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Genetic Studies on *Triticum timopheevi* Based Cytoplasmic Genetic Male Sterility (CGMS) System in Relation to Hybrid Seed Production in Wheat (*T. aestivum* L.).

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ABSTRACT

Forty-five F_1 hybrids were produced by crossing fifteen elite wheat cultivars used as pollen donor/ male parents with three *T. timopheevi* based male sterile lines used as female parents. Self-fertility status of these F_1 hybrids was studied. Two indexes, viz., number and percentage of seed development in bagged spikes of these forty-five F_1 hybrids, bagged before starting of anthesis, were used to determine the degree of fertility restored in the F_1 hybrids. Based upon a scale devised using the second index, i.e. percentage of seed development in bagged spikes, corresponding pollen parents were identified and classified as maintainer, partial maintainer, partial restorer or restorer. In the present study, WH 542 and KRL 99 were identified as partial restorers for CMS line WH 416A. Agronomic performances of the forty-five hybrids produced were also studied for days to heading, plant height, number of tillers per plant, number of florets per spike. F_1 cross-combinations, WH 416A X CBW 38, WH 416A X DBW 39, WH 416A X DBW 50 and WH 416A X NIAW 1188 were found remarkable for their agronomic performance.

***Keywords*:** CMS, hybrid seed production, *Triticum timopheevi*, fertility restoration.

Introduction

Wheat is one of the most important cereal crops of the world. It's wide adaptability to the different agro-climatic conditions and unique property of wheat flour and dough which allow its processing into a range of food products (Kant et al., 2014). With the increase in global population it is becoming of utter importance to produce sufficient amount of food grains for ensuring food security. Since the scope of area expansion is very limited, vertical expansion of productivity seem to be only solution to achieve our target of increased production of food grains. In this endeavour, exploitation of heterosis in crop plants seems to provide the needful respite. Although hybrid technology has remained very successful for several crops like rice (Singh et al., 2015), maize (Arya et al., 2015), pearl millet (Kumar et al., 2013) etc. but, successful exploitation of heterosis/ hybrid vigour on commercial basis is still awaited in important crop like wheat.

Since the autogamous nature of reproduction in wheat makes hybrid seed production a challenging task, it continues to be a major constraint in commercial level production of hybrid seed. Several types of pollination control strategies including genetic, cytoplasmic-genetic and chemically induced male sterility were tested for their use in hybrid seed production of wheat at commercial level.

With the discovery of cytoplasmic genetic male sterility (CGMS) in wheat by Kihara in 1951, hybrid wheat has attracted considerable research efforts

spanning several decades (reviewed by Blouet et al., 1999). Later, Wilson and Ross (1962) establish the existence of usable male sterility in wheat from the interaction of common wheat nucleus with *Triticum timopheevi* cytoplasm. Since then much work has been done using this species for the development of commercial hybrid. With the progress of research on cytoplasmic male sterility, male sterile cytoplasms of around 35 species were transferred to bread wheat genetic background. Among these, complete to partial sterility was induced by as many as, 20 species (Adugna et al., 2004). To date *Triticum timopheevi* cytoplasm (identified by Wilson & Ross, 1962) is considered the most effective one and has been used commercially (Mukai & Tsunewaki 1979), although to a limited extent. *T. timopheevi* zhuk. cytoplasm (also known as G cytoplasm) is now most widely used, because compared to other sources of cytoplasmic male sterility *T. timopheevi* provides several comparative advantages for it's uses in development of CGMS lines to be utilized for development of hybrid wheat. Most important of all is it's superior stability (Wilson & Ross, 1962) and high survival due to it's existence in nature for a very long time. Besides, it is unlikely for this cytoplasm to become vulnerable to biotic or abiotic stress compared to other sources of male sterile cytoplasms. An advantage of no ill effect of cytoplasm on agronomic performances of the hybrid also makes it better suited for it's uses in development of CGMS lines (Sage, 1976).

Wilson and Ross (1962) reported that the interaction of *T. timopheevi* zhuk. cytoplasm and the T. aestivum nucleus resulted in a complete and stable male sterility. *T. timopheevi* cytoplasm had no apparent adverse effects on plant growth and development. Nearly all hybrid wheat breeding motioned to be based on the *T. timopheevi* and its wide spread use has been largely the result of its apparently neutral effect on agronomic and quality characters. Only drawback is that it requires more than one gene for fertility restoration (Zhao, 1988).

Materials and Methods

The study was executed in two separate experiments. The first experiment dealt with detection of ability of male parents for their ability of fertility restoration and classification of fifteen male parents into four categories *viz.,* maintainer, partial maintainer, partial restorer or restorer, based on the ability of these parents to induce development of seed in selfed spikes of corresponding F_1 hybrids. This experiment was carried out during the crop season, November, 2011-April, 2012. These forty-five hybrids were produced during crop season, November, 2010- April, 2011. The second experiment that was aimed at studying agronomic characteristics of the F_1 hybrids was carried out during November 2011-April, 2012. The hybrids were produced and both the experiments were performed at the experimental farm area of CCS Haryana Agricultural University, Hisar (29.1700° N, 75.7200° E), India, in sandy loam soil under normally grown, irrigated conditions.

For the first experiment forty-five F_1 hybrids were produced by crossing fifteen elite wheat cultivars used as pollen donor/ male parents and three *T. timopheevi* based male sterile lines used as female parents. Fifteen male parents were WH 147, WH 542, HD 2967, CBW 38, DBW 31, DBW 39, KRL 99, KRL 284, HD 2964, NIAW 1188, MP 1194, LB-PY 05-02, LBPY 05-04, RWP 206-33 and DBW 50. Three CMS lines used were WH 416A, Atilla A and PBW 445A. For the second experiment these forty-five F_1 hybrids were used as experimental materials.

Forty-five hybrids were grown in randomized block design with three replications in normal (timely sown) environment. Each genotype was grown in a plot of two meter long single row. The rows were placed 30 cm apart keeping 10 cm plant to plant distance. The other packages of practices were the same as recommended for normal environment. The fifteen male parents WH 147, WH 542, HD 2967, CBW 38, DBW 31, DBW 39, KRL 99, KRL 284, HD 2964, NIAW 1188, MP 1194, LB-PY 05-02, LBPY 05-04, RWP 206-33 and DBW 50 were grown in randomized block design with three replications in normal environment. Each genotype was grown in a plot of two meter long single row. The row was placed 30 cm apart keeping 10 cm plant to plant distance. The other packages of practices were the same as recommended for normal environment.

To produce F_1 hybrids, spikes were first covered with butter paper bags before their complete emergence from the leaf sheath of the flag leaf. The bags were then removed, lemma and palea of two lateral florets were trimmed, central florets were removed, and ¼ portion of the tip of spikes were removed. After these operations, the spikes were again covered with butter paper bags for ensuring parental identity of the hybrid seeds. After 3-4 days bags were opened and pollens from inflorescence of respective male parents were applied on them. After application of pollens externally, bags were again sealed to restrict entry of pollens from any undesired sources.

Five competitive plants from each F_1 hybrids were taken randomly from each replication and observations were recorded and mean values of each replication were calculated for number of grains per spike (bagged),

percentage of seed set (bagged), percentage of seed set (unbagged) and outcrossing (%). Percentages of seed set in bagged spikes were calculated as number of seeds setting per hundred bagged florets. Outcrossing percentage was calculated as the number of seeds developed in open spike per hundred florets, excluding the number of seeds developed in selfed spike of the same F_1 hybrid plant. Agronomic performance of the F_1 hybrids were also studied and observations were recorded for the following parameters *viz.,* days to heading, plant height, number of tillers per plant, number of florets per spike.

The mean values for these observations in each replication were calculated and subjected to analysis of variance (ANOVA) under Randomized Block Design (RBD) using statistical software OPSTAT (developed by Dr. O. P. Sheoran). For the purpose of suitability of applying ANOVA, to the percentage data, square root transformation and arc sine transformation was applied. Genetic variability for these agronomic traits was worked out in terms of range, mean, genotypic and phenotypic coefficient of variation (GCV and PCV), broad sense heritability, genetic advance under selection and genetic advance under selection as percentage of character mean.

Results and Discussion

Data recorded in these two experiments are presented in Table 1 and Table 2 respectively for agronomic parameters and fertility restoration related parameters. Sum of square values from the analysis of variance for agronomic parameters of the hybrids are presented in table 3, which clearly revealed that all these characters varied significantly among the hybrids. Genotypic differences were significant for days to heading, plant height, number of tillers per plant and number of florets per spike.

Although the character days to heading had significant variability as tested in ANOVA, it showed a narrow range of 100.7 days to 103.07 days among the hybrids. This character showed low broad sense heritability and thus genetic advance under selection was less for this trait. Similarly, plant height also showed low broad sense heritability and genetic advance under selection. Plant heights of the hybrids were mostly around 100 cm, and it ranged between 90.4 cm to 146.4 cm. Number of tillers per plant and number of florets per spike showed high broad sense heritability and thus had high genetic advance under selection. This implies that there are scopes for further improvements for these traits and can be achieved by simple selection. Numbers of tillers per plants ranged from 16.06 to 35.06, while number of florets per spike among the hybrids ranged from 63.2 to 76.46. For the agronomic performances studied F_1 cross-combinations WH 416A X CBW 38, WH 416A X DBW 39, WH 416A X DBW 50 and WH 416A X NIAW 1188 was remarkable for their agronomic performance.

In the present study, floret fertility in terms of seed set under selfed condition was considered as an index to classify pollen parents among test crosses, into different categories. During this present study, it was fund that WH 542 and KRL 99 restored fertility but only partially, for CMS line WH 416A. Percentages of seed development in selfed spikes of test cross progenies from the crosses involving WH 542 and KRL 99 as pollen donor were 29.87% and 20.78% respectively. This difference between percentages of seed development in selfed spikes clearly indicates that there was difference between the two male parents for their ability of fertility restoration. The occurrence of fertility restoration to different degrees suggests that the importance and need for selection of satisfactory restorer lines for hybrids seed production, and at the same time indicates that fertility restoration in this case is not under control of a single dominant gene. Several research workers working on genetics of fertility restoration of wheat with sterile cytoplasmic background of *T. timopheevi* also reached similar conclusion (Mukai and Tsunwaki, 1979; Ikaguchi et al*.,* 1999; Tsunewaki et al*.,* 2002).

Another interesting point noted during the present study regarding the effect of fertility restorers. Although all the three CMS lines were derived from *T. timopheevi* cytoplasmic background, WH 542 and KRL99 showed partial fertility restoration against only one CMS line WH 416A. This unexpected result of fertility restoration only in WH 416A may be due to certain changes in it's organeller genome. Molecular studies related to endosymbiotic co-evolution of organeller genomes and nuclear genome revealed that several retrograde and anterograde regulatory systems and cross talks between several biochemical pathways in response to the external stimuli ultimately results in expression of male sterility and fertility restoration (Linke et al*.,* 2005; Chase et al*.,* 2006). Although determination of exact reason behind this weird behaviour was beyond the scope of present investigation, comparative analysis of mt DNA of these three lines and dissection of the mechanism of fertility restoration at molecular level might suggest probable reason.

Lack of satisfactory restoration is the major limiting factor to the development of wheat hybrids, which emphasizes the need to screen wheat germplasm in order

to identify effective restorers. In the present investigation WH 542 and KRL 99 behaved as partial fertility restorers, whereas rest thirteen behaved as sterility maintainer for CMS lines WH 416A, while all fifteen pollen parents behaved as sterility maintainers for CMS lines Atilla A and PBW 445A. Comparative representation of fertility restoration and sterility maintenance in the fifteen male parents are presented in Table 4.

Figure 1. Development of F_1 hybrid seeds. Florets were trimmed for easy access of externally applied pollens to the stigma. Photograph was taken after removing butter paper bag, used to ensure parental identity of the hybrid seeds produced

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Table 1. Mean values for agronomic traits of F_1 hybrids

Male parents	CMS lines											
	WH 416A				Atilla A				PBW 445A			
	DTH	PH	NTP	NFS	DTH	PH	NTP	NFS	DTH	PH	NTP	NFS
WH 147	101.8	97.7	34.2	65.5	102.4	103.1	22.1	65.3	102.0	95.4	24.9	66.3
WH 542	103.1	95.7	24.3	65.1	101.6	125.6	21.3	63.5	102.6	90.4	26.9	64.5
HD 2967	100.9	106.8	24.4	65.1	102.8	105.6	21.7	63.4	101.0	113.4	27.9	65.3
CBW 38	100.7	101.8	27.9	63.6	101.0	109.4	26.3	63.9	102.7	130.3	18.1	63.2
DBW 31	102.6	113.0	23.5	63.5	102.4	106.7	24.5	65.5	102.6	105.6	32.0	65.3
DBW 39	100.7	110.5	21.0	64.7	102.6	144.8	18.3	65.3	102.7	113.3	16.2	66.3
KRL 99	101.0	108.0	25.3	63.5	102.4	97.88	25.0	64.0	102.6	107.6	25.8	64.5
KRL 284	100.8	103.6	18.9	64.4	101.8	111.5	23.7	66.3	102.4	131.7	30.7	64.0
HD 2964	101.4	115.5	25.5	63.4	102.8	105.0	26.6	66.4	102.7	106.3	18.7	65.5
NIAW 1188	102.5	119.1	26.7	76.5	102.7	103.4	31.1	65.3	102.8	146.4	27.9	65.3
MP 1194	102.8	124.0	21.5	65.3	102.4	107.6	29.6	66.5	102.4	114.4	26.3	63.40
LB-PY 05-02	102.2	139.5	24.3	73.2	102.8	120.6	16.3	63.4	101.0	141.8	18.1	63.5
LBPY 05-04	101.8	134.6	31.9	64.5	101.8	136.0	19.1	64.0	102.4	124.7	21.1	65.3
RWP 206-33	102.0	105.9	22.2	66.3	101.0	98.2	17.5	65.5	102.4	115.4	24.1	65.3
DBW 50	102.7	101.0	35.1	64.0	102.2	106.3	16.1	63.2	102.7	111.6	23.4	64.4
CD at 5%	1.01	12.67	2.01	0.39	1.01	12.67	2.01	0.39	1.01	12.67	2.01	0.39

DTH = Days to heading, PH = Plant height (cm), NTP = Numbers of tillers per plant, NFS = Number of florets per spike

Table 2. Mean values for fertility restoration related traits of F_1 hybrids

Male parents	CMS lines											
	WH 416A				Atilla A				PBW 445A			
	NGS (UB)	NGS (B)	SSP (B)	SSP (UB)	NGS (UB)	NGS (B)	SSP (B)	SSP (UB)	NGS (UB)	NGS (B)	SSP (B)	SSP (UB)
WH 147	12.93	0.56	0.86	19.74	17.47	0.00	0.00	26.76	17.73	0.00	0.00	26.73
WH 542	28.27	19.46	29.87	43.40	11.80	0.00	0.00	18.56	12.20	0.00	0.00	18.91
HD 2967	19.33	0.00	0.00	29.71	14.00	0.25	0.39	22.08	14.03	0.00	0.00	21.51
CBW 38	18.80	0.00	0.00	29.57	10.33	0.00	0.00	16.20	21.93	0.00	0.00	34.72
DBW 31	1.60	0.00	0.00	2.52	15.33	0.00	0.00	23.43	2.07	0.00	0.00	3.17
DBW 39	9.73	0.00	0.00	15.05	16.40	1.05	1.61	25.15	10.40	0.00	0.00	15.69
KRL 99	26.67	13.20	20.78	41.99	17.13	0.00	0.00	26.77	18.33	0.00	0.00	28.41
KRL 284	12.13	0.00	0.00	18.84	10.13	0.00	0.00	15.28	16.47	0.00	0.00	25.74
HD 2964	15.07	0.00	0.00	23.77	13.93	0.00	0.00	20.98	14.33	0.00	0.00	21.89
NIAW 1188	11.07	0.78	1.02	14.47	14.47	0.00	0.00	22.16	17.93	0.00	0.00	27.50
MP 1194	13.73	0.00	0.00	21.04	18.93	0.00	0.00	28.47	19.60	0.00	0.00	30.92
LB-PY 05-02	20.90	0.00	0.00	28.55	11.93	0.00	0.00	18.82	15.63	0.50	0.79	24.62
LBPY 05-04	15.33	0.00	0.00	23.81	15.80	0.00	0.00	24.70	18.20	0.00	0.00	27.89
RWP 206-33	15.40	0.00	0.00	23.26	8.60	0.00	0.00	13.13	14.87	0.00	0.00	22.78
DBW 50	16.73	0.00	0.00	26.14	19.73	0.00	0.00	31.23	16.57	0.35	0.54	25.72

NGS (UB) = Number of grains/spike (un-bagged), NGS (B) = Number of grains/spike (bagged), SSP (B) = Seed setting Percentage (bagged), SSP (UB) = Seed setting Percentage (un-bagged)

Table 3. Analysis of variance for agronomic traits of F_1 hybrids

Sources of variation (SOV)	Degrees of freedom (df)	Mean squares			
		Days to heading (days)	Plant height (cm)	Number of tillers per plant	Number of florets per spike
Replication	2	1.09	77.52	1.30	1.02
Genotype	44	1.50**	700.08**	69.25**	16.67**
Error	88	0.39	60.72	1.52	0.61

** $p < 0.01$

Table 4. Classification of wheat cultivars for their ability of fertility restoration

Pollen parents	Seed setting under selfing (%) in CMS lines		
	WH 416A	Atilla A	PBW 445A
WH 147	0.86 (PM)	0.00 (M)	0.00 (M)
WH 542	29.87 (PR)	0.00 (M)	0.00 (M)
HD 2967	0.00 (M)	0.39 (PM)	0.00 (M)
CBW 38	0.00 (M)	0.00 (M)	0.00 (M)
DBW 31	0.00 (M)	0.00 (M)	0.00 (M)
DBW 39	0.00 (M)	1.61 (PM)	0.00 (M)
KRL 99	20.78(PR)	0.00 (M)	0.00 (M)
KRL 284	0.00 (M)	0.00 (M)	0.00 (M)
HD 2964	0.00 (M)	0.00 (M)	0.00 (M)
NIAW 1188	1.02 (PM)	0.00 (M)	0.00 (M)
MP 1194	0.00 (M)	0.00 (M)	0.00 (M)
LB-PY 05-02	0.00 (M)	0.00 (M)	0.79 (PM)
LBPY 05-04	0.00 (M)	0.00 (M)	0.00 (M)
RWP 206-33	0.00 (M)	0.00 (M)	0.00 (M)
DBW 50	0.00 (M)	0.00 (M)	0.54 (PM)

Note:

Nuclear cytoplasmic interaction	Percentage of seed setting in selfing
Maintainer (M)	0%
Partial maintainer (PM)	>0% to 20%
Partial restorer (PR)	>20% to 80%
Restorer (R)	>80% to 100%

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