



## EVALUATION OF TERMINAL HEAT TOLERANCE OF BREAD WHEAT ON THE BASIS OF PHYSIOLOGICAL TRAITS

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### ABSTRACT

Wheat (*Triticum aestivum* L.), an important cereal crop for the majority of the world's population, is a staple diet for around two billion people. Wheat produces roughly 55% of the carbs and 20% of the dietary calories consumed worldwide. Relative water content has been used to assess the impact of abiotic conditions on wheat, such as drought and heat stress. Plant vigor is a comprehensive measure of plant health and performance, and its decline suggests that heat stress can have detrimental effects on plant physiology, possibly due to water loss. Under heat stress, chlorophyll loss in leaves occurs due to the fast degradation of chlorophyll. chlorophyll content is related to heat tolerance and stay green traits. A high chl conc. in wheat can be a criterion for selecting heat-resistant wheat. The calculated chlorophyll concentration can be compared with the standard values for the plant species being analyzed to determine if the plant is healthy or stressed. Higher chlorophyll levels indicate healthier plants, while lower levels may indicate stress or disease. Average value of relative water content 83.0 % in TS and 69.6% in LS conditions crop season 2021-2022. And in crop season 2022-2023, 81.6% in TS and 72.0% in LS conditions respectively. Average value of chlorophyll content 30.0mg/g in TS and 21.0mg/g in LS conditions, in crop season 2021-2022. And in crop season 2022-2023, 26.6mg/g in TS and 18.0mg/g in LS conditions respectively.

**KEYWORDS:** Wheat, Relative water content (RWC), Chlorophyll content, Heat stress.

### INTRODUCTION

Wheat (*Triticum aestivum* L.), an important cereal crop for the majority of the world's population, is a staple diet for around two billion people. Wheat produces roughly 55% of the carbs and 20% of the dietary calories consumed worldwide.<sup>[1]</sup> It is the largest source of vegetal protein in human meals on a global scale, with a protein level of roughly 13%, which is relatively high when compared to other major cereals.<sup>[2]</sup> Wheat is indeed considered the world's most important food crop. It is a staple for a large portion of the global population, providing a substantial portion of their daily caloric intake. Wheat is a primary source of energy and nutrients for people in many countries.<sup>[3]</sup>

Relative water content (RWC) has been used to assess the impact of abiotic conditions on wheat, such as drought and heat stress. Tolerant cultivars retain their dry shoot weight and RWC under stress circumstances.<sup>[4]</sup> There is a correlation between a reduction in plant vigor and a decrease in RWC under heat stress conditions. This implies that as plants experience heat stress, there is a negative impact on their water status, as reflected by a reduction in RWC. Plant vigor is a comprehensive measure of plant health and performance, and its decline

suggests that heat stress can have detrimental effects on plant physiology, possibly due to water loss.<sup>[5]</sup>

Osmotic potential and emphasized the importance of water potential and grain yield as key indicators of heat tolerance in wheat. Osmotic potential is related to the ability of plant cells to retain water under osmotic stress, and it is closely tied to water potential. The conclusion suggests that maintaining optimal water potential and grain yield are critical factors in determining a wheat plant's ability to tolerate heat stress.<sup>[6]</sup> This aligns with the idea that water-related parameters play a crucial role in heat tolerance, and maintaining water balance is essential for crop productivity under such conditions. The significance of water-related parameters, especially RWC, osmotic potential, and water potential, in assessing the impact of heat stress on wheat. These parameters provide insights into the physiological responses of plants to heat stress and are valuable indicators of heat tolerance in crops. Understanding these relationships is crucial for developing strategies to enhance the heat resilience of crops like wheat, which is important for global food security in the face of changing climate conditions. Investigated the usage of RWC as a

useful selection criterion for various stress resistance methods.<sup>[7]</sup>

Suggests that grain yield and RWC are reliable criteria for evaluating tolerance to both heat and drought stress in crops.<sup>[8]</sup> Grain yield is a direct measure of a crop's productivity and economic value, while RWC provides insights into the water status and hydration levels of plant tissues. Together, these parameters offer a comprehensive assessment of a plant's ability to withstand the combined stresses of heat and drought.<sup>[9]</sup> The substantial gap between the potential productivity of crops and their actual production. This gap suggests that, despite the genetic and environmental potential for high yields, various factors, including abiotic stresses like heat and drought, may be limiting actual production.

Grain yield and RWC emerge as crucial indicators for assessing stress tolerance in crops, providing a basis for developing strategies and breeding programs aimed at narrowing the gap between the potential and actual productivity of crops. This holistic approach is essential for ensuring sustainable and resilient agricultural practices in the face of ongoing climate changes. Wheat varieties that can tolerate various pressures, such as abiotic stresses, notably heat tolerance, must be created in order to meet future food demand.<sup>[10]</sup> It is necessary to identify genotypes that not only have the ability to avoid, escape, and exhibit resistance to various stresses, but also have the potential to offer improved grain yields under heat stress circumstances.

Under heat stress, chlorophyll loss in leaves occurs due to the fast degradation of chlorophyll.<sup>[11]</sup> In wheat, chl content is related to heat tolerance and stay green traits.<sup>[12,13]</sup> A high chl conc. in wheat can be a criterion for selecting heat-resistant wheat.<sup>[14,15]</sup> A high chl conc. under thermal stress has a low photo inhibition.<sup>[16,17]</sup> The measurement of chlorophyll content is an important parameter in assessing plant health, photosynthetic activity, and its potential contribution to heat tolerance.<sup>[18]</sup> The chlorophyll content in plant leaves is often associated with various aspects of plant performance, including transpiration efficiency and heat tolerance. Higher chlorophyll levels can indicate better photosynthetic capacity, which can contribute to improved plant resilience to heat stress. The study suggests that chlorophyll content may be a contributing factor to heat tolerance in plants.<sup>[18]</sup> To determine the relative amount of chlorophyll in plant leaves, researchers commonly use a method based on absorbance measurements at specific wavelengths, typically at 665nm and 645nm. This method relies on the fact that chlorophyll absorbs light in these wavelength ranges, and the absorbance values can be used to estimate the chlorophyll concentration in the leaf tissue. Absorbance at 665nm and 645nm, these specific wavelengths are chosen because chlorophyll a and chlorophyll b, the two primary types of chlorophyll found in plants, have distinct absorption peaks in this

range. By measuring the absorbance at these wavelengths, researchers can estimate the levels of these chlorophyll types and, consequently, the total chlorophyll content.<sup>[19]</sup>

Overall, measuring chlorophyll content using absorbance at specific wavelengths is a valuable technique for assessing a plant's photosynthetic capacity and, by extension, its ability to cope with heat stress. Higher chlorophyll content often indicates healthier and more photosynthetically active plants, which can better withstand environmental stressors like high temperatures. Several studies have successfully used chlorophyll content assessment to screen for heat-tolerant wheat genotypes. Heat-tolerant genotypes have been found to maintain high chlorophyll content. It is important to note that while chlorophyll estimation can provide valuable information about plant health, it should not be the sole method used to assess heat tolerance or any other plant trait.

## MATERIALS AND METHODS

### Field trials and trait evaluation

A field trial was carried out to investigate the terminal heat tolerance of ten wheat genotypes. The trial was designed with a precise phenotyping technique, which may have included careful measurement and monitoring of the plants' numerous physical and biochemical properties. The genotypes were chosen from several agro-climatic zones in India, implying that the trial attempted to examine wheat performance under various environmental conditions. The trial was carried out under two conditions: timely sown (TS) and late sown (LS). The TS were completed between the third and fourth weeks of November and the LS between the third and fourth weeks of December.<sup>[20]</sup> This shows that the researchers were interested in determining how wheat genotypes responded to high temperatures, which can alter crop output and quality. Three replications were employed, which indicates that the experiment was done three times to guarantee that the results were reliable.

PBW752, PBW644, PBW757, DBW14, DBW71, AKW1071, DBW168, GW273, K9644, and HD2967 were the ten wheat genotypes utilized in the trial. The genotypes were grown in three rows of 72 square meter plots.<sup>[21]</sup> The experiment was set up in a precise phenotyping technique with three replications under TS and LS conditions throughout the rabi seasons of 2021-2022 and 2022-2023. These genotypes were obtained from the Indian agricultural research institute ICAR-IIWBR, Karnal.

## ENVIRONMENTS

Field trials were carried out on sandy soil at Banasthali Vidyapith's Krishi Vigyan Kendra's research farm. Which is located in a specific geographical position (25°41'N, 76°19'E) and may have specific environmental variables that could affect the performance of the wheat genotypes. The trial's overall goal was to evaluate the

performance of various wheat genotypes under high temperature circumstances.<sup>[21]</sup>

### PLANTING METHODS

The genotypes were cultivated in three replications on plots. In a replication, the genotypes were grown in four-row plots. Rectangular plots, each 30 cm long and 50 cm wide. The plots were utilized to grow seedlings, with 24 of them being saved following germination and the rest being removed. The seedlings were planted at a depth of 5 cm, with a gap of 10 cm between rows and plants. Each allotment included four rows of six plants each. To avoid boundary effects, the inner four plants from the second and third rows were chosen for data collection.<sup>[20]</sup>

### RELATIVE WATER CONTENT (RWC)

The RWC of a cell is a measure of its water state that has been linked to biotic and abiotic conditions such as heat stress. RWC was analyzed following the previously reported method<sup>[22,23]</sup>, with some modifications. In this experiment leaf samples were collected 12 days after anthesis in timely and late sown conditions. RWC & water potential are thought to be more desirable indicators of drought stress than other biochemical and physiological properties of the plants.<sup>[22,23]</sup>

Determination of RWC (relative water content) in flag leaves. RWC is a measure of the water content of plant tissue relative to the maximum amount of water it can hold, and it is often used as an indicator of plant water status. Determining RWC can be useful for understanding how plants respond to changes in water availability or other environmental stressors. Based on the information provided, The RWC in the flag leaves appears to have been established 12 days after anthesis (which refers to the time when a flower opens and becomes receptive to pollination). The flag leaf is typically the last leaf to emerge from the plant and is an important source of photosynthate (sugar produced through photosynthesis) for grain filling in cereal crops such as wheat.

The leaf was instantly taken from the field, packed in plastic bags right away, and brought to the lab. This suggests that the researchers wanted to minimize any potential water loss from the leaf during transport or storage, which could affect the accuracy of RWC measurements. Once in the laboratory, the leaf would likely have been weighed to determine its fresh weight, then dried in an oven to determine its dry weight<sup>[24]</sup>, then all leaf put in water for overnight after that next morning leaf would likely have been weighed to determine its turgid weight. Based on these measurements, the RWC could be calculated using the formula:

$$RWC = (FW - DW) / (TW - DW) \times 100\%$$

where FW is the leaf's fresh weight, DW is its dry weight, and TW is the leaf's turgid weight (i.e., its weight following a complete rehydration).

### CHLOROPHYLL CONTENT

Chlorophyll estimation, was give the relative indication of chlorophyll within the plant. Chlorophyll readings have to be taken at the absorbance of 645 and 665 nm.<sup>[19]</sup> Several studies have successfully used chlorophyll content assessment to screen for heat-tolerant wheat genotypes. Heat-tolerant genotypes have been found to maintain high chlorophyll content, which is essential for photosynthesis. Higher chlorophyll content and lower reduction due to drought stress in wheat genotypes have been identified as tolerant genotype. It is important to note that while chlorophyll estimation can provide valuable information about plant health, it should not be the sole method used to assess heat tolerance or any other plant trait. It should be used in conjunction with other methods to obtain a comprehensive understanding of the plant's physiology and response to stress.

The chlorophyll content was measured at 8 and 24 days after anthesis (DAA) in the flag leaf. To measure the chlorophyll content, leaf samples were taken from different positions on the flag leaf and ground using a mortar and pestle. The chlorophyll was extracted from the ground samples using aqueous acetone that was 80% concentration. After that, the suspension was put into centrifuge tubes and centrifuged for five minutes. Then, a colorless residue was converted into a clear green solution. The optical density of the mixture was then determined using a spectrophotometer and two separate wavelengths, 645 nm and 665 nm, along with 80% acetone.

The calculated chlorophyll concentration can be compared with the standard values for the plant species being analyzed to determine if the plant is healthy or stressed. Higher chlorophyll levels indicate healthier plants, while lower levels may indicate stress or disease.

### STATISTICAL ANALYSIS

The statistical analyses of relative water content (RWC) and chlorophyll content using SPSS software version 20. Additionally, the analysis of variance (ANOVA) was performed and indicated significance for both timely sown and late sown conditions. The analysis of data revealed that both conditions (TS & LS) are significant. This suggests that there are statistically significant differences in either RWC, chlorophyll content, or both, between the timely sown and late sown conditions.

### RESULTS

In this graph showed that relative water content (RWC). In crop season 2021-2022, Relative water content under TS & LS conditions is shown in figure 1(a). Genotype K9644 showed that highest value that is 88.56% and genotype HD2967 showed lowest value that is 75.69% in TS conditions. Genotype K9644 showed highest value that is 80.21% and HD2967 showed lowest value that is 65.91% in LS condition.

In crop season 2022-2023, Relative water content under TS & LS conditions is shown in figure 1(b). Genotype

K9644 showed that highest value that is 90.22% and genotype HD2967 showed lowest value that is 77.35% in TS condition. Genotype PBW644 showed that highest value that is 74.10% and genotype HD2967 showed lowest value that is 58.24% in LS condition because of terminal heat stress.

K9644 performed well in terms of water retention, while HD2967 showed lower RWC. In late sown conditions,

PBW644 exhibited better water retention compared to HD2967, which had the lowest RWC, likely due to the impact of terminal heat stress. It's important to note that maintaining higher RWC values is generally associated with better plant hydration and stress tolerance. The differences in RWC among genotypes can be attributed to their genetic characteristics and how they respond to environmental conditions, particularly heat stress in the case of late sown conditions.

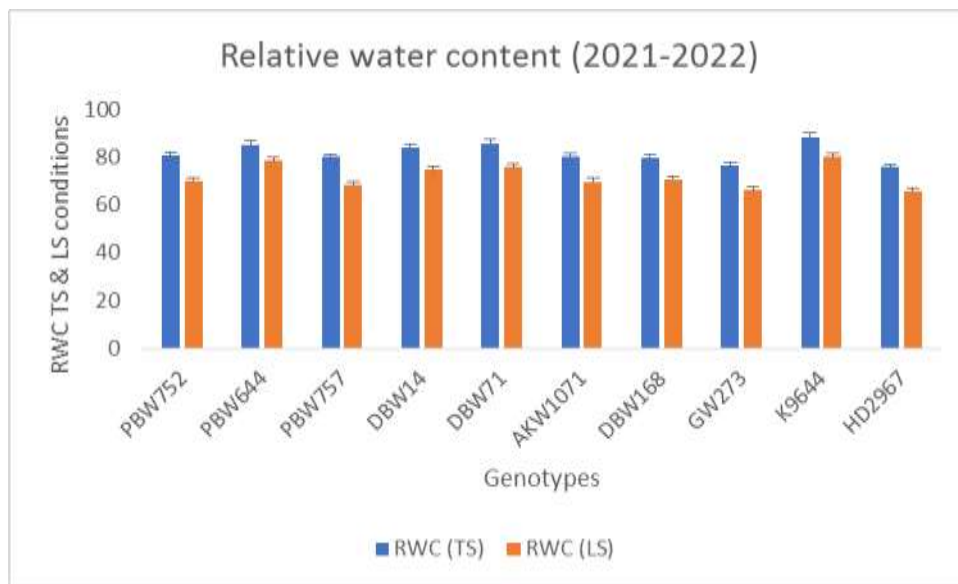


Fig 1(a): Graph showed relative water content (RWC) in 2021-2022.

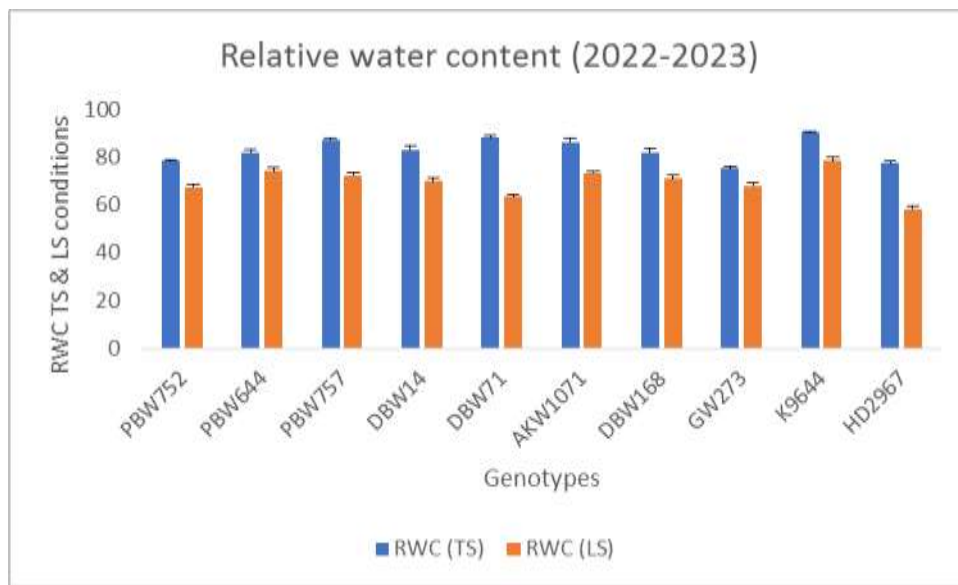


Fig 1(b): Graph showed relative water content (RWC) in 2022-2023.

In crop season 2021-2022, chlorophyll content under TS & LS conditions is shown in figure 2(a). Genotype HD2967 highest chlorophyll content of 33.81mg/g. Genotype AKW1071 lowest chlorophyll content of 20.31mg/g in TS condition. Genotype HD2967 highest chlorophyll content of 24.18mg/g. Genotype AKW1071 lowest chlorophyll content of 11.74mg/g in LS condition. It appears that under both TS and LS conditions,

genotype HD2967 consistently exhibited higher chlorophyll content compared to genotype AKW1071. Additionally, the LS condition led to a decrease in chlorophyll content for both genotypes, with genotype HD2967 still maintaining a higher value compared to AKW1071. Chlorophyll content is an important indicator of plant health and photosynthetic activity. The decrease in chlorophyll content under late sown conditions,

attributed to terminal heat stress, suggests that environmental factors can significantly impact crop performance.

In crop season 2022-2023, chlorophyll content under TS & LS conditions is shown in figure 2(b). Genotype K9644 exhibited the highest chlorophyll content at 30.48mg/g. Genotype AKW1071 displayed the lowest chlorophyll content at 14.54mg/g in TS condition. Genotype PBW752 showed the highest chlorophyll content at 21.04mg/g. Genotype AKW1071 had the

lowest chlorophyll content at 9.39mg/g in LS condition. The lower chlorophyll content in late sown condition for genotype AKW1071 is attributed to terminal heat stress. The genotypes responded differently to the sowing conditions, with some showing higher chlorophyll content in TS conditions and others in LS conditions. Additionally, the impact of terminal heat stress, especially in the case of genotype AKW1071, highlights the influence of environmental factors on chlorophyll levels.

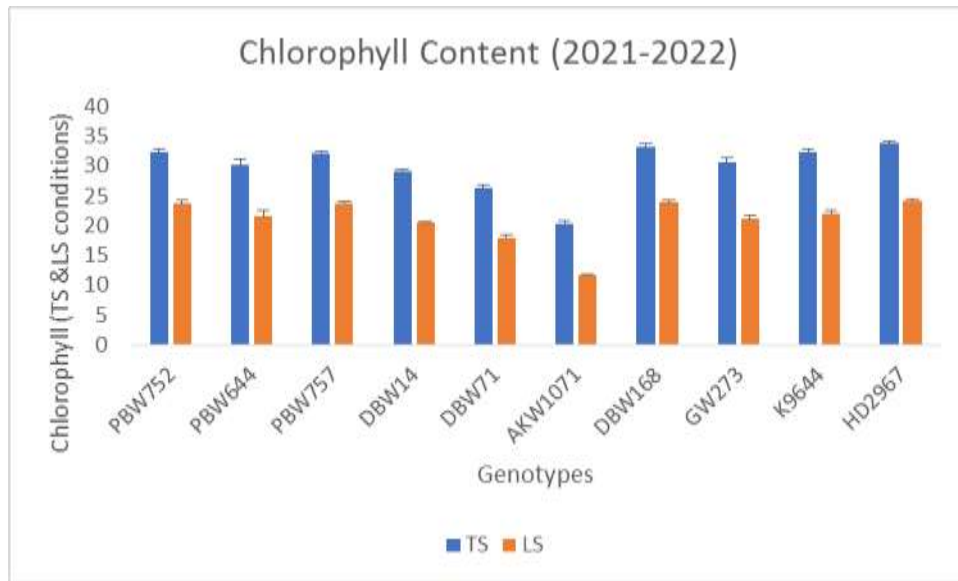


Fig 2(a): Graph showed chlorophyll content (Chl) in 2021-2022.

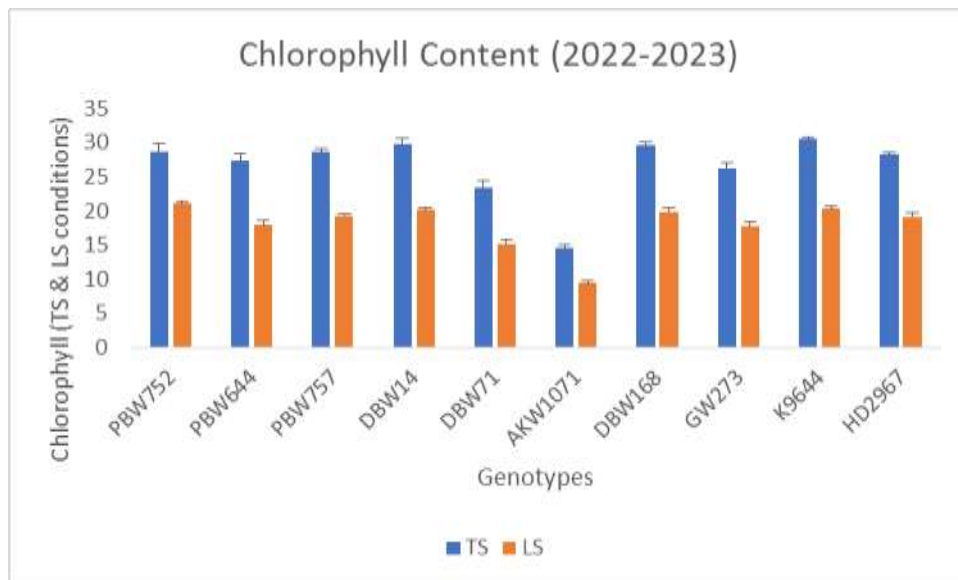


Fig 2(b): Graph showed chlorophyll content (Chl) in 2022-2023.

**DISCUSSION**

Relative Water Content (RWC) in late-sown conditions, coupled with terminal heat stress, likely exacerbate water scarcity for plants. High temperatures during the terminal stages of plant development can increase evaporation and transpiration rates, leading to water loss from the plant.

The combination of late sowing and terminal heat stress can result in reduced water uptake and retention by the plants, leading to a lower RWC. Insufficient water availability during crucial growth stages can negatively impact plant turgor pressure, cellular expansion, and overall hydration status. Relative Water Content (RWC)

in timely sowing, on the other hand, provides plants with a better chance to establish robust root systems before encountering terminal heat stress. This early establishment allows plants to access water more efficiently and maintain higher RWC even under heat stress conditions. The plants in timely-sown conditions may exhibit greater resilience to water loss, contributing to their overall better hydration status.

Chlorophyll Content in late-Sown Conditions, terminal heat stress, especially during the late stages of plant development, can negatively impact chlorophyll stability and synthesis. High temperatures can accelerate the breakdown of chlorophyll molecules, leading to lower chlorophyll content in late-sown conditions. Additionally, heat stress may hinder the plant's ability to carry out photosynthesis optimally, resulting in decreased chlorophyll production. This reduction in chlorophyll content indicates a compromised photosynthetic capacity, which can affect the plant's ability to generate energy and sustain growth. Chlorophyll Content, timely sowing provides plants with a longer growing period under more favorable conditions, allowing for adequate chlorophyll development. Plants in timely-sown conditions are likely to experience less heat stress during critical growth stages, preserving chlorophyll integrity and promoting optimal photosynthetic activity. The higher chlorophyll content observed in timely-sown conditions reflects the plants' ability to maintain a healthier photosynthetic apparatus.

RWC is proposed to be a more important indicator of water status than other water potential parameters under heat stress conditions. This suggests that RWC is a valuable metric for assessing how well plants are coping with water availability, especially when subjected to high temperatures. High RWC is directly associated with the resistance mechanism to heat stress. This implies that plants with a higher RWC are more likely to exhibit resilience or resistance to the adverse effects of high temperatures, increased osmotic regulation in plants leads to higher RWC. Osmotic regulation involves the control of water movement into and out of cells through the regulation of solute concentrations. This process helps plants maintain adequate water content, contributing to higher RWC. RWC can provide valuable

insights into how well plants are managing water under heat stress conditions. A higher RWC is indicative of effective osmotic regulation and is associated with the plant's ability to resist the negative impacts of heat stress. This information is crucial for understanding and potentially improving the resilience of plants in the face of environmental challenges.<sup>[25]</sup>

According to the current study, high RWC is associated with plant resilience to heat stress. Plant RWC decreased during heat stress.<sup>[26,27,6,28]</sup> The findings of our investigation agreed with the findings of these scientists.

The comparison of calculated chlorophyll concentration with standard values for a specific plant species serves as a valuable indicator of the plant's health status. This approach is grounded in the understanding that chlorophyll, the green pigment in plants, plays a crucial role in photosynthesis, the process through which plants convert light energy into chemical energy. As such, chlorophyll concentration is often considered a reliable proxy for assessing a plant's overall well-being. When the calculated chlorophyll concentration is higher than the standard values established for a particular plant species, it generally suggests that the plant is in a robust and healthy condition. Healthy plants with optimal chlorophyll concentrations are more likely to exhibit vigorous growth.

Lower chlorophyll levels in comparison to the established standards may signal potential stress or disease in the plant. Various factors can contribute to reduced chlorophyll concentrations, such as nutrient deficiencies, pathogen infections, environmental extremes. It's important to note that while chlorophyll concentration provides valuable insights into a plant's condition, it is just one aspect of a comprehensive assessment of plant health.

The comparison of calculated chlorophyll concentration with standard values serves as a practical and effective method for gauging the health status of plants. This approach enables researchers, agronomists, and farmers to proactively identify and address issues affecting plant health, contributing to more informed decision-making in agriculture, horticulture, and ecological studies.

**Table 1(a): showed ANOVA for all genotypes 2021-2022.**

Genotypes	RWC (Control)	RWC (Stress)	Chl (Control)	Chl (Stress)
PBW752	80.76±1.2	70.26±1.3	32.33±0.5	23.68±0.6
PBW644	85.30±1.5	78.44±1.4	30.14±1	21.53±0.9
PBW757	80.11±1.1	68.66±1.1	32.06±0.4	23.76±0.3
DBW14	83.76±1.4	74.65±1.6	29.11±0.3	20.51±0.1
DBW71	85.72±1.6	75.85±1.4	26.29±0.5	18.00±0.4
AKW1071	80.51±1.3	69.73±1.5	20.31±0.5	11.74±0.1
DBW168	79.72±1.1	70.66±1.2	33.13±0.5	23.83±0.3
GW273	76.38±1.6	66.04±1.5	30.64±0.7	21.13±0.5
K9644	88.56±1.5	80.21±1.3	32.41±0.3	22.00±0.6
HD2967	75.69±1.2	65.91±1.4	33.81±0.2	24.18±0.3

Sig	0.00	0.00	0.00	0.00
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**Table 1(b): showed ANOVA for all genotypes 2022-2023.**

Genotypes	RWC (Control)	RWC (Stress)	Chl (Control)	Chl (Stress)
PBW752	78.42±0.7	67.59±1.0	28.67±1.1	21.04±0.4
PBW644	81.97±1.2	74.10±1.2	27.27±1	17.88±0.6
PBW757	87.43±0.6	72.22±1.3	28.27±0.5	19.30±0.1
DBW14	82.99±1.8	69.98±1.3	29.86±0.8	20.18±0.3
DBW71	88.06±1.1	63.51±1.1	23.41±0.9	15.13±0.6
AKW1071	86.84±1.7	73.39±0.6	14.54±0.5	9.39±0.2
DBW168	82.06±1.9	70.99±1.5	29.51±0.5	19.89±0.6
GW273	75.38±0.9	68.04±1.2	26.27±0.7	17.77±0.6
K9644	90.22±0.7	78.55±1.3	30.48±0.2	20.33±0.3
HD2967	77.35±1.2	58.24±1.3	28.26±0.3	19.07±0.5
Sig	0.00	0.00	0.00	0.00

## CONCLUSION

Timely sown conditions have a positive impact on plant physiology, specifically with higher relative water content and chlorophyll content compared to late sown conditions. This information can be valuable for agricultural practices, emphasizing the importance of planting crops within an optimal timeframe to enhance water status and photosynthetic activity, ultimately influencing overall plant health and productivity.

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## AUTHOR CONTRIBUTIONS

AT performed the experiments and design by GCP. GCP and AT wrote and edited the manuscript.

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## DECLARATIONS

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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