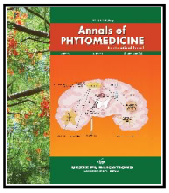
Annals of Phytomedicine 12(1): 856-864, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.1.87

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Evaluation of physiological, enzymatic, biochemical, nutritional parameters and stress tests in wheat genotypes

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Article Info

Article history
Received 12 May 2023
Revised 18 June 2023
Accepted 19 June 2023
Published Online 30 June-2023

Keywords
Physiological
Biochemical
Enzymatic
Nutritional
Wheat

Abstract

Climatic changes are found to have deleterious effects on crop growth and development. Global warming is causing frequent rise in ambient temperature rapidly for last two decades. The increased temperature during grain formation and filling phase of crop growth has emerged as a serious problem all over the world. The grain-filling rate of wheat, like other cereals, depends largely on leaf photosynthesis and water soluble carbohydrates (WSCs) transported to the grain from leaves, stem and ear reserves. The present study entitled evaluation of physiological, enzymatic, biochemical, nutritional parameters and stress tests in wheat genotypes was carried out during rabi 2018. Geographically, Rahuri is situated at 19034 N latitude and 74064 E longitudes with an altitude of 536 meters above mean sea level. Two genotypes with two checks were grown in field conditions with varying sowing dates, *viz*., 21st November 2017 (D1), 21st December 2017 (D2) and 21st January 2018 (D3) and physiological, enzymatic, biochemical and nutritional parameters analysis were conducted timely. Results clearly revealed that all the characters recorded and analysed found to be affected by elevated temperature. Genotypes, viz., NIAW-3033 and NIAW-1994 (Phule Samadhan) were found to be more efficient for withstanding this stressful elevated temperature condition.

1. Introduction

Across the globe, temperature is increasing continuously at the rate of 0.15-0.17°C per decade since the industrial revolution. It is influencing agricultural crop productivity. Though, heat stress affects the metabolic pathways at every stage of life of wheat leading to yield reduction, the effect of high temperature is particularly severe during grain filling; these losses may be up to 40% under severe stress (Hays *et al*., 2007). Other effects of high temperatures are decreased grain weight, early senescence, shriveled grains, reduced starch accumulation, altered starch-lipid composition in grains, lower seed germination and loss of vigour (Balla *et al*., 2012). In late sown wheat, terminal heat stress is the main cause of yield reduction which is responsible for shortening of grain growth period and improper grain filling (Reynolds *et al*., 2001; Rane *et al.,* 2007). Every 1°C rise in temperature above 28°C during grain filling, results in yield reduction by 3-4% (Reynolds *et al*., 1994). Heat stress during the postanthesis grain-filling stage affects availability and translocation of photosynthates to the developing kernel, and starch synthesis and deposition within the kernel, thus resulting in lower grain weight and altered grain quality.

The most significant factors for heat stress-related yield loss in cereals include the high-temperature-induced shortening of developmental phases, reduced light perception over the shortened life cycle and perturbation of the processes associated with carbon assimilation (transpiration, photosynthesis and respiration). Increased respiration requires greater carbon fixation for sustained growth and survival. The damage caused by high temperature is greatly dependant on the developmental stage of the plants when subjected to the stress. Heat stress at the beginning of flowering or during spikelet development reduces the number of potential grains. Heat stress during grain filling, on the other hand, influences the translocation of photoassimilates, starch synthesis, and the accumulation of starch in the grains, thus causing changes in grain quality and weight. High temperature was also reported to cause reductions in the weight of individual grains. The weight of mature grains was most sensitive to heat stress when this occurred early in grain filling, becoming progressively less sensitive in later stages.

2. Materials and Methods

2.1 Experimental design

The research was conducted at Post Graduate Institute Farm, Mahatma Phule Krishi Vidyapeeth, Rahuri, District Ahmednagar (MH). The tested crop was two genotypes of wheat along with one heat tolerant check and one heat susceptible check, *viz*., NIAW-3033, NIAW- 3161, NIAW-1994 (Tolerant check) and MACS-6222 (Susceptible check), respectively. The soil was with good condition with 7.6 pH. The crop was sown at 22.5 cm spacing between two rows and total land used was 4R. Experimental design used was split plot design. Seed material was obtained from Agriculture Research Station, Niphad, District Nashik (MH). The experiment was conducted with staggered sowing dates as 21st November 2017, 21st December 2017 and 21st January 2018.

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2.2 Meterological data

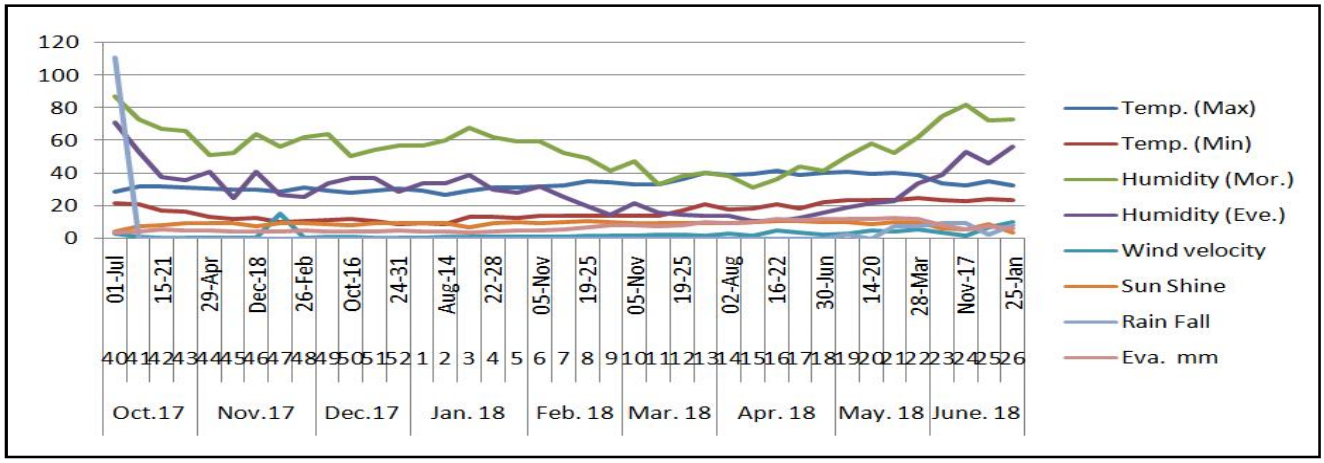
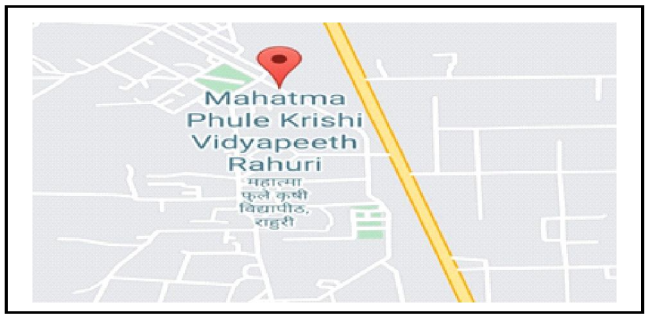


Figure: Temperature and other environmental variations during wheat growth cycle.

2.3 Experimental field location



2.4 Methods used

2.4.1 Leaf area and lag leaf area (dm^2)

Leaf area and flag leaf area were measured at the time of flowering. To determine the area of photosynthetic leaves, five plants were randomly selected and uprooted from each net plot and their green leaves were separated from the stem and leaf area and flag leaf area measured by scanning the leaves on automatic leaf area meter. The mean values of leaf area and flag leaf area (dm^2) $plant^{-1}$ were calculated.

2.4.2 Enzymatic activities

2.4.2.1 Sucrose synthase (µmole sucrose synthesized g^{-1} wt. of sample)

The activity of sucrose synthase assayed (SuSy) as per the modified method of Hawker (1967).

I. Extraction of enzyme

The grains from all the genotypes brought and cleaned separately and crushed, homogenized and extracted in a minimum volume of extraction buffer containing 0.1 M Tris-HCl buffer (pH, 7.6) containing 0.3 M mannitol, 0.01 M $MgCl_2$, 0.02 M EDTA, 0.02 M cystein-HCl, 0.02 M sodium diethyl-dithiocarbamate (DIECA) and 1% Triton-X100 and the homogenate was filtered through two layers of cheese cloth and centrifuged at 15,000 rpm for 10 min. To the known volume of supernatant ammonium sulphate and different fraction 0-30%, 30-60% and 60-80% were tested for activity of sucrose synthase (SuSy). The fraction 30-60% saturation centrifuged at 20,000 x g for 15 min. The supernatant was discarded and the precipitate was dissolved in minimum volume of extraction buffer and used for assay of sucrose synthase.

II. Enzyme Assay

The reaction mixture for sucrose synthase contained 125 µl 0.015 M UDPG, 125 µl 0.05 M fructose, 700 µl 0.2 M Tris-HCl buffer (pH 8.2) containing 0.025 M $MgSO_4$ and 50 µl of enzyme preparation in total volume of 1.0 ml.

III. Estimation of sucrose

Sucrose was determined as per the method of Roe (1934) with some modifications.

Reagents

i. Recorcinol solution: 0.1% (w/v) recorcinol in glacial acetic acid containing 0.25 g thiourea.

ii. 6% KOH solution: 6 g KOH in 100 ml distilled water.

iii. 75% (v/v) H_2SO_4

Procedure

The reaction mixture of sucrose synthase was incubated at 37°C for 30 min and subsequently the tubes were kept in boiling water bath for 10 min and cooled. After cooling the tubes, 0.5 ml 6% KOH was added and again kept in boiling water bath for 20 min. The cooled test extract was then used for sucrose estimation. To suitable aliquots of the extract, 1 ml recorcinol solution and 3 ml of 75% H_2SO_4 were added and then incubated at 80°C for 10 min. The intensity of pink colour was measured at 490 nm by preparing the control and expressed the enzyme activity as µmoles of sucrose formed mg^{-1} protein min^{-1} and the concentration of sucrose was calculated from the standard curve prepared by using sucrose standard (0-100 µg ml^{-1}).

2.4.2.2 Acid invertase (µg sucrose hydrolyzed min^{-1} g^{-1} FW)

Acid invertase was extracted by the method of Vattuone *et al.* (1981).

I. Extraction of enzyme

Grains separately collected crushed and extracted in minimum volume of 50 mM sodium phosphate buffer (pH 7.5) containing 1 mM β-mercaptoethanol and 5 µM $MnSO_4$ and filtered the content through two layers of cheese cloth and centrifuged the homogenate at 10,000 x g for 10 min. The supernatant and pellet were carefully separated. To the measured volume of the supernatant solid ammonium sulphate was added to get 80% saturation and kept for 4 h after which the contents were centrifuged at 10,000 x g for 10 min. The supernatant was discarded and the pellet was dissolved in minimum volume of 50 mM sodium phosphate buffer (pH 7.0) and used for determination of acid invertase activities.

II. Enzyme assay

Acid invertase activity was assayed by adding 50 µl enzyme to 750 µl of 50 mM sodium acetate buffer (pH 5.5). The enzyme reaction was started by addition of 0.2 ml 0.5 M sucrose solution and the reaction was terminated after 30 min by adding 1 ml of alkaline copper reagent and kept the mixture exactly for 20 min in boiling water bath. The tubes were cooled under running tap water or using ice and the reducing sugar produced was assayed by the method of Nelson (1944). The activity of enzymes was expressed as mole glucose formed mg^{-1} protein min^{-1}.

2.4.3. Determination of WSC and fructans from internodes

2.4.3.1. Internodes WSC content (mg g^{-1} dry weight)

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630 nm.

i. Materials

1. 2.5 N HCl
2. Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice cold 95% H_2SO_4. Prepare fresh before use.
3. Standard glucose: Stock- Dissolve 100 mg in 100 ml of distilled water. Working standard- 10 ml of stock diluted in 100 ml with distilled water. Store refrigerated after adding a few drops of toluene.

ii. Procedure

1. Weigh 100 mg of sample into a boiling tube.
2. Hydrolyse by keeping it in a boiling water bath for 3 h with 5 ml of 2.5 N-HCl and cool to room temperature.
3. Neutralise it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume 100 ml and centrifuge.
5. Collect the supernantent and take 0.5 and 1 ml aliquots for analysis.
6. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1of the working standards. '0' serves as blank.
7. Make up the volume to 1 ml and in all the tubes including the sample tubes by adding distilled water.
8. Then add 4 ml of anthrone reagent.
9. Heat for 8 min in a boiling water bath.
10. Cool rapidly and read the green to dark green colour at 630 nm.
11. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance at the Y-axis
12. From the graph calculate the amount of carbohydrate present in sample tube.

2.4.3.2 Internodes fructans content (mg g^{-1} dry weight)

Fructan contents in stem internodes were determined by using the acid recorcinol thiourea reagent as per the method described by Petkova and Denev (2012). The total fructan content from various internodes was expressed as mg g^{-1} dry weight.

i. Reagents

1. Recorcinol solution: 0.1% (w/v) recorcinol in glacial acetic acid containing 0.25 g thiourea.
2. 6% KOH solution: 6 g KOH in 100 ml distilled water.
3. 75% (v/v) H_2SO_4.

ii. Procedure

1. Fructans were determined, after destroying free fructose by adding 0.5 ml of 30% NaOH to 0.5 ml of sugar extract.
2. 100 microliters extract were place in glass tube of 10 ml, and 100 µl resorcinol (1% ethanol solution), 100 µl thiourea (0.1% ethanol solution), 800 µl 95% ethanol and 900 µl HCl were added to them.
3. The sample was heated 8 min at 80°C, cooled and filled with water until 10 ml.
4. Then the absorbance was measured spectrophotometrically at 480 nm against blank sample prepared at the same procedure with distilled water.

2.4.4 Nutritional parameters

Parameters such as gluten, iron and zinc were analyzed mechanically on Near Infra Red (NIR) spectralizer.

3. Results

3.1 Physiological parameters

3.1.1 Leaf area $plant^{-1}$ (dm^2)

Leaf area among all the genotypes, showed significant variation for all three sowing dates and it was observed that leaf area found to be lower as the sowing dates delayed. Interaction between sowing dates and genotypes found to be non-significant for this trait. Genotype NIAW-3033 (2.93 dm^2) exhibited maximum leaf area as compared to NIAW-1994 (Phule Samadhan) (2.90 dm^2), NIAW-3161 (2.58 dm^2) and MACS-6222 (2.71 dm^2) for all the sowing dates in present study.

3.1.2 Flag leaf area $plant^{-1}$ (dm^2)

Significant variation observed among all the genotypes in all three sowing dates for flag leaf area based on data obtained and it was reported that the area of flag leaf decreased as the sowing dates delayed. Interaction between sowing dates and genotypes found to be non-significant for number of tillers. Among the genotypes NIAW-1994 (Phule Samadhan) (0.81 dm^2) exhibited highest number of tillers as compared to NIAW-3033 (0.79 dm^2), MACS-6222 (0.65 dm^2) and NIAW-3161(0.71 dm^2) in three sowing dates.

Table 1: Leaf area plant^{-1} (dm^2) and flag leaf area plant^{-1} (dm^2)

	Leaf area plant^{-1} (dm^2)				Flag leaf area plant^{-1} (dm^2)			
Genotypes	**SD-1**	**SD-2**	**SD-3**	**Mean**	**SD-1**	**SD-2**	**SD-3**	**Mean**
NIAW-3033	3.76	3.34	1.69	2.93	0.93	0.86	0.58	0.79
NIAW-1994	3.58	3.39	1.74	2.90	0.91	0.89	0.62	0.81
NIAW-3161	3.22	3.06	1.47	2.58	0.79	0.73	0.44	0.65
MACS-6222	3.43	3.19	1.52	2.71	0.87	0.77	0.49	0.71
Mean	3.50	3.25	1.61	2.78	0.88	0.81	0.53	0.74
	D	**G**	**D×G**		**D**	**G**	**D×G**	
SE	0.020	0.076	0.132		0.026	0.029	0.050	
CD at 5%	0.077	0.227	NS		0.101	0.086	NS	

*SD-Sowing date

Table 2: Enzymatic activities from grains

	Sucrose synthase (µmole sucrose synthesized g^{-1} wt. of sample)				Acid invertase (µg sucrose hydrolyzed min^{-1} g^{-1} FW)			
Genotypes	**SD-1**	**SD-2**	**SD-3**	**Mean**	**SD-1**	**SD-2**	**SD-3**	**Mean**
NIAW-3033	287.64	266.39	236.49	263.51	221.27	236.65	247.30	235.07
NIAW-1994	310.84	283.62	246.51	280.32	274.82	263.26	265.28	267.79
NIAW-3161	220.63	212.65	186.65	206.64	134.87	209.84	210.67	185.13
MACS-6222	242.31	238.57	209.57	230.15	161.36	219.67	214.51	198.51
Mean	265.36	250.31	219.81	245.16	198.08	232.36	234.44	221.63
	D	**G**	**D×G**		**D**	**G**	**D×G**	
SE	0.523	0.912	1.579		2.027	2.558	4.430	
CD at 5%	2.054	2.709	4.692		7.959	7.599	13.163	

*SD-Sowing date

3.2 Enzymatic activities

3.2.1 Sucrose synthase (µmole sucrose synthesized g^{-1} wt. of sample)

Activity of sucrose synthase found to be higher in genotype NIAW-1994 (Phule Samadhan) (280.32 µmole sucrose synthesized g^{-1} wt. of sample) which was comparable with rest of the genotypes, *viz.,* NIAW-3033 (263.51 µmole sucrose synthesized g^{-1} wt. of sample), MACS-6222 (230.15 µmole sucrose synthesized g^{-1} wt. of sample) and NIAW-3161 (206.64 µmole sucrose synthesized g^{-1} wt. of sample) for all three sowing dates. Significant reduction in activity of this enzyme found as sowing date delayed, *viz.,* first sowing date (265.36 µmole sucrose synthesized g^{-1} wt. of sample), second sowing date (250.31 µmole sucrose synthesized g^{-1} wt. of sample) and third sowing date (219.91 µmole sucrose synthesized g^{-1} wt. of sample). Significant positive interaction found between sowing dates and genotypes for sucrose synthase activity.

Table 3: Stem water soluble carbohydrates (mg g^{-1} dry weight)

	Basal				Middle				Apical				Peduncle			
Genotypes	**SD-1**	**SD-2**	**SD-3**	**Mean**	**SD-1**	**SD-2**	**SD-3**	**Mean**	**SD-1**	**SD-2**	**SD-3**	**Mean**	**SD-1**	**SD-2**	**SD-3**	**Mean**
NIAW-3033	278.66	252.37	234.16	255.06	296.59	263.54	236.42	265.52	367.42	277.56	251.24	298.74	326.16	289.65	254.32	290.04
NIAW-1994	264.55	255.84	240.17	253.52	384.63	315.66	276.59	325.63	412.36	285.62	252.66	316.88	426.56	296.26	275.61	332.81
NIAW-3161	239.57	208.56	178.43	208.85	257.68	251.06	221.67	243.47	306.55	246.57	216.54	256.55	284.22	254.29	224.59	254.37
MACS-6222	255.69	216.44	176.55	216.23	266.84	251.74	223.50	247.36	324.62	254.26	219.67	266.18	311.47	261.27	226.44	266.39
Mean	259.62	233.30	207.33	233.42	301.44	270.50	239.55	270.49	352.74	266.00	235.03	284.59	337.10	275.37	245.24	285.90
	D	**G**	**D×G**		**D**	**G**	**D×G**		**D**	**G**	**D×G**		**D**	**G**	**D×G**	
SE	6.67	3.13	5.42		2.89	4.42	7.65		2.18	4.38	7.59		4.09	5.10	8.83	
CD at 5%	26.19	9.30	16.10		11.34	13.13	22.74		8.58	13.03	22.57		16.06	15.16	26.26	

*SD-Sowing date

3.2.2 Acid invertase (µg sucrose hydrolyzed min^{-1} g^{-1} FW)

Acid invertase activity observed higher in genotype NIAW-1994 (Phule Samadhan) (267.79 µg sucrose hydrolyzed min^{-1} g^{-1} FW) which was comparable with remaining genotypes, *viz.,* NIAW-3033 (235.07 µg sucrose hydrolyzed min^{-1} g^{-1} FW), MACS-6222 (198.51 µg sucrose hydrolyzed min^{-1} g^{-1} FW) and NIAW-3161 (185.13 µg sucrose hydrolyzed min^{-1} g^{-1} FW) for all three sowing dates. Significant rise in activity of this enzyme found as sowing date delayed, *viz*., first sowing date (198.08 µg sucrose hydrolyzed min^{-1} g^{-1} FW), second sowing date (232.36 µg sucrose hydrolyzed min^{-1} g^{-1} FW) and third sowing date (234.44 µg sucrose hydrolyzed min^{-1} g^{-1} FW). Significant positive interaction found between sowing dates and genotypes for acid invertase activity.

3.3 Biochemical parameters

3.3.1 Stem water soluble carbohydrates (mg g^{-1} dry weight)

Significantly higher amount of water soluble carbohydrates were found in genotype NIAW-1994 (Phule Samadhan), *viz.*, basal (253.52), middle (325.63), apical (316.88) and peduncle (332.81) which was followed by NIAW-3033, *viz.*, basal (255.06), middle (265.52), apical (298.74) and peduncle (290.04), whereas NIAW-3161 exhibited least amount of WSC content in basal (208.85), middle (243.47), apical (256.55) and peduncle (254.37).

Table 4: Stem fructans content (mg g^{-1} dry weight)

	Basal				Middle				Apical				Peduncle			
	SD-1	SD-2	SD-3	Mean	SD-1	SD-2	SD-3	Mean	SD-1	SD-2	SD-3	Mean	SD-1	SD-2	SD-3	Mean
NIAW-3033	153.42	149.85	133.26	145.51	236.23	226.79	189.55	217.52	236.56	231.29	211.52	226.46	156.48	156.12	139.64	150.75
NIAW-1994	186.59	183.54	152.74	174.29	261.47	241.56	213.41	238.81	284.53	251.27	218.63	251.48	187.65	177.52	152.48	172.55
NIAW-3161	142.57	142.57	114.55	133.23	204.86	186.59	162.35	184.60	211.39	211.39	188.65	203.81	121.74	121.74	114.52	119.33
MACS-6222	165.29	148.68	119.63	144.53	221.56	212.39	165.74	199.90	232.41	223.46	203.78	219.88	126.59	122.26	118.95	122.60
Mean	161.97	156.16	130.04	149.39	231.03	216.83	182.76	210.21	241.22	229.35	205.64	225.41	148.12	144.41	131.40	141.31
	D	G	D×G		D	G	D×G		D	G	D×G		D	G	D×G	
SE	1.67	2.84	4.93		4.88	4.06	7.04		3.69	5.45	9.44		2.94	4.65	8.05	
CD at 5%	6.54	8.45	NS		19.17	12.08	NS		14.48	16.20	NS		11.53	13.81	NS	

*SD-Sowing date

3.3.2 Stem fructans content (mg g^{-1} dry weight)

All the four internodes were found significantly comparable for internodal fructans content based on data recorded. Interaction between sowing dates and genotypes found to be statistically non significant for all internodes, *viz*., basal, middle, apical and peduncle. Mean values of fructans were found decreased as sowing date delayed.

Maximum amount of fructans content were found in genotype NIAW-1994 (Phule Samadhan), *viz*., basal (174.29 mg g^{-1} dry weight), middle (238.81 mg g^{-1} dry weight), apical (251.48 mg g^{-1} dry weight) and peduncle (172.55 mg g^{-1} dry weight) which was followed by NIAW-3033, *viz.,* basal (145.51 mg g^{-1} dry weight), middle (217.52 mg g^{-1} dry weight), apical (226.46 mg g^{-1} dry weight) and peduncle (150.75 mg g^{-1} dry weight), whereas NIAW-3161 exhibited least amount of WSC content in basal (133.23 mg g^{-1} dry weight), middle (184.60 mg g^{-1} dry weight), apical (203.81 mg g^{-1} dry weight) and peduncle (119.33 mg g^{-1} dry weight).

Synthesis, accumulation and transport of WSC and fructans is affected due to deleterious effects of heat stress. The pre-anthesis high temperature retards the pollen viability, seed formation, and embryo development and post-anthesis high temperature declines the starch granules accumulation, stem reserve carbohydrates, and translocation of photosynthates into grains. A high temperature above 40°C inhibits the photosynthesis by damaging the photosystem-II, electron transport chain, and photosystem-I.

Table 5: Nutritional parameters

	Gluten (%)				Iron (mg/100 g)				Zinc (mg/100 g)			
Genotypes	SD-1	SD-2	SD-3	Mean	SD-1	SD-2	SD-3	Mean	SD-1	SD-2	SD-3	Mean
NIAW-3033	29.36	26.84	24.86	27.02	5.32	5.04	4.86	5.07	3.54	3.21	3.07	3.27
NIAW-1994	28.64	25.74	24.45	26.28	5.86	5.61	5.03	5.50	3.69	3.46	3.16	3.44
NIAW-3161	26.87	23.21	22.84	24.31	4.87	4.68	4.23	4.59	3.12	3.01	2.84	2.99
MACS-6222	25.97	23.09	21.74	23.60	4.39	4.08	4.00	4.16	3.09	2.97	2.41	2.82
Mean	27.71	24.72	23.47	25.30	5.11	4.85	4.53	4.83	3.36	3.16	2.87	3.13
	D	G	D×G		D	G	D×G		D	G	D×G	
SE	0.286	0.689	1.194		1.778	1.038	1.797		0.358	0.726	1.258	
CD at 5%	1.123	2.049	NS		6.982	3.083	NS		1.406	2.158	3.737	

*SD-Sowing date

3.4 Nutritional parameters

Gluten is a protein naturally found in some grains including wheat. It acts like a binder, holding food together and adding a "stretchy" quality. Gluten may also act as a prebiotic, feeding the "good" bacteria in our bodies. Genotype NIAW-3033 was found to have maximum amount of glutent content than remaining genotypes. Relatively less amount of gluten was found in genotype MACS-6222. Genotype NIAW-1994 was at par with genotype NIAW-3033 for gluten content in grain. Gluten consist of two main groups of proteins, gliadins and glutenins in approximately equal proportions. When gliadin is mixed with starch and water a purely viscous material is formed. In contrast, glutenin forms a rubbery texture (provided it is above its transition temperature) with low extensibility (Singh and MacRitchie, 2004). Both of these groups attribute to the excellent viscoelastic properties of gluten when hydrated.

Iron is essential in making hemoglobin, a protein in red blood cells. These red blood cells help carry oxygen throughout your body. About 70% of the iron in your body can be found in a protein in red blood cells called hemoglobin. Zinc is a major player in the creation of DNA, growth of cells, building proteins, healing damaged tissue, and supporting a healthy immune system. Because it helps cells to grow and multiply, adequate zinc is required during times of rapid growth, such as childhood, adolescence, and pregnancy.

3.5 Stress tests

3.5.1 Heat tolerance test (Membrane injury index %)

Membrane injury index at 50% flowering and at dough stage exhibited highest significant variation among all the genotypes under investigation in varying sowing dates. It was observed that membrane injury was increased from flowering to dough stage as the sowing dates delayed. Interaction between sowing dates and genotypes found to be significant for membrane injury at both stages. Among the genotypes, minimum injury at both the stages was recorded by NIAW-1994 (Phule Samadhan) (8.78% and 10.15%, respectively), which was followed by NIAW-3033 (10.44% and 11.69% respectively), MACS-6222 (18.52% and 21.89%) and highest membrane injury was found in NIAW-3161 (23.30% and 26.09% respectively) in three sowing dates.

Table 6: Heat tolerance test (membrane injury index %)

	Heat tolerance test (at 50% flowering)				Heat tolerance test (at dough stage)			
Genotypes	**SD-1**	**SD-2**	**SD-3**	**Mean**	**SD-1**	**SD-2**	**SD-3**	**Mean**
NIAW-3033	8.59	9.47	13.26	10.44	10.08	10.42	14.58	11.69
NIAW-1994	7.15	7.86	11.34	8.78	9.54	8.16	12.74	10.15
NIAW-3161	13.84	19.21	36.86	23.30	16.37	22.39	39.51	26.09
MACS-6222	11.74	15.26	28.56	18.52	13.65	17.72	34.31	21.89
Mean	10.33	12.95	22.51	15.26	12.41	14.67	25.29	17.46
	D	**G**	**D×G**			**D**	**G**	**D×G**
SE	0.157	0.746	1.292			0.358	0.726	1.258
CD at 5%	0.616	2.216	3.838			1.406	2.158	3.737

*SD-Sowing date

Table 7: Membrane stability index (%)

Membrane stability index (at 50% flowering)				Membrane stability index (at 50% flowering)		
SD-2	**SD-3**	**Mean**	**SD-1**	**SD-2**	**SD-3**	**Mean**
69.48	59.93	66.93	62.37	60.18	59.37	60.64
69.83	61.84	68.31	62.86	61.85	60.72	61.81
53.61	48.61	52.88	54.07	51.26	50.63	51.99
56.04	50.74	54.29	52.75	52.31	52.23	52.43
62.24	55.28	60.61	58.01	56.40	55.74	56.72
D	**G**	**D×G**		**D**	**G**	**D×G**
0.429	0.596	1.032		0.424	0.742	1.284
1.684	1.770	NS		1.665	2.203	NS

*SD-Sowing date

3.5.2 Membrane stability index (%)

Membrane stability index at 50% flowering and at dough stage exhibited highest significant variation among all the genotypes under investigation in varying sowing dates. It was observed that membrane stability was decreased from flowering to dough stage as the sowing dates delayed. Interaction between sowing dates and genotypes found to be significantly negative for membrane stability at both stages. Among the genotypes maximum membrane stability at both the

stages was recorded by NIAW-1994 (Phule Samadhan) (68.31% and 61.81%, respectively), which was followed by NIAW-3033 (66.93% and 60.64% respectively), MACS-6222 (54.29% and 52.43%) and lowest membrane stability was found in NIAW-3161 (52.88% and 51.99%, respectively), in three sowing dates.

3.5.3 Chlorophyll stability index (%)

According to data on chlorophyll stability index at 50% flowering and at dough stage exhibited highest significant variation among all the genotypes under investigation in varying sowing dates. Chlorophyll stability index was found to be decreased from 50% flowering to dough stage as the sowing dates delayed. Interaction between sowing dates and genotypes found to be non-significant for chlorophyll stability index at both stages. Among the genotypes, highest chlorophyll stability at both the stages was recorded by NIAW-1994 (Phule Samadhan) (76.60% and 66.93%, respectively), which was followed by NIAW-3033 (74.90% and 65.74% respectively), MACS-6222 (64.66% and 55.43%) and lowest chlorophyll stability was found in NIAW-3161 (59.70% and 53.58% respectively) in three sowing dates.

Table 8: Chlorophyll stability index (%)

	Chlorophyll stability index (at 50% flowering)				Chlorophyll stability index(at dough stage)			
Genotypes	**SD-1**	**SD-2**	**SD-3**	**Mean**	**SD-1**	**SD-2**	**SD-3**	**Mean**
NIAW-3033	76.56	76.56	71.59	74.90	75.63	62.55	59.04	65.74
NIAW-1994	78.12	78.12	73.56	76.60	77.56	62.50	60.72	66.93
NIAW-3161	61.37	61.37	56.37	59.70	58.86	51.26	50.63	53.58
MACS-6222	67.28	67.28	59.42	64.66	61.74	52.31	52.23	55.43
Mean	70.83	70.83	65.24	68.97	68.45	57.16	55.65	60.42
	D	**G**	**D×G**			**D**	**G**	**D×G**
SE	0.286	0.689	1.194			1.778	1.038	1.797
CD at 5%	1.123	2.049	NS			6.982	3.083	NS

*SD- Sowing date

4. Discussion

Leaf is one of the most important parts of plant body which is responsible for photosynthesis, transpiration and gaseous exchange. High temperature (41°C) causes a decrease in the potential photosynthetic rate in both heat resistant and susceptible variety (Volkova and Koshin, 1984). Ubaidullah *et al*. (2006) concluded that heat stress causes reduction in leaf area which is the ultimate result of reduced leaf length and leaf breadth. In the study leaf area (LA) was significantly affected by late sowing (high temperature or heat stress). Heat stress accelerated the decline in viable leaf blade area and photosynthetic activity per unit leaf area (Al-Khatib and Paulsen, 1984). Higher temperature enhances leaf senescence causing reduction in green leaf area and number during reproductive stages (Al-Khatib and Paulsen, 1984). The leaf area that is the photosynthetic area has a great effect on the amount of photosynthesis. So, the varieties with higher leaf area even in high temperature will be benefited in this sense.

Carbohydrates serve as a source of energy and act as signalling molecule in regulation of metabolic pathway under normal and stressed conditions. During germination, starch present in the endosperm is hydrolysed to glucose by the amylases and then converted to sucrose by the sucrose phosphate synthase. Sucrose, thus formed is then transported to the growing embryonic axis, where it is hydrolysed and the product so formed is used as energy source for growth of seedlings. Sucrose helps in the osmotic adjustment. Biochemical conversion of sucrose to starch is one of the most important components of sink strength. Photosynthates accumulation and transport to sink is adversaly affected by elevated temperature stress because rate of photosynthesis is get affected.

The main function of sucrose synthase is to catalyze the sucrose cleaving to produce UDPG and fructose and provide glycosyl for starch synthesis rather than sucrose synthesis during grain filling. It showed that the sucrose synthase activity in grains of all the genotypes was even lower under late sowing condition, suggesting that the ability of decomposing sucrose through sucrose synthase pathway had the tendency to decline on the whole under high temperature. Invertase is also named sucrose enzyme and its function is to produce glucose and fructose by hydrolyzing sucrose. Activity of enzymes sucrose synthase and acid invertase showed significant differences among the genotypes studied for all the three sowing dates. However, high temperature decreased CWI activity in rice grains compared with the control, indicating that the high temperature is unfavorable for the degradation of sucrose. NIAW-1994 (Phule Samadhan) exhibited highest activity for both sucrose synthase and acid invertase for all the three staggered sowing dates than genotypes NIAW-3033. Similarly genotype NIAW-3161 showed reduced activity of both the enzymes than MACS-6222 for three sowing dates. The results obtained are in accordance with the results of Tian *et al*. (2006).

Irrespective of normal and late sown conditions, there was continuous decline of total carbohydrates in all the genotypes which may be correlated to their rapid utilization for the synthesis of carbohydrate polymer mainly starch. In late sown genotypes there was more decline in total carbohydrates content. Since, temperature was also high during late sown condition, so it is quite likely that higher utilization of sugars prevails during stress conditions. It has also been reported that free sugars decline during biotic or abiotic stresses to overcome stress and sugars are essential to plant growth and metabolism both as energy source and structural components. An increase in assimilate availability around anthesis is able to improve the distal grain weight.

Plaut *et al*. (2004) reported that high temperature reduced the rate of transport of dry matter from vegetative organs to kernel, but sensitivity to high temperature stress differed with varieties. Our data indicated variations in days to anthesis for normal and late sown wheat genotypes, which was reflected in their stem WSC synthesis and remobilization towards grains.

Genotypes NIAW-1994 and NIAW-3033 were found to have higher iron and zinc content than rest of the genotypes. During delayed sowing gluten, iron and zinc content of wheat grains were decreased. Both of these minerals are too impotant for our body, so we need adequate quantity of these through our diet and wheat is a supplier of iron and zinc. Minerals (including Fe and Zn) in the aleurone layer and scutellum of wheat and other cereals are largely present as salts of phytic acid (inositol hexakisphosphate) (Neal *et al*., 2013; Persson *et al*., 2009). High temperature reduces the end use quality of protein (Li *et al.,* 2013; Castro *et al.,* 2007).

Genotype NIAW-1994 (Phule Samadhan) is found to have more chlorophyll stability index, membrane stability index and less membrane injury index (%) than rest of the genotypes. In contrast genotype NIAW-3161 had relatively less chlorophyll stability index, membrane stability index and more heat tolerance index than among all the genotypes. Genotypes with less membrane injury will show tolerance to any environmental stress by avoiding less membrane integrity loss and electrolyte leakage. Under stress conditions, the tolerant genotypes activated their protection mechanisms faster and more efficiently than the sensitive ones.

5. Conclusion

The maximum and minimum temperatures during flowering for delayed sowing dates were higher when compared with normal sowing, so there ware significant changes in all the parameter related to physiological, enzymatic, biochemical, nutritional and stress tests for membrane stability, injury and chlorophyll. Significant and noticeable reduction in above mentioned parameters were found in third sowing date D_3 21/1/2018. The overall reduction in growth, physiological characters along with other characters found in this sowing because delayed sowing coincides with elevated temperature which subsequently affected all the genotypes from starting growth stages.

Stem/internodes water soluble carbohydrates and fructans are crucial for stem reserve mobilization and grain filling in wheat, but at elevated temperature stress total WSCs and fructans were found to be decreased so, grain filling is markly affected under delayed sowings than timely. All the parameters recorded under all three sowing dates showed variations because weather parameters were changing which were not favourable for late sown crop. High temperature stress causes changes in protein denaturation which subsequently causes reduction in enzymatic activities which affects grain filling and total protein content in grains. Nutritional quality of delayed sown wheat will be poor than timely sown.

Elevated temperature stress at the time of flowering and grain filling is deleterious which affects reproductive growth of wheat which can cause significant yield reduction too when compared with timely sown crop. Improving thermotolerance in the crop is a challenging task for crop scientists. Genotypes NIAW-1994 and NIAW-3033 were found to be tolerant to elevated temperature stress compared to remaining ones. Both of these genotypes can be used as parent for future breeding programme for developing temperature stress tolerant varieties.

Acknowledgements

We are thankful to Department of Agricultural Botany, MPKV, Rahuri for providing laboratory facilities and research grant to carry out this research work and authors cited in references for proving necessary literaturary material.

Conflicts of interest

The authors declare no conflicts of interest relevant to this article

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Citation **Suvarna Gare, R. S. Wagh and A. U. Ingle (2023). Evaluation of physiological, enzymatic, biochemical, nutritional parameters and stress tests in wheat genotypes. Ann. Phytomed., 12(1):856-864. http://dx.doi.org/10.54085/ap.2023.12.1.87.**