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Response of foliar spray of synthetic compounds on wheat (*Triticum aestivum* L.) grain quality parameters during heat stress

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Abstract
Wheat (*Triticum aestivum* L.) plants in many locations of the world face terminal high temperature stress during the grain filling stage, which is a major contributor to a decrease in single seed weight and texture and, as a result, a reduction in grain quality and yield. To mitigate terminal heat stress of wheat strategies are followed like foliar application of nitrogenous compound, polyamines, sulfhydryl compounds and different osmoprotectants. The influence of foliar applied exogenous osmoprotectants on terminal heat stress in wheat (*Triticum aestivum* L.) was investigated in the current study under field conditions and it also alter quality of grain. Synthetic osmoprotectants shows varying results on different quality attributes. Like protein content increases in compared to control when SNP (400 µg/ml and 800 µg/ml) and DTT (50 ppm) applied at different concentration while for starch, moisture content, sedimentation value and hectolitre weight best results were observed in thiourea (20 mM and 40 mM) and KNO_3 (1%) treatment. Same as KNO_3 and DTT increases gluten content of the grains.

Keywords: Heat, mitigation, protein, starch, gluten, hectolitre weight, moisture, SNP, DTT, KNO_3, thiourea, sedimentation value

Introduction

Climate change and global warming have direct impact on agricultural production as well as on quality by increasing the frequency and magnitude of several stresses. Cereals are mostly affected under this changing environment of earth and had effect adverse effect on yield and quality. When it comes to wheat, it has been asserted that the flowering, fertilisation, and grain-filling stages are crucial, and even a small change in temperature during these stages can significantly reduce the output. Wheat under harsh terminal heat stress has serious impairments in the photosynthetic process and starch metabolism (Cao *et al.* 2019) [1]. Due to the incomplete transit of photosynthates, it causes denaturation/aggregation of critical enzymes linked with pathways and the creation of tiny starch granules in the endospermic tissue (Kumar *et al.* 2018) [5]. Due to domestication and its role as the world's main crop for staple foods, wheat is placed first among important cereals (Iqbal *et al.* 2021) [3]. It also contains a lot of vitamins, minerals, and fibre aside from the carbohydrates. In addition to disrupting the ratios of amylose to amylopectin in the endospermic tissues, it has been discovered that elevated environmental temperature significantly lowers the nutritional density of the grains. Under HS, even the cereals' storage proteins' quality is severely diminished (Kumar *et al.* 2018) [5].

Terminal heat stress affects the late-planted wheat crop during the anthesis and grain filling period (Hays *et al.* 2007) [2]. Farmers, particularly those who are growing wheat in the same field after harvesting of previous crop, are facing this problem very frequently due to the fact that they often get compelled to sow the wheat under delayed condition after harvesting of long duration previous crop and it also effects the quality of grain. When salt concentrations or temperatures are adverse, osmoprotectants help to increase osmotic pressure in the cytoplasm and can also stabilise proteins and membranes (Yancey, 1994) [9]. Mitigation of terminal heat stress improved by exogenous application of various synthetic compounds. These comprises of inorganic salts like potassium nitrate, sulfhydryl compounds like thiourea, thioglycolic acid (TGA), dithiothreitol (DTT), secondary metabolites like putrescine and salicylic acid and NO donor like sodium nitroprusside (SNP). Literature says that these compounds mitigate the adverse effects of high temperature stress in plants through various mechanisms like preventing the degradation of chlorophyll, reducing electrolytic leakage from cells etc. and it also increases the quality of wheat grain *viz.* protein, starch, gluten, moisture, sedimentation value and hectolitre weight.

Materials and Methods

Experimental design and establishment

To determine the role of Osmo-protectant in response to mitigate terminal heat stress and their effect on quality of wheat grain was conducted at Wheat Research Station, Junagadh Agricultural University, Junagadh (21.5 °N latitude and 70.5 °E longitudes) during *rabi* season, 2019-20. At the site of experiment soil was medium black and calcareous varying from 25 to 75 cm in depth. The annual temperature ranges from 15 °C in winter to 45 °C in summer with an average minimum of 17.9 °C and maximum of 34.8 °C.

Treatment description and experimental design

The experiment was laid out in a split-plot design with three replications. The dimension of each sub-plot was 2.5 m × 1.0 m. The wheat crop was sown on 18th December, 2019 which is considered as late-sown. In this study, two wheat cultivars, viz., GW 11 and GW 496 were taken as main-plot treatment and foliar application of synthetic compounds viz., thiourea (TU), sodium nitroprusside (SNP), potassium nitrate (KNO_3), thioglycolic acid (TGA), dithiothreitol (DTT), salicylic acid (SA) and Putrescine were randomized in the subplots with two different concentrations. These synthetic compounds applied at flowering and grain filling stages (Table 1).

Table 1: Treatment Details

Genotypes and Treatments	
Genotypes	
V_1: GW 11	V2: GW 496
Treatments	
T_1- Thiourea @ 20 mM	T_9- Salicylic acid @ 100 ppm
T_2- Thiourea @ 40 mM	T_{10}- Salicylic acid @ 200 ppm
T_3- KNO_3 @ 1%	T_{11}- TGA @ 200 ppm
T_4- KNO_3 @ 2%	T_{12}- TGA @ 250 ppm
T_5- SNP @ 400 µg/ml	T_{13}- Putrescine @ 4 mM
T_6- SNP @ 800 µg/ml	T_{14}- Putrescine @ 6 mM
T_7- DTT @ 25 ppm	T_{15}- No spray(control)
T_8- DTT @ 50 ppm	T_{16}- Spray of water

Quality characters of wheat such as protein (%), moisture (%), starch (%), gluten (%) and sedimentation value (ml) were determined by Infratec™ 1241 Grain Analyzer at Main Wheat Research Station, Vijaypur (Gujarat).

Results

The data regarding quality characters as influenced by genotype, treatments and their interaction effects are presented in Table 2 and Table 3.

Protein (%)

The data indicated that wheat genotypes and treatments and their interaction found significant influence on protein (%). The higher and lower protein (%) was found in the genotype GW-11 (12.6750%) and GW-496 (12.0313%) respectively. Among foliar treatments T_5 (SNP @ 400 µg/ml), T_6 (SNP @ 800 µg/ml) and T_8 (DTT @ 50 ppm) which was 12.45 (%); while significantly lowest protein (%) was recorded in no spray (control) T_{15} (12.30%). But in interactions highest protein (%) was found in genotype GW-11 with T_1 (Thiourea @ 20 mM) (13.00%) while lowest protein (%) was found in T_1 (Thiourea @ 20 mM) (11.80%) of GW-496.

Moisture %

Genotypes found non-significant influence on moisture (%) but treatments have significant effect on moisture (%). The highest moisture (%) was found in the treatment T_{10} (Salicylic acid @ 200 ppm) (11.25%); while significantly the lowest moisture (%) was recorded in T_2 (Thiourea @ 40 mM) and T_3 (KNO3 @ 1%) which was 10.70 (%). And for treatment and genotype interactions shows highest moisture (%) in genotype GW-496 with T_{10} (Salicylic acid @ 200 ppm) and T_{11} (TGA @ 200 ppm), which was 11.60 (%); while lowest moisture (%) found in T_2 (Thiourea @ 40 mM) (10.50%) of GW-11 and T_3 (KNO_3 @ 1%) (10.50%) of GW-496.

Starch (%)

The highest starch (%) was found in the treatment T_2 (Thiourea @ 40 mM) and T3 (KNO_3 @ 1%) which was 63.45 (%); while significantly the lowest starch (%) was recorded in T_{13} (Putrescine @ 4 mM) (62.80%). While for effect of genotype on starch (%) shows non-significant results but starch (%) showed significant interaction effect between genotype and treatments. Interaction was highest for starch (%) in genotype GW-496 with T_3 (KNO_3 @ 1%) (64.40%); while lowest starch (%) found in T_5 (SNP @ 400 µg/ml) (61.80%) of GW-11.

Gluten (%)

Effect of genotype on gluten (%) shows non-significant results but treatment shows significant results with highest gluten (%) was found in the treatment T_3 (KNO_3 @ 1%) and T_8 (DTT @ 50 ppm) which was 31.90 (%); while significantly the lowest gluten (%) was recorded in no spray (control) T_{15} (31.40%). Furthermore, interactions showed significant results and it was highest in genotype GW-11 with T_4 (KNO_3 @ 2%) (32.90%); while lowest gluten (%) found in T_1 (Thiourea @ 20 mM) and T_7 (DTT @ 25 ppm) which was 30.70 (%) of GW-496.

Table 2: Ameliorative response of different synthetic compounds on protein (%), moisture (%) and starch (%) of wheat genotypes under terminal heat stress

Genotypes and Treatments	Protein %	Moisture %	Starch %
Genotypes			
V_1: GW 11	12.6750	10.8438	62.7125
V_2: GW 496	12.0313	11.0250	63.5125
S.Em. ±	0.0505	0.0773	0.4463
C.D. at 5%	0.3072	NS	NS
C.V. %	2.8313	4.8990	4.8990
Treatments			
T_1- Thiourea @ 20 mM	12.40	10.80	63.25
T_2- Thiourea @ 40 mM	12.40	10.70	63.45
T_3- KNO_3 @ 1%	12.40	10.70	63.45
T_4- KNO_3 @ 2%	12.40	10.85	63.05
T_5- SNP @ 400 µg/ml	12.45	11.00	62.90
T_6- SNP @ 800 µg/ml	12.45	10.75	63.35
T_7- DTT @ 25 ppm	12.30	10.95	62.95
T_8- DTT @ 50 ppm	12.45	10.95	63.05
T_9- Salicylic acid @ 100 ppm	12.25	10.95	63.05
T_{10}- Salicylic acid @ 200 ppm	12.35	11.25	62.85
T_{11}- TGA @ 200 ppm	12.25	11.20	62.95
T_{12}- TGA @ 250 ppm	12.20	11.00	63.15
T_{13}- Putrescine @ 4 mM	12.35	11.15	62.80
T_{14}- Putrescine @ 6 mM	12.35	11.05	63.05
T_{15}- No spray(control)	12.30	10.85	63.25
T_{16}- Spray of water	12.35	10.80	63.25
S.Em. ±	0.0007	0.0011	0.0019
C.D. at 5%	0.0019	0.0030	0.054

Quality parameters	Protein %		Moisture %		Starch %	
Interaction (T x V)	**V1**	**V2**	**V1**	**V2**	**V1**	**V2**
T_1- Thiourea @ 20 mM	13.00	11.80	10.70	10.90	62.50	64.00
T_2- Thiourea @ 40 mM	12.70	12.10	10.50	10.90	63.40	63.50
T_3- KNO_3 @ 1%	12.90	11.90	10.90	10.50	62.50	64.40
T_4- KNO_3 @ 2%	12.90	11.90	10.90	10.80	62.10	64.00
T_5- SNP @ 400 µg/ml	12.90	12.00	11.20	10.80	61.80	64.00
T_6- SNP @ 800 µg/ml	12.80	12.10	10.80	10.70	62.80	63.90
T_7- DTT @ 25 ppm	12.70	11.90	11.00	10.90	62.30	63.60
T_8- DTT @ 50 ppm	12.80	12.10	11.10	10.80	62.20	63.90
T_9- Salicylic acid @ 100 ppm	12.40	12.10	10.90	11.00	62.70	63.40
T_{10}- Salicylic acid @ 200 ppm	12.60	12.10	10.90	11.60	62.80	62.90
T_{11}- TGA @ 200 ppm	12.60	11.90	10.80	11.60	63.00	62.90
T_{12}- TGA @ 250 ppm	12.40	12.00	10.70	11.30	63.20	63.10
T_{13}- Putrescine @ 4 mM	12.50	12.20	11.00	11.30	62.70	62.90
T_{14}- Putrescine @ 6 mM	12.60	12.10	10.70	11.40	63.10	63.00
T_{15}- No spray(control)	12.60	12.00	10.70	11.00	63.00	63.50
T_{16}- Spray of water	12.40	12.30	10.70	10.90	63.30	63.20
S. Em. ±	0.0010		0.0015		0.0027	
C.D. at 5%	0.0027		0.0042		0.0077	
C.V. %	0.0134		0.0238		0.0074	

Table 3: Ameliorative response of different synthetic compounds on gluten (%), sedimentation value (ml) and hectolitre weight (kg/hl) of wheat genotypes under terminal heat stress

Genotypes and Treatments	Gluten (%)	Sedimentation value (ml)	Hectoliter weight (kg/hl)
Genotypes			
V_1: GW 11	32.3563	45.1250	76.9938
V_2: GW 496	31.0188	41.8750	78.7188
S.Em. ±	0.2241	0.1780	0.3179
C.D. at 5%	NS	1.0829	NS
C.V. %	4.8993	2.8343	2.8289
Treatments			
T_1- Thiourea @ 20 mM	31.75	44.50	78.60
T_2- Thiourea @ 40 mM	31.65	46.00	77.75
T_3- KNO_3 @ 1%	31.90	46.00	77.55
T_4- KNO_3 @ 2%	31.75	43.50	76.05
T_5- SNP @ 400 µg/ml	31.65	44.00	78.50
T_6- SNP @ 800 µg/ml	31.70	45.00	77.60
T_7- DTT @ 25 ppm	31.55	42.50	77.90
T_8- DTT @ 50 ppm	31.90	42.50	78.40
T_9- Salicylic acid @ 100 ppm	31.55	42.50	78.15
T_{10}- Salicylic acid @ 200 ppm	31.75	43.00	78.55
T_{11}- TGA @ 200 ppm	31.70	41.50	77.65
T_{12}- TGA @ 250 ppm	31.65	42.00	78.45
T_{13}- Putrescine @ 4 mM	31.75	42.50	77.65
T_{14}- Putrescine @ 6 mM	31.60	43.50	77.30
T_{15}- No spray(control)	31.40	44.00	78.45
T_{16}- Spray of water	31.75	43.00	77.15
S.Em. ±	0.0012	0.0070	0.0032
C.D. at 5%	0.0034	0.0197	0.0090

Quality parameters	Gluten (%)		Sedimentation value (ml)		Hectolitre weight (kg/hl)	
Interaction (T x V)	**V1**	**V2**	**V1**	**V2**	**V1**	**V2**
T_1- Thiourea @ 20 mM	32.80	30.70	48.00	41.00	76.80	80.40
T_2- Thiourea @ 40 mM	32.40	30.90	49.00	43.00	77.00	78.50
T_3- KNO_3 @ 1%	32.70	31.10	47.00	45.00	76.80	78.30
T_4- KNO_3 @ 2%	32.90	30.60	45.00	42.00	75.60	76.50
T_5- SNP @ 400 µg/ml	32.50	30.80	45.00	43.00	77.70	79.30
T_6- SNP @ 800 µg/ml	32.20	31.20	45.00	45.00	76.90	78.30
T_7- DTT @ 25 ppm	32.40	30.70	44.00	41.00	77.50	78.30
T_8- DTT @ 50 ppm	32.70	31.10	43.00	42.00	77.70	79.10
T_9- Salicylic acid @ 100 ppm	31.90	31.20	43.00	42.00	77.80	78.50
T_{10}- Salicylic acid @ 200 ppm	32.60	30.90	45.00	41.00	77.70	79.40
T_{11}- TGA @ 200 ppm	32.40	31.00	44.00	39.00	76.30	79.00
T_{12}- TGA @ 250 ppm	32.00	31.30	44.00	40.00	77.00	79.90
T_{13}- Putrescine @ 4 mM	32.30	31.20	43.00	42.00	76.90	78.40
T_{14}- Putrescine @ 6 mM	32.10	31.10	46.00	41.00	76.40	78.20
T_{15}- No spray(control)	31.90	30.90	47.00	41.00	77.50	79.40
T_{16}- Spray of water	31.90	31.60	44.00	42.00	76.30	78.00
S.Em. ±	0.0017		0.0099		0.0045	
C.D. at 5%	0.0049		0.0289		0.0127	
C.V. %	0.0094		0.0393		0.0100	

Sedimentation value (ml)

The data indicated that wheat genotypes found significant influence on sedimentation value (ml). The higher and lower value was found in the genotype GW-11 (45.1250 ml) and GW-496 (41.8750 ml) respectively. And effect of treatments shows highest sedimentation value (ml) was in the treatment T_2 (Thiourea @ 40 mM) and T_3 (KNO_3 @ 1%) which was 46.00 (ml); while significantly the lowest sedimentation value (ml) was recorded in T_{11} (TGA @ 200 ppm) (41.50 ml); while interaction was higher for sedimentation value (ml) in genotype GW-11 with T_2 (Thiourea @ 40 mM) (49.00 ml); but lowest sedimentation value (ml) found in T_{11} (TGA @ 200 ppm) (39.00 ml) of GW-496.

Hectolitre weight (kg/hl)

The highest hectolitre weight (kg/hl) was found in the treatment T_1 (Thiourea @ 20 mM) (78.60 kg/hl); while significantly the lowest hectolitre weight (kg/hl) was recorded in T_4 (KNO_3 @ 2%) (76.05 kg/hl) while for genotype and treatment interaction it shows higher hectolitre weight (kg/hl) in genotype GW-496 with T_1 (Thiourea @ 20 mM) (80.40 kg/hl); while lowest hectolitre weight (kg/hl) found in T_4 (KNO_3 @ 2%) (75.60 kg/hl) of GW-11. But effect of genotype on hectolitre weight (kg/hl) shows non-significant results.

Discussion

The quality character of wheat was adversely affected by high temperatures. The variation of some quality traits it could be attributed to grain filling process that is harmfully affected by high temperatures and grains reaching to maturity stage before complete filling (Subedi *et al.,* 2007) [8]. Quality characters such as protein (%), gluten (%), and sedimentation value (ml) were higher in heat-tolerant variety (GW-11) than heat susceptible variety (GW-496). The favourable growing condition during timely sown crop induces more of an increase in nitrogen accumulation than in dry matter leading to higher protein content. Similar results have been reported earlier by Jat *et al.* (2013) [4]. But moisture (%), starch (%) and hectolitre weight (kg/hl) was more in GW-496 than GW-11.

The data shows that protein content of grain significantly influenced as a result of all synthetic compounds treatments as compared with control (no spray) and there was a significant difference between treatments and all the treatments increases the quality of grain over control. The highest value of grain protein content was obtained from foliar application of SNP @ 400 µg/ml, SNP @ 800 µg/ml, and DTT @ 50 ppm. This increase in protein content might be the outcome of increased concentration of nitrogen in the grain of wheat by foliar application of synthetic compounds which promote protein synthesis, similar findings was observed by Kousar *et al.* (2018) [6] and Sofy (2015) [7].

High moisture content leads to storage problems because it encourages fungal and insect problems, respiration and germination. However, moisture content in the growing crop is naturally high and only starts to decrease as the crop reaches maturity and the grains are drying. The moisture content of wheat grain was significantly lowest in treatment thiourea @ 40 mM and KNO_3 @ 1%. Slow settling (higher sedimentation values) indicates a high protein quantity and stronger gluten protein. Sedimentation values usually correlate positively with protein content and loaf volume. So, higher sedimentation value represents greater volume of baked bread. Starch content and sedimentation value were significantly highest in treatments thiourea @ 40 mM and KNO_3 @ 1%. Gluten as the miraculous net that holds bread together; it helps dough rise by trapping gas bubbles during fermentation and gives bread its unique texture for bread making gluten content is important. The gluten content of wheat grain was significantly highest in KNO_3 @ 1% and DTT @ 50 ppm. While hectolitre weight is good indication of grain-soundness and the higher the hectoliter weight of wheat, the higher the amount of dry matter and flour yield. Hectolitre weight was found significantly highest in thiourea @ 20 mM.

Conclusion

Based on current study it can be concluded that terminal heat stress adversely affects the grain quality of the both wheat genotypes. While susceptible genotype affected more by terminal heat stress but tolerance genotype not affected as much, so that protein (%), gluten (%) and sedimentation value were higher in heat tolerant genotype (GW-11). When different synthetic compounds applied on plant for heat stress mitigation it also alters grain quality like protein (%) and gluten (%) were recorded highest in (SNP, DTT) and (KNO_3, DTT) respectively; while starch and sedimentation value were recorded highest in thiourea and KNO_3. For hectolitre weight highest value was recorded in thiourea treatment. So based on all the observation we can revealed that these osmoprotectants not only increases the heat stress mitigation but also beneficial to plants for quality increment.

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