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# Heterosis in wheat hybrids derived from *Triticum timopheevi* based cytoplasmic male sterility system

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Wheat (*Triticum aestivum* L.) is one of the principal cereal crops of world. India will require 109 million tonnes of wheat annually by the year 2025 (Nagarajan *et al.* 1998) which is a quantum leap beyond the current productivity level of about 80 million tonnes. To meet this demand is an enormous challenge, as we do not foresee any expansion of wheat acreage. Moreover, the rate of growth in productivity has shown a decline over the last one decade. Hybrids represent a plant breeding option for surpassing the current productivity level. The efforts to develop hybrid wheat started in early sixties, propelled by identification of cytoplasmic male sterility (CMS) but were sidelined by the advent of semi-dwarf cultivars. The problems encountered in the successful production of hybrids in wheat including seed production, environmental sensitivity of fertility restorer genes, shriveled grains and a genetically complex system of fertility restoration remain inadequately addressed. Renewal of interest in hybrid wheat in 1990s may be explained by: (a) gains from the conventional approach tending to plateau, (b) availability of new chemical hybridization agents (CHAs), (c) availability of diverse male sterility systems and (d) availability of new germplasm, requiring fresh exploration of heterosis. Recently improved fertility restoration systems (Chen 2003), potential for high yield heterosis (Kindred and Gooding 2005) and also new systems such as 4E-ms (Zhou *et al.* 2006) have been reported to address the above mentioned problems.

Wheat is a self-fertilizing species with a floral morphology, which does not favour cross-pollination. Since the seed rate for commercial planting is also high, the economic viability of hybrid cultivars would, by and large be decided by the possibility of producing adequate amounts of hybrid seed. Apart from efficiency of hybrid seed production, heterosis is critical and most of the empirical studies have shown its levels to be rather modest. Earlier studies to assess the levels of heterosis in wheat were based on small quantity of seed produced by hand pollination and evaluated in single row or small plots with widely spaced plants. Often the studies on heterosis were a byproduct of analysis of genetic variance based on sets of $F_1$s and the best released varieties were generally not included as checks. Thus this information on heterosis has little relevance with regard to commercial exploitation.

At Punjab Agricultural University, Ludhiana both CGMS and CHA approach were initiated in the late 1990s. A comparative study these two approaches was made and CGMS approach was found to be about three times more effective for hybrid seed production attributes (Adugna *et al.* 2004). The CHA's were also observed to adversely affect the $F_1$ yield.

The present study explores the CGMS approach with the first large batch of CMS and restorer lines available at our centre. The main objective of the study was to access the levels of standard heterosis in a set of CMS × R crosses derived from this germplasm.

The experiment was conducted at the experimental farm of the Department of Plant Breeding, Genetics and Biotechnology, PAU, Ludhiana, during the crop seasons 2002-03 and 2003-04 and at PAU Research Station, Keylong (Himachal Pradesh) during off-season 2003. The plant material consisted of: (a) 5 CMS lines with *Triticum timopheevi* cytoplasm in 8$^{th}$ backcross generation. These lines were chosen for this experiment from a large set of CMS lines on the basis of their complete and stable sterility, phenotypic uniformity and desirable agronomic characters. The lines used in the study were in the background of PBW 343, F6.74/BUN//S15/3/VEE#7, KAUZ*2/MNV//KAUZ, OPATA M85 and VARONA/CNO//KAUZ. (b) 50 restorer lines (numbered as R1 to R 50) derived from crosses of a restorer in winter wheat background with adapted, high yielding lines: HD 2687, PBW 343, Chilero and Chuan Mai

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18. The restoration factor is designated as *Rfx*.

In crop season 2002-03, fifty small manual crossing units were planted for seed production. Each unit consisted of the five CMS lines sown in 1m rows and two plots of a restorer parent on either side of CMS lines, one sown at the same time as the female and the other 10 days later so as to facilitate synchronization of flowering. Each restorer plot comprised 8 rows of 1m each. Twenty CMS line ears were used for each cross combination. The ears on male sterile lines (A-lines) were clipped prior to opening, bagged, manually pollinated with restorer (R-line), and appropriately tagged. The target was to produce adequate crossed seeds per combination. Shortfall in the number of required grains was made up at Keylong during the 2003 off-season. Finally the desired number (400) of seeds could be obtained for 199 of 250 all possible crosses.

In the crop season 2003-04, the hybrid combinations obtained were evaluated for yield and other economic traits. The genotypes were sown in a randomized complete block design and were replicated twice. The plot size for each genotype consisted of 4 rows of 2 m lengths each. Row to row spacing was 23 cm and 50 seeds were hand-sown per row of 2 m, so as to maintain plant-to-plant distance of 4 cm. This plant-to-plant spacing corresponds to approximately half the commercial seed rate (i e 20 kg/acre). The crop was raised following agronomic practices as per the package recommended by PAU, Ludhiana. The hybrids along with 50 restorer lines, 5 maintainers of the CMS lines and three commercial checks, viz. PBW 343, PBW 502 and WH 542 were evaluated in randomized complete block design. The checks were repeated at regular intervals and were used in the analysis as such. Observations were recorded for yield/plot, grain weight/spike, 1 000-grain weight, number of grains/spike, tillers/meter, number of spikelets/spike, plant height and days to heading.

The analysis of variance for different traits was done as per the following model:

$$Yij = m + ti + bj + eij$$

where, Yij, observation obtained from the i-th treatment and j-th block, m, general mean, ti, the effect of i-th treatment, bj, the effect of j-th block, eij, error associated with i-th treatment and j-th block.

Standard heterosis was worked out for yield and yield components.

$$\text{Standard heterosis (per cent)} = \frac{\text{F1 hybrid} - \text{value of best commercial variety}}{\text{Value of best commercial variety}} \times 100$$

Significant genotypic differences were observed for yield components (viz. Grain weight/spike, 1 000-grain weight, no. of grains/spike, tillers/meter and number of spikelets/spike), in the set of 262 genotypes including $F_1$ and parents. The mean grain yield/plot ranged from 357.50 to 1205.00 g among $F_1$ hybrids. The mean grain yield/plot for maintainer lines ranged from 632.50 to 1100.00 g, while for restorer lines the range observed was from 577.50 to 1130.00 g. The cross Kauz*2/MNV//Kauz × R19 had the highest yield (1205.00g) followed by Varona/Cno//Kauz × R30 (1170.00g) and Varona/Cno//Kauz × R44 (1162.50 g). The maintainer line Kauz*2/MNV//Kauz showed the highest yield (1100.00 g) followed by Varona/Cno//Kauz (1012.50 g). The restorer R31 showed highest grain yield (1130.00 g) followed by R4 (1090.00 g). The extent of heterosis for yield was calculated over the best value of the best check, i e PBW 502 (standard or commercial heterosis). The standard heterosis for yield over best value of PBW 502 ranged from –58.96 (PBW 343 × R32) to 38.51 percent (Kauz*2/MNV//Kauz × R19). On the basis of standard heterosis for yield over best value of best check (PBW 502), a total of 16 hybrid combinations (Table 1), were found to be significantly better than PBW 502 at 5 percent level of significance and 7 hybrid combinations were found to be significantly better than PBW 502 at 1 percent level of significance. Standard heterosis for yield in this set of 16 hybrids ranged from 20.11 to 38.51%. Out of the five CMS lines used as parents, Varona/Cno//Kauz gave highest number of superior combinations (9), followed by Kauz*2/MNV//Kauz (3), PBW 343 (2) and F6.74/BUN//S15/3/VEE#7 (2), whereas Opata M85 gave non significantly better combination. Restorers R14 and R44 gave two significantly better combinations each whereas other 12 gave one better combination each.

The minimum and maximum number of grains were 23.10 (Kauz*2/MNV//Kauz × R9) and 76.00 (Kauz*2/MNV//Kauz × R25) among hybrids. The standard heterosis for number of grains/spike varied from –51.47 (Kauz*2/MNV//Kauz × R9) to 59.66 percent (Kauz*2/MNV//Kauz × R25). In the present study the minimum and maximum values of average tillers/meter were observed as 35.25 (PBW 343 × R32) and 111.50 (F6.74/BUN//S15/3/VEE#7 × R1). Standard heterosis for tillers/meter ranged from –51.88 (PBW 343 × R32) to 52.22% (F6.74/BUN//S15/3/VEE#7 × R1). Thousand-grain weight unlike tillering is a component that has got depressed in the modern day varieties. Bolder grains besides contributing to greater yield also have a quality enhancing effect. The maximum and minimum values of standard heterosis for 1 000-grain weight observed were 38.41 (Varona/Cno//Kauz × R32) and –34.82 percent (F6.74/BUN//S15/3/VEE#7 × R7), whereas the mean weight of 1 000-grains ranged from 24.41 (F6.74/BUN//S15/3/VEE#7 × R7) to 51.82 g (Varona/Cno//Kauz × R32). Another major yield component is spike yield, which is reflected in the number of spikelets/spike, number of grains/spike and weight of grains/spike. This is regarded as a major target for hybrid wheat as the tillering component is already well exploited by present day high tillering pure line varieties (which also have the advantage of greater seed rate). Average grain yield/spike ranged from 1.05 (PBW 343 × R 21) to 2.65g (PBW 343 × R33 and Kauz*2/MNV//Kauz × R 19). Standard heterosis for grain yield/spike was found to range from 51.16 to 23.26

Table 1 Heterosis for yield and other traits in hybrid combinations showing significant standard heterosis for yield

| Entry | Standard heterosis (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Yield/plot | Days to heading | Plant height | No. of spikelets/spike | Tillers/ meter | No. of grains/ spike | Grain weight/spike | 1 000 grain weight |
| C8 × R19 | 38.51** | –4.62** | –3.07 | 3.35 | 11.95 | 25.63** | 23.26** | 14.72 |
| C12 × R30 | 34.48** | –2.05* | 6.91 | 7.01* | –1.88 | 24.79** | 9.30 | 15.48 |
| C12 × R44 | 33.62** | –5.13** | 4.22 | 0.61 | 6.83 | 38.45** | 13.95 | 11.43 |
| C12 × R27 | 29.02** | –2.56** | 4.80 | 5.49 | 7.17 | –1.47 | –13.95 | 5.25 |
| C6 × R35 | 27.30** | –5.64** | 0.77 | 8.23** | –15.70 | 40.55** | 9.30 | –2.22 |
| C12 × R7 | 26.72** | 6.67 | 11.71 | 9.45** | 18.26* | 34.66** | 0.00 | –9.74 |
| C12 × R29 | 25.00** | –5.13** | 3.27 | 6.71* | –10.07 | 44.75** | 9.30 | 5.60 |
| C12 × R31 | 24.71* | 2.05 | 1.35 | 4.57 | –0.34 | 33.61** | 13.95 | 3.07 |
| C12 × R18 | 24.43* | –3.59** | 4.80 | 8.84** | –5.97 | 33.40** | 6.98 | –3.10 |
| C4 × R46 | 23.56* | 3.08 | 7.49 | 3.66 | 29.35** | 8.19 | –27.91 | –19.63 |
| C4 × R44 | 23.28* | –3.08** | 3.07 | 3.05 | 23.21** | 26.47** | –9.30 | –3.90 |
| C12 × R14 | 22.41* | 1.03 | 5.57 | 10.37** | 13.14 | 45.38** | 13.95 | –0.95 |
| C8 × R14 | 21.55* | –2.56** | 0.20 | 11.28** | –13.99 | 2.94 | –6.98 | 9.07 |
| C6 × R47 | 21.26* | –5.13** | 1.34 | 1.83 | 1.19 | 13.45 | 4.65 | 11.53 |
| C12 × R16 | 20.40* | –1.03 | 6.33 | 8.84** | –3.24 | 43.49** | 4.65 | –12.29 |
| C8 × R41 | 20.11* | –1.54 | 3.46 | 6.40* | 4.44 | 26.47** | –4.65 | –6.64 |

* Significant heterosis in the desirable direction (at 5% level of significance), ** significant heterosis in the desirable direction (at 1% level of significance)

percent (Kauz*2/MNV//Kauz × R19 and PBW 343 × R33). Average value of number of spikelets/spike ranged from 19.38 (PBW 343 × R17 and Kauz*2/MNV//Kauz × R10) to 24.81 (Kauz*2/MNV//Kauz × R50). Standard heterosis for number of spikelets/spike ranged from –5.49 (PBW 343 × R17 and Kauz*2/MNV//Kauz × R10) to 21.04% (Kauz*2/MNV//Kauz × R50).

Along with the above-mentioned traits, plant height and days to heading were also studied for all the 262 genotypes. The analysis of variance indicated significant differences among genotypes for these characters. Yield heterosis accompanied by raised plant height or delayed maturity would not be desirable. Thus in both these cases negative heterosis is considered to be beneficial. The observations recorded in the present set show a desirable trend with many combinations exhibiting a negative heterosis for these traits. The female lines had a similar number of days to heading as the standard checks, whereas restorer lines showed a wide range of variation. The hybrids also showed a variation in the value of number of days to heading from 89.00 to 106.50 days. The average plant height of the hybrids recorded at maturity varied from 76.00 (OpataM85 × R24) to 107.00cm (Kauz*2/MNV//Kauz × R35). The value of standard heterosis for plant height ranged from –45.68 (Varona/Cno//Kauz × R3) to 23.22% (Kauz*2/MNV//Kauz × R35).

The levels of heterosis in the present study are much higher than those generally observed in wheat. The reasons for this may lie in the small plot formats, with large number of genotypes being tested with only two replications. These limitations were unavoidable in view of the exploratory nature of this experiment involving a large number of hybrid combinations. As Picket (1993) reported on the basis of large number of trials conducted in Europe during 1980s, the amount of heterosis observed in small trials was not replicated in large scale trials. The repeated use of checks in present study may have helped to mitigate this limitation to some extent. The set of restorers used had a winter wheat (the restorer gene donor) in their immediate parentage while the CMS lines represented the best agronomic genotypes in the spring wheat programme. It is noteworthy that some of the restorers out yielded the maintainer lines tested in the trial. The parents thus had high per se performance and were potentially diverse and the high expression of heterosis may not be altogether unjustified. Confirmatory evaluation of a smaller set of hybrids with larger plot size would be a logical follow up of this experiment on heterosis in wheat. The importance of the present study lies in the fact that heterosis was evaluated using materials already converted to CGMS system which would allow rapid commercial application. For the hybrid combination high tillering potential is a must as they are to be sown at a lowered seed rate (half the commercial seed rate in the present study). Previous studies in the department have shown that both the parents of hybrid need to be high tillering as low tillering was dominant. This observation was made with hybrids having the high tillering. Veery derivatives (CIMMYT lines) as one parent and lines from Indian programme, which generally possess bolder grains, were used as the other parent. Consequently, for the present study high tillering lines were preferred for use as males as well as females. Experiments over the last five years have shown that genotypes like PBW 343 and WH 542 which were included as checks perform equally well at half seed rate provided the seed is evenly distributed.

The yield components that contributed to the highest yielding hybrid included number of grains/spike, tillers/m, number of spikelets/spike and 1000-grain weight, with maximum contribution from number of grains/spike, 1000-grain weight and no. of spikelets/spike. As indicated by earlier workers (Shebeski 1996, Yadav and Murty 1976, Snape 1982, Gale *et al.* 1987, Morgan *et al.* 1989), grain weight and number of grains are the principle sources of heterosis in wheat hybrids. Recently similar types of reports have been published from hybrid wheat breeding programmes of China (Zhang *et al.* 2001). These findings acquire a great significance if we consider the ideotype of the highest yielding present day wheats such as PBW 343 and WH 542. These wheats have maximized their tillering ability whereas ear size is medium and 1000-grain weight, particularly in case of WH 542 is rather low. Heterosis for these deficient attributes can have a yield enhancing influence. This could also be used as an indication that floral biology may be playing a critical role in the development of parental lines of wheat hybrids. Singh *et al.* (2007) studied the floral biology of Indian spring wheats and suggested their use in hybrid breeding programmes. But the high amounts of heterosis observed in this study; need to be studied again at large scale and in different environments. This will ensure proper estimates and also nullify the environmental influence if any.

## SUMMARY

Heterosis and fertility restoration in wheat (*Triticum aestivum* L.) were studied in the context of *Triticum timopheevi* based cytoplasmic genetic male sterility system. Five elite CMS lines were crossed with 50 restorers in line × tester fashion and sufficient seed of 199 out of all possible 250 combinations could be generated. The $F_1$s along with parental lines (restorers, maintainers) and commercial checks were evaluated using two replications in a randomized complete block design. The trial was sown using half the commercial seed rate with all other agronomic practices being followed as per the standard package. Observations were recorded on yield; yield components, and other agronomic attributes. Genotypic differences for all these traits were shown to be significant by analysis of variance. Standard heterosis for yield (over best commercial check, PBW 502) ranged from –58.96 (PBW 343 × R 32) to 38.51% (Kauz*2/MNV//Kauz × R 19). Sixteen hybrids out yielded PBW 502, at 5% level of significance, with a significant margin ranging from 20.11 to 38.51%. Number of grains/spike and number of spikelets/spike were the main yield components contributing to heterosis.

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