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HEAT STRESS INDUCED MALE STERILITY OF WHEAT VARIETIES AT ANTHESIS IN RELATION TO HIGH TEMPERATURE

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ABSTRACT

To elucidate the effect of high temperature on male sterility or pollen sterility, we have established an experiment with 28 common wheat (Triticum aestivum L.) varieties under two environmental conditions (normal and heat stressed) during winter season of 2010-11 and 2011-12 at Pusa farm, Bihar. Cytological observations revealed that pollen viability in plants exposed to high temperatures was lower than those of normal plants. Pollen sterility was significantly influenced by seasons, varieties and temperature and significant varietal differences were recorded in both seasons. The higher number of male sterile wheat varieties were found in heat stressed than the normal condition in both seasons. Two check varieties used namely Sonalika and HD 2733 was performed well at heat stressed conditions than other varieties. Varieties, Raj 3765 (65.48% and 67.11%), HI 1539 (64.34% and 65.51%) and YL 914 (64.28% and 64.96%) showed maximum pollen sterility and minimum in KYP 637 (12.64%), Iepaca rabe (16.40%) and Sonalika (21.45%). Some varieties (Raj 3765, HI 1539 and VL 914) were more sensitive to heat stress and showed higher male sterility. The results indicated that the increase in sterility percentage of pollen grains was directly proportional to increasing temperature.

Key words: Wheat, pollen sterility, high temperature.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crop, which is cultivated over a wide range of climatic conditions and it is very sensitive crop in relation to temperature (Barnabas *et al.,* 2008). High temperature after drought stress is major abiotic factor which limits physiological processes, crop productivity globally in several ways, deviation from normal temperature or the periodicity of day/night around reproductive stages of crop affecting the seed yield and seed quality (Murthy *et al.,* 2008). Terminal heat stress frequently limits wheat production worldwide and one of the reasons is poor seed setting at higher temperature and higher humidity. This may be due to abnormal microsporogenesis and microgametogenesis at increased temperature. In cereal like wheat higher temperature during meiosis mainly affects the pollen functionality (Stone, 2001). Low temperature induced pollen sterility is non pronounced in case of wheat and is less sensitive at lower temperature as compared to other crop like rice (Endo *et al.*, 2009). By sudden increasing of 4-7 ^{0}C corresponding minimal temperature, creates ‘heat stress’ on crops which leads to affecting timing and rate of physiological process, respiration and accelerated maturity. The development of male reproductive during young microspore stage is very sensitive in relation to abiotic stress in higher plant, particularly; temperature (high or low) stress results in problem in seed setting due to male sterility in wheat (Saini *et al.*, 1984). In wheat plant, high temperatures reduce biomass and adversely affect the crop reproductive efforts (Polowick and Sawhney, 1988) particularly during flowering and fertilization. The heat stress physiological affects fertility traits viz. male sterility, number of spikelet fertility, number of grain per ear head and number of spike per plant. Most of the studies focused on heat stress especially in or around anthesis stage and studies ensure that the development of male reproductive is prone to be more damage than female (Monterroso and Wien, 1990). Heat stress at or just before anthesis reduced pollen fertility, number of grain per ear head and number of spikelet fertility of wheat lines, but did not change number of spike per plant and in addition pre-fertilization stages are more sensitive to heat stress than post-fertilization stages of the development (Gross and Kigel, 1994). Heat stress before anthesis and after anthesis decreases crop yield (Wardlaw *et al.*, 1989) and rate of grain filling duration (GFD) (Wardlaw and Moncur, 1995), so there is need to develop more sustainable and eco-friendly approaches for better performances in stressed conditions. The present investigation was carried out to identify specific changes in pollen morphology leading crop sterility under heat stress which may account for at least some of the differences between yields of heat-tolerant and heat-sensitive wheat genotypes.

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MATERIALS AND METHODS

Plant materials: The experimental materials consisted of 28 common wheat (*Triticum aestivum* L.) varieties (Table 3), among them HD 2733, Sonalika, Pusa gold and PBW 343 often serve as standards in wheat breeding programmes for developing superior genetic material in many parts of India and Nepal. The varieties were sown during winter season of 2010-11 and 2011-12 and normal agronomical practices were followed during crop growth period to till maturity. Monthly meteorological data during crop growth period belonging to two experimental years for plant growth were recorded (Table 1).

Field experiments: The experiments were conducted at Rajendra Agricultural University, Pusa farm which is geographically situated in Indo-Gangatic plain at a North latitude of 25.984, East longitude of 85.674 and altitude of 52.20 m above mean sea level during 2010-11 and 2011-12 in field as normal and in pot followed by polyhouse as heat stressed condition using statistical design i.e. randomize block design and completely randomize design with three replications, respectively. The wheat plants were grown in pots under normal condition was transferred to polyhouse and subjected to stress treatment with high temperature during the time of anthesis. After two to three days in the high temperature environment, the anthers were collected from some of the treated plants, while other plants leave for seed maturity. The temperature and relative humidity of stressed condition were relatively higher (5-7^{0}C and 1-3%) than the normal condition in both seasons.

Cytological analysis: For sectioning, anthers were fixed in fixative, absolute alcohol and glacial acetic acid (3:1) (Nassar *et al.*, 2000). To estimate pollen viability anthers before anthesis were stained with 2% acetocarmine and observed under Olympus compound microscope (Devasirvatham *et al.*, 2013). All the data were recorded from randomly selected five plants from each replication under both conditions using 45 spikelets for each treatment for determination of pollen viability by their stainability pattern. The determination of pollen sterility was calculated as suggested by Waheed *et al.* (2013).

Pollen sterility (%)

$$= \frac{\text{Number of unstained pollen grains}}{\text{Total number of pollen grains}} \text{X } 100$$

On the basis of stainability the data were scored for (i) stained for fertile and (ii) sterile for unstained or lightly stained pollen grains. The data were statistically evaluated by the student's T-test and analysis of variance (ANOVA) by using SPSS for Windows ver. 16.0.

RESULTS AND DISCUSSION

To investigate the effects of high temperatures on male sterility or pollen sterility, wheat plants were subjected to high temperatures stress at anthesis or flowering stage. The result clearly indicated that high temperature during the time of anthesis significantly affected sterility of the pollen grains and spikelet fertility is completely lost at this stage. Wheat plants were exposed to high temperatures for 2-5 days, and were subsequently transferred back to a normal condition until seed maturity. In high temperature-treated plants, the spikelets had a significant amount of pollen grains with reddish purple stained. We recorded a notable difference in the numbers of pollen grains between high temperature-stressed and normal plants.

Table 1: Monthly minimum, maximum and average temperature, rainfall and relative humidity

Months		**Normal (field)**					**Stressed (polyhouse)**			
		Temp. (^{0}C)		**RH (%)**		**Rainfall (mm)**	**Temp. (^{0}C)**		**RH (%)**	
		2010-11	**2011-12**	**2010-11**	**2011-12**		**2010-11**	**2011-12**	**2010-11**	**2011-12**
December	Max	19.3	20.1	89.6	88.2	Nil	24.5	25	90.7	91.6
	Min	11	11.2				11.7	11.9		
	Avg	14.8	15.1				18.6	19.2		
January	Max	20.5	19.9	89.4	89.1	5.5	26.3	26.5	90.1	91
	Min	8.9	9				10.3	11		
	Avg	13	13.2				18.3	19.1		
February	Max	25	25.8	89	88.9	Nil	30.2	29.8	89.6	90.1
	Min	10.3	11				10.3	11.4		
	Avg	17	17.9				20.3	21.3		
March	Max	30.1	30.3	84.3	86.9	0.9	36.5	37	84.5	84
	Min	12.7	13				13.6	12.9		
	Avg	17.9	18.5				25	26.1		
April	Max	35.5	36	78.6	77.2	1.7	42.5	43.1	79.6	78.8
	Min	20.7	19.7				22	23		
	Avg	26.3	27				32.4	33.1		

Under stressed condition the higher percentage of pollen sterility was recorded in case of varieties, Raj 3765 (65.48% and 67.11%) followed by HI 1539 (64.34% and 65.51%) and YL 914 (64.28% and 64.96%) than the normal condition, that may be due to premature abortion of tapetum cells following high temperature stress (Sakata, 2008) while, minimum percentage of pollen sterility was observed in case of varieties Sonalika and Iepaca rabe (28.06% and 29.73%) and was better performed in both conditions normal as well as stressed. Only variety SAWSN 3106 showed higher percentage of pollen sterility (47.66% and 48.91%) in normal as far as both years is concerned. Minimum percentage of pollen sterility was observed in case of KYP 637 (12.64%), Iepaca rabe (16.40%) and Sonalika (21.45%) varieties in normal condition in both years. In some varieties like SAWSN 3106, Raj 3765, PBW 343 and VL 914 where anthesis took place earlier then the control and showed higher pollen sterility percentage in both years (2010-11 and 2011-12) as compared to other varieties. Similar observations were recorded by Dorion *et al.* (1996). Mean of varieties showed they were more fertile may be due to relatively low temperature and low relative humidity. Our result show that period of high temperature imposed during stress significantly reduce the sterility of wheat varieties, however sensitive genotype encountered more sever reduction.

Most of the varieties except Sonalika and Iepaca rabe suffer from sterility as high (>36%) in such condition. In all wheat varieties the sterility of pollen grains in normal was significantly (.01 and .05) lower than the stressed. The analysis of variance (Table 2) revealed that highly significant differences between genotypes and seasons were obtained. These results were accordance to findings of Wheeler *et al.* (2000) in which severe yield reduction due to pollen sterilization was observed during application of transitory high temperature near or around to the flowering stages. These results indicate that wheat varieties respond differently under different environmental conditions necessitating identification of better suited varieties for a particular environment. Similar results were also observed by earlier workers (Menshawy, 2007).

Table 2: Mean squares for pollen sterility measured in 28 wheat cultivars for 2-years

Source of variation	**Environment**	**2010-11**	**2011-12**
Mean squares			
Treatment	Normal	175.440**	172.008**
	Stressed	372.249**	355.335**
Error	Normal	6.721	7.164
	Stressed	12.680	12.212

Two check varieties (Sonalika and HD 2733), also had the highest pollen fertility under field condition than the other varieties except KYP 637 and Iepaca rabe. It is apparent (Table 3) that the pollen grains were more sterile in stressed condition in both years which may be due to increasing temperature and relative humidity (form December to April months) than normal in almost all varieties. Harsant *et al.* (2013) found that with rise in temperature, pollen sterility percentage in anther also increased. However Subedi *et al.* (1998) reported that low temperature near heading stage prolonged the time to anthesis and led to greater chance of sterility in wheat cultivars. In some cases, temperature, relative humidity and photoperiod also affect the stability of cytoplasmic male sterile lines in case of rice (Ali *et al.*, 1998). In addition, season and varieties interactions were also significant due to different varietal response to climatic changes by the years.

It could be concluded that the high temperatures around anthesis affected most wheat cultivars due to disruption of tapetal functions that is required for pollen either adhere to stigma or for germination on stigma. However, the varietal differences to tolerate heat stress were observed with increasing of months and years. Check varieties were better performed in varying environment and season and some varieties Raj 3765, HI 1539 and VL 914 were more sensitive to heat stress and showed higher male sterility, so there is need to improve for better performance.

Table 3: Mean performance and student t-test (test value =0) of pollen sterility in normal and stress condition

Varieties	2010-11 &2011-12(Normal)				2010-11 &2011-12 (Stressed)			
	t	Mean (%)	t	Mean (%)	t	Mean (%)	t	Mean (%)
VL 914	49.09	35.5	67.52	36.4	60.76	64.2	99.76	64.9
Mons alds	13.65	24.7	13.91	25.3	10.15	54.6	10.75	56.6
SAWSN 3101	13.38	26.5	13.88	28.1	12.44	56.6	13.98	58.0
Pusa gold	19.67	31.6	17.08	31.8	28.97	42.2	26.00	43.4
HD 2733	65.28	33.7	39.68	34.4	22.96	38.4	26.28	40.4
Halna	27.72	33.5	33.41	35.1	78.76	61.3	59.20	63.5
AKAW 4008	9.89	32.4	10.47	33.6	37.92	41.9	46.77	45.4
HD 2285	25.96	24.0	23.79	25.2	10.97	36.7	10.53	39.1
AKAW 4189-3	8.01	23.2	8.90	24.7	20.25	36.9	18.88	37.6
CUO/79/Prulla	12.94	26.7	12.62	28.0	55.34	40.2	67.65	41.8
K 0583	23.25	21.6	31.89	23.1	16.06	41.8	16.57	43.4
Sonalika	18.37	20.7	21.62	21.4	31.19	25.5	23.67	28.0
C 306	10.64	24.4	10.09	25.6	28.59	49.4	27.89	50.6
HD 2888	57.66	22.5	29.43	23.6	18.82	51.7	20.43	53.0
SAWSN 3010	17.60	26.8	18.73	27.8	83.73	63.1	70.15	63.9
HD 2824	23.32	21.3	31.44	22.2	27.33	56.4	29.18	57.3
KYP 637	9.01	12.6	12.35	14.3	49.55	45.3	45.09	46.7
Euganda 3	18.24	26.8	18.69	27.9	53.85	54.0	57.67	55.1
Raj 3765	45.13	45.2	57.15	45.7	93.00	65.4	63.15	67.1
SAWSN 3106	56.61	47.6	30.75	48.9	94.26	59.0	95.74	60.6
HI 1539	101.38	34.8	63.98	36.8	24.95	64.3	29.48	65.5
Iepaca rabe	17.25	16.4	16.26	17.5	15.65	27.5	17.50	29.7
KYP 516	20.88	28.0	18.58	29.6	79.05	58.3	51.38	59.7
Kauz/AA/Kauz	32.49	31.2	30.92	32.0	61.70	37.6	60.93	38.8
VL 514	24.56	31.8	26.61	33.1	61.62	46.8	54.07	47.7
F5-995	108.82	27.7	50.53	28.9	41.09	50.7	58.48	51.7
AKAW 8189-3	19.11	26.3	18.05	27.4	21.05	57.6	22.28	58.9
PBW 343	101.29	36.1	70.15	37.9	68.06	58.8	100.37	59.8
Mean		28.3		29.5		49.5		51.0

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