The Pharma Innovation Journal 2022; SP-11(10): 728-732

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(10): 728-732

www.thepharmajournal.com
Received: 10-08-2022
Accepted: 13-09-2022

Bharat Garg
Department of Molecular Biology, CCS Haryana Agricultural University, Biotechnology and Bioinformatics, CCS HAU, Hisar, Haryana, India

Shikha Yashveer
Department of Molecular Biology, CCS Haryana Agricultural University, Biotechnology and Bioinformatics, CCS HAU, Hisar, Haryana, India

Jayanti Tokas
Department of Biochemistry, CCS HAU, Hisar, Haryana, India

Ashmita
School of Agricultural Biotechnology, PAU Ludhiana, Punjab, India

Prakhar Singla
Department of Genetics & Plant Breeding, College of Agriculture, CCS HAU, Hisar, Haryana, India

Jyoti Duhan
Department of Biochemistry, CCS HAU, Hisar, Haryana, India

Corresponding Author:
Bharat Garg
Department of Molecular Biology, CCS Haryana Agricultural University, Biotechnology and Bioinformatics, CCS HAU, Hisar, Haryana, India

Best stage to study stress conditions biochemically: Vegetative or maturity stage?

Bharat Garg, Shikha Yashveer, Jayanti Tokas, Ashmita, Prakhar Singla and Jyoti Duhan

Abstract
The present study was conducted at CCS Haryana Agricultural University, Hisar, during *rabi* 2020-21 to estimate and compare the biochemical characteristics in the vegetative and maturity stage using 58 wheat genotypes. Biochemical screening is vital to know the content of antioxidants, osmolytes, and enzymes, which play a critical role in stress conditions. Varieties that show more range of these biochemical traits have more potential to resist the stress conditions, so these varieties are also included in the selection process of fungal disease-resistant types. In the present study, five biochemical traits were used for biochemical screening that was total phenolic content (TPC) determined by using the Folin-Ciocalteu method, total flavonoid content (TFC) by using aluminum chloride method, proline content, glycine betaine, and tyrosine ammonia lyase (TAL). These biochemical tests were done at vegetative (VS) and maturity stages (MS). In the vegetative stage, the range for total phenols was found to be 0.41 to 4.74 mg CE/g, for total flavonoids 0.30 to 6.97 mg QE/g, for proline content 0.07 to 0.45 mg /g, for glycine betaine 0.59 to 9.59 mg /g DW and TAL enzyme 1.45 to 12.43 µmole/h/g. In the maturity stage, the range for total phenols was found to be 1.07 to 6.17 mg CE/g, for total flavonoids 1.07 to 9.18 mg QE/g, for proline content 0.08 to 0.55 mg /g, for glycine betaine 2.67 to 4.24 mg /g and TAL enzyme 1.74 to 12.10 µmole/h/g. Results showed that the maturity stage is characterized by the highest contents of biochemical parameters and is directly associated with grain yield. Therefore it could be considered the best stage for studying stress conditions.

Keywords: Antioxidants, osmolytes, enzymes, wheat genotypes

Introduction
Wheat, one of the world's three most important cereal crops, is the staple food of about 40% of the world's population (Li *et al*., 2019) [7]. The global population will exceed 9.5 billion by 2050, and global wheat yield will need to grow by 60% to satisfy food requirements (Savadi *et al*., 2017) [1].

Several pathogens are harmful to wheat, although rust infections are the most common. They are a severe threat to global wheat production and have been documented in every country that grows wheat (Roelfs *et al*., 1992) [9]. It includes leaf rust (*Puccinia triticina*), stem rust (*P. graminis triticina*), and stripe rust (*P. striiformis*). Every rust pathogen is a parasite that can only infect specific host plants or animal species. In ideal conditions, all three rusts can spread rapidly and cause severe epidemics, as was seen in some counties (Roelfs *et al*., 1992) [9]. Worldwide, rust diseases cause a loss of grain yield between 20 and 30 mt and 15 to 20 percent (Hanson *et al*., 1982) [4].

In abiotic stress, wheat cultivars are affected by stress due to moisture, salt, temperature, and micronutrients. Continued experience of high temperatures in rain fed areas of the globe may lead to drought stress.

Screening at biochemical levels is essential to know the internal system of antioxidants and enzymatic activity. When the plants get infected or wounded by any microbe or fungi, a combination of is flavones and some other flavonoids are induced (Mierziak *et al*., 2014, Schulz *et al*., 2016) [8, 11]. Phenolic compounds are important in defense against a pathogen in plants and are generally correlated with resistance against pathogens. The phenylalanine ammonia-lyase (PAL) and tyrosine ammonia lyase (TAL) are essential enzymes in the phenylpropanoid pathway that lead to biosynthesis of lignin and produce phenols as byproducts. The concentration of PAL and TAL enzymes varies at each stage of the plant relative to any fungal infection. Phenylalanine converts into cinnamic acid by PAL through the phenylpropanoid pathway, and then phenolic compounds are formed from cinnamic acid, Which fights against biotic stress (Heleno *et al*., 2015) [5].

In addition, proline and glycine betaine, produced during stress conditions, act as osmolytes. The present study is conducted to investigate at which stage there is the highest range of antioxidants, osmolytes, and enzyme activity to study stress conditions.

Material and Methods

A total of 58 wheat genotypes were used in the present study (Table 1). These genotypes were sown at the Wheat & Barley Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar, India, during the *Rabi* season of 2020-21. Biochemical analysis was done at the vegetative stage and maturity stage. For the vegetative stage leaf sample was taken at 60 days after sowing (DAS), and for the maturity stage, 120 DAS. All biochemical parameters with their extraction buffers and their respective wavelengths are listed in Table 2.

Table 1: List of wheat genotypes used in the present study

S. No.	Genotype	S. No.	Genotype	S. No.	Genotype
1	PBW 725	21	WH 1268	41	DBW 17
2	DBW 71	22	WH 1271	42	PBW 709
3	WH 1124	23	WH 1272	43	WH 147
4	WH 1105	24	WH 1274	44	PBW 527
5	DPW 621-50	25	WH 1276	45	WH 1137
6	DBW 88	26	WH 1277	46	WH 1152
7	DBW 187	27	WH 1278	47	PBW 373
8	WH 1025	28	WH 1279	48	WH 789
9	WB 2	29	WH 1283	49	PBW 762
10	DBW 90	30	HD 2967	50	WH 1100
11	UP 2565	31	HD 3059	51	PBW 158
12	WH711	32	HD 3086	52	PBW 677
13	WH 1257	33	WH 1142	53	PBW 714
14	WH 1258	34	WH 1252	54	PBW 695
15	WH 1259	35	WH 1080	55	PBW 698
16	WH 1261	36	WH 283	56	LOK 54
17	WH 1264	37	PBW 723	57	WH 1129
18	WH 1263	38	PBW 752	58	HUW 540
19	WH 1265	39	PBW 763		
20	WH 1266	40	PBW 706		

Total phenolic content (TPC)

The total phenolic content of extracts prepared in different solvents was determined using the Folin-Ciocalteu method using catechol as standard (Swain & Hillis, 1959) [12].

Calculations

$$\text{TPC (mg CE g}^{-1}\text{ DW)} = \frac{C \times V}{M}$$

Where,
C = Concentration of catechol (CE) established from the calibration curve in ($\mu g/ml$)
V = Volume of the extract solution in ml
M = Weight of the sample in g

Total flavonoid content

The total flavonoid content of leaves from different genotypes was determined by the method described by Kalita *et al.* (2013) [6].

Calculations

$$\text{TFC (mg QE g}^{-1}\text{ DW)} = \frac{C \times V}{M}$$

Where,
C = Concentration of Quercetin (QE) established from the calibration curve in ($\mu g/ml$)
V = Volume of the extract solution in ml
M = Weight of the sample in g

Proline content

The proline content in the leaf sample was analyzed by Bates *et al.* (1973) [1].

Calculations

$$\text{Proline content (mg / g FW)} = \frac{C \times V}{M}$$

Where,
C = Concentration of proline established from the calibration curve in ($\mu g/ml$)
V = Volume of the extract solution in ml
M = Weight of the sample in g

Glycine-betaine (GB)

GB was estimated by Grieve and Grattan (1983) [3] method in dry leaf powder.

Calculations

$$\text{Glycine betaine (mg / g DW)} = \frac{C \times V}{M}$$

Where,
C = Concentration of glycine betaine established from the calibration curve in ($\mu g/ml$)
V = Volume of the extract solution in ml
M = Weight of the sample in g

M = Weight of the sample in g

Tyrosine ammonia lyase (TAL)

TAL was estimated as per the procedure adopted by Dickerson *et al.* (1984) [2].

Calculations

$$\text{TAL (µmole/h/g)} = \frac{C \times D}{MW}$$

Where,

C = Concentration of t coumaric acid established from the calibration curve in ($\mu g/ml$)
D = Dilution factor
MW = Molecular weight of tyrosine

Table 2: Material and methods for Biochemical parameters

	Parameters	Extraction buffer	Wavelength	Standard	Method
1	**TPC**	Methanol	725	Catechol	Folin-Ciocalteu method
2	**TFC**	Methanol	415	Quercetin	Kalita *et al*., 2013 [6]
3	**PC**	3% sulphosalicylic acid	520	Proline	Bates *et al*; 1973 [3]
4	**GB**	Water (deionized)	365	Glycine betaine	Greive & Grattan, 1983 [3]
5	**TAL**	Borate Hcl buffer	290	Coumaric acid	Dickerson *et al*.;1984 [2]

TPC: Total phenolic content; TFC: Total flavonoids content; PC: Proline content, GB: Glycine betaine; TAL: Tyrosine ammonia lyase

Results

All biochemical parameters of wheat genotypes were done at both vegetative and maturity stages. The results obtained are described below and given in table 3.

Total phenolic content

Total phenolic content of all wheat genotypes for both vegetative and a maturity stage is depicted in Figure 1. For the vegetative stage, it ranged from 0.41 to 4.74 mg CE/g with an overall mean value of 2.05 mg CE/g. The maximum value for total phenolic content was found in genotype PBW 714 (4.74) and the minimum in WH 1252 (0.41). For the Maturity stage, it ranged from 1.07 to 6.17 mg CE/g with an overall mean value of 3.11 mg CE/g. The maximum value for total phenolic content was found in genotype WH 1271 (6.17) and the minimum in WH 1129 (1.07).

Total Flavonoids

Total Flavonoids of all wheat genotypes for both vegetative and maturity stage is depicted in Figure 2. For the vegetative stage, it ranged from 0.30 to 6.97 mg QE/g, with an overall mean value of 2.33 mg QE/g. The maximum value for total flavonoids was found in genotype WH 1263 (6.97) and the minimum in WH 1268 (0.30). For the Maturity stage, it ranged from 1.07 to 9.18 mg QE/g with an overall mean value of 3.72 mg QE/g. The maximum value for total flavonoids was found in genotype PBW 723 (9.18) and the minimum in WH 1258 (1.07).

Proline content

Proline content of all wheat genotypes for both vegetative and a maturity stage is depicted in Figure 3. For the vegetative stage, it ranged from 0.07 to 0.45 mg/g FW with an overall mean value of 0.27 mg /g FW. The maximum value for total phenolic content was found in genotype PBW 762 (0.45) and the minimum in PBW 706 (0.07).For maturity stage, it ranged from 0.08 to 0.55 mg/g FW with overall mean value 0.29 mg/g. The maximum value for proline content was found in genotype WH 789 (0.55) and the minimum in WH 1100 (0.08).

Glycine betaine

Glycine betaine of all wheat genotypes for both vegetative and maturity stage is depicted in Figure 4. For the vegetative stage, it ranged from 0.52 to 9.59 mg/g DW, with an overall mean value of 2.67 mg /g DW. The maximum value for glycine betaine was found in genotype PBW 706 (9.59) and the minimum in WH 1252 (0.52). For the maturity stage, it ranged from 0.83 to 11.13 mg/g DW with an overall mean value of 4.24 mg/g DW. The maximum value for glycine betaine was found in genotype WH 1265(11.13) and the minimum in PBW 714 (0.83).

Tyrosine Ammonia Lyase (TAL)

TAL of all wheat genotypes for both vegetative and maturity stage is depicted in Figure 5. For vegetative stage, it ranged from 1.45 to 12.43 umole /h/g with overall mean value 5.31 µmole/h/g. Maximum values for TAL were found in genotype PBW 762(12.43) and minimum in WH 1271(1.45). For the maturity stage, it ranged from 1.74 to 12.10 umole/h/g with an overall mean value of 5.11 µmole/h/g. The maximum value for TAL was found in genotype HVW 540 (12.10) and the minimum in PBW 527(1.74).

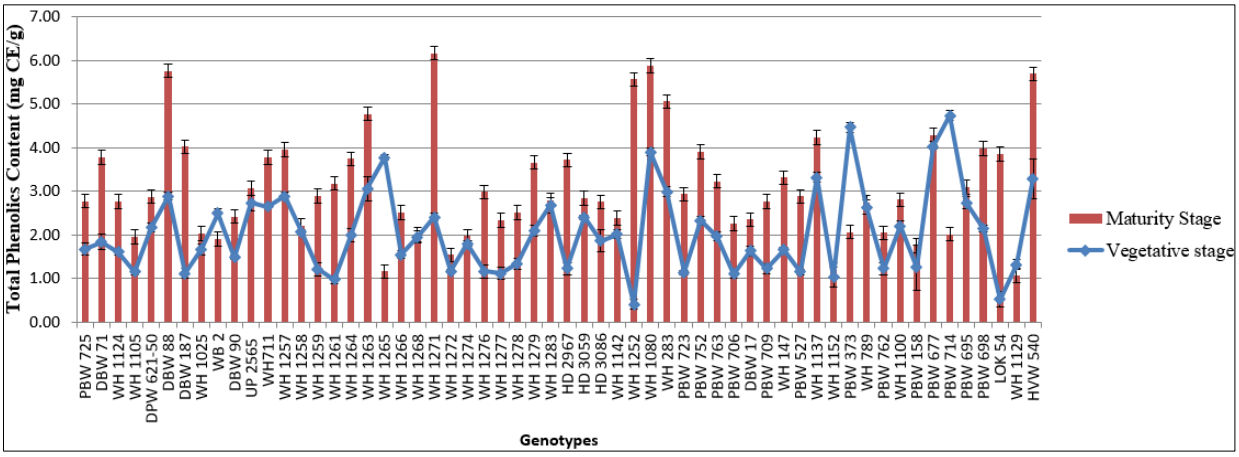

Fig 1: Total Phenolic Content (mg CE/g) of all Wheat Genotypes
For Vegetative stage [C.D at 5 percent = 0.382]
For Maturity stage [C.D at 5 percent = 0.424]

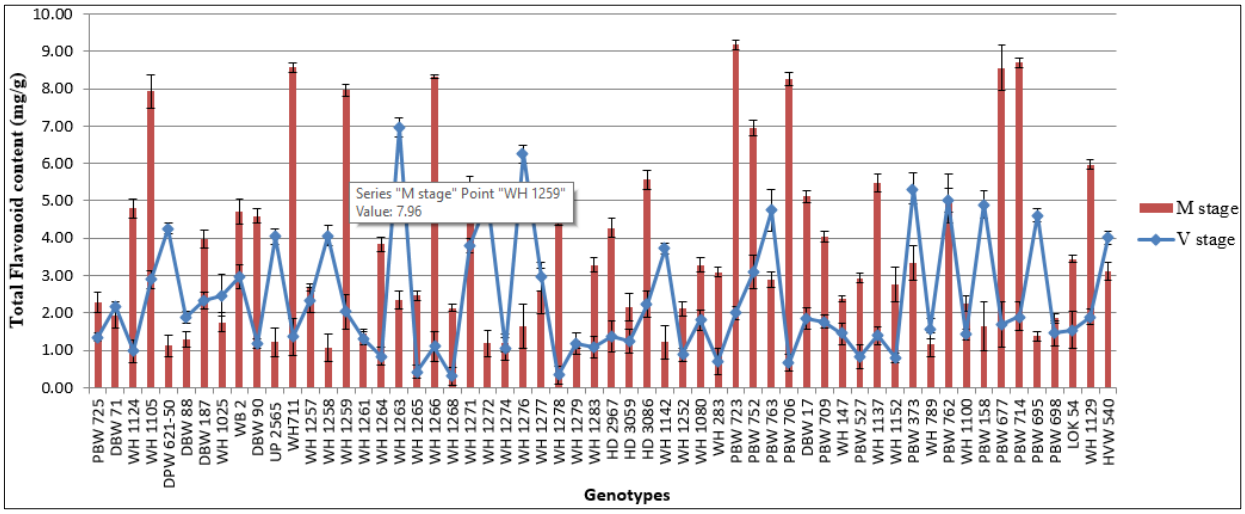

Fig 2: Total Flavonoids (mg QE/g) of all Wheat Genotypes
For Vegetative stage [C.D at 5 percent = 0.831]
For Maturity stage [C.D at 5 percent = 0.871]

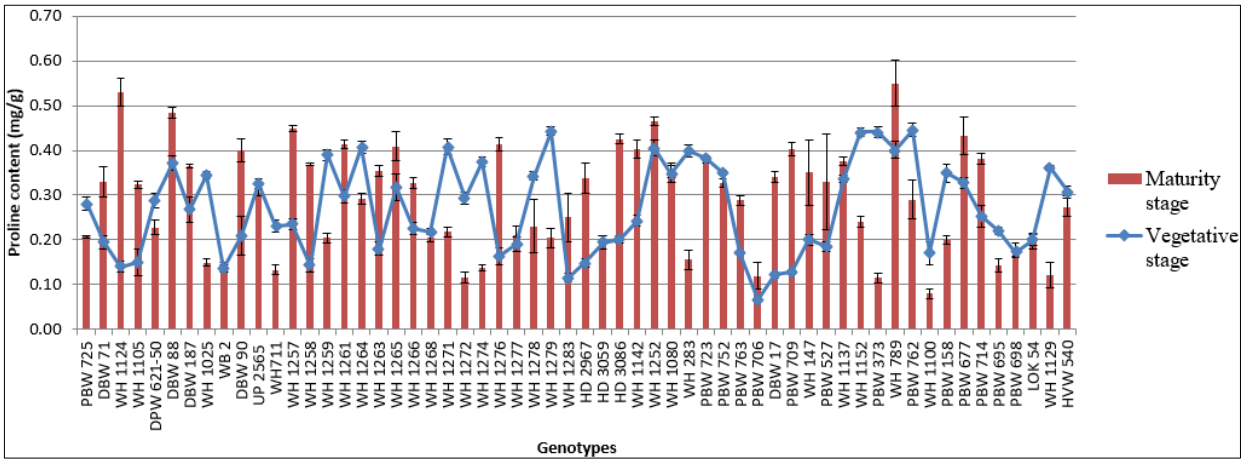

Fig 3: Proline content (mg/g FW) of all Wheat Genotypes
For Vegetative stage [C.D at 5 percent = 0.044]
For Maturity stage [C.D at 5 percent = 0.076]

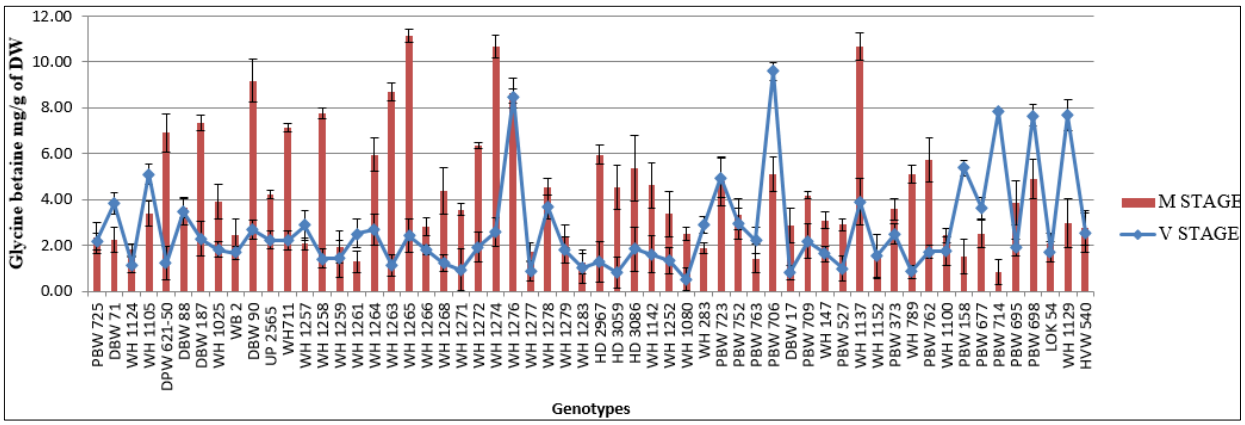

Fig 4: Glycine betaine (mg/g DW) of all Wheat Genotypes
For Vegetative stage [C.D at 5 percent = 2.583]
For Maturity stage [C.D at 5 percent = 1.641]

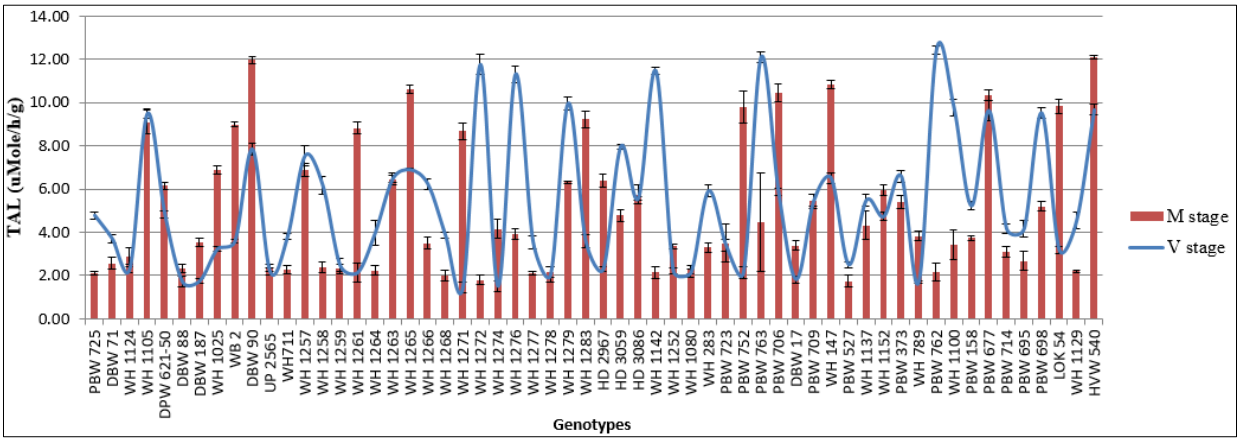

Fig 5: TAL activity (µmole/h/g) of all Wheat Genotypes
For Vegetative stage [C.D at 5 percent = 0.762]
For Maturity stage [C.D at 5 percent =1.197]

Table 3: Biochemical parameters of wheat genotypes at both vegetative and maturity stage

	Parameters	Stage	Range	Mean
1	TPC	Vegetative	0.41 - 4.74	2.05
		Maturity	1.07 - 6.17	3.11
2	TFC	Vegetative	0.30 - 6.97	2.33
		Maturity	1.07 - 9.18	3.72
3	PC	Vegetative	0.07 - 0.45	0.27
		Maturity	0.08 - 0.55	0.29
4	GB	Vegetative	0.52 - 9.59	2.67
		Maturity	0.83 - 11.13	4.24
5	TAL	Vegetative	1.45 - 12.43	5.31
		Maturity	1.74 - 12.10	5.11

Conclusion

The biochemical parameters like antioxidants, osmolytes, and enzyme activity were more at the maturity stage than the vegetative stage. The maturity stage is closely associated with grain yield, our ultimate crop output. Therefore, biochemically studying plant reaction to stress conditions at the maturity stage can significantly contribute to our understanding of biochemical mechanisms underlying plant stress tolerance. A better understanding of the underlying biochemical processes in response to different biotic and abiotic stresses can drive the selection of the appropriate promoter or transcription factor to be used for transformation.

Acknowledgement

The authors wish to acknowledge Indian Council of Agricultural research (ICAR), India for providing National Talent Scholarship to the first author.

References

1. Bates LS, Waldren RP, Teare ID.Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39(1):205-207.
2. Dickerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL. Phenylalanine ammonia-lyase and hydroxycinnamates: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiological Plant Pathology. 1984;25(2):111-123.
3. Grieve CM, Grattan SR. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant and Soil. 1983;70(2):303-307.
4. Hanson H, Borlaug NE, Anderson RG. Wheat in the third world. Boulder, CO, USA; c1982.
5. Heleno SA, Martins A, Queiroz MJR, Ferreira IC. Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. Food Chemistry. 2015;173:501-513.
6. Kalita P, Tapan BK, Pal TK, Kalita R. Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. Journal of Drug Delivery and Therapeutics. 2013;3 (4):33-37.
7. Li CX, Xu WG, Guo R, Zhang JZ, Qi XL, Hu L, *et al*, Author Correction: molecular marker assisted breeding and genome composition analysis of Zhengmai 7698, an elite winter wheat cultivar. Sci. Rep. 2019;8:322. https://doi:10.1038/s41598-017-18726-8.
8. Mierziak J, Kostyn K, Kulma A. Flavonoids as important molecules of plant interactions with the environment. Molecules. 2014;19(10):16240-16265.
9. Roelfs AP. Rust Arabidopsis thaliana. Scientific Reports, Diseases of wheat: concepts and methods of disease management. Cimmyt. 1992;6(1):1-10.
10. Savadi S, Prasad P, Kashyap P, Bhardwaj SC. Molecular breeding technologies and strategies for rust resistance in wheat (*Triticum aestivum*) for sustained food security. Plant Pathol. 2017;67:771-791. https://doi:0.1111/ppa.12802.
11. Schulz E, Tohge T, Zuther E, Fernie AR, Hincha DK. Flavonoids are determinants of freezing tolerance and cold acclimation in Arabidopsis thaliana. Scientific Reports. 2016;6(1):1-10.
12. Swain T, Hillis WE. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture. 1959;10(1):63-68.