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Research Article

Evaluation of biochemical changes in wheat varieties as influenced by terminal heat stress under varying environments

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Abstract

Climate is considered as the major uncontrollable factor in crop production. Alternation in the climate worldwide is predicted to have critical impact on crop production. High temperature negatively affects growth process of wheat by upsetting various physiological and biochemical processes and thereby affecting the nutritional quality of the crop. Heat stress during reproductive growth stages of wheat is detrimental to chloroplast activity, reducing activity of source organs and thereby reducing its sink capacity. Reduction in starch content is the major reason leading to yield loss during heat stress and this is because of accumulation of starch is associated with activities of various enzymes like sucrose synthase, soluble starch synthase, etc which are highly heat labile. Metabolic pathways are most altered during heat stress followed by secondary metabolite biosynthesis pathways. A field experiment was conducted during the Rabi season of 2020-21 at Student's Instructional Farm of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh to evaluate the biochemical changes in wheat varieties as influenced by terminal heat stress. The treatment for the experiment consisted of sowing on three different dates i.e. D_1 (30th November), D_2 (15th December), D_3 (30th December). It was observed that delayed sowing decreased substantially total chlorophyll content, total soluble sugar content in leaves of V_1 (PBW-343) and V_2 (HD-2967). This reduction was caused due to onset of high temperature during reproductive stage of wheat crop. Variety V_3 (Halna) reduced the detrimental effect of heat stress and thus showed greater total chlorophyll content, total soluble sugar content. Proline content in leaves increased with age of crop up to 75 DAS in all the sowing dates this varying dates of sowing because proline is thought to play an adaptive role in mediating osmotic adjustment and protecting sub cellular structure in stressed plants.

Keywords: wheat, heat stress, total chlorophyll content, total soluble sugar, proline content.

1. Introduction

Wheat is regarded as the 'King of cereals' because of its cultivation on large area and its potential to give high productivity and its prominent position in international food grain trade. It contributes about 30% of world food grain production and 50% of the world grain trade (Akter *et al*., 2017). Wheat is regarded as staple food in more than 40 countries of the world and provides basic calories for 85% and protein for 82% of the world population (Sharma *et al*., 2019; Chaves *et al*., 2013). It is an annual, long day and self-pollinated plant. The major cultivated varieties of wheat belong genus *Triticum* i.e. the hexaploid, *Triticum aestivum* L. (bread wheat), and the tetraploid, *T. durum*, *T. dicoccum* and *T. monococcum* (Kimber *et al*., 1987). Wheat has a very good nutritional status with 12.1% protein, 1.8% lipids, 1.8% ash, 2.0% reducing sugars, 6.7% pentosans, 59.2% starch, 70% total carbohydrates and it also provides 314 K cal/100 g of food. It also serves as a fairly good source of minerals and vitamins i.e. calcium (37 mg/100 g), iron (4.1 mg/100 g), thiamine (0.45 mg/100 g), riboflavin (0.13 mg/100 g) and nicotinic acid (5.4 mg/100 g) (Lorenz *et al*., 1991).According to FAO's estimate the annual cereal production has to be increased by almost one billion to feed the projected population of 9.1 billion by 2050. In order to fulfill these increased food demands, increase in crop production and productivity is the need of the hour (Iqbal *et al*., 2017).

Climate is the major factor that influences crop yield and productivity (Godden, 1998). Climate change refers to "change in climate due to natural or anthropogenic activities and this change remain for a long period of time" (Solomon *et al*., 2007). Adverse climatic conditions significantly reduce crop production (Rahaie *et al*., 2013). Wheat is primarily grown in tropical and sub-tropical areas of the world which experiences various abiotic stresses throughout the growing season. The major abiotic stresses include heat, drought, salinity, cold, chemicals and excess water. Heat and drought however are the main abiotic stresses affecting the wheat production worldwide (Lesk *et al*., 2016; Leu *et al*., 2016). Wheat crop production is highly sensitive to heat stress. According to the global climate model, the mean ambient temperature is likely to rise by 6°C by the end of 21st century (De Costa WAJM, 2011). It has been estimated that even a slight increase of 1°C in temperature can lead to a decrease of 6% in global wheat production (Asseng *et al*., 2011). High temperature affects the various physiological, biological and biochemical process in wheat (Asseng *et al*., 2015). Heat stress in wheat cause poor seed germination, decrease in duration of grain filling, reduction in grain number, deactivation of Rubisco enzyme, decrease in photosynthetic capacity, reduction in rate of assimilate translocation, premature leaf senescence, decrease chlorophyll content and ultimately decrease in yield (Hossain *et al*., 2013; Din *et al*., 2010). Starch and protein content in grain is also severely affected by heat stress.Heat stress causes photo-inhibition and photo-destruction of pigments and related protein complexes, and also disruption of photosynthetic membrane (Hussain *et al*., 2018; Kumari *et al*., 2018). Decreased chlorophyll content under heat stress is attributed to destroying of thylakoid membranes (Zhang *et al*., 2006; Prasad *et al*., 2007). Therefore, minimum build-up of chlorophyll contents in plants might be due to increased degradation or decreased biosynthesis of chlorophyll contents or integrated effect of both under heat stress. Plants affected by heat stress also showed an increase of compatible solute such as soluble sugars and proline (Rivero *et al*., 2014) as they help to stabilize membranes, sub-cellular structures and cellular redox potential by destroying the free radicals (Kishor *et al*., 2005; Demir *et al*., 2002). Thus considering the detrimental effect of high temperature on the various biochemical attributes of wheat crop it is important that sowing of wheat crop be don at the optimum timing to avoid heat encountering heat stress and also development of varieties which can withstand this effect without the yield being compromised. The present study was conducted keeping in view the effect of elevated temperature on various biochemical attributes of prominent varieties of wheat.

2. Materials and Methods

The current field study was conducted during the Rabi season of 2020-21at Student's Instructional Farm of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh. Geographically the experimental site is situated 42km away from Ayodhya between latitude of 26.47 North and longitude of 81.12 East at an elevation of 113 meters in the gangetic alluvium of eastern Uttar Pradesh. Climate of Ayodhya district falls under semi-arid zone, receiving a mean annual rainfall of 1001.7 mm, of which about 80% occurs during monsoon season (November to April) with few showers in winter. Meteorological data i.e. temperature, rainfall, relative humidity and sunshine hours related to experimental site were all collected from meteorological observatory situated at Kumarganj, the main campus of University. The experiment was conducted using three varieties V_1 (PBW-343), V_2 (HD-2967) and V_3 (Halna) in field containing silt loam soil. PBW-343 and HD-2967 are popular timely sown high yielding wheat varieties in the North Eastern Plain Zone

(NEPZ) and they are susceptible to temperature stress but Halna is a popular high yielding variety for late sown conditions, which is tolerant to high temperature stress. The whole experiment was planned under split plot design with three replications along with three treatments. The treatment given were namely; D_1 (30th November), D_2 (15th December), D_3 (30th December). The three dates were selected based on the optimum dates recommended for sowing of timely sown and late sown varieties of wheat due to rise in temperature in later developmental stages heat stress may be experienced by the varieties. In case of timely sown varieties, the optimum time of sowing is mid-November and for late sown varieties, it is first fortnight of December. A total of 27 plots were taken under observation during the study with plot size of 5m*4m and spacing of 20cm*10cm. A total of five plants were taken from each plot as sample for recording the observations. All the biochemical studies were done on leaves at three different stages of crop growth i.e. 60, 75 and 90 days after sowing (DAS). The total chlorophyll content was estimated by following the method of Arnon (1949) and expressed as mg g^{-1} fresh weight.Total soluble sugar was determined according to the method described by Yemm and Wills (1954) and expressed as mg g^{-1} fresh weight. Free proline content in leaves was estimated spectrophotometrically according to the method described by Bates *et al.* (1973). The data recorded on various biochemical parameters was subjected to statistical analysis by Fisher method of analysis of variance. Significance of various treatments was judged by comparing calculated, F value with Fisher's F value at 5 percent level.

3. Results & Discussion

The meteorological data on weather conditions prevailing during Rabi season of year 2020-21 has been illustrated in Figure1.

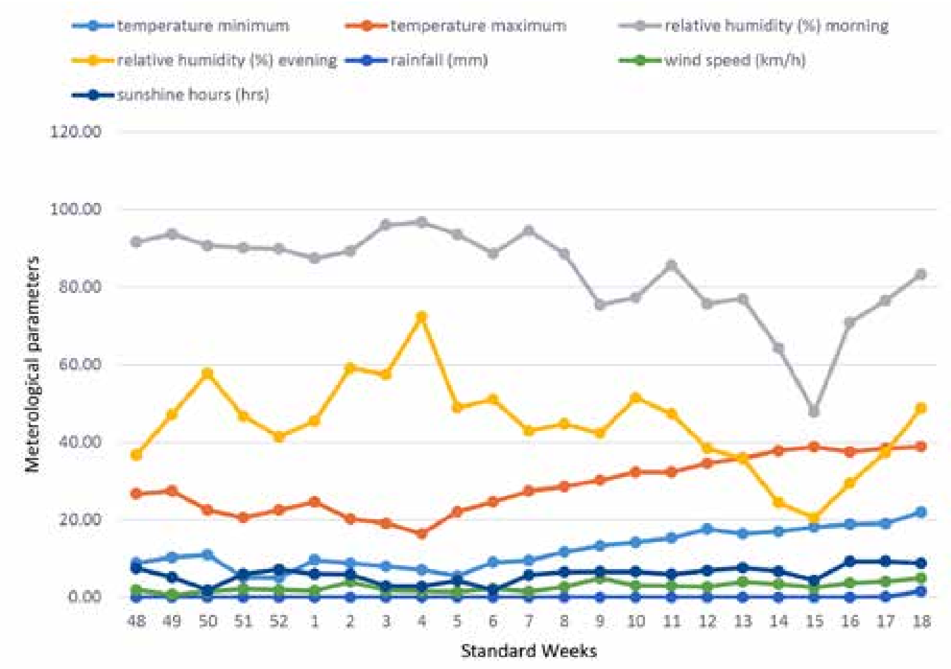

Figure 1: Meteorological data during the crop season 2020-21

Total chlorophyll content

The data related to total chlorophyll content at various crop growth stages as influenced by time of sowing is represented in Table 1. The chlorophyll progressively increased with plant age up to 75 DAS after which a decrease in chlorophyll content was observed at 90 DAS in all varieties. At D_1 and D_2 the total chlorophyll content showed a maximum value for V_2, while for D_3 a maximum value was obtained for V_3 at 60, 75 and 90 DAS. Minimum chlorophyll content was recorded for V_3 at D_1 and D_2. It is clear from the data that the three dates of sowing D_1, D_2, D_3 significantly affected the total chlorophyll content of all the varieties i.e. V_1, V_2, V_3.

Table 1: Effect of different time of sowing on total chlorophyll content (mg/g fresh weight) recorded at 60, 75, 90 Days After Sowing (DAS) on different wheat varieties

Treatments	60 DAS			75 DAS			90 DAS		
	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
D_1	3.46	4.12	2.96	3.54	4.18	3.06	1.61	2.23	2.03
D_2	3.42	4.06	3.08	3.52	4.14	3.18	1.58	2.17	2.14
D_3	3.1	3.20	3.38	3.16	3.30	3.46	2.17	2.23	2.38
	S.Em±	CD at 5%		S.Em±	CD at 5%		S.Em±	CD at 5%	
Variety	0.14	0.44		0.15	0.45		0.14	0.43	
Treatment	0.13	0.52		0.12	0.48		0.12	0.49	
V*T	0.25	0.77		0.25	0.78		0.24	0.74	

Note: the values were analyzed with analysis of variance (ANOVA); S.Em± represents standard error of mean; CD represent the critical difference value to test the level of significance between means (P>0.05).

Total soluble sugar

Data recorded at different crop growth stages for total soluble sugar content in leaves is presented in Table 2. It showed that total soluble sugar content was affected due to time of sowing at different stages of observation. The perusal of data indicated that total soluble sugar content was increased with increase in plant age. At D_1 and D_2 maximum value for total soluble sugar content was observed for V_2 while at D_3 the maximum value was obtained for V_3 at 60,75 and 90 DAS. Minimum total soluble sugar content was obtained in V_3 at D_1 and D_2. It is evident from the data that late variety V_3 showed reduced total soluble sugar content in D_1 and D_2 and increased value in D_3 at all stages of observation.

Table 2: Effect of different time of sowing on total soluble sugar content (mg/g fresh weight) recorded at 60, 75, 90 Days After Sowing (DAS) on different wheat varieties

Treatments	60 DAS			75 DAS			90 DAS		
	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
D_1	63.12	64.15	56.52	83.17	84.23	76.56	91.60	92.20	84.10
D_2	62.99	63.13	57.11	82.99	83.17	77.19	91.10	91.90	85.50
D_3	60.96	61.63	62.33	79.01	79.69	82.35	84.80	85.80	91.80
	S.Em±	CD at 5%		S.Em±	CD at 5%		S.Em±	CD at 5%	
Variety	0.59	1.81		0.58	1.79		0.81	2.50	
Treatment	0.24	0.96		0.23	0.91		0.60	2.37	
V*T	1.02	3.13		1.01	3.10		1.40	4.33	

Note: the values were analyzed with analysis of variance (ANOVA); S.Em± represents standard error of mean; CD represent the critical difference value to test the level of significance between means (P>0.05)

Proline content

The data pertaining to proline content is represented in Table 3. Critical analysis of the data revealed that proline content was influenced by different time of sowing at different crop growth stages. A perusal of data showed that proline content progressively increased with the increase of plant age in all the treatments. At D_1 and D_2 highest proline content was obtained in V_2 while at D_3 it was obtained at par in V_2 and V_3 at 60, 75 and 90 DAS. Minimum proline content was obtained in V_3 at D_1 and D_2. Data pertaining to proline content indicated that delayed sowing of early varietiesV_1 and V_2 significantly increased proline content accumulation inside the plant as it is believed to provide heat tolerance to susceptible varieties.

Table 3: Effect of different time of sowing on proline content (mg/g fresh weight) recorded at 60, 75, 90 Days After Sowing (DAS) on different wheat varieties

Treatments	60 DAS			75 DAS			90 DAS		
	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
D_1	204.9	206.1	212.6	224.8	226.8	226.6	246.0	245.0	238.0
D_2	210.3	211.2	210.1	226.8	228.9	227.1	118.0	116.0	117.0
D_3	210.5	210.8	212.6	255.7	257.3	257.1	101.0	113.0	111.0
	S.Em±	CD at 5%		S.Em±	CD at 5%		S.Em±	CD at 5%	
Variety	0.99	3.04		0.11	0.35		1.32	4.05	
Treatment	1.21	4.74		0.15	0.57		1.32	5.18	
V*T	1.71	5.26		0.20	0.61		2.28	7.02	

Note: the values were analyzed with analysis of variance (ANOVA); S.Em± represents standard error of mean; CD represent the critical difference value to test the level of significance between means (P>0.5)

Continual heat stress (mean daily temperature of over 17.5° C in the coolest month of the season) affects approximately 7 million hectares of wheat in developing countries, while terminal heat stress is a problem in 40% of temperate environments, which cover 36 million hectares (Reynolds *et al.* 2010). For a healthy and good wheat crop production the range of the optimum temperature is around 18 to 24°C. A temperature rise above 28 to 32°C for short periods i.e. 5 to 6 days was shown to cause about 20% or more wheat yield losses (Stone and Nicolas 1994). The loss of yield is might be due to heat stress causes an array of physiological, biochemical and morphological changes in wheat which reduces the plant's photosynthetic capacity through metabolic limitation and oxidative damage to chloroplasts with concomitant reduction in dry matter accumulation and yield (Farooq *et al.* 2011). This ultimately reduces tillering capacity, shortens grain filling period and accelerates crop senescence (Elbashier *et al.* 2012).

In the present study it was observed that total chlorophyll content increased up to 75 DAS and then decreased at 90 DAS for all the treatments and for all the varieties. The effect of temperature on total chlorophyll content of V_1, V_2, V_3 for various treatments at various growing stages is depicted in Figure 2.

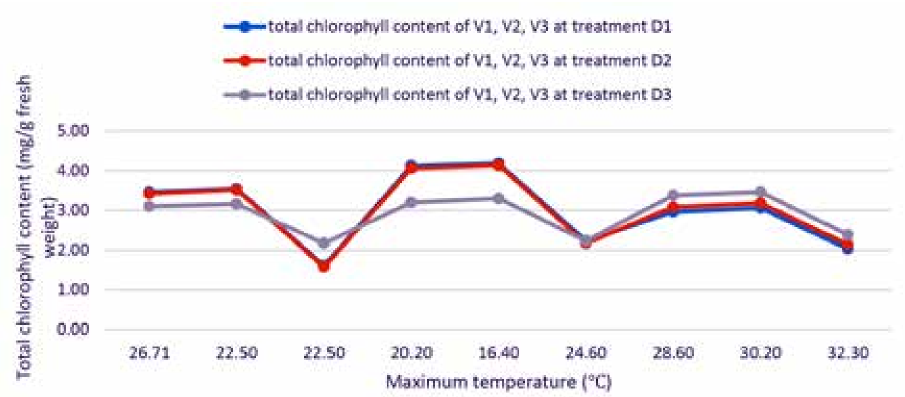

Figure 2: Effect of temperature on total chlorophyll content of V_1, V_2, V_3 for various treatments at various growing stages

It was further observed that V_2 had maximum chlorophyll content for D_1 and D_2 but a decrease in total chlorophyll content was observed for V_1 and V_2 as these varieties are susceptible to high temperature experienced by them due to delayed sowing. High temperature causes reduction in chlorophyll content due to damage caused to thylakoids. However, in case of V_3 since it is temperature stress resistant variety it showed a significant increase in the total chlorophyll content for D_3. Similar findings were reported by Almeselmani *et al.* (2006), they also reported a significant reduction in chlorophyll content with age of crop and under late sown condition in wheat crop.

Critical examination of data for total soluble sugar content revealed that changes in sowing time from optimum exposed the varieties to high temperature stress. The effect of temperature on total soluble sugar of V_1, V_2, V_3 for various treatments at various growing stages is depicted in Figure 3.

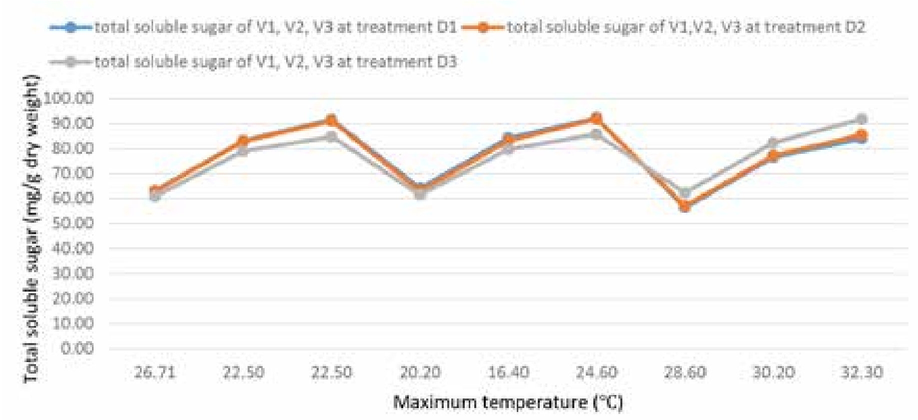

Figure 3: Effect of temperature on total soluble sugar of V_1, V_2, V_3 for various treatments at various growing stages

It is evident from the data that late variety V_3 showed reduction in total soluble sugar content in D_1 and D_2 and an increased value in D_3 at all stages of observations. On the contrary late sown condition i.e. D_3 caused a significant reduction in the total soluble sugar of V_1 and V_2 in comparison to when they are sown early. This is because of high temperature stress imposed at the early growth stages significantly reduces soluble sugar accumulation in wheat. Similar findings were also reported by Wang *et al* (2016) and Sumesh *et al* (2008).

It is also evident from the study that proline content of all the varieties increased up to 75 DAS after that it showed a decline in the value for 90 DAS. Proline is thought to play adaptive role in osmotic adjustment and protecting subcellular structure in stressed plants (Foolad*et al.,* 2007). Data pertaining to proline content indicated that delayed sowing of early varieties V_1 and V_2 significantly increased the proline content when compared to variety V_3 thereby indicating that the varieties are facing heat stress as more proline in accumulated in susceptible varieties to mitigate adverse effects of heat stress than in the tolerant ones. The maximum proline content was observed under late sown condition in comparison to early sown condition this is might be due to late sowing exposes the plants to higher temperature range than optimal required for its proper functioning. These results were at par with the findings of Ahmed and Hasan (2011) who mentioned that the increment of proline in different wheat genotypes were different and higher in heat sensitive genotypes. In this situation of heat stress, plant accumulates proline that helps to stabilize membranes, sub-cellular structures and cellular redox potential by destroying the free radicals(Hussain *et al.*, 2018; Loutfy *et al.*, 2012).The effect of temperature on proline content of V_1, V_2, V_3 for various treatments at various growing stages is depicted in Figure 4.

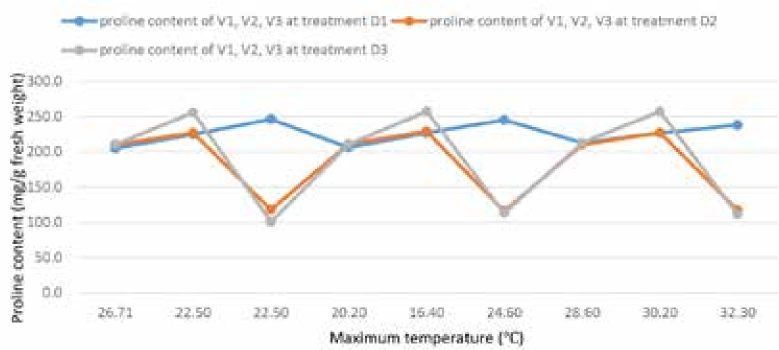

Figure 4: Effect of temperature on proline content of V_1, V_2, V_3 for various treatments at various growing stages

The present investigation makes it explicit that biochemical attributes like total chlorophyll content, total soluble sugar content and proline content for V_1, V_2, and V_3 was found maximum in D_1, D_1 and D_3 respectively at 60, 75 and 90 DAS. Significant reduction in chlorophyll content with age and also under delayed sowing was observed. Reduction in total soluble sugar content may be due to onset of high temperature which deteriorates photosynthetic activity and crop proceeds towards senescence or forced maturity. V_1 and V_2 being heat stress susceptible varieties when grown under delayed sowing condition showed a significant decline in all the biochemical attributes. However, V_3 being heat tolerant is not affected by delayed sowing and thereby overcomes the high temperature stress easily. Based on the analysis from the current study it can be suggested that optimum time for sowing of V_1 and V_2 is around mid-November, later than this will affect the biochemical processes and ultimately cause a reduction in the yield due to the crop facing heat stress. In areas facing high temperature stress frequently V_3 can be recommended for cultivation as it can be sown till 30th December and it withstands high temperature stress without the biochemical processes being affected.

Author contributions

Conceptualization of research (LK, AKS & AKS); Designing of the experiments (LK, SS & NPV); Contribution of experimental materials (LK, AKS & DT); Execution of field/lab experiments and data collection (LK, SS, RKY & SRM); Analysis of data and interpretation (AKS & SP); Preparation of the manuscript (LK & AKS).

Conflict of interest: No

Declaration

The authors declare no conflict of interest.

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