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FAST-TRACK TO BIOMASS: PROTEOMICS ANALYSIS DECIPHERS ENERGY SYNTHESIS IN SPEED-BRED WHEAT

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ABSTRACT

Wheat, a primary staple food in numerous countries, is extensively cultivated worldwide due to its adaptability. It constitutes a significant portion of the global calorie intake, accounting for approximately 20% of the total. Wheat cultivation spans a vast area of 215 million hectares, resulting in a worldwide production of 772 million metric tons. One of the major challenges in wheat breeding is the lengthy process of selecting homozygous genotypes. However, a valuable technique called speed breeding has been developed to address this bottleneck. Speed breeding significantly reduces the time required for variety development and selection, allowing the production of 5 to 6 wheat generations per year. Despite the accelerated growth achieved through speed breeding, the size of wheat plants remains shorter compared to traditional methods, although biomass has a positive correlation with plant yield. Biomass accumulation is influenced by the activity of the vital enzyme RuBisCO. An experiment was conducted on five wheat genotypes under normal sunlight and controlled conditions using light-emitting diodes (LEDs) to evaluate RuBisCO activity. The experiment followed a factorial, completely randomized design with three replications. Various traits, including plant height, flag leaf area, spike length, stomata size, chlorophyll content, biomass, and 1000-grain weight, were measured and showed significant differences among the genotypes and treatments. Proteomics analysis using SDS-PAGE revealed variability in RuBisCO protein expression between normal and controlled plants. Light intensity was also measured for sunlight and the speed of breeding chamber lights. The findings highlight the critical role of light intensity, which remains an indispensable factor in studies about biomass-related research.

***Keywords*:** Speed breeding, Wheat, RuBisCO, SDS-PAGE

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1. INTRODUCTION

Wheat, as one of the significant cereal crops, holds immense value and has played a pivotal role in the advancement of human civilization. Serving as a staple food for half of the global population, wheat has been integral to human sustenance for thousands of years. Its cultivation dates back nearly 10,000 years ago in the Fertile Crescent, an area known for its rich agricultural history. Even before the formal development of human societies, wheat was being utilized and appreciated for its nutritional benefits and versatility (Yang et al. 2022; Haroon et al. 2022). With the adaptation of wheat for domestication purposes, there was a significant surge in the demand for wheat, spanning both domestic and industrial sectors. This increase in demand for wheat coincided with the development and progression of human civilization (Bonjean and Angus 2001; Huang et al. 2022). In present times, wheat cultivation has expanded on a global scale, with its growth encompassing almost every region except Antarctica. The widespread cultivation of wheat highlights its crucial role as a vital crop supporting food security and socioeconomic development worldwide (Yang et al. 2022; Haroon et al. 2023).

Wheat grains are categorized according to their color and strength, which can vary in the seed bran layers. Different color variations observed in wheat grains include red, blue, amber, and purple (Garg et al. 2016). The characteristics of wheat grains are further classified into types such as durum, bread, and soft wheat, each with its own distinct properties and applications. Another classification of wheat is based on its growing seasons, distinguishing between winter wheat and spring wheat (Macholdt et al. 2021). Winter wheat undergoes its life cycle from fall to summer, while spring wheat grows from spring and reaches maturity in the fall. These seasonal distinctions play a significant role in determining the appropriate cultivation practices and optimal growth

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conditions for different types of wheat (Cheng et al. 2022). While wheat is often used as an energy source, it is also rich in important dietary factors such as carbohydrates, proteins, fiber, and various micronutrients, which are highly valuable (Carcea 2020; Pandey et al. 2020).

A plant breeder with expertise in promoting the efficiency of desirable plant traits or introducing new features plays a crucial role in the development of economically valuable plants (Weedon and Finckh 2019). Selection was the initial method employed by early humans to improve plant traits. To obtain desirable plant features, the finest specimens were always crossed with the most superior ones (Altieri and Nicholls 2017). Several methods are implemented to enhance the quantity and quality of plants, such as selection, hybridization, mutation, bridge crossing, and tissue culturing. However, due to the long life cycle of plants, approximately 10 to 12 years are required for variety development (Harkness et al. 2020). The current situation has prompted breeders to adopt modern techniques, such as speed breeding, to expedite plant development (Gill et al. 2015). The rapid rate of population growth and the changing climate have raised significant concerns regarding the need for an adequate food supply. The slow rate of crop improvement is primarily attributed to the long generation time required to complete the plant life cycle (Panhwar et al. 2019).

Speed breeding is a technique that accelerates the life cycle of plants by controlling light, temperature, and humidity. In the late 20th century, the US National Aeronautics and Space Administration (NASA) conducted a wheat growth experiment in space, successfully shortening the plant life cycle by growing plants under continuously controlled lights, which triggered early reproduction (Ahmar et al. 2020). In 2003, Dr. Hickey introduced a new technique called 'Speed breeding' to further reduce the plant life cycle. Through normal growing, one generation can be achieved, while shuttle breeding allows for 2-3 generations, and speed breeding enables the production of 5-6 generations within a year (Bhatta et al. 2021; Ali et al. 2023). Speed breeding utilizes sodium vapor lamps (SVL) or specific high-intensity lights like blue, red, and white in combination, such as light-emitting diodes (LEDs) (Cazzola et al. 2020).

Different wavelengths of light, such as blue, green, and far-red light, are essential for completing the life cycle of a plant. These wavelengths play a role in plant stem elongation (Ngilah et al. 2018). Each plant species has its specific photoperiod duration, and in speed breeding, the photoperiod is artificially controlled using additional lamps to promote early maturity (Mickens et al. 2019). Wheat is grown under speed breeding conditions with optimized temperature, humidity, and nutrient levels, with a light period of 22 hours. While the plant completes its life cycle within a short duration, it does not necessarily increase its total biomass. If plants undergo photosynthesis for 22 hours, their biomass and yield are expected to be enhanced. A higher photosynthesis rate leads to increased activity of Ribulose 1,5-bisphosphate Carboxylase/Oxygenase (RuBisCO), associated with more CO_2 assimilation, resulting in greater biomass and yield. In this research, we analyzed the difference in RuBisCO protein expression between plants grown under normal and controlled light conditions using protein profiling with SDS-PAGE to assess its activity level.

Plants can fix approximately 30% of the total atmospheric CO_2 through photosynthesis. The excessive amount of CO_2 in the atmosphere has prompted scientists to develop efficient climate-smart cultivars (Rosenzweig et al. 2014). An excess of available CO_2, combined with light and water, promotes the production of carbohydrates. However, the rate of CO2 fixation relies on the activity of RuBisCO in the Calvin Cycle (Aranjuelo et al. 2008). RuBisCO is an enzyme that constitutes 50% of the leaf's soluble protein and plays a key role in the process of CO_2 fixation during the Calvin Cycle by capturing atmospheric CO_2 (Ding et al. 2016). Enhancing the activation of RuBisCO has become a focal point for improving photosynthetic rates (Carmo-Silva et al. 2015). RuBisCO has an evolutionary history dating back approximately 3 billion years when the environment was rich in CO_2. It triggers carbon fixation through the carboxylation of ribulose-bisphosphate. Carbon assimilation promotes plant biomass, which is directly correlated with plant yield. A more complex form of RuBisCO, comprising 16 subunits, has existed for around 500 million years. It consists of 8 large subunits with a protein size of 55kDa and 8 small subunits with a protein size of 15kDa (Andersson and Backlund 2008). There is potential to enhance RuBisCO efficiency in major crops like wheat, maize, and rice through conventional breeding and genetic engineering (Bathellier et al. 2018). The primary objective of each strategy is to enhance CO2 fixation through RuBisCO. Moreover, the activity of the RuBisCO enzyme is increased under high-intensity light conditions (Greco et al. 2012; Ahmed et al. 2023).

This experiment aims to utilize the speed breeding method to investigate five different wheat genotypes. The primary focus of this study is to examine the factors responsible for the reduction in biomass and to explore the morphological characteristics associated with biomass in wheat. To achieve this, the experiment will employ analysis of variance to assess the significance of various factors, correlation analysis to identify relationships between traits, and proteomics analysis to gain insights into the proteomic profile related to biomass. Through these analytical approaches, a comprehensive understanding of the factors influencing reduced biomass and the morphological features associated with biomass in wheat will be obtained.

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2. MATERIALS AND METHODS

The experimental research was conducted at two specific labs: the Wheat Lab of the Plant Breeding and Genetics (PBG) Department and the Proteomics Lab of the Center of Biochemistry and Biotechnology (CABB) at the University of Agriculture (UAF) in Faisalabad. The experimental material, comprising the wheat genotypes, was obtained from the Ayub Agriculture Research Institute (AARI), also located in Faisalabad. The study involved the use of five specific wheat genotypes: Akbar-2019 (a1), Anaj-2017 (a2), Ujala-2016 (a3), Galaxy-2013 (a4), and LU-26 (a5).

2.1. Experimental Description

The seeds were stratified to simulate germination by soaking the genotypes in distilled water overnight. Following stratification, the seeds were sown in pots under a completely randomized design (CRD) factorial arrangement with 3 replications and 2 treatments: normal (under sunlight) and controlled (under speed breeding) conditions for each genotype. Each pot contained 2kg of media, consisting of 70% loamy soil and 30% peat moss. In the controlled conditions, the temperature was maintained at 22°C using an air conditioner and heater, while the humidity was controlled between 65 and 75% through water spray in the room. After the emergence of wheat seedlings, only 10 seedlings were retained in each pot to ensure consistency. Standard agronomic measures were carefully implemented to minimize experimental error in both conditions. Light intensity was measured using a photometer (PM6612L) for both treatments, with sunlight measuring approximately 76,000 lux and the LED panel measuring 24,400 lux. At the maturity stage, data was collected for various characteristics under both normal and controlled conditions, including plant height (cm), flag leaf area (cm_2), spike length (cm), chlorophyll content, stomata size (µm), biomass (g), and 1000-grain weight (g).

2.2 Agronomic, Yield and Physio-bio Traits

2.2.1. Plant height (H): At maturity, the plant height was measured in centimeters (cm). Plants were selected from each replication in both the normal and controlled experiments. The plant height was determined by measuring from the plant base to the tip of the spike using a meter rod, with awns excluded from the measurement.

2.2.2. Flag leaf area (A): The flag leaf area was measured in square centimeters (cm^2) when the flag leaf of the main shoot had fully expanded and developed. Plants were selected from each replication under both normal light and speed breeding conditions. The flag leaf area was calculated using a formula that involved measuring the total width and length of the flag leaf and multiplying the result by 0.75 (Aldesuquy et al. 2014).

2.2.3. Spike length (S): After the completion of the vegetative phase but before maturity, the spike length was measured in centimeters (cm) under normal and controlled conditions. A mother tiller was used to estimate the spike length, with the assistance of a scale. The measurement involved determining the distance from the base of the spike to the tip of the spike. The spike length for each specific experimental unit was estimated without including the awns.

2.2.4. Plant biomass (Q) and 1000-grain weight (W): Each plant in the experiment was thoroughly sun-dried until all moisture content was removed. The total dry biomass of the plants was measured using a weighing balance (de Lima et al. 2021). The weight of a thousand grains was determined using an electric balance, and the measurement was recorded in grams under normal and controlled conditions.

2.2.5. Measurement of chlorophyll contents (C) and stomata size (T): The chlorophyll contents were measured during the heading stage in wheat when the leaves were fully mature and greenish. The chlorophyll content was estimated using a chlorophyll meter (leaf +) to measure the greenish pigments. Fresh flag leaves from the middle of each replication were selected for the analysis of total chlorophyll contents (Novichonok et al. 2016). The stomata size of the fresh mature leaf was measured using the micrometry technique and recorded in micrometers (µm). A light microscope was utilized to analyze the stomata. At a magnification of 400X, one stage piece unit corresponded to 0.01mm, and one stage piece was equivalent to 25 eyepiece units. Additionally, one eyepiece unit was equal to 0.4µm in size.

2.2.6. Proteomics analysis: Fresh flag leaves of the plants were used for protein extraction using standard procedures. The collected leaves were thoroughly washed with distilled water until the leaf surface was completely clean. After washing, the leaves were dried in the open air on tissue paper. Once dried, 300mg of leaf sample was collected for each genotype under normal and controlled conditions using a weighing balance. Protein extraction

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was then carried out from the leaf samples of each genotype under both conditions using a standard protocol (Wang et al. 2006). Proteomics analysis was performed using SDS-PAGE (Abbas et al. 2020) to assess the variability in RuBisCO protein expression on the gel for normal and controlled plants.

3. RESULTS

In this study, several parameters were used to evaluate the reasons for the reduction in plant size under controlled conditions. These parameters included plant height (H), flag leaf area (A), chlorophyll contents (C), spike length (S), stomata size (T), biomass (Q), and 100g grain weight (W). The data were analyzed using Statistics 8.1.

Significant differences were observed among all the traits studied in normal and controlled plants. Five genotypes were evaluated, and the interaction among the genotypes for each trait under both conditions showed significant differences at a significance level of 0.01. Two treatments were compared for different traits, and it was found that there were significant differences between plants grown under sunlight and those grown under LEDs for most of the traits. However, one trait, stomata size (S), did not show significant differences between the two treatments. The significance level for all the significant traits was 99%.

3.1. Interaction among the Genotypes and Treatments

The primary purpose of studying the interaction between genotypes and treatments was to analyze the effects of treatments on the selected traits. The significance observed for all the characters indicates that the traits were significantly influenced by the treatments. Table 1 presents the F-values for genotypes, treatments, and the interaction between genotypes and treatments.

Table 1: Analysis of variance (ANOVA) table for different traits in wheat genotypes

Traits	Significance Level	H	A	C	S	T	Q	W
Genotypes	F-value$^{0.01}$	32.79**	38.66**	28.63**	17.74**	28.87**	18.8**	20.2**
Treatments	F-value$^{0.001}$	2351.04***	1035.5***	1173.5***	0.84$^{(Ns)0.05}$	3420.2***	8041.3***	3211.5***
Genotypes × Treatments	F-value$^{0.01}$	5.8**	6.29**	9.3**	5.1**	7.42**	9.35**	13.94**

P≤0.001=***, P≤0.01=**, P≤0.05=*, P>0.05=non-significant.

3.1.1. Response of all the traits under Tukey pair-wise comparison: The Tukey HSD test was used to evaluate the differences among the genotypes for each trait. For plant height, the genotypes were divided into four groups, with Galaxy-2013 showing significant differences from all other genotypes. The mean values of the flag leaf area indicated that Ujala-2016 had greater variability. Chlorophyll contents were categorized into four groups, with genotypes Anaj-2017, Galaxy-2013, and Ujala-2016 showing significant differences from each other. Spike length was divided into three groups, with significant differences observed between genotypes Galaxy-2013 and Ujala-2016. No significant differences were found among the genotypes for stomata size. Galaxy-2013, LU-26, and Ujala-2016 exhibited differences in 1000-grain weight. Table 2 compares traits among wheat genotypes, and Fig. 1 illustrates the differences in mean values for all the traits.

Table 2: Tukey HSD all-pairwise comparison test for wheat genotypes traits

Genotypes	Galaxy- 2013	LU-26	Akbar-2019	Anaj-2017	Ujala-2016
Mean values (P)	84.26	82.25	81.31	79.01	78.9
Homogenous group	A	B	C	D	D
Mean values (A)	18.1	17.5	17.2	15.7	19.5
Homogenous group	A	AB	B	C	D
Mean values (C)	45.83	45.01	44.63	42.85	40.93
Homogenous group	A	AB	BD	C	D
Mean values (S)	9.01	8.93	8.73	8.50	8.05
Homogenous group	A	AB	AB	BC	C
Mean values (T)	9.26	8.71	8.65	8.61	8.58
Homogenous group	A	A	A	A	A
Mean values (Q)	3.39	3.28	3.23	3.02	2.93
Homogenous group	A	AB	ABC	BC	C
Mean values (W)	25.11	23.85	23.30	23.16	22.30
Homogenous group	A	B	BC	BC	C

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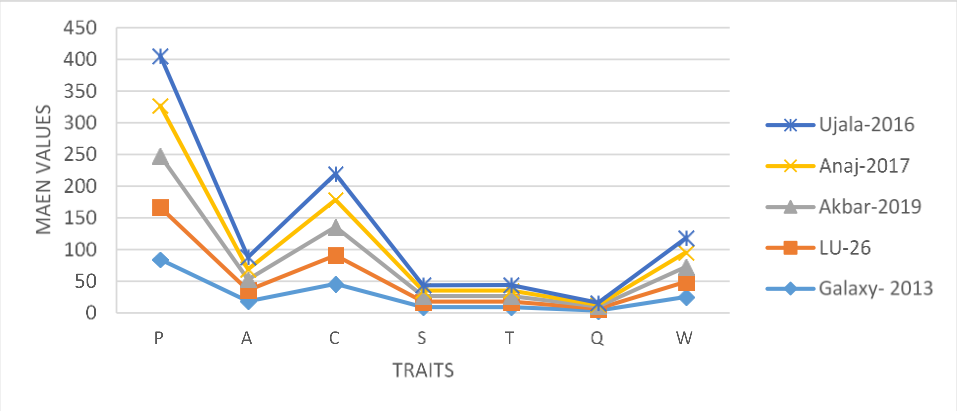

Fig. 1: Mean values variability among the wheat genotypes relating to the traits in Tukey HSD.

3.2 Correlation of the Traits among Normal and Controlled Plants

Correlation analysis among variable photosynthetic traits measures the association between them. The correlation analysis of any two traits determines whether they are genetically linked or not. The data for seven traits were evaluated through correlation analysis under both normal and controlled treatments. The values of all traits are shown in Fig. 2. Under normal conditions, plant height (H) showed a highly significant positive correlation with plant biomass (0.841**), as well as a significant positive correlation with flag leaf area (0.779*) and 1000 grain weight (0.711*). It showed a significant negative correlation with stomata size and a non-significant correlation with spike length and chlorophyll content. Under controlled conditions, plant height revealed a highly significant positive correlation with spike length (0.897**), while showing a significant positive correlation with flag leaf area (0.734*) and plant biomass (0.755*). It revealed a non-significant correlation with chlorophyll content, stomata size, spike length, and 1000-grain weight. All of the correlation values for the traits are shown in Table 3.

Table 3: Correlation table for wheat genotype traits

Traits	H	A	S	C	T	Q
A	0.779*	I				
	0.734*					
S	0.299**	0.710*	I			
	0.897**	0.331				
C	-0.379	0.893**	-0.824**	I		
	-0.055	-0.383	0.303			
T	-0.799*	0.043	0.103*	-0.197	I	
	-0.114	-0.824	-0.897	-0.552		
Q	0.841	-0.095	-0.217	0.367*	0.797*	I
	0.755	0.472	-0.281	0.989**	0.499	
W	0.711*	0.127	-0.495	0.752*	-0.607	-0.189
	0.152	-0.395	0.484	0.989*	-0.591	0.972**

P≤0.001=***, P≤0.01=**, P≤0.05=*, P>0.05=non-significant.
Plant height (H), Flag leaf area (A), Spike length (S), Chlorophyll contents (C), Stomata Size (T), Biomass (Q), 1000 grain weight (W).

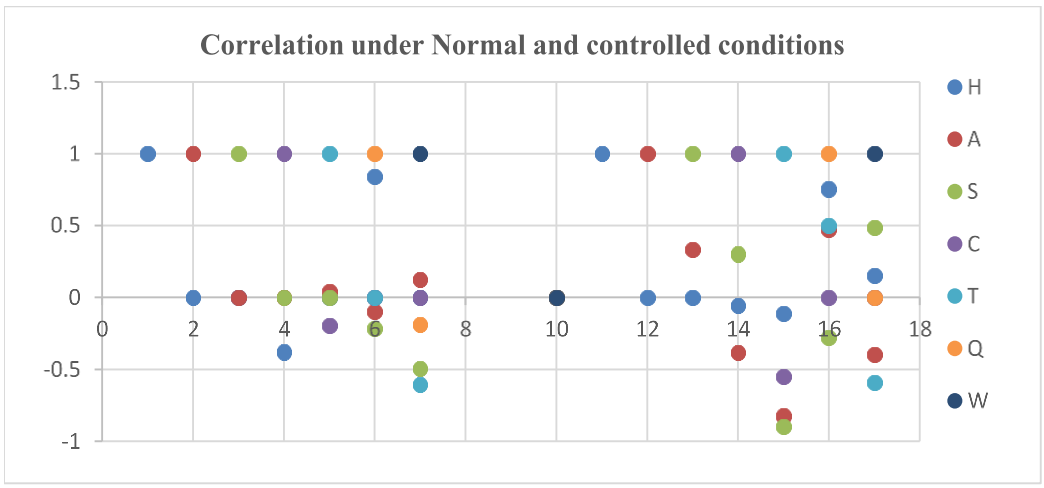

Fig. 2: Correlation analysis of the wheat genotype traits under normal and control conditions

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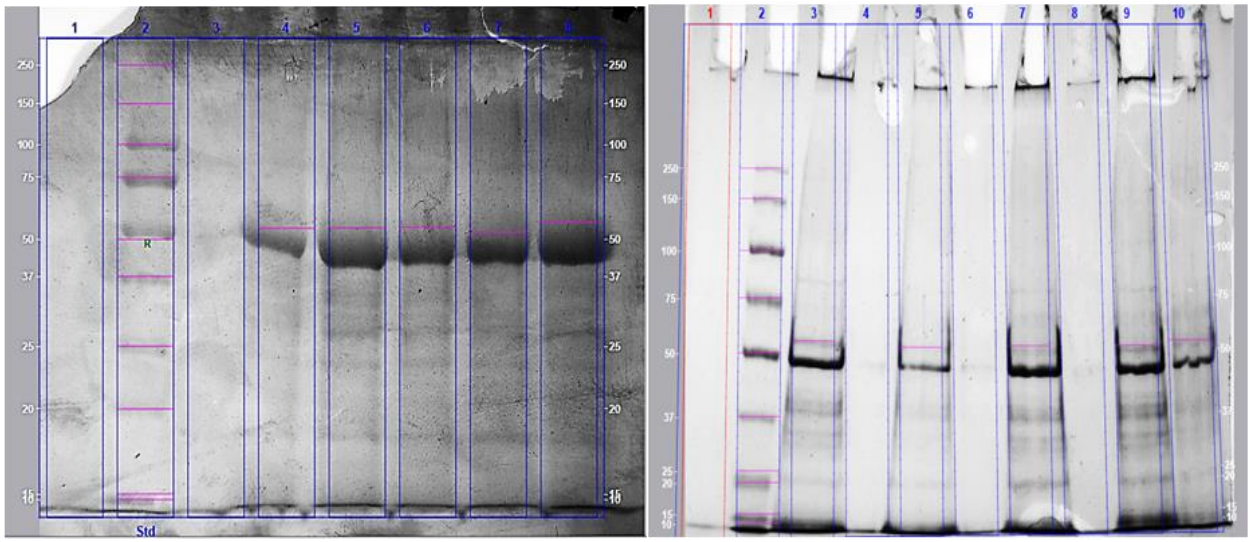
3.3. RuBisCo Protein Expression in Normal Plants

For proteomics analysis, SDS-PAGE gel was used to analyze the protein expression, specifically the RuBisCo protein band, using the Quantity One software by Bio-Rad (Demirevska et al. 2009). The gel analysis of the normal plants showed expression of RuBisCO protein bands for five genotypes within the range of 53 to 55kDa. In the gel, Lane 2 represented the Bio-Rad Precision Plus marker (250-10kDa). Lane 4 represented the genotype Akbar-2019, which exhibited RuBisCO protein expression at 53.7kDa with a total adjusted volume intensity of 34,161. Lane 5 represented the genotype Anaj-2017, which showed RuBisCO protein expression at 53.8kDa with a total adjusted volume intensity of 7,080. Lane 6 represented the genotype Ujala-2016, revealing RuBisCO protein expression at 54.1kDa with a total adjusted volume intensity of 12,319. Lane 7 represented the genotype LU-26, exhibiting RuBisCO protein expression at 52.4kDa with a total adjusted volume intensity of 114,342. Lane 8 represented the genotype Galaxy-2013, showing RuBisCO protein expression at 55.7kDa with a total adjusted volume intensity of 6,726 Fig. 3a.

3.3.1. RuBisCo protein expression in controlled plants: The gel analysis showed expression of RuBisCO protein bands for five genotypes within the range of 53 to 55kDa. In the gel, Lane 2 represented the Bio-Rad Precision Plus marker (250 - 10kDa). Lane 3 represented the genotype Akbar-2019, which exhibited RuBisCO protein expression at 55.3kDa with a total adjusted volume intensity of 384. Lane 5 represented the genotype Anaj-2017, which showed RuBisCO protein expression at 53.5kDa with a total adjusted volume intensity of 576. Lane 6 represented the genotype Ujala-2016, revealing RuBisCO protein expression at 54.7kDa with a total adjusted volume intensity of 2,304. Lane 9 represented the genotype LU-26, exhibiting RuBisCO protein expression at 54.9kDa with a total adjusted volume intensity of 3,072. Lane 10 represented the genotype Galaxy-2013, showing RuBisCO protein expression at 55.1kDa with a total adjusted volume intensity of 960 Fig. 3b.



(3 A) (3 B)

Fig. 3: SDS-PAGE gel shows the expression of normal treatment (3a) and the expression of controlled treatment of wheat (3b).

3.3.2. The difference between the expression of normal and controlled plant proteins: The adjusted volume value represents the expression intensity of protein bands on SDS-PAGE. In this study, five wheat genotypes were analyzed using Quantity One software to assess their expression intensity under both sunlight and speed breeding conditions. The genotype Akbar-2019 (V1) exhibited a volume intensity difference of 19,357 between normal and controlled conditions, as shown in Fig. 4. Similarly, the genotype Anaj-2017 (V2) displayed a volume intensity difference of 9,004 under normal conditions compared to controlled conditions, as depicted in Fig. 5. For the genotype Ujala-2016 (V3), the volume intensity difference between normal and controlled conditions was 10,015, as shown in Fig. 6. The genotype LU-26 (V4) exhibited a volume intensity difference of 22,362 under normal conditions compared to controlled conditions, illustrated in Fig. 7. Lastly, the genotype Galaxy-2013 (V5) displayed a volume intensity difference of 15,585 between normal and controlled conditions, as depicted in Fig. 8.

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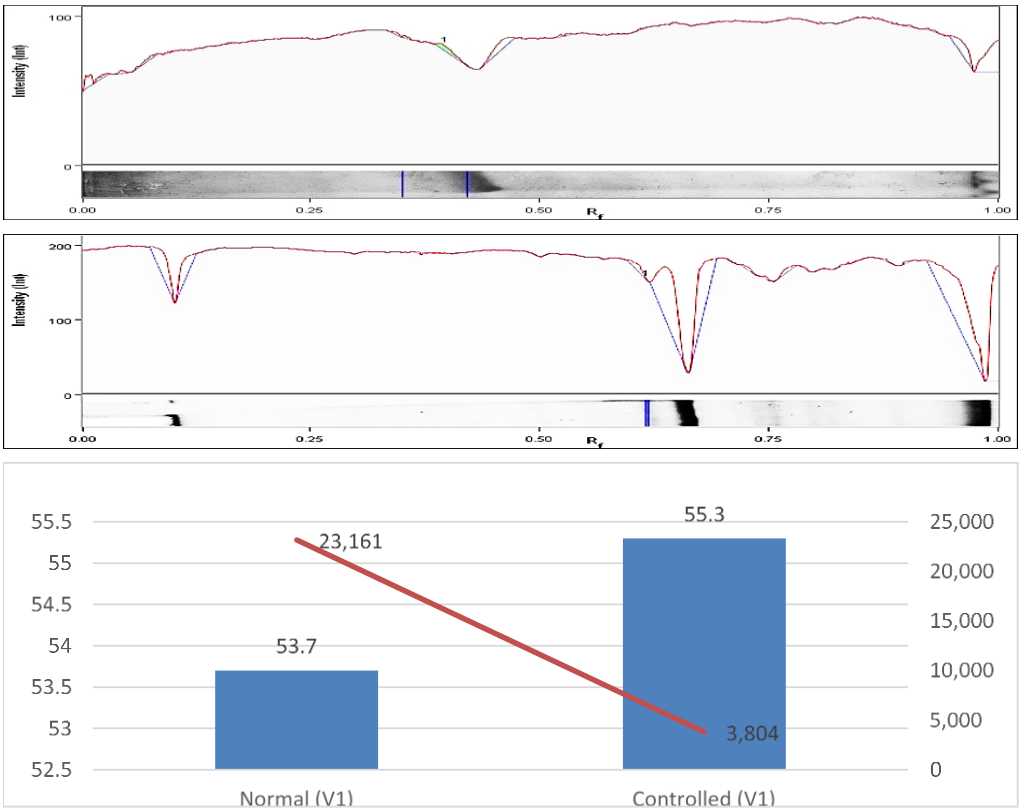

Fig. 4: Expression of the values of genotype V1 (Akbar-2019) under normal and controlled conditions.

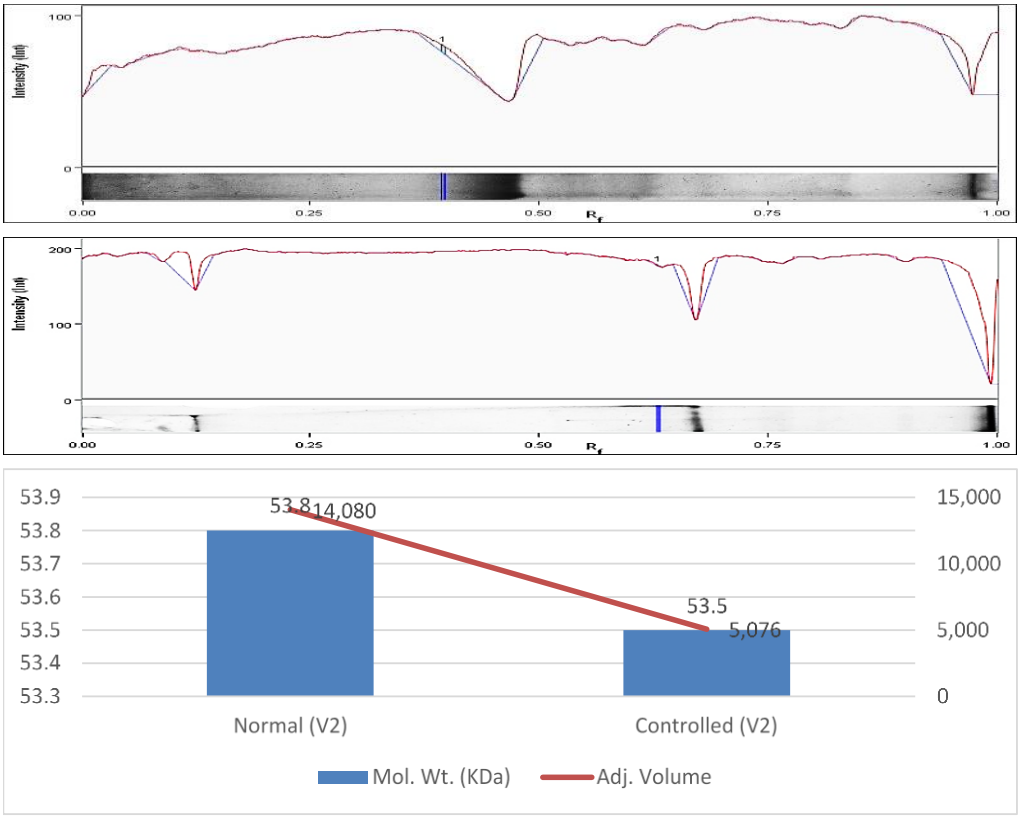

Fig. 5: Expression of the values of genotype V2 (Anaj-2017) under normal and controlled conditions.

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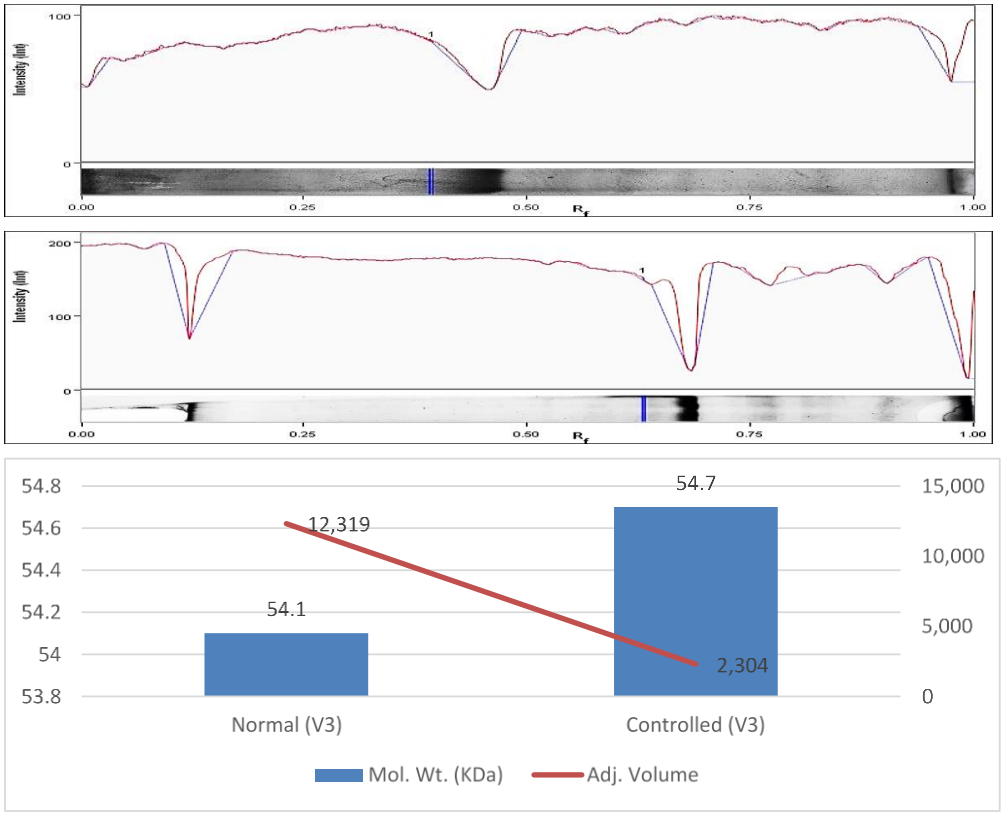

Fig. 6: Expression of the values of genotype V3 (Ujala-2016) under normal and controlled conditions.

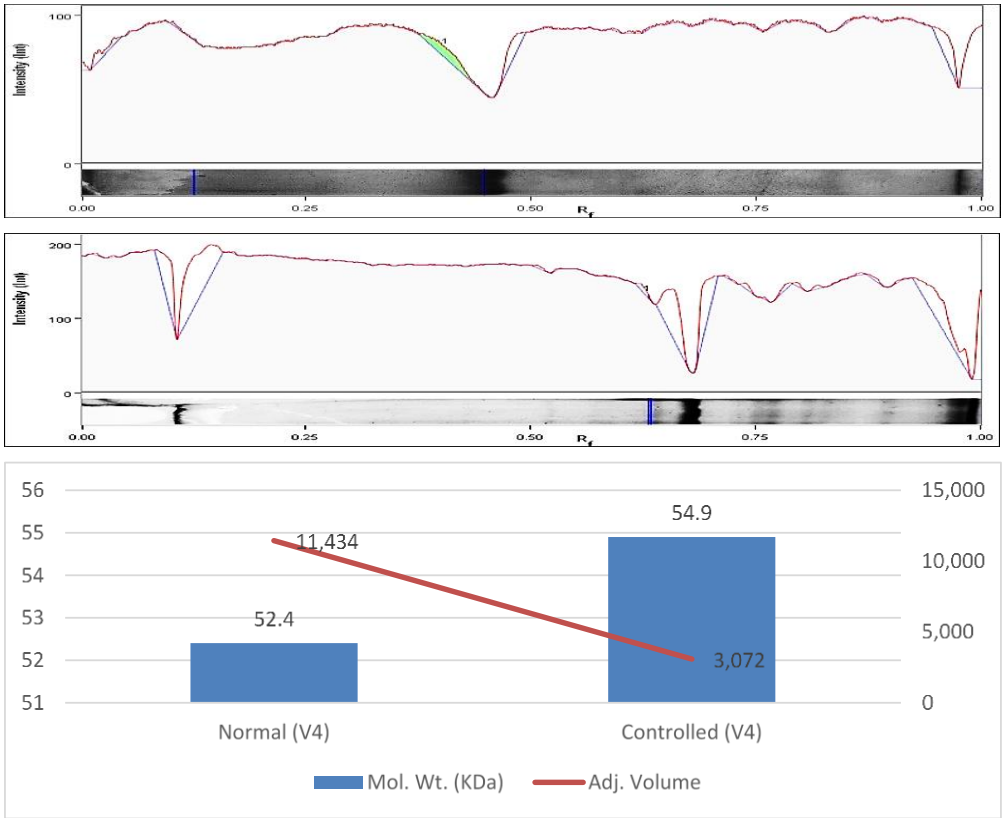

Fig. 7: Expression of the values of genotype V4 (LU-26) under normal and controlled conditions.

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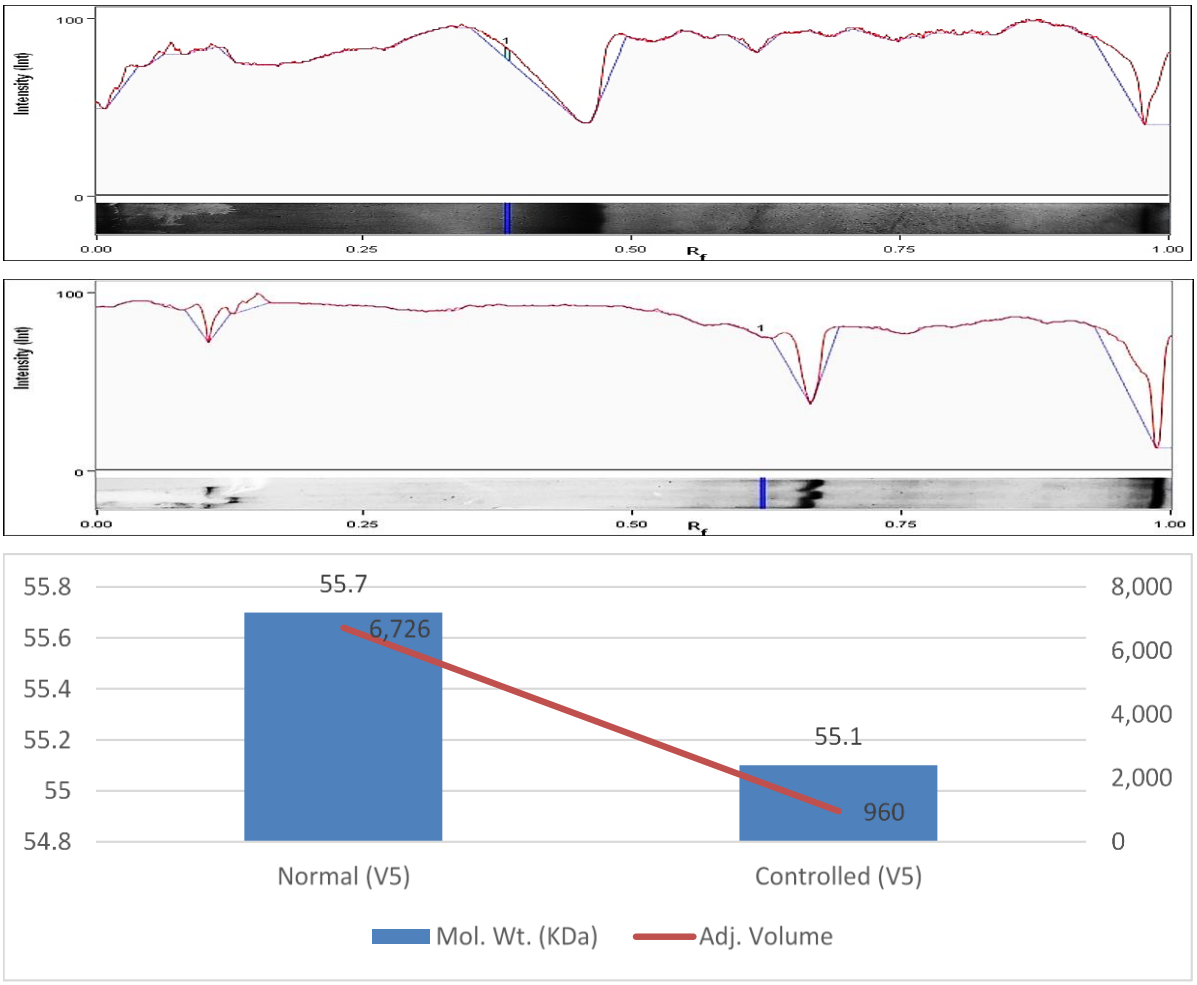

Fig. 8: Expression of the values of genotype V5 (Galaxy-2013) under normal and controlled conditions.

4. DISCUSSION

The characteristics related to the energy synthesis pathway and plant biomass were evaluated using analysis of variance (ANOVA). All the studied traits exhibited differences under normal and speed breeding conditions. Dong et al. (2014) conducted an estimation of plant height under normal conditions and different combinations of LED lights, and significant differences were observed (Dong et al. 2014). In this experiment, the values of plant height exhibited significant fluctuations among all the genotypes under normal and controlled conditions. Flag leaf area played a significant role in enhancing biomass because the size of the flag leaf and photosynthetic activity are positively correlated. Molero and Reynolds (2020) conducted an experiment on wheat and observed changes in the photosynthetic activity of the flag leaf under varying light intensities. The analysis of variance revealed highly significant differences in the size of the flag leaf under sunlight and LEDs (Molero and Reynolds 2020). Ghosh et al. (2018) observed variations in the spike length of wheat when subjected to speed breeding. They noted that the length of the spike was considerably reduced under controlled conditions compared to normal conditions. This reduction in spike length can be attributed to the significant impact of light intensity on the overall height of the plants (Ghosh et al. 2018). Craft et al. (2017) conducted a study investigating the role of chlorophyll content in photosynthetic activity (Croft et al. 2017). Leaf chlorophyll content is influenced by leaf area, stomata size, carbon dioxide availability, and nitrogen levels. Fluctuations in these traits can impact the efficiency of photosynthesis. In our study, we noticed differences in the ANOVA values across all genotypes and between the normal and controlled conditions. However, when it comes to stomata size, it is primarily determined by genetic factors. As a result, we did not find any significant variations in our results in relation to stomata size. Monda et al. (2016) performed a study focusing on the analysis of stomata size and its impact on gaseous exchange in leaves. In their research, they investigated different Arabidopsis ecotypes to assess their carbon dioxide uptake activity. Through their investigation, they found that the Arabidopsis ecotype Me-0 exhibited high gaseous exchange activity, which could be attributed to its larger stomata size compared to other ecotypes (Monda et al. 2016).

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In a study conducted by Yamori et al. (2016), the researchers focused on analyzing biomass variations among different genotypes. Their findings indicated a positive correlation between photosynthesis and biomass at the leaf level. This correlation suggests that enhancing photosynthesis could be a valuable target for improving yield in crop plants (Yamori et al. 2016). Our results demonstrated significant variations among the treatments when examining the variance for biomass and 1000-grain weight. The differences observed in all traits related to biomass indicated fluctuations between the results obtained under normal conditions and those obtained in the speed breeding chamber. For traits that exhibited significant differences in their mean values, we conducted further analysis using a mean comparison test, such as Tukey's Honestly Significant Difference (HSD) test, to determine specific pairwise differences between treatment groups.

The trait data were subjected to correlation analysis to investigate the relationships between the traits under normal and controlled conditions. Plant height showed a significant positive correlation with flag leaf area, spike length, stomata size, and 1000-grain weight at both the genotypic and phenotypic levels. This suggests that taller plants tend to have larger flag leaf areas, longer spikes, larger stomata size, and higher 1000-grain weight. Anwar et al. (2013) conducted correlation studies on the relationship between flag leaf area and other component characteristics. They found a significant positive correlation between flag leaf area and spike length and between flag leaf area and chlorophyll content. Additionally, spike length exhibited a strong negative correlation with chlorophyll content and a significant positive correlation with stomata size at the phenotypic level (Anwar et al. 2013). In a study conducted by Masood et al. (2014), similar findings were reported, supporting our observations (Masood et al. 2014). The researchers found that chlorophyll content significantly impacts enhancing plant biomass. They also identified a highly significant correlation between chlorophyll content and both plant biomass and 1000-grain weight, indicating the importance of chlorophyll in determining these traits under both normal and controlled conditions. Furthermore, the study revealed a significant positive correlation between stomata size and plant total biomass, suggesting that stomata size and biomass production are associated with each other. Additionally, plant biomass exhibited a highly significant correlation with 1000 grain weight, indicating that biomass accumulation plays a crucial role in determining grain yield. These findings further support the importance of these traits in crop productivity.

Feng et al. (2019) conducted a study to investigate the impact of light intensity on the plant's photosynthetic pathway, specifically focusing on the activity of RuBisCO. They designed five treatments with varying light intensities ranging from 100 to 500 µmol/s. The researchers observed that the activity of RuBisCO was significantly influenced by the changing intensity levels of light (Feng et al. 2019). In the experiment, differences in light intensity were observed between the normal and controlled treatments. Yamori et al. (2010) conducted a study to investigate the impact of light intensity on the photosynthesis pathway, with a specific focus on the activity of RuBisCO in the Calvin cycle. They utilized lights of different colors to examine the effects on RuBisCO. The researchers found that high light intensity, when combined with optimal temperature conditions, improved RuBisCO expression and carbon dioxide assimilation performance. The results indicated that the interplay between light intensity and temperature is crucial for enhancing the efficiency of RuBisCO and facilitating the process of carbon fixation in the Calvin cycle (Yamori et al. 2010).

In our research, we put forward the hypothesis that the biomass of plants is closely linked to the energy synthesis activity occurring in the Calvin cycle. Photosynthetic processes significantly influence this activity, with light intensity playing a particularly important role. Consequently, the light intensity pattern for each crop may undergo alterations when subjected to speed breeding conditions. By acknowledging the significance of photosynthetic activity and its relationship to biomass production, we anticipate that the manipulation of light intensity during speed breeding techniques may substantially impact the growth and development of plants. The alterations in light intensity patterns under speed breeding conditions are expected to influence the overall energy synthesis activity in the Calvin cycle and subsequently impact plant biomass.

5. CONCLUSION

Plant breeders face significant challenges in achieving desirable quantities and qualities of wheat, particularly due to climate-related obstacles. Speed breeding has emerged as a valuable technique to address these challenges, enabling breeders to develop new wheat varieties within a shorter timeframe. However, one major hurdle in speed breeding is the reduced biomass of plants, which leads to decreased crossing efficiency and a lower seed yield per spike. To gain insights into the factors contributing to reduced biomass, our research employed a combination of methods, including analysis of variance, correlation analysis of morphological traits, and proteomics analysis. Through these analyses, we discovered that high light intensity, coupled with optimized temperature conditions, plays a crucial role in enhancing the expression of RuBisCO and facilitating carbon dioxide assimilation. From our findings, we concluded that the biomass of plants is intricately linked to the energy synthesis activity in the Calvin cycle and the photosynthetic process, with light intensity being a primary influencer. Understanding these relationships is crucial for addressing the biomass reduction issue in speed breeding and improving the overall

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success and efficiency of the technique. As a result, the light intensity patterns for each crop need to be adjusted and optimized under speed breeding conditions. It is important to acknowledge that the light intensity parameter should not be disregarded in future studies to improve biomass. By considering and optimizing light intensity, we can further enhance biomass production and address the challenges faced by plant breeders.

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