to less radiant heat loss because of increased cloudiness (Alward *et al.* 1999, Braganza *et al.* 2004, Huang *et al.* 2006), precipitation and evaporation (Chahine 1992, Dai *et al.* 1997), aerosols (Henderson-Sellers 1992, Ramanathan *et al.* 2007) and desertification (Karl *et al.* 1991, Gallo *et al.* 1996). A glance at the the past 40 years of temperatures in the Indian region highlights an increase of 0.17 and 0.29°C/decade in the maximum and minimum temperatures, respectively (INCCA 2010).

Studies on high night temperature have shown that quality traits are adversely affected with increase in night temperature, viz. grain weight, head rice recovery, percentage of chalky rice grains, amylose content and packing of starch granules (Ambardekar *et al.* 2011 , Shi *et al.* 2016, Song *et al.* 2015). The decrease in grain weight under high night temperature in *japonica* rice cultivars has been explained on the basis of two hypothesis (i) deficit in carbohydrate supply to the developing grains (Kobata and Uemuki 2004). (ii) reduction in grain growth rate in the early or middle stages of grain filling. Information is lacking on grain growth and its relation to quality traits in *indica* cultivars under higher degree of minimum temperatures (>23°C) compared

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to experiments with lesser degree minimum temperature (20°C) in *japonica* types (Song *et al.* 2013). Therefore, this study was undertaken to evaluate the grain growth of early (90-95 days), mid-early (105 days) and medium (115 days) duration *indica* cultivars under elevated minimum temperatures (>23°C) and its effect on grain quality traits.

MATERIALS AND METHODS

A preliminary experiment conducted at ICAR – Indian Agricultural Research Institute, New Delhi with 20 *indica* rice cultivars (unpublished) led to selection of cultivars in the different maturity groups, viz. early- Vandana, Parijat, Bakal and Nagina 22 (90-95 days), mid-early-Sahbhagi Dhan (105 days) and medium- Gotrabidhan and Abhishek (115 days). The present investigation was carried out in the pot culture at ICAR – Indian Agricultural Research Institute, New Delhi during *kharif* (rainy/wet) seasons of 2014 and 2015**.** Grain quality was determined for the two seasons and grain growth measurements recorded in *kharif* 2014 for assessing the relationship between grain growth and quality attributes under ambient and elevated minimum temperature conditions(ET_{min}). Average minimum temperatures under ambient and treatment conditions during first fortnight after anthesis for early duration cultivars were 23 and 25°C (2014); 25 and 26°C (2015) and for mid-early 23 and 24°C (2014); 24 and 25°C (2015) and medium duration cultivars 22 and 23°C (2014); 23 and 24°C (2015). Besides these, it was observed that the difference between treatments was in the range of 2-4°C soon after entrapment on different days, which narrowed down to give the above mentioned minimum temperatures.

Crop husbandry: Seeds were sown during the last week of June at the nursery of ICAR–Indian Agricultural Research Institute, New Delhi. Twenty one days old seedlings were transplanted in 14 replicate pots of 12" diameter containing 14 kg of soil, with five seedlings per pot that were thinned to three seedlings per pot after establishment. Nitrogen was applied in the form of urea at the rate of 120 kg N/ha as three split doses (50:25:25) as basal dose at transplanting, 20 days after transplanting (DAT) and primordial initiation stage respectively, while phosphorus was applied as P_2O_5 in the form of SSP @ 60 kg P/ha and potassium as K_2O in the form of muriate of potash @ 40 kg K/ha at transplanting stage. One set each consisting of seven pots was shifted at anthesis to two polyhouses, one for ambient and other for high night temperature exposure, respectively.

Temperature treatment: Two polyhouses were used for the experiment; (i) polyhouse for high night temperature treatment had PVC sheet covering on the four sides which were pulled down at 1800 hr each day to trap the day temperature in order to increase the night temperature. (ii) polyhouse with ambient temperature was with open sides. The PVC sheets were rolled upward during the daytime for both the experimental polyhouses to ensure similar daytime temperature. Maximum and minimum thermometers were used to record the temperature, starting from the time of shifting the pots at anthesis to maturity.

Grain growth rate: Panicles were tagged at 50% exsertion and grains were collected at an interval of 5 days after anthesis for each cultivar as also observed by Yamakawa *et al*. (2007). Fifty grains per replicate were weighed for fresh weight and then oven dried at 80°C till constant weight to obtain the dry weight. Grain filling rate was calculated as /mg DW grain/day. Percent moisture was also calculated from fresh and dry weight of the grains at different time of sampling.

Hulling (%): One gram grain sample of each variety was subjected to manual dehusking in a palm dehusker. The dehusked kernels were weighed and percentage grains dehusked was calculated as

$$\text{Hulling (\%)} = \frac{\text{Weight of dehusked kernel}}{\text{Weight of paddy}} \times 100$$

Head rice recovery: Whole grains and grains more than three fourth size were separated from the dehusked grains and weighed. Head rice recovery was calculated as:

$$\text{Head rice recovery (\%)} = \frac{\text{Weight of more than three fourth size grains}}{\text{Weight of dehusked grains}} \times 100$$

High density grains: Specific gravity method was used to determine high density grains by immersing 100 seeds in 25% sodium chloride solution. The number of grains that settled at the bottom of the solution was counted to calculate the percentage of high density grains (Krishnan and Rao 2005).

Alkali spreading value: Ten whole dehusked grains in three replicates per treatment were uniformly spread in a petri-plate containing 20 ml of 1.7% KOH. Using 7 point scale, spreading and clearing values of kernels were recorded (Cruz and Khush 2000). Grains with low score remain unaffected, whereas with high score disintegrated completely.

Chalkiness (Endosperm appearance): Five gram rice grains in triplicate were manually separated for the presence of >75% opaque or chalky presence and weighed. The weight of the chalky grains was expressed as a percentage of total weight (Adu-Kwarteng *et al.* 2003).

Amylose content: Rice flour (100 mg) was boiled for 10 min after adding 1 ml of 95% ethanol and 9 ml of 1N NaOH. To an aliquot of 5 ml, 1 ml of 1 N acetic acid and 2 ml of freshly prepared iodine solution (0.2 g iodine in 2% KI solution) was added and kept at room temperature for 20 min followed by measurement of absorbance at 620 nm. A standard curve with potato starch was prepared to estimate the amylose content (Nagarajan *et al.* 2010).

Statistical analysis: Anaysis of variance (ANOVA) was performed for each season and Duncan's mutiple range test was used to compare the effect of temperature treatment on each cultivar at significance level $P< 0.05$ y using the software - Statistical Tool For Agricultural Research (STAR) version 2.0.1 developed by IRRI. The data for each quality trait was tabulated as mean of two seasons and significance denoted by a and b.

RESULTS AND DISCUSSION

Grain growth under ambient and ET_{min} treatment: The final grain weight is influenced by the rate and duration of movement of assimilates to the developing grains (Kobata and Uemuki 2004). Data analysis on grain growth showed that all cultivars recorded an increase in grain dry weight uptil 15 DAA irrespective of the anthesis. The early duration cultivars, Vandana and Parijat were found to be susceptible, compared to Bakal and Nagina 22. The grain growth in both the cultivars was 34 and 25% less under ET_{min} condition compared to ambient control throughout the grain growth period (Fig 1a-b). On the other hand, Bakal and Nagina 22 did not show much difference in grain dry matter accumulation under both the growing conditions, albiet in Bakal at 10 DAA where 16% less dry matter was observed under ET_{min} (Fig 1c-d). No remarkable difference was seen in dry matter accumulation in the mid-early cultivar, Sahbhagi dhan under ambient and ET_{min} conditions (Fig 1e). Gotrabidhan showed reduced dry matter accumulation (42% less under ET_{min} than ambient at 10 DAA) in comparison to relatively tolerant Abhishek (Fig 1f-g). The reduced dry matter accumulation in all the susceptible cultivars irrespective of their duration brought out an interesting observation that early grain filling period was affected in the *indica* rice under ET_{min} which varied in the first fortnight for early, mid-early and medium duration varieties. These observations found concurrence in earlier studies on grain development under high night temperature where grain growth rate in the early or middle stages of grain filling was affected through a reduction in cell enlargement in the region at which the cells enlarge during these stages (Morita *et al.* 2005). Earlier studies have confirmed that the endosperm cell count increases until about 10 days after anthesis and then they begin to accumulate starch (Hoshikawa 1967). Therefore, any environmental change during this phase can have a negative effect on test weight. High temperature at night also enhances respiratory losses of carbon that may lead to lesser accumulation of dry matter (Wardlaw *et al.* 1980) as there is more investment of photoassimilates towards maintenance respiration. Grain filling rate was in the range of 0.66 to 2.96 under ET_{min} compared to 1.13 to 2.00 mg/g/day during the intial phase of grain growth in

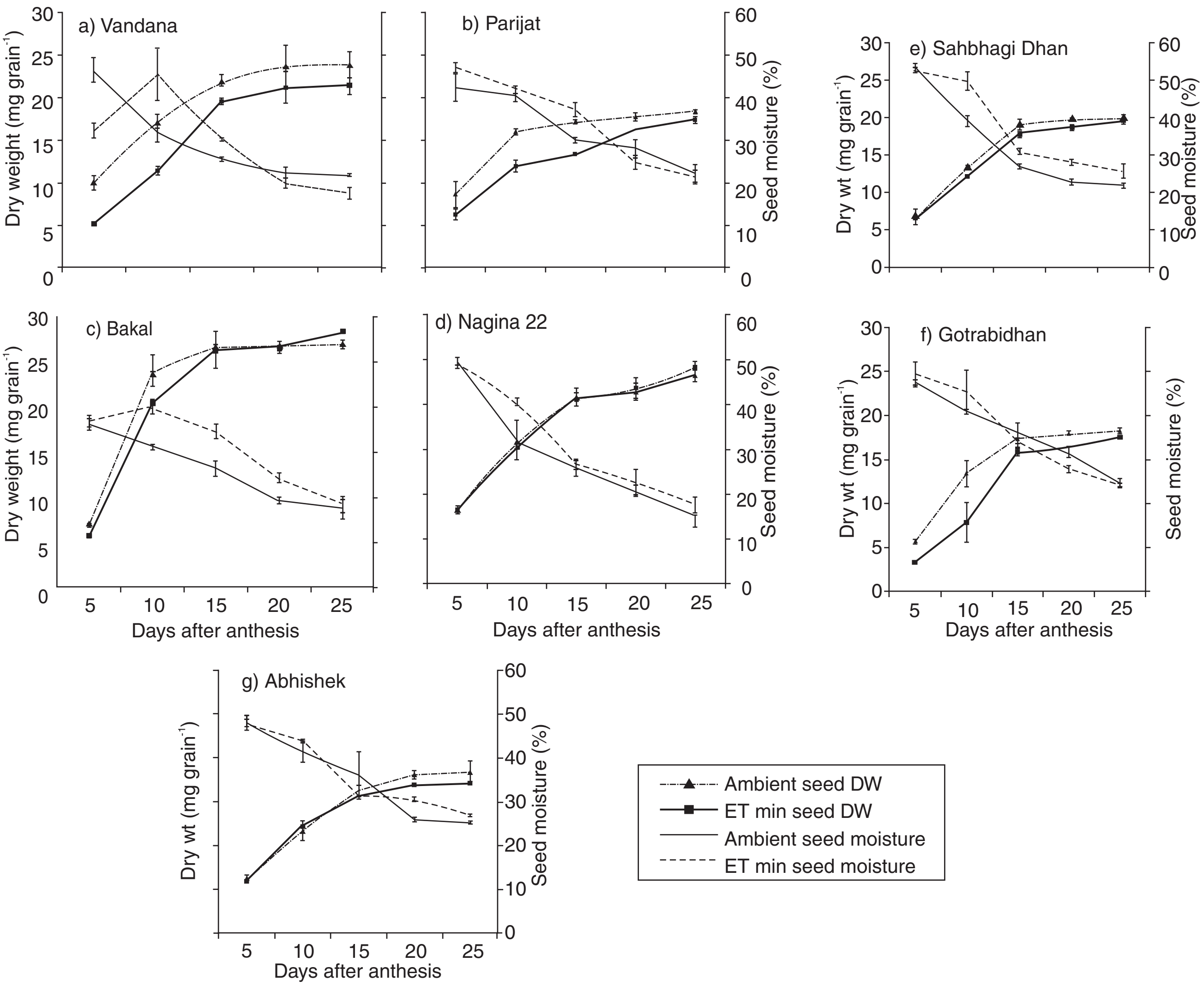

Fig 1a-g Grain growth and moisture content in early, mid-early and medium duration rice cultivars under ambient and ET_{min} conditions early and medium duration susceptible cultivars (Table 1). One of the strategies followed by the tolerant genotypes to escape high temperature stress is hastening the rate of grain growth so as to accumulate more dry matter under stressed environment (Tashiro and Wardlaw 1989, Wilhelm *et al.* 1999) but in our study the slower rate of grain growth in the early phase determined the reduction in test weight of susceptible cultivars. Tolerant cultivars recorded similar grain growth rate throughout the grain development period. Unlike our observation, Morita *et al*. (2005) reported that high night temperatures accelerated the rate of increase of grain dry matter, but the consequent supply of photosynthates to sustain this increase is not met, as photosynthesis is dormant during night time.

Loss of grain moisture with grain maturity revealed that grain moisture reduced faster in early duration Vandana and Nagina 22 (slope ≥10) compared to medium duration, Gotrabidhan and Abhishek (slope = ~6). Higher grain moisture under ET_{min} could be explained due to higher relative humidity (67%) in treatment polyhouses compared to ambient (56%). Gotrabidhan and Abhishek did not show this difference in the rate of moisture loss as relative humidity decreased to average 52% during grain filling in these two cultivars.

Table 1 Grain growth rate in early, mid-early and medium duration rice cultivars under ambient and high night temperature

Cultivar		Grain growth rate (mg/grain/day)				
		5 DAA	10 DAA	15 DAA	20 DAA	25 DAA
Vandana	Ambient	2.00 ± 0.17	1.44 ± 0.13	1.96 ± 0.10	0.32 ± 0.54	0.09 ± 0.54
	ET_{min}	1.04 ± 0.03	1.24 ± 0.08	1.64 ± 0.12	0.32 ± 0.32	0.04 ± 0.47
Parijat	Ambient	1.72 ± 0.31	1.49 ± 0.34	0.24 ± 0.01	0.09 ± 0.11	0.14 ± 0.01
	ET_{min}	1.27 ± 0.13	1.13 ± 0.03	0.31 ± 0.15	0.55 ± 0.02	0.22 ± 0.06
Bakal	Ambient	1.36± 0.05	3.44± 0.37	0.53 ± 0.33	0.01 ± 0.10	0.03 ± 0.10
	ET_{min}	1.12± 0.04	2.96 ± 0.07	1.20 ± 0.47	0.05 ± 0.05	0.36 ± 0.10
Nagina 22	Ambient	1.72 ± 0.01	1.37 ± 0.08	1.08 ± 0.13	0.12 ± 0.11	0.36 ± 0.10
	ET_{min}	1.64 ± 0.07	1.52 ± 0.06	0.96 ± 0.14	0.24 ± 0.35	0.44 ± 0.25
Sahbhagi Dhan	Ambient	1.35 ± 0.2	1.34 ± 0.25	1.11 ± 0.14	0.14 ± 0.20	0.03± 0.10
	ET_{min}	1.32± 0.02	1.16 ± 0.01	1.13 ± 0.10	0.16 ± 0.17	0.11 ± 0.10
Gotra-bidhan	Ambient	1.13 ± 0.04	1.55 ± 0.34	0.73 ± 0.31	0.19 ± 0.04	0.05 ± 0.03
	ET_{min}	0.66± 0.01	0.91 ± 0.45	1.61 ± 0.42	0.09 ± 0.09	0.23 ± 0.04
Abhishek	Ambient	1.24 ± 0.09	1.09 ± 0.16	0.91 ± 0.11	0.36 ± 0.06	0.06 ± 0.32
	ET_{min}	1.18± 0.01	1.39 ± 0.13	0.57 ± 0.14	0.24 ± 0.07	0.03± 0.07

Grain quality traits

Test weight and high density grains under ambient and ET_{min} conditions: Grain quality traits like test weight and high density grains were maximally affected by the increase in temperature during the early phase of grain growth (Table 2). It was observed that the susceptible genotypes like Vandana, Parijat and Gotrabidhan showed a reduction of 5, 6 and 4% respectively, in test weight under ET_{min}. Similar trend was also observed in proportion of high density grains with a significant reduction of 12- 18% in the susceptible cultivars. The reduced grain weight under high minimum temperature may be attributed to the excessive energy consumption to meet the respiratory demand of the seeds (Tanaka *et al.* 1995) or decrease in endosperm cell size during early phase of grain development (Morita *et al.* 2005).

Chalkiness in rice grains under ambient and ET_{min} conditions: Chalkiness in rice grains is controlled by genetic and environmental factors (Lisle *et al.* 2000). Chalky grains are formed due to irregular packing of starch during the grain filling stage that generates air spaces in the endosperm forming opaque white regions in the translucent kernel (Ashida *et al.* 2009). High temperature during grain-filling period accelerate the demand for more assimilates to avoid milky white kernels (Kobata and Uemuki 2004). In a recent study, Kaneko *et al*. (2016) showed that increased expression of α amylases in the endosperm causes starch degradation forming chalky rice kernels under high night temperature. Conflicting reports on the impact of ET_{min} suggest an increase in chalkiness (>75% chalky grains) in rice with an increase in night temperature (Ambardekar *et al.* 2011) vis-a-vis chalk formation to be independent of increase in night temperature in other experiments (Dai *et al*. 2009, Li *et al*. 2011, Shi *et al.* 2013). Our study, however, revealed that all the cultivars had a low percentage of chalky grains (Table 2) at ET_{min} of 23-25^{o}C which was infact less than that used in other experiments (Shi *et al*. 2016). Despite the low values, genotypic variation existed within the *indica* cultivars as Vandana showed the highest chalk formation under both growing conditions (Table 2). Similar variation was also recognised in earlier studies where medium-grain cultivars were less susceptible than long-grain cultivars (Cooper *et al.* 2008, Lanning *et al.* 2011).

Head rice recovery under ambient and ET_{min} conditions: Head rice recovery determines the milling quality of rice as it refers to the proportion of rice kernels which are three-fourth of the kernel size after dehusking. All the cultivars had more than 45% head rice recovery under ambient and ET_{min} conditions (Table 2). Increased night temperature did not cause a significant change in head rice recovery values which was in agreement with the earlier results obtained with early maturing varieties under high night temperature

Table 2 Mean values for rice grain quality traits in early, mid-early and medium duration cultivars under ambient and ET_{min} during the *kharif* season 2014 and 2015

Cultivar	Test weight (g)		HDG (%)		Chalkiness (%)		Head rice recovery (%)		Amylose (%)		ASV	
	Ambient	ET_{min}	Ambient	ET_{min}	Ambient	ET_{min}	Ambient	ET_{min}	Ambient	ET_{min}	Ambient	ET_{min}
Vandana	19.20[a2]	18.32[b2]	62.33[a2]	49.92[b2]	12.77[b2]	14.28[a2]	61.67[a2]	56.65[b2]	24.93 [a2]	23.53 [b2]	2.00	1.00
Parijat	16.60[a1]	15.54[b1]	50.62[a1]	33.00[b1]	1.03[b1]	1.73[a1]	50.68	52.24	26.89	25.96	5.00	3.00
Bakal	21.86	21.40	25.33[a2]	24.80[b2]	1.77	1.93	71.80	72.51	22.45[a1,a2]	20.78[b1,b2]	2.00	2.00
Nagina 22	19.44	19.68	53.17	50.17	2.53	2.63	65.67	64.10	19.44	19.44	6.00	6.00
Sahbhagi Dhan	17.71	17.60	57.00	51.80	2.10[b1,b2]	3.47[a1,a2]	52.67	47.72	20.35	19.23	3.0	3.00
Gotrabidhan	18.22	17.44	60.33[a2]	48.33[b2]	3.91	4.64	48.93	50.74	16.03	15.04	2.00	2.00
Abhishek	18.17	18.21	63.75	64.45	2.73	2.78	64.03	61.15	16.28	15.39	2.00	2.00

Data are means of the experiment for the two seasons with three replicated measurement and the letters a1, b1 and a2, b2 indicate significant difference between treatments during first and second season respectively, according to Duncan's Multiple Range Test.

(Dai *et al.* 2009). Contrary to these observations, Shi *et al.* (2016) reported a reduction in recovery of head rice but the night temperature used in their study was 29°C which were higher than our minimum temperature (23-25°C).

Amylose content and alkali spreading value under ambient and ET_{min} conditions: Consumer preference is altered by a change in amylose content as a reduction in amylose content makes the cooked rice kernel sticky (Jennings *et al.* 1979). Earlier studies have shown that high night temperature reduces the amylose content in the grains (Nagarajan *et al.* 2010, Song *et al.* 2015). Our experiment showed that irrespective of the duration the early and medium duration cultivars showed approximately 1% decline in amylose content under elevated temperature. Only early duration variety Vandana and Bakal recorded significant reduction in first season when latter phase of grain growth was affected under increased temperature (Table 2). Song *et al*. (2015) reported that amylose content in rice kernel exhibited an "increase decrease stabilization pattern" in *japonica* rice cultivar along with amyloplast maturation.

Alkali spreading Value (ASV) indicates the cooking temperature at which starch grains swell and simultaneously lose their crystallanity and birefringence (Song *et al.* 2013). Higher ASV is inversely related to the gelatinization temperature. Early duration susceptible cultivars, Vandana and Parijat had lower ASV under HNT compared to ambient conditions (Table 2). This suggests that the cooking temperature for grains of these cultivars will tend to increase with the increase in night temperature.

Our results showed cultivar specific effect of elevated minimum temperature during the early phase of grain filling in rice. This study will help in identification of cultivars that can be used in breeding for tolerance to elevated minimum temperature stress. It is imperative to understand the mechanistic basis of quality deterioration under elevated minimum temperature to breed tolerant cultivars with superior quality traits.

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