

Full Length Research Article

## Improving resistance against terminal heat stress in wheat (*Triticum aestivum* L.) through seed priming with allelopathic water extracts

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### Abstract

Crop yield is very sensitive to climatic conditions such as increasing temperature. This study was conducted to evaluate the role of allelopathy in improving terminal heat stress during reproductive stages (booting and anthesis) of wheat. Seeds of two wheat cultivars (Mairaj-2008 and Faisalabad-2008) were primed with allelopathic water extracts of sorghum, sunflower, moringa and brassica (each at 3% either alone or in combination) for 12 h, keeping seed:water ratio of 1:5. Seeds of wheat cultivars were sown in 10 kg soil filled pots on November 21, 2013. The heat stress was imposed separately at booting and anthesis by placing pots in glass canopies with temperature of 4-5°C above than the ambient until maturity. Controlled pots were maintained under well-watered conditions at ambient temperature. Heat stress negatively impeded the performance of both wheat cultivars by enhancing the leaf malondialdehyde contents and reducing the membrane stability, which drastically reduced the grain weight and yield. However, seed priming with all the allelopathic crop water extracts, either used alone or in combination, significantly improved plant height, spike length, 100-grain weight, biological yield, grain yield, harvest index, chlorophyll *a*, chlorophyll *b*, free leaf proline, total soluble phenolics, leaf glycinebetaine membrane stability, and reduced the leaf malondialdehyde contents under terminal heat stress conditions. Seed priming with combined sorghum and moringa water extracts was more beneficial than other allelopathic water extracts in this regard. Among wheat cultivars, the performance of wheat cultivar Mairaj-2008 was better than Faisalabad-2008.

**Keywords:** Heat stress, chlorophyll, Wheat, allelopathy.

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### Introduction

Climate change is severely affecting cereal production across the world (Qin *et al.*, 2002), through increase in CO<sub>2</sub> concentration and temperature, resulting in heat stress (Farooq *et al.*, 2011). Climate model predicts that temperature will increase by 1.8-5.8°C at the end of this century (IPCC, 2007) and terminal heat stress will increase in wheat growing regions in near future (Mitra and Bhatia, 2008). Heat stress is more detrimental especially when it occurs at reproductive and grain filling stages (Farooq *et al.*, 2011). Heat stress affects photosynthetic capacity of plants, causes metabolic limitations, promotes the production of oxidative reactive species (Wang *et al.*, 2011), reduces pollen tube development, causes pollen mortality (Saini *et al.*, 2010), encourages ethylene production thus increasing grain abortion and causes oxidative damage to the chloroplast resulting in minimum grain yield (Farooq *et al.*, 2011).

Terminal heat stress adversely affects grain weight and number of grains (Wollenweber *et al.*, 2003). At grain filling stage, reduction in grain weight by 1.5 mg per day occurs if temperature increases just 1°C above 15–20°C (Streck, 2005). Heat stress may enhance the rate of grain filling but reduces the grain filling period (Dias and Lidon, 2009).

Seed priming with allelopathic extracts have been reported to improve the stand establishment of wheat (Farooq *et al.*, 2008). At lower concentration, allelochemicals or secondary metabolites act as growth regulators while they have inhibitory effect at higher concentrations (Cheema *et al.*, 2013). Though improving resistance against terminal heat stress in wheat considering different traits has been done in the past but none is available with allelochemicals as potential heat suppressor in wheat. Further, a screening of wheat cultivars for heat resistance at reproductive stage especially based on their stay green character and grain-filling

rate is lacking. Thus, objective of this study is to examine the role of different allelochemicals against terminal heat stress in different wheat cultivars.

## Materials and Methods

The experiment was conducted in soil-filled pots placed in the glasshouse of the University of Agriculture, Faisalabad, Pakistan (latitude 31°N, longitude 73°E and altitude 184.4 masl), during 2013-2014. Seed of wheat cultivars viz., Mairaj-2008 and Faisalabad-2008 were obtained from Wheat Research Institute, AARI, Faisalabad, Pakistan. Individual pot was weighted and filled with 10 kg soil. Experiment was laid down following completely randomized design (CRD) in factorial arrangement with three replications. Crop was sown on 21<sup>st</sup> November, 2013. Seeds were subjected to osmopriming, while untreated seeds were taken as control. Seed of two wheat cultivars viz. Faisalabad-2008 and Meraj-2008 were primed with sorghum, sunflower, brassica and moringa water extract each at 3% either alone or in various combinations and were sown in 10 kg plastic pots (29 cm × 18 cm) placed in a net house under ambient conditions. In this case, soaking was done for 12 h in aerated solution (osmopriming) keeping seed to solution ratio 1:5 (w/v). Aeration was provided by aquarium pump. After removing from the respective solution, seeds were thoroughly rinsed with water and dried in forced air under shade till original weight.

Initially, 10 seeds were sown in each pot, which were thinned to six seedlings per pot after complete emergence. Heat stress was imposed at booting and anthesis by placing pots in glass canopies with temperature 4-5°C above than the ambient temperature until maturity. Pots under control treatment were maintained under well watered conditions at ambient temperature.. Chlorophyll contents were measured with the help of chlorophyll meter (CCM 200 plus). Data collected on all parameters was analyzed statistically by using MSTAT-C software on computer (Crop and Soil Sciences Department of Michigan University, USA). Least significance difference (LSD) test at 5% probability level was applied to compare the treatments means (Steel *et al.*, 1997).

## Results

Among the growth stages, grains per spike, 100-grain weight, biological yield, grain yield and HI, were recorded low when heat stress was imposed at anthesis stage, while all these

studied traits were lowest when heat stress was imposed at booting stage. However, chlorophyll *b* contents and TSP were highest when heat stress was imposed at booting (Table 1). Regarding cultivars, maximum grains per spike, 100-grain weight, grain yield, HI, chlorophyll *a* contents, chlorophyll *b* contents and TSP was recorded in Mairaj-2008, (Table 1). Combine application of sorghum and moringa water extracts through seed priming significantly improved the spike length, 100-grain weight, biological yield, grain yield, chlorophyll *a* contents, chlorophyll *b* contents and TSP than other allelopathic water extracts. Under this treatment, spike length, 100-grain weight, biological yield, grain yield, chlorophyll *a* contents and chlorophyll *b* contents were statistically similar with the well-watered treatment (Table 1). Likewise, combine application of sorghum and moringa water extract through seed priming was statistically similar with sole application of moringa water extract for spike length, 100-grain weight, biological yield, grain yield and chlorophyll *b* contents (Tables 1). Highest harvest index was recorded when seeds were primed with individual moringa water extract and that was statistically similarly with the combine application of sorghum and moringa water extract and well-watered treatment (Table 2)

The interaction of allelopathic water extract and growth stages showed that highest chlorophyll *a* and *b* contents were recorded for seed priming with sorghum and moringa water extract and heat imposition at booting stage; it is statistically similar with the sole application of moringa water extract application, and heat imposition at the same stage (Table 2). Interestingly, both allelopathic water extract treatments produced statistically similar chlorophyll *b* contents as they were observed in well-watered treatment at boot stage (Table 2). Likewise, maximum TSP accumulation was recorded for seed priming with combined sorghum and moringa water extracts with heat imposition either at booting or anthesis stage (Table 2). Two way interactions of wheat cultivars and growth stages showed that 100-grain weight was highest of both cultivars when heat stress was imposed at anthesis (Table 3). Highest leaf MDA contents were recorded in Faisalabad-2008 with heat imposition at booting stage and they were lowest in Mairaj-2008 with heat imposition at anthesis stage (Table 3).

## Discussion

Heat stress censored substantially the spike length when stress forced at early stage of

reproduction and it affected to laser extent at grain filling stage. The grain weight, number of grains per spike, biological and grain yield of wheat were severely affected under stress (Table 1). The reason for this reduction in spike length is the detrimental effects of heat on plant growth and development. Growth is comprised of three main processes i.e. cell division, cell elongation and differentiation (Farooq *et al.*, 2009).

In this study, heat stress reduce the wheat grain yield by enhancing the lipid peroxidation and damaging the biological structure of membrane, these adverse effects possibly reduce the grain weight and number under heat stress. Heat stress decreases the transpiration in plants by closing the stomata to maintain internal environment, the uptake of minerals from soils through roots, which are not only essential parts of important structural and functional cellular constituents but also important in osmotic adjustment. Though heat stress accelerates the growth but it reduces the phenology and the desired material is not prepared for new dividing cell at enhanced pace (Zahedi and Jenner, 2003). Heat stress between spike initiation and anthesis stage of wheat affects the number of grains. Heat stress accelerates the spike development and reduces the number of grains per spike. Elevated temperature causes shrinkage of grains due to ultra-structure of aleurone layer (Dias *et al.*, 2008). Plants have adaptive mechanism to cope with stresses including heat stress. Plants adopt certain morphological and physiological strategies to survive and produce economic yield under stress. In most cases, the plants enhance the accumulation of compatible solutes under stress which help in osmotic adjustment in plants. In this study, seed priming with allelopathic water extracts

improved the accumulation of proline, phenolics, GB, and reduced the MDA contents which resulted in better grain weight and stability of biological membranes under heat stress. Indeed, crop water extract contains phenolics and other secondary metabolites that act as growth promoters in low concentration (Farooq *et al.*, 2013). Sunflower water extract contains chlorogenic, caffeic, syringic, ferulic and vinillic acids (Ghafar *et al.*, 2001). Moringa water extract contains cytokinin that is important in shoot growth and flowering by direct shoot initiation, axillary branching and bud formation. Brassinolide is a natural plant hormone which promotes the growth of plants that is present in *Brassica napus* L. (Rehman *et al.*, 2013).

Seed priming of allelopathic crop water extracts improved the growth possibly through induction of certain metabolites that might have functioned as osmoprotectants or osmoregulators (secondary metabolites). Secondary metabolites protect the enzymes and helped in expression of higher photosynthetic rate (He *et al.*, 2009). The application of these allelopathic compounds increased the stay green character of the crop resultantly continues its growth and maintenance to longer period under stress. At exposure to heat stress at different growth stages, grain yield reduced by 13% over control (no stress). Detrimental effect of heat stress is compensated by application of allelopathic water extracts as grain yield improved by 11% when combined application of moringa and sorghum extracts was applied through seed priming.

In crux, combine application of moringa and sorghum water extracts may be adopted as useful strategy to enhance the performance of wheat exposed to terminal heat stress.

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**Table 1: Mean comparison of wheat cultivars, growth stages and water extracts for the morphological/yield related traits under terminal heat stress conditions**

WHEAT CULTIVARS	SL (CM)	GPS	HGW (G)	GY	BY (G PER POT)	HI (CM)	CHL A	CHL B	TSP
MAIRAJ-2008	13.12A	29.18A	2.69A	7.85 A	24.69	36.30B	0.212 A	0.267 A	3.81A
FAISALABAD-2008	12.98B	28.03B	2.62B	7.77 B	24.61	38.22A	0.204 B	0.251 B	3.72B
LSD (P 0.05)	0.10	1.02	0.02	0.03	Ns	1.71	5.18×10 <sup>-3</sup>	4.53×10 <sup>-3</sup>	0.03
GROWTH STAGES									
BOOTING STAGE	12.83	28.97 B	2.63 B	6.98B	24.41B	35.82B	0.213	0.313 A	3.81A
ANTHESIS STAGE	13.05	30.06 A	2.68A	8.65A	24.89A	38.69A	0.211	0.225 B	3.72B
LSD P<0.05	ns	1.11	0.02	0.23	0.42	1.79	ns	4.88×10 <sup>-3</sup>	0.03
WATER EXTRACTS									
CONTROL (NO STRESS)	13.58a	30.88	2.83a	8.25a	24.65a	34.79ab	0.263 a	0.313a	2.991
CONTROL (HEAT STRESS)	11.94g	27.48	2.45g	7.32d	25.00f	28.36b	0.152 h	0.191 k	3.35 k
HS+SWE (3%)	13.41a-c	30.67	2.75b	8.13b	24.66ab	33.88a-c	0.234 bc	0.291 bc	3.46 j
HS +BWE (3%)	12.58d-g	28.25	2.54d-f	7.47cd	21.70c-e	35.06b-d	0.203 f	0.223 ij	4.04cd
HS +SUWE (3%)	13.04a-f	28.70	2.63c	7.73a-d	24.36bc	31.87b-d	0.204 ef	0.262 ef	3.78gh
HS +MWE (3%)	13.51ab	30.60	2.83a	8.16a	21.03a	37.61a	0.241 b	0.302 ab	3.47 j
HS +SWE (3%) +BWE (3%)	12.78b-f	29.10	2.61c-e	7.67a-d	24.36bc	31.08b-d	0.192 fg	0.241 gh	3.90ef
HS +SWE (3%) +SUWE (3%)	12.68c-g	28.95	2.58c-e	7.50cd	23.03cd	31.81b-d	0.183 g	0.232 hi	3.96de
HS +SWE (3%) +MWE (3%)	13.57a	31.73	2.84a	8.24a	23.53a	34.83ab	0.251 a	0.314 a	4.39 a
HS +BWE (3%) +SNWE (3%)	12.47e-g	27.43	2.53ef	7.51b-d	24.02d-f	30.75b-d	0.192 fg	0.213 j	4.10bc
HS +BWE (3%) +MWE (3%)	13.25a-d	30.49	2.73b	8.09ac	24.35ab	32.89a-d	0.224 cd	0.281 cd	3.56 i
HS +SNWE (3%) +MWE (3%)	12.88a-f	29.18	2.61cd	7.68a-d	24.02cd	31.98b-d	0.203 f	0.252 fg	3.84fg
HS +SWE (3%) +BWE (3%) +SNWE (3%)	12.31fg	27.51	2.49fg	7.29d	24.68ef	29.03cd	0.204 f	0.241 gh	4.15 b
HS +SWE (3%) +BWE (3%) +MWE (3%)	13.13a-e	29.99	2.72b	8.08a-c	24.68a	32.38bd	0.213 de	0.274 de	3.77 gh
HS +SNWE (3%) +BWE (3%) +MWE (3%)	13.12a-e	30.36	2.71b	8.08a-c	24.68a	32.38b-d	0.214 de	0.271 de	3.69 h
LSD (P ≤ 0.05)	0.76	Ns	0.07	0.63	2.31	3.91	0.01	0.01	0.09

SWE= Sorghum water extract; SUNWE= Sunflower water extract; BWE= Brassica water extract; MWE=Moringa water extract; Spikelets per spike; GPS= Grains per spike; HGW= 100-grain weight; BY= Biological yield; GY= Grain yield; HI= Harvest index; TSP=Total Soluble Phenolics; Chl a= Chlorophyll a; Chl b= Chlorophyll b

**Table 2: Influence of seed priming with allelopathic water extracts on chlorophyll *a*, *b* and total soluble phenolics of wheat under terminal heat stress imposed at different growth stages.**

	CHLOROPHYLL A		CHLOROPHYLL B		TOTAL SOLUBLE PHENOLICS	
	Booting	Anthesis	Booting	Anthesis	Booting	Anthesis
CONTROL (NO STRESS)	1.251 ab	0.262 a	1.361 a	0.272 gh	3.01 q	2.98 q
CONTROL (HEAT STRESS)	1.163m	0.141o	1.232 lm	0.151 r	3.34 p	3.32 p
HS+SWE (3%)	1.222 e-g	0.241 b-d	1.334 bc	0.254 i-k	3.60 mn	3.35 p
HS +BWE (3%)	1.214 e-h	0.181 j-n	1.261 hi	0.183 pq	4.05 b-e	4.02 c-e
HS +SUWE (3%)	1.193 h-l	0.212 g-i	1.303 de	0.224 mn	3.86 g-j	3.70 k-m
HS +MWE (3%)	1.234 c-f	0.251 a-c	1.342 ab	0.261 h-j	3.52 no	3.42 op
HS +SWE (3%) +BWE (3%)	1.181 k-n	0.204h-k	1.282 e-g	0.202 no	3.95 e-g	3.86 g-j
HS +SWE (3%) +SUWE (3%)	1.171 l-m	0.191 i-m	1.271 f-h	0.192 op	3.99 d-f	3.94 e-h
HS +SWE (3%) +MWE (3%)	1.242 a-c	0.261 a-b	1.352 a	0.271 gh	4.45 a	4.34 a
HS +BWE (3%) +SNWE (3%)	1.212 e-h	0.172 mn	1.251 ij	0.172 qr	4.10 b-d	4.11 b-d
HS +BWE (3%) +MWE (3%)	1.214 e-h	0.231 c-e	1.324 c	0.243 j-l	3.70 k-m	3.42 op
HS +SNWE (3%) +MWE (3%)	1.191 h-m	0.203 g-j	1.291 ef	0.212mn	3.90 f-i	3.78 i-k
HS +SWE (3%) +BWE (3%) +SNWE (3%)	1.213 f-i	0.191 i-m	1.312 cd	0.172 qr	4.14 bc	4.17 b
HS +SWE (3%) +BWE (3%) +MWE (3%)	1.203 g-i	0.221 b-g	1.312 cd	0.233 k-m	3.81 h-k	3.72 k-m
HS +SNWE(3%) +BWE (3%) +MWE (3%)	1.202g-l	0.221 b-g	1.314 cd	0.234 lm	3.74 j-l	3.65 l-n
LSD (P ≤ 0.05) =	0.02		0.13		0.13	

HS = HEAT STRESS; SWE= SORGHUM WATER EXTRACT; BWE= BRASSICA WATER EXTRACT; SUNWE= SUNFLOWER WATER EXTRACT; MWE= MORINGA WATER EXTRACT; MEANS SHARING THE SAME LETTER, DO NOT DIFFER SIGNIFICANTLY AT P ≤ 0.05

**Table 3: Influence of heat stress at different growth stages on productive tillers, 100-grain weight, chlorophyll *a* and malondialdehyde contents of wheat cultivars**

Treatments	Mairaj-2008	Faisalabad-2008
	Productive tillers	
Booting	5.80 bc	6.31 a
Anthesis	5.95 b	5.53 c
Means sharing the same letter, do not differ significantly at p ≤ 0.05; lsd (p ≤ 0.05) = 0.32		
	100-grain weight	
Booting	2.64 b	2.57 c
Anthesis	2.69 a	2.69 a
Means sharing the same letter, do not differ significantly at p ≤ 0.05; lsd (p ≤ 0.05) = 0.0		
	Water potential	
Booting	1.13 c	1.27 a
Anthesis	1.14 c	1.20 b
Means sharing the same letter, do not differ significantly at p ≤ 0.05; lsd (p ≤ 0.05) = 0.04		
	Malondialdehyde	
Booting	9.12 ab	8.97 b
Anthesis	9.31 a	8.62 c
Means sharing the same letter, do not differ significantly at p ≤ 0.05; lsd (p ≤ 0.05) = 0.32		