INVESTIGATIONS ON CYTOPLASMIC MALE STERILITY AND FERTILITY RESTORATION IN BREAD WHEAT (*Triticum aestivum* L.) FOR EXPLOITATION OF HETEROSIS

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INVESTIGATIONS ON CYTOPLASMIC MALE STERILITY AND FERTILITY RESTORATION IN BREAD WHEAT (*Triticum aestivum* L.) FOR EXPLOITATION OF HETEROSIS

A Thesis

by

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CERTIFICATE

This is to certify that the thesis entitled, **"INVESTIGATIONS ON CYTOPLASMIC MALE STERILITY AND FERTILITY RESTORATION IN BREAD WHEAT (*Triticum aestivum* L.) FOR EXPLOITATION OF HETEROSIS",** submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy in Genetics,** embodies the results of *bona fide* research work carried out by **S. ANBALAGAN** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

All the assistance and help received during the course of these investigations, have been duly acknowledged by him.

Date: July 28, 2003

Place: New Delhi

(S.M.S.TOMAR)
Chairman,
Advisory Committee

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"Were I so tall to reach the pole,
Or grasp the ocean with my span,
I must be measured by my soul,
The mind's the standard of the man".

- ISAAC WATTS

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I hold in respect, this simple work of mine to be holier than the Holy Texts and Scriptures, for, this has been aimed to serve the humble peasants.

As a prime duty in whatever I do, I pay my obeisance to my parents who have not only been the animated force behind me ever since I saw the light of the day, but were also instrumental in my learning, 'Honesty and discipline always wins'.

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(S.Anbalagan)

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ABSTRACT

"In art, economy is always beautiful".

ABSTRACT

The present study on, "Investigations on cytoplasmic male sterility and fertility restoration in bread wheat (*Triticum aestivum* L.) for exploitation of heterosis", was undertaken to characterize CMS lines, identify maintainers and restorers, study the inheritance of fertility restoration and kernel colour and identify heterotic combinations in wheat. The studies were undertaken at the Division of Genetics, Indian Agricultural Research Institute, New Delhi-110 012.

The experimental materials consisted 39 CMS lines carrying seven different types of cytoplasms. The cytosterility systems were developed through repeated backcrossing with the cytoplasms of wild species like *T. timopheevi*, *T. araraticum* and *Ae. speltoides* and unknown sources which have been named TMS 8, TMS 9, TMS 11 and TMS 20.

The characterization of CMS lines included study on floral and agronomic traits and assessment of stability of CMS lines in three environments. In general, the CMS lines had values less than or equal to that of their fertile counterparts with respect to all the floral traits. However, genetic variation between CMS (A) and maintainer (B) lines were observed for only four of the six floral characters *viz.*, anther size, filament length, ovary size and length of style.

None of the six CMS lines with *T. timopheevi* cytoplasm showed higher mean values than their respective B lines for any of the floral trait. Similarly, a majority of CMS lines carrying *T. araraticum* cytoplasm had significantly lower mean values than B lines for anther size, filament length, ovary size and length of style. Only *arar* 2687 A had higher significant value for ovary size. Unlike CMS lines with *timopheevi* and

araraticum cytoplasm, the male sterile lines possessing *Ae. speltoides* cytoplasm had significantly higher mean values in a majority of the cases for all the four floral traits, *e.g.*, *spelt* 2038 A and *spelt* 2643 A had larger anthers than the B lines while *spelt* 2038 A possessed longer styles. Significant differences in floral traits were observed in the CMS lines with TMS 8 cytoplasm, with inferior and superior performances by A lines when compared to B lines. The CMS lines with TMS 9 cytoplasm showed significant difference for anther size and filament length but showed poor variation for ovary size. In contrast only two out of six male sterile lines possessing TMS 11 cytoplasm showed significant differences for anther size and a majority of the CMS lines had significantly lower values for filament length, ovary size and stylar length. The CMS lines derived from TMS 20 cytoplasm also exhibited significant variation for the floral traits.

Studies on out-crossing clearly indicated the need for assured pollination in CMS lines to enhance seed setting. The maximum out crossing was 13.38 % (*arar* 2022 A) where the supply of pollen to A line was not assured (Method I) whereas in the case of assured pollen supply (Method II), there was about 3.5 fold increase (38.36 percent) in TMS 8/2046 A.

The effect of male sterility inducing cytoplasm derived from the various sources on agronomic characters *viz.*, number of tillers, plant height, main spike length, number of spikelets, days to 50% flowering and synchrony of tillers, exhibited differential interaction. Unlike the floral traits, the general trend with respect to agronomic traits was not only a depression of performances but also an enhancement as an exception such as the increased spike length of *timo* 2338 A, taller plants in *spelt* 2042 A, increased number of tillers in TMS 11 / 2046 A, increased number of spikelets in TMS 11 / 2338 A. The

CMS lines generally flowered later when compared to their respective B lines will exception and there was no significant variation observed for synchrony of tillers.

The stability of sterility in CMS lines was judged on the basis of pollen fertility and seed setting under different environments. All the 39 CMS lines exhibited complete spikelet sterility as evidenced by the absence of seed setting in bagged spikes. In addition, among the four categories of pollen grains distinguished on the basis of shape and staining pattern, the proportion of Unstained Spherical Sterile (USS) was found to be high followed by Unstained Withered Sterile (UWS) pollen. These two categories contributed to more than 97 percent; while the other two categories *viz.*, Stained Round Fertile (SRF) and Stained Round Sterile (SRS) contributed only up to 3 percent. Owing to the production of remarkably high proportion of USS and UWS pollen, 29 CMS lines were placed in completely sterile group and the remaining 10, due to production of SRF pollen, though at an extremely low frequency were placed under highly sterile group.

To identify maintainers and restorers, 521 testcrosses were made and evaluated, of which a very high frequency (87.14 percent) of them were male sterile and therefore classified as complete maintainers. Only two pollinators namely, PWR 4099 and PWR 4101 were able to restore complete fertility in the testcrosses. PWR 4099 restored complete fertility in *T. araraticum*, TMS 8, TMS 9, TMS 11 and TMS 20 cytoplasms while PWR 4101 was able to restore complete fertility only in two cytoplasms *viz.*, TMS 9 and TMS 20. The studies on inheritance of fertility restoration of the two exotic lines PWR 4099 and PWR 4101 revealed that two and one dominant gene(s) controlled fertility restoration, respectively. The F_2 population of the crosses involving PWR 4099 as restorer segregated in the modified Mendelian ratio of 9:6:1 while the F_2 population of

crosses involving PWR 4101 segregated into a monogenic 3:1 ratio. Studies on genetics of kernel colour involving the red-grained exotic line PWR 4099 revealed that a single dominant gene controlled this trait. The segregation pattern in six F_2 population revealed a monogenic pattern of 3 red :1 amber. The study further led to the conclusion that genes for fertility restoration and kernel colour in PWR 4099 were not linked.

Heterosis was estimated for eleven hybrids namely, PHW 1, PHW 2, PHW 3, PHW 4, PHW 5, PHW 6, PHW 7, PHW 8, PHW 9, PHW 10 and PHW 11 over mid-parent, better parent and standard check for various yield and yield components *viz.*, number of productive tillers, plant height, main spike length, number of spikelets, number of grains per spike, 1000-grain weight, yield per spike and yield per plant. Seven hybrids namely, PHW 2, PHW 3, PHW 4, PHW 5, PHW 6, PHW 7, and PHW 10 showed significant positive heterosis for number of productive tillers over the standard check PBW 343. Similarly a high number of hybrids *viz.*, PHW 2, PHW 3, PHW 5, PHW 6, PHW 7, PHW 9, PHW 10 and PHW 11 exhibited significant positive heterosis for plant height over the standard check, PBW 343. Three hybrids *viz.*, PHW 1, PHW 7 and PHW 9 registered positive significant standard heterosis for spike length while six hybrids, registered standard heterosis for number of spikelets. They were PHW 1, PHW 2, PHW 3, PHW 6, PHW 7, and PHW 8. Four hybrids *viz.*, PHW 1, PHW 2, PHW 3, and PHW 7 exhibited standard heterosis for number of grains per spike. None of the 11 hybrids registered better 1000-grain weight over the standard check PBW 343. Four hybrids PHW 1, PHW 2, PHW 3 and PHW 7 recorded significant positive heterosis over the standard check variety PBW 343 for yield per spike, while a similar number of

hybrids PHW 2, PHW 6, PHW7 and PHW 9 showed significant positive standard heterosis for yield per plant.

The study on the yield performance of five selected hybrids, PHW 1, PHW 2, PHW 3, PHW 12 and PHW 13 revealed the need for assessment of yield on a larger plot area. This was confirmed by the yield performance of the hybrid PHW 2 on per plant basis (26.12 percent) over the standard check and on a larger plot area (-1.76 %, -6.29 % and –6.72 % over the standard checks PBW 343, HD 2329 and HD 2687, respectively). Therefore, appropriate plot size is essential in order to assess the real performance of the hybrids.

INTRODUCTION

"The by product is sometimes more valuable than the product".

- HAVELOCK ELLIS

1. INTRODUCTION

The cereal demand situation, particularly in developing world does not lend itself to complacency because the demand for basic cereals like rice, wheat and maize is anticipated to grow at least 2% per annum. The overall demand for rice and wheat in Asian countries will continue to rise because of continuous population growth. The global requirement for wheat will also increase not because of it being a staple food for millions, but also as a result of substitution of rice and coarse grain cereals. The other major factors include improvement in living standards, increased income, urbanization, development of modern high yielding varieties, their free global exchange along with infra structural investments and a conducive policy environment.

The world food production has to be enhanced by 40 % from the level of 1996 of 600 million tonnes to 775 million tonnes to meet the projected demand by 2020 and in future will need to reach 850 million tonnes by the year 2030 (Curtis *et al.* 2002) to keep abreast of population growth. The demand for wheat in India is expected to be 109 million tonnes by the year 2020 due to increasing population growth. However, to date, the contribution of wheat to meet the food security in India has been quite impressive. The wheat yield in India rose steeply by manifolds during the initial phase of green revolution, which was immensely, contributed by the two important traits, *e.g.*, photoperiod insensitivity and dwarfing genes. During the period 1994-95 to 2001-02, growth rate in production and productivity at the national level was 2.90 and 2.47 %, respectively, in comparison to 1.65 and 1.22 % in rice. India could produce 71.45 million tonnes of wheat in 2001-02 from around 25.5 million hectares (Anonymous, 2002).

However, it remained low as compared to 1999-2000, a year, when an all time record of 76.37 million tonnes was realized, but productivity remained constant at the level of 2.7 t ha^{-1}.

Slow increase in productivity level indicates the plateauing of wheat yield level. Therefore, the projected target can only be achieved by increasing average yield of wheat annual production of one percent or slightly less has been maintained. As the projected demand of wheat in India by the year 2020 has been put at 109 million tonnes (Anonymous,1997 a, b), it has to achieve more than 30 % enhanced production at the rate of 2% annual increase in production from now. In the absence of a significant increase in wheat productivity beyond current levels, the challenge of meeting future demands appear more daunting. Therefore both public and private sectors have to intensify their agricultural research and research activities to meet the challenges. Virtually the increased wheat production growth must come from increased yield per unit of land, time and input used since the chances of further area expansion are exhausted in India.

Productivity increases are essentially needed in both the high potential and the marginal wheat production environments to meet the future needs. However, the opportunities for further yield growth are also limited in case of wheat due to lack of diversity in germplasm, in-built heterosis in wheat and gap between the technology frontier and performance on farmers' field. To bridge this gap, new technology designed to significantly reduce the cost per unit of output, either through genetic manipulation or through enhanced input use efficiency would substantially enhance the level of productivity and profitability. This fact emphasizes the long-term commitment needed to

introduce genes that may radically alter the conventional phenotype of a wheat plant. Exploitation of heterosis or hybrid vigour, the benefits of which have been amply demonstrated in several crops, including rice, a strictly self-pollinated crop appears to be another important approach, which could help in dramatically reducing the cost of production by enhanced levels of productivity under optimum input condition.

Heterosis, synonymous with hybrid vigour, and a manifestation of the superiority of F_1 performance over the parents is basically concerned with in-breeding and out- breeding. While working on effects of inbreeding in maize and its role in hybrid vigour, Shull (1908) clarified that selfing isolated homozygous lines and the hybridization of inbreds exhibited positive benefits. He later coined the term "heterosis" in 1914 (Goldman, 1997). Over the past 75 years since the hybridization of maize, numerous field crops and vegetables have been benefited from this phenomenon. Example includes plants, characterized by both perfect and imperfect flowers, with genetic and cytoplasmic genetic sterility, protogyny, and incompatibility allowing natural cross pollination by wind, insect and other means. Wheat, an allohexaploid, with chasmogamous flowers, makes it a strictly self-pollinated crop. However, the natural hybrid in wheat has several inherent limitations in heterosis breeding. The possibility of developing hybrids in wheat attracted the attention of several breeders for quite long time. Since then extensive efforts have been made to exploit heterosis in wheat. Manual emasculation for the production of F_1 hybrids had been under investigation since long. Another viable approach, employed in self-pollinated crops like rice and millets, is the induction of male sterility in the seed line on the basis of sterile cytoplasm. This type of male sterility is conditioned by hereditary particles in the cytoplasm. Later, systems for

cross-pollination like the production of cytoplasmic male sterility-fertility restoration, nuclear or genetic male sterility were developed.

Genetic male sterility is a widely occurring phenomenon in crop plants and is governed by major gene(s). The expression of *ms* gene(s) is influenced by the environment. While the environment insensitive *ms* gene is less affected by the environment, the environment sensitive genes express within a specified range of temperature and photoperiod regimes. Both temperature-sensitive genetic male sterility (TGMS) and photoperiod-sensitive genetic male sterility (PGMS) may have complete pollen sterility, which makes them useful in hybrid breeding. In rice, EGMS lines *viz.*, X-88, M 201, Nongken 58 S, Annong S *etc.*, have been used in hybrid seed production (Ahmed and Siddiq, 1998). Transgenic genetic male sterility has also been developed. In such cases a gene is introduced into the genome of an organism by recombinant DNA technology or genetic engineering, which caused genetic male sterility. The fertility behaves as recessive to male sterility and consequently it is essential to develop effective fertility-restorer system, if these are to be utilized for hybrid seed production. An effective fertility-restoration system is available in at least one case called Barnase/Barstar system in *Brassica*. Although this system has not been utilized in wheat, it provides an ample scope in hybrid wheat technology. Chemicals are also being used for a very long time to induce male sterility in wheat. A gametocide is sprayed on seed line to make the pollen sterile and the system does not need any restorer. Production of hybrids through manual emasculation is not economically and practically feasible to produce large quantities of F_1 hybrid seed. Similarly development of hybrids through the use of chemical hybridizing agents (CHAs) may suffer from problems associated with

quality of seed production, higher costs and environmental hazards. Genetic male sterility also could not be exploited due to the disadvantage it has in maintaining the male sterile line. Interest in hybrid wheat is still present; particularly in the regions where wheat yields and commercial values are relatively high (Duvick, 1997; Bruns and Peterson, 1998) but successful commercialization is still conjecture.

Work on development of hybrid wheat in India began in the mid 1960s, with the introduction of seed of male sterile lines carrying *T. timopheevi* cytoplasm. The cytoplasmic male sterile stocks of *T. timopheevi* (*T. timopheevi* X Marquis^2) F_4 X Pembina^3 and (*T. timopheevi* X Marquis^2) F_4 X Selkirk^3 were received from Canada (Miri, 1968). With the introduction of high yielding dwarf wheat varieties during late 1960's, the efforts of hybrid wheat development were diluted in India. However, in other countries like Australia, USA and France, the work on hybrid wheat development continued and commercial hybrids were released in 1980s in Australia, but it was not until the release of hybrids Mercury and Apollo in 1994, the hybrids were not perceived by wheat growers as offering a viable alternative to pure line varieties, due to the high cost of hybrid seed and moderate levels of its advantage (approximately 10 – 15%) over the best variety (Wilson, 1997).

Wheat hybrids could yield up to 30% more than their parents but lacked desired milling and baking quality. However, the hybrids developed with desired quality had yield advantage of 5-15 % only (Sun *et al.* 1997). The higher cost of hybrid seed had to be compensated through either increasing the hybrid yield advantage or reducing relative seed cost by improving seed production procedures and reducing seeding rates. Other than in Australia, hybrids have been released for cultivation in South Africa, USA and

France. The first attempt to make hybrid wheat in the USA dates back to the 1940s. But around 1970s, attempts at commercializing hybrid wheat were abandoned after public and private seed producing agencies became convinced that commercial production of hybrid seed could not generate profit for farmers and seed companies simultaneously (Wijk, 1994). However, it has been shown lately that wheat hybrids yield significantly higher and are more consistent in their performance than conventional cultivars, with the increased responsiveness to input management and adaptability to adverse conditions (Peterson *et al.* 1997; Jordan, 1996).

India's private sector MAHYCO (Monsanto) has developed two wheat hybrids, namely, Pratham I and Pratham II for Central and Peninsular India. However, the hybrids are yet to be adopted on large scale. Whatever may be the status of wheat hybrids today, the possibility to produce hybrid seed in wheat, as in other self-pollinated crops, is met with enthusiasm. Notwithstanding the success, which was dramatic in other crops including rice, a self-pollinating crop, wheat hybrids have been successfully commercialized though at a slower rate. Concerted efforts with more public support with respect to infrastructure, human resource and free flow of scientific information are needed to strengthen the case of hybrid wheat.

The key to the successful production of hybrid seed in any crop is sufficient control of the pollination process. Self-pollination in the seed line (female) plants must be prevented. To satisfy the primary criteria for the success of hybrids, the following steps must be taken into consideration (i) the effective pollination control system, (ii) magnitude of heterosis, (iii) identification of useful traits (yield or quality or both) and environment for which hybrids are required, and, (iv) enhanced seed yields in A X B

and A X R and the reduced seeding rates. With the availability of synthetic wheats, which offer great opportunity for diversifying the genetic base of hybrid wheat breeding programme in order to develop diverse parents with better combining ability to enhance the level of heterosis and availability of alternative sources of CMS and gametocides, the future of hybrid wheat looks bright. However, it requires concerted efforts.

However, the constraints if any, can be remedied through strong research and development efforts in the long run. As stated earlier that hand emasculation and pollination for commercial production of hybrid seed in wheat is impractical and use of chemical hybridizing agents is not eco-friendly, the viable option to produce hybrid seed on large scale remains with the exploitation of cytoplasmic male sterility-fertility restoration system (CGMS). The CGMS system may provide useful and eco-friendly approach, if the technology is made easy and efficient. The present study was, therefore, undertaken with the following major objectives in wheat:

1. Characterisation of CMS lines
2. Identification of maintainers and restorers
3. Genetics of fertility restoration and kernel colour
4. Identification of heterotic combinations

REVIEW OF LITERATURE

"The thing that teases the mind over and over for years, and at last gets itself put down rightly on paper – whether little or great, it belongs to Literature".

- SARAH ORNE JEWETT.

2. REVIEW OF LITERATURE

The production of hybrid depends on a system where a male sterile female parent is cross-pollinated by the male parent and the hybrids are fully self-fertile. To produce fully fertile hybrids, cytoplasmic male sterile A lines are crossed with fertility restorer R-lines. The B-line provides nuclear genes to produce A lines. With the development of stable cytoplasmic male sterile and restorer lines, the three-line method of developing hybrid wheats seems to be one of the science based technological approaches available for commercial exploitation of heterosis. The identification of divergent heterotic groups is the most important aspect to realise the high level of heterosis in hybrid wheat. Further, information regarding the number and diversity of restorer genes available in the germplasm contributes to effective restorer breeding programme. The review of literature on male sterility systems in wheat, instability of CMS lines, limitations of CMS, fertility restoration, development of fertility restorer lines, chemical hybridizing agents (CHAs), CMS utilization in hybrid seed production and heterosis in wheat is presented below.

2.1. Cytoplasmic male sterility

Cytoplasmic male sterility in wheat is a derived trait, first described by Kihara (1951). He transferred the genomic complement of one species into the cytoplasm of a second species by backcrossing, using the nuclear donor parent as pollen parent in the initial cross and in successive backcrosses (substitution backcrossing). He found that plants in which the nucleus of common wheat (*Triticum aestivum* L.) had been substituted into the cytoplasm of *Aegilops caudata* were male sterile. Subsequently, Fukasawa (1953) backcrossed the *Triticum durum* L. genomes into the cytoplasm of

T. ovatum L. The resulting plants were male sterile and had delayed heading. Other studies by Japanese researchers established the presence of CMS plants in inter generic crosses, in which the sterility persisted through backcross generations (Fukasawa, 1957, 1958, 1959; Kihara and Tsunewaki, 1961). These findings and their potential use in hybrid wheat breeding prompted Wilson and Ross (1962) to seek alternative cytoplasm that would cause male sterility without causing deleterious effects. They reported that the interaction of *T. timopheevi* zhuk. cytoplasm and the *T. aestivum* nucleus resulted in a complete and constant male sterility. Furthermore, the *T. timopheevi* cytoplasm had no apparent adverse effects on plant growth and development. Maan and Lucken (1967) reported two additional cytoplasmic male sterility-fertility restorer systems in *Triticum*. When the nucleus of the common wheat variety Justin was substituted into *T. zhukovskyi* cytoplasm, male sterile plants were produced. The *T. durum* nucleus substituted into a diploid wheat cytoplasm (*T. boeoticum* type of unknown origin with 2n = 2x = 14, genome AA) produced male sterile 2n = 4x = 28 chromosome plants.

Maan (1973) and Sage (1976) reviewed cytoplasmic male sterility in wheat. Over 15 different cytoplasms have been recognised, several of which induce male sterility in common wheat and could form a basis for alternative systems of hybrid production (Maan, 1975; Mukai and Tsunewaki, 1980). Suemoto (1978) reported that the cytoplasm of *T. timopheevi* has been derived from *Ae. speltoides* and the cytoplasm of *T. araraticum* has been also derived from the same donor. Nearly all hybrid wheat breeding continued to be based on the *T. timopheevi* system, and its widespread use has been largely the result of its apparently neutral effect on agronomic and quality characters. Most other cytoplasms from *Triticum* and *Aegilops* have deleterious effects on various traits (Maan, 1973; Sage, 1976).

Ghiasi and Lucken (1982 b) compared the reactions of *Ae. speltoides* and *T. timopheevi* cytoplasms to various restorer combinations and examined for a number of agronomic and quality traits. They concluded that *Ae. speltoides* cytoplasm can be used interchangeably with that of *T. timopheevi* in hybrid wheat breeding providing an alternative that can broaden genetic variability. Pickett (1993 a) studied the effects of 20 different cytoplasms from *Aegilops*, one each from *Haynaldia* and *Secale* and five cytoplasms from the genus *Triticum* and concluded that CMS derived from *Triticum* appeared to present fewer problems. Additional cytosterility systems that supply male sterile plants with good vigour and female fertility have been produced with the cytoplasms of Zhukovskyi (2n=42; AAA'A'GG), *Triticum araraticum* (2n=28: AAGG), and *T. dicoccoides var nudiglumis* (2n=28: AAGG) (Maan, 1975; Maan and Lucken, 1971).

Franekowiak *et al.* (1976) proposed a proposal for hybrid wheat using *Aegilops squarrosa* cytoplasm that seeks to avoid the breeding of restorer lines. The D genome of common wheat contains genetic factor(s) for restoration of fertility of *T. aestivum* with *Ae. squarrosa* cytoplasm. The nucleus of *T. aestivum* was substituted into *Ae. squarrosa* cytoplasm and the seed was treated with a mutagenic agent (ethyl methanesulfonate, EMS) to inactivate the critical gene(s) that caused fertility. Ten male sterility mutants from an M_2 population of 45,000 plants expressed sterility in F_1 or F_2 generation, which indicates control by a single recessive gene. Crosses with four spring wheat produced completely fertile F_1 progeny. However, the major weakness of the system was that no male-sterile genes that function specifically in the *Ae. squarrosa* cytoplasm were found. The development of fertile *T. aestivum* B lines with homozygous recessive genes for maintenance of the A lines was not completed.

The Sv type of cytoplasm of *Aegilops kotschyi* was proposed to be an alternate candidate for male sterile cytoplasm to be exploited in hybrid wheat breeding by Mukai and Tsunewaki (1979). They found that the Sv type cytoplasm in a 6x strain Salmon derived from an 8x triticale expressed male sterility. However, much success has been recorded in use of chemical hybridizing agents and two-line system involving photo-thermo sensitive male sterile wheats.

The male sterility employed in developing hybrid wheat results from the interaction between the nuclear and cytoplasmic genes of different genomes involved. Okocha (1999) observed that interaction between nucleus and cytoplasm in the F_1 hybrids varied depending on the nuclear genotype. The cytoplasms of *Aegilops squarrosa* (*Ae. tauschii*), *Aegilops speltoides*, *Triticum timopheevi* and *Haynaldia villosa* (*Dasypyrum villlosum*) depressed plant height while positively influencing main ear length, grains per plant and fresh weight. Simultaneously he also pointed out that extra nuclear DNA was largely under the control of the nuclear genotype.

Edwardson (1956, 1970) reported that cytoplasmic male sterility occasionally arises from intergeneric crosses and more frequently from interspecific crosses. The intergeneric hybridization maximises the differences between nucleus and cytoplasm and may increase the likelihood of obtaining a new CMS system due to incompatible nuclear – cytoplasmic interactions. He further noted that there existed a relationship between the width of crosses attempted and the likelihood of CMS's being produced. Incompatible nuclear cytoplasmic interactions were more likely to occur in wide crosses and thus more are the likelihood of the discovery of cytoplasmic male sterility.

In contrast, the interspecific cross produces smaller differences between the nucleus and the cytoplasm and, therefore, somewhat decrease the likelihood of obtaining a new CMS system. Nonaka *et al.* (1993, 1994) and Toriyama *et al.* (1993) proposed a new breeding scheme to develop hybrid wheat cultivar using male sterility caused by interaction between the Sv type cytoplasm of *Aegilops kotschyi* and the 1BL-1RS chromosome. Also Takayama and Taya (1998) obtained F_1 hybrids by utilizing male sterility induced by interaction between Sv type cytoplasm and the 1BL-1RS translocations. The hybrids were compared with standard varieties and Norin 61 with respect to agronomic and quality characteristics. Average yield of F_1 hybrids was not higher than that of Norin-61; In fact the yields of F_1 hybrids derived from Australian varieties was much lower than that of Norin 61. Lower fertility restoration was thought to be major cause of low yield, although hybrids were superior in 1000-grain weight.

Similarly, Murai *et al.* (1991 a) produced male sterile lines using *Aegilops crassa* cytoplasm. Murai (1997) also studied the effect of *Ae. crassa* cytoplasm on the agronomic characters in photoperiod-sensitive CMS wheat lines and F_1 hybrids. Although the *Ae. crassa* cytoplasm reduced selfed and open pollinated grain fertilities in the PCMS lines and averaged 69% grain fertility under open pollination, it indicates that the PCMS lines can be maintained by self pollination under the natural conditions at specific location(s). In the F_1 hybrids selfed and open pollinated grain fertilities were also reduced by the effect of *Ae. crassa* cytoplasm. The reduced seed fertility resulted in decreased grains per ear, and grain weight per plant, but increased 1000-grain weight. No significant effect of cytoplasm was detected on volume weight of the grains producing the F_1 hybrid, suggesting no deleterious effect of *Ae. crassa* cytoplasm on grain quality.

The studies on the inter-relationship among different sources of male sterility are many and Kumari and Mahadevappa (1998) viewed that any two cytosterile sources are genetically different and diversity within the CMS lines of rice derived from WA sources with regard to their fertility restoration suggested differential interaction of both the cytoplasm as well as the nuclear background of the female parent with the pollinator varieties. While the magnitude of cytoplasmic diversity between *Triticum* and *Aegilops* species have been demonstrated, Tsunewaki (1999) identified the methods of conservation of cytoplasmic diversity in the form of extracted chloroplast and mitochondrial DNA.

2.1.1. Instability of cytoplasmic male sterile lines

During the 1980's hybrid wheats produced using the CMS system of *T. timopheevi* cytoplasm were released in the USA, Australia and Argentina. However, they were not widely adopted by farmers because of instability of fertility restoration caused by environmental influences (Pickett, 1993 b). Stable expression of sterility in cytosterile lines has also been observed to be significantly affected by temperature in rice (Ali *et al.* 1998). The stage at which temperature affects pollen mother cells is not precisely known, however, pre-meiotic stage is considered to be most vulnerable stage to temperature in CMS lines of different crops including rice and wheat. In rice, high temperature (35-41°C) during anthesis induces pollen sterility (Satake and Yoshida, 1977) while low temperature induced sensitivity is maximum at microspore release stage (Satake and Hajase, 1974). On the other hand, Murai (2001) observed that the male sterility in photoperiod-sensitive cytoplasmic-male sterile (PCMS) wheat lines with *Aegilops crassa* cytoplasm depended on the genotype.

The stability of sterility in cytosterile lines depends on the stage at which pollen abortion takes place. Male sterility of sporophytic system in which pollen grains mostly abort at uninucleate stage has been found to be more stable than gametophytic system wherein abortion occurs at bi-or-tri nucleate stage (Lin and Yuan, 1980; Chaudhury *et al.* 1981). The stage at which pollen abortion occurs depends upon genetic distance between cytoplasmic donor and recipient parents (Chaudhury *et al.* 1981). In rice, the cross involving narrow genetic base between cytoplasm and nuclear donor parents produced gametophytic unstable male sterile lines in China, whereas wider crosses between a primitive female line and an advanced male line more frequently resulted into sporophytic and stable CMS system (Virmani and Edwards, 1983).

Wu *et al.* (1994) studied the abortive process of pollen in genetic and cytoplasmic male sterile lines with cytoplasm from wild rice. The cytoplasmic male sterile lines had typical nuclear degenerative pollen abortion, which began at late uninucleate stage while the genetic male sterile lines were all typical nuclear proliferative types. The membrane between two daughter nuclei at telophase in the first meiotic division failed to form. This type of male sterility was stable, producing flowers with dehiscent anther. According to Maan and Mc. Cracken (1968), the external environment did not appear to influence meiotic instability after the onset of meiosis. The environment during pre-meiotic phase determined the extent of meiotic regularity or irregularity. Powers (1932 a, 1932 b) reported that meiotic instability persisted in common wheats of hybrid origin.

Based on the field observations of fertility restoration, Wilson (1968 a) classified environment into three categories – 'shallow sterile', 'sterile' and 'deeply sterile'. He suggested that restorers adequate in the shallow sterile environment may be inadequate in deep-sterile environments. Using his classification, Tsunewaki *et al.* (1976) while

studying the expressions of male sterility in different localities inferred that stability of male sterility apparently depends on genetic-environmental interactions. Unstable male sterile lines can be used commercially only in 'sterile' environments.

2.1.2. Limitations of CMS

Many CMS systems have limitations that make it difficult or impossible to use them as pollination control system in the production of commercial quantities of hybrid seed. These limitations are (i) pleiotropic negative effects of the CMS on agronomic performance of plants in the CMS cytoplasm, (ii) enhanced disease susceptibility, (iii) complex and environmentally unstable maintenance of male sterility and/or male-female restoration and, (iv) inability to produce commercial quantities of hybrid seed economically because of poor floral characteristics for cross-pollination (Mc.Vetty, 1999). In addition, the dangers of monoculture of crop production in one CMS cytoplasm have been documented for corn (Levings, 1993).

For common wheat with the *timopheevi* CMS, the principal problems are high hybrid seed production costs, caused by a low seed multiplication rate (approximately 35%) and by the limited extent of cross-pollination in seed production fields (Edwards, 1987). In addition, male-fertility restoration for the *timopheevi* CMS system is complex, with up to four major restorer genes and one or more minor modifier genes required to effect full male-fertility in some environments; even then, the restorer system is sensitive to environmental stresses (Stroike, 1987). It has been observed that the F_1 kernels produced from male-sterile lines (*timopheevi* cytoplasm) of red winter wheats were found to be shrivelled when pollinated either by their respective normal fertile counterparts (B lines) or a fertility restorer (R line) as compared to well filled kernels produced from self-fertile B and R lines (Rai, 1978). The shrivelling of kernels appeared to be associated

with the interaction between *T timopheevi* cytoplasm and nuclei from *T. aestivum*. Panayotov (1983) reviewed 129 cytoplasms of species and subspecies in five different genera and found that 37 of them caused male sterility. He also reported that several of the cytoplasms depress growth and development in wheat.

Comparison of the nuclear-cytoplasm hybrids to the corresponding normal wheat lines disclosed genetic differences between the alien and wheat cytoplasm (Mukai *et al.* 1978). They found intra specific cytoplasmic differentiations in four species, *Ae. caudata*, *Ae. speltoides*, *Ae. triuncialis* and *Ae. ovata*. The distinct differences between the *caudata*-K and *caudata*-N cytoplasm occurred in anther form: The former induced pistillody of anther, while the latter did not cause pistillody but resulted in degenerated anther, which is similar to the effect of the *timopheevi* cytoplasm. Suemoto (1978) compared the effects of the cytoplasms of *Aegilops* and einkorn species on the genomes of *timopheevi* group. She found that those lines having the cytoplasm of *Ae. comosa*, *caudata*, *heldreichii*, *T. monococcum* and *boeticum* show high male sterility and severe weakness in growth. Sasaki *et al.* (1978) observed that the lines of Chinese Spring (CS) with each *Ae. caudata*, *T. timopheevi*, *Ae. umbellulata* and *S. cereale* cytoplasms were higher than CS with the original one in the % grain protein and the CS line with *Ae. caudata* cytoplasm lower in the dibasic amino acid per gram protein. They further explained these variations to be secondary effects and were affected by alien cytoplasms.

2.2. Fertility restoration

After Wilson and Ross (1962) bred wheat male-sterile lines with *Triticum timopheevi* cytoplasm, and successfully transferred *Rf* genes from *T. timopheevi* to common wheat by substitution backcrossing, wheat breeders began to attach importance

to wheat breeding. Restoration of wheat pollen fertility in strains with foreign cytoplasm is an extremely complex problem. Transmission of restoration factors from one variety to another is possible but complicated and requires several generations of selection. Fertility restoration is controlled by a complex system of factors. External factors (light, temperature, *etc.*,) also influence restoration of pollen fertility. Moreover restoration factors transferred to a different variety enter a new genetic environment, which, together with external conditions, has a detrimental influence on their expression and effectiveness.

Several workers have investigated fertility restoration of male sterile lines. Yang *et al.* (1989) observed that the cytoplasmic factors controlling male sterility in different cytosterility systems are different; therefore, differential fertility restoration will be shown by different CMS systems in testcrosses. Fertility restoring genes (*Rf*) for cytoplasm *i.e.*,, male sterile lines with *T. timopheevi* cytoplasm have been found in various tetraploid and hexaploid wheats and the fertility restoration capacity primarily depends on the effect of *Rf* genes present and their interaction with the cytoplasm of the CMS lines (Kihara, 1968); it can also be influenced by the environment and the nuclear genes of the CMS lines (Wilson, 1968 b); he also reported that complete fertility restoration for a male-sterile line needed joint effects of several parts of restoring genes. The male fertility restoration for the *timopheevi* CMS system in complex, with up to four major restorer genes and one or more minor modifier genes required to effect complete male-sterility restoration in some environments, even then, the restorer system in sensitive to environmental stresses (Stroike, 1987). According to Johnson and Patterson (1973), mutual action of fertility restoring genes from different sources enables the

restorer lines to exceed their parent varieties in amount of pollen produced, pollen dispersal ability and seed yield.

Sage (1976) reviewed the literature on fertility restoration and pointed out that in many studies, the initial genetic explanation was proved by subsequent work to be an oversimplification. For example, restoration of *T. timopheevi* cytoplasm by Primepi has been reported to be caused by a single dominant gene (Oehler and Ingold, 1966) two incompletely dominant genes with a major and minor effect, respectively (Miller *et al.* 1974), two incompletely dominant genes with the epistatic action of a single recessive gene and one major and one minor dominant gene which act in a complementary manner together with modifier gene and one inhibitor gene (Virmani and Edwards, 1983). Using monosomic analysis, it was shown that two *Rf* genes on chromosomes 1B and 5D controlled male fertility restoration in Primepi (Bahl and Maan, 1973).

On the basis of the established correlations between the ability of pollen fertility restoration and anther size, or number of pollen grains per anther and that these characters could be used in the process of breeding R-lines for identification of plants with *Rf*-genes from segregating progenies, Jost and Glatki-Jost (1978) recorded a positive correlation between the ability of fertility restoration and the number of pollen grains per anther, and also recommended that the number of pollen grains per anther to be a subsidiary criterion for determining the presence of *Rf*-genes in plants of segregating progenies.

Nonaka *et al.* (1993) while proposing a new breeding scheme to develop hybrid wheat, observed that one dose of *Rfv*1 gene was enough to restore complete fertility. Earlier Mukai and Tsunawaki (1979) had identified the fertility restorer gene *Rfv*1 to be located on the short arm of 1B chromosome and also established the genetics of fertility

restoration. The segregation of fertiles and steriles in their F_2 generation followed simple Mendelian fashion *i.e.*, 3 fertile: 1 sterile indicating that a single dominant gene controlled fertility restoration. Curtis and Lukaszewski (1993) also confirmed this by identifying *Rfc*3 and *Rfc*4 on the chromosome 6R and 4R respectively of hexaploid wheat with *T. timopheevi* cytoplasm. Contrary to this Ikaguchi *et al.* (1994, 1999) stated that single dose of *Rfv*1 on the 1BS arm of wheat was insufficient to restore a high level of fertility.

Genetic and cytogenetic analyses of R-lines have indicated that one or two major *Rf* genes, one or two minor *Rf* genes and several negative and positive factors modify conditions of the *T.timopheevi* sterility-fertility restoration system of wheat hybrids (Robertson and Curtis, 1967; Maan *et al.* 1984; Samad *et al.* 1997). Expression of major *Rf* genes on chromosomes 1A, 6B and 1B in different R-lines are modified by genes located on other 18 chromosomes (Mann *et al.* 1984; Mann, 1985). The inter relationship of other nuclear genes and individual *Rf* genes has not been investigated. The restoring genes of the restorer line Kansas, bred by Wilson, were located on chromosomes 1A and 7D (Bahl and Maan, 1973) and the restoring genes of Primepi were located on chromosomes 1B and 5D (Bahl and Maan, 1973; Ma and Sorrells, 1995). Ma *et al.* (1991) were successful in transferring a fertility restorer gene from *Aegilops umbellulata* to wheat, where it was found to be located on chromosomes 6 AS and 6 BS.

According to Ganesan and Rangaswamy (1997) fertility restoration in rice lines was governed by two independent dominant genes and the segregation pattern showed semi epistatic (additive 9:6:1 ratio) interaction and dominant epistatic (12:3:1 ratio) interaction. The difference in type of gene interaction could be due to the influence of female parent genotype and modifier genes in female parents. Tahir (1969) noted that

there was not a single common restorer to *Ae. ovata* and *T. timopheevi* cytoplasms. The restorer that induced partial or complete fertility in *Ae. ovata* cytoplasm did not function so in *T. timopheevi* cytoplasm and *vice versa*, indicating the interaction between restorer and male sterile cytoplasm to be undoubtedly very specific. Singh and Rathore, (1992) supported this view and further added that the genotype of the CMS line influences the fertility restoration by the restorer line suggesting the existence of modifier genes, in addition to major genes.

2.2.1. Development of fertility restorer lines

The availability of effective restorer lines is an essential pre-requisite for the exploitation of heterosis in cereal crops using cytoplasmic male sterility systems. In wheat, few studies have reported economically significant yield advantages of F_1 hybrids over the best conventional varieties. However, much of the research on the hybrid wheat has been directed at perfecting the genetic systems. Only since the early 1970's have the leading hybrid programmes devoted more research effort towards the agronomic improvement of male inbred (restorer lines). The identification of varieties potentially useful as female inbreds, their rapid incorporation into a male sterile conversion programme and the maintenance of pure seed have provided a management challenge to hybrid breeders (Virmani and Edwards, 1983). A good restorer should possess satisfactory and stable restoring ability over seasons and locations in addition to superior general combining ability. Knowledge on the nature and mode of gene action for fertility restoration in a CMS system facilitates breeding and selection of restorers used in hybrid programme. Such information help in tailoring the genotypes into effective restorers, which could be used in hybrid breeding programme.

Potential sources of fertility restoring factors have been found among wild tetraploid, as well as hexaploid wheats. Breeding to achieve complete fertility restoration in F_1 hybrids has set the pace of hybrid wheat breeding for many years. It has been pointed out that all species capable of donating a cytoplasm that causes male sterility in common wheat are also sources of corresponding *Rf* genes (Virmani and Edwards, 1983). This is to be expected if it is the absence of specific genes, caused by their elimination during backcrossing that causes pollen development to breakdown in male-sterile nucleocytoplasmic combinations. The initial *Rf* genes were derived from *T. timopheevi* and from a *T. aestivum* line with *T. timopheevi* as one of the parents (Wilson, 1962). List of common wheats that carry *Rf* genes have been provided by several researchers (Zeven, 1967; Porter and Merkle, 1967; Johnson and Schmidt, 1968; Jost and Milohnic, 1976; Ghiasi and Lucken, 1982 a) and it is now a comparatively common occurrence for hybrid wheat breeders to encounter *Rf* genes in common wheat varieties during substitution backcrossing into *T. timopheevi* cytoplasm.

The attempts to introduce *Rf* genes into agronomically adapted wheats by backcrossing almost invariably failed to produce R-lines that would confer complete male fertility to their F_1 hybrids with male steriles having *T. timopheevi* cytoplasm. The R-line breeding programmes therefore must be designed to select for complementary *Rf* gene combinations to accumulate favourable modifier genes and to eliminate inhibitory genes. A preliminary report on the utilization of V-type male sterility in spring wheat (a new type of male sterility in wheat discovered in China) was given by Zong *et al.* (1996) when used for studying sterility rate, anther characteristics and allogamy rate of the sterile lines, agronomic characters of their maintainers and fertility restoration rate in F_1 s of the cross combinations. Of the 100 combinations, 45 % maintained highly sterile

while 55 % restored to different degrees, the fertility, suggesting the importance of selection for satisfactory restorer lines for hybrid seed production

Chinese rice breeders have developed new elite restorer lines through hybridization between restorers/restorers, restorers/maintainers, maintainers/restorers and male sterile lines/restorers besides screening and isolation of restorer on the basis of fertility restoration in test cross progenies. Similarly in wheat, pedigree test cross system is normally used in breeding R-lines; with crosses made so that segregating populations have male-sterile cytoplasm. Over the years, with the identification of several restorer genes, their locations known, and genetics studied, transfer of these genes from one species to another has been adequately demonstrated. Ma *et al.* (1991) transferred a restorer gene from *Aegilops umbellulata* to wheat. Zhou *et al.* (1999) developed a series of restorer lines using a fertility restorer line R16 by accumulating fertility restoring genes and genetically improving them. These workers have demonstrated that fertility restoration is no longer an obstacle for commercial utilization of hybrid wheat.

2.3. Chemical Hybridizing Agents (CHAs)

A more recent and alternate viable system for producing hybrid wheat is through the use of chemicals that induce male sterility, thus circumventing the use of genetic pollination control systems. This approach using CHAs neither requires neither restoration nor conversion of parental lines to a CGMS background. Male sterility induced by CHAs is relatively convenient to use because there is no need to maintain it. Compared with CMS systems, an effective CHA allows the production of large numbers of parental combinations and permits the evaluation of a number of inbreds for combining ability and/or breeding value. This substantially reduces the time required for

hybrid development as noted by a number of authors (Bruns and Peterson, 1998; Wilson, 1984).

The earliest report on the effect of a CHA on wheat was by Hoagland *et al.* (1953), who investigated the effects of maleic hydrazide on spring wheat. Chopra *et al.* (1960) and Porter and Weise (1961) reported the use of maleic hydrazide (250-1000 ppm): both of these groups obtained complete pollen sterility, but considerable female sterility and plant damage was also encountered. Foliar application of 2-chloroethyl phosphoric acid (ethapon or ethrel) induced male sterility in wheat without significantly affecting female fertility (Law and Stoskopf, 1973; Hughes *et al.* 1974; Rowell and Miller, 1974). However, restricted spike emergence (phyto toxicity) and the need for precision in the time of application (narrow target period) have limited the commercial utilization of this chemical. Fairey and Stoskopf (1975) have since reported that soil application of granular ethaphon overcome the phytotoxic effects associated with foliar application, although high rates were required and average sterility was less than 100%. Liable (1974), Virmani and Edwards (1983) and Pickett (1993 a) have suggested the attributes of an ideal CHA.

Jan *et al.* (1974) found that treatment with the compound RH 531 [Sodium 1 – (p-chlorophenyl) – 1, 2 – dihydro – 4, 6- dimethyl – 2-oxinicotinate] several days prior to meiosis at a rate of 2.0 kg ha^{-1} active ingredient (ai) gave maximum reduction in fertility with the spring wheats 'Anza' and 'Yecora 70'. Floral opening was not conducive to cross pollination, and very low seed set resulted. Oxanilates have been reported to selectively impair the pollen formation in monoecious and hermaphrodite plants. Two of the formulations code-named as Compound No.20 and No.60 have been found highly effective in inducing male sterility in barley and corn. (Batch *et al.* 1980).

The synthesis and screening of anilates as chemical hybridsing agents have been successful in rice (Ali *et al.* 1990, 1999) and wheat (Chakraborty *et al.* 2000). Virmani and Edwards (1983) reported that two chemicals-zinc methyl arsenate and sodium arsenate have been used to develop two rice hybrids. However, they also reported that the consensus in development of hybrid rice favours the CMS system. Hybrids in wheat have been released through the use of chemical hybridising agent, SC 2053 in USA and France (Streiff *et al.* 1997). Selective and high induction of male sterility in two cultivars of wheat was observed when fluoro analogues were used (Chakraborty *et al.* 2001a, 2001b). No serious adverse effects on growth and yield parameters were observed. Wilson (1984), Virmani and Edwards (1983) and others have summarized the advantages of using CHA in the development and production of hybrid wheat. The advantages include simplification of breeding procedures, evaluation of large number of lines for general and specific combining ability, development and improvement of heterogeneously diverse breeding populations *etc.* However, despite the extensive works on development of gametocides, 100 per cent rate sterility has still not been achieved. Other disadvantages encountered are reduced seed set of CHA females, difficulty in optimum field application of the CHA, high costs *etc.*, (Wilson, 1984; Virmani and Edwards, 1983). Clearly, the effective use and versatility of CHAs depend on genotypes-chemical, environment-chemical, genotype-environment-chemical interactions.

2.4. CMS utilization in hybrid seed production

The long term sustained interest in CMS is related to the fact that it provides a possible mechanism of pollination control in plants to permit the easy production of commercial quantities of hybrid seed. A CMS system consisting of a male sterile line (the

A-line) an isogenic maintainer line (the B-line) and for crops where male-fertility restored F_1's are required, a restorer (the R-line) must be developed (Allard, 1960).

Schmidt *et al.* (1962) for the first time pointed out the possibility of producing hybrid seed in wheat. This was the result of the development of male sterility and fertility restoration system among *T. timopheevi* derivatives. Nonaka *et al.* (1993) produced male sterile lines caused by the interaction between the Sv type of cytoplasm of *Ae. kotschyi* and the 1BL-1RS chromosome. Based on this interaction, a new breeding scheme was to develop hybrid wheat cultivars was proposed. Shimada *et al.* (1994) proposed the use of anther culture for the same.

The XYZ system, involving three lines, all of which are homozygous for the male sterility mutant and two of these possessing a twenty second chromosome, present in two doses in the X and in one dose in the Y line, was proposed by Driscoll (1972). Later, Driscoll (1978) produced a mutant stock 'Cornerstone' and used it in examining the XYZ system of producing hybrid wheat. This mutant produced male sterility in all environments and in all genotypes tested. Panayatov and Panaiotov (1980) identified promising male sterile cytoplasms for hybrid wheat production in *Ae. mutica* *Ae. triuncialis* and *T. dicoccoides* and obtained several male-sterile alloplasmic lines by backcrossing the nuclei of *T. aestivum* into these.

2.5. Heterosis in wheat

Over the past 75 years since the experiments on artificial hybridization in maize, numerous field crops and vegetables, have benefited from the phenomenon coined by Shull in 1914 as "heterosis" (Goldman, 1997). The definition of heterosis and the explanation of its genetic basis are important for heterosis theory. In practice, however, hybrids must out perform conventional varieties to be commericialised. Infact, heterosis

s a phenomenon by which the performance of an F_1, generated by crossing of different ndividuals, is superior to that of the better parent. The economic significance of heterosis in crop breeding was widely appreciated after the successful exploitation of hybrid vigour in maize. Expression of an adequate vigour of F_1 hybrid is one of the important economic properties, whereas yield and quality are considered as fundamental requirements for the successful use of hybrids in wheat. Heterosis may give hybrids a yield advantage over normal wheats.

Observations of heterosis in wheat dates back to 1919 when Freeman (1919) studied date of first head, height and leaf width in crosses involving a *T. durum* wheat and three common wheats. It was believed that the extent of heterosis, which can appear in wheat as a self-pollinated polyploid species, was insufficient. Briggle (1963) reviewed heterosis in wheat and cited yields up to 84% greater than those of the higher yielding parent. Later, Briggle *et al.* (1967 a, b) demonstrated heterosis for yield in both spring and winter wheat hybrids. Moll *et al.* (1965) in their study on the relationship between the magnitude of heterosis and the degree of divergence concluded that although the amount of heterosis is a linear function of differences in allele frequency for loci having dominance or over dominance effects, the linearity disappears when highly differentiated populations or inbreds or pure breeding lines are crossed.

Yield advantages as high as 10-17% over the leading check cultivars have been reported for hybrid wheat in Italy and the UK. The possibility of introducing F_1 seed into practical cultivation has been greatly enhanced by the discovery of affective chemical hybridising agents (CHAs), although some technical and economic problems concerning the use of CHAs for large-scale production of F_1 seed remain to be solved. However, some gains in heterosis of F_1 hybrids have been claimed in several European countries

i.e., France, U.K. and Italy. A positive trend in F_1 yield was observed, that certain combinations yielded 15% more than the best standard checks (Perenzin *et al.* 1997). Singh *et al.* (1997) assessed 100 F_1 combinations and reported more than 20% heterosis for grain yield in two crosses, *viz.*, CPAN 3048 X HUW 206 and CPAN 4007 X HUW 206, evaluated under high input environment. In China, a yield advantage upto 30% has been reported for hybrids produced using CHAs.

In order to develop commercial hybrids, a number of researchers have reported varying degree of heterosis for grain yield in wheat ranging from 0 to 90% (Sharma and Menon, 1996; Weginaar *et al.* 1998; Prasad *et al.* 1998). Singh and Rathore (1996) studied hybrid wheat seed production based on *T. timopheevi* cytoplasmic male sterile lines and observed that the degree and direction of mid-parent and better parent heterosis varied greatly for different characters and crosses. A greater range of heterosis reported in literature is based on the observation on a limited number of plants (5-10). This unfortunately has misled the breeders about proper interpretation and the commercial exploitation of heterosis. Wheat breeders dealing with various aspects of hybrid wheat found that the standard heterosis for grain yield on large plot basis ranged from 6% (Borghi *et al.* 1986) to as high as 41% (Zehr *et al.* 1997). Other advantages have also been reported such as hybrids' higher yield stability combined with yield potential compared to purelines (Peterson *et al.* 1997).

Plant height is an important character helping in heterotic expression regarding grain yield in wheat. Many scientists have studied the heterosis and genetic features of plant height in hybrid wheat. Li *et al.* (1997) reported heterosis of about 8-10% for plant height and also observed an increase in grain weight per spike. It was suggested that there were relatively high levels of heterosis and heterobeltiosis of plant height (Virmani and

Edwards, 1983; Li *et al.* 1995, 1997), which is controlled by genetic system involved in additive and dominance effects of genes. Jost and Glatki-Jost (1976) noted that exceptional hybrids could produce more tillers per unit area than inbreds regardless of the seeding rate.

High parent heterosis for seed yield in wheat is approximately 20-30% whereas standard heterosis is approximately 10-20% (Edwards, 1987). High parent heterosis is also displayed for plant height, for earlier heading and maturity (Virmani and Edwards, 1983). Nettevich (1968) concluded that the sharpest rise in yield of the F_1 is manifested by crossing high yielding varieties, which differ in origin and agronomic characters. Hybrid wheats tended to have high protein content (Uddin *et al.* 1992; Perenzin *et al.* 1992), dough extensibility and weak gluten compared with parents (Perenzin *et al.* 1992). Other advantages of hybrid wheat include better disease and insect resistance, wide environmental adaptability, better milling and baking traits, seedling vigour and improved root system development (Stroike, 1987). It has been shown lately that wheat hybrids yield significantly more and are more consistent than conventional cultivars, with an increased responsiveness to higher yield potential conditions (Peterson *et al.* 1997 and Jordaan, 1996).

He *et al.* (1998) reported that three hybrids based on *T. timopheevi* and six hybrids based on *Ae. kotschyi* had a 15 percent advantage over commercial pureline cultivars. Heterosis for both grain yield and quality can also be obtained. Livers and Heyne (1968) pointed out that each yield component was important at that no single one was predominant in determining yield. The results obtained by Zhao *et al.* (1997) show that good level of heterosis for grain yield and protein concentration can be achieved in wheat but achieving them simultaneously is likely to be difficult. Corbellini *et al.* (2002)

detected significant heterotic effects for grain yield and other quality attributes in wheat. Epistatic effects, particularly non-fixable types were the contributors to significant and positive heterosis for tillers per plant (Sharma and Sain, 2002) and grain yield per spike (Sharma *et al.* 2002) in *durum* wheat. They further reported a different genetic system governing grain yield per spike.

MATERIALS AND METHODS

"Physiological experiment on animals is justifiable for real investigation, but not for mere damnable and detestable curiosity".

- CHARLES ROBERT DARWIN

3. MATERIALS AND METHODS

The experiments were conducted at the Experimental Farm of the Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi during *rabi* 1999-2000, 2000-2001, 2001-2002 and 2002-2003. Geographically, the experimental site is situated at an altitude of about 228 m above mean sea level with 28° 40' North latitude and 77° 13' E longitude. This region has a semi-arid subtropical climate with alluvial soil, which is slightly alkaline with clay loam texture and low organic matter. Materials were also grown at IARI Regional Station, Wellington during the off-season (July-Oct) 2000, and at Lahaul. However, the data collected from Wellington were not considered due to heavy incidence of rusts and foliar diseases, which affected several agronomic traits including seed set and its quality.

The details of the materials used and methods employed for analysis of data under different experiments have been presented.

EXPERIMENT I

3.1. Characterization of cytoplasmic male sterile lines and their evaluation for stability

3.1.1. Experimental materials

The experimental materials consisted 40 CMS lines (Table 3.1) carrying eight different types of cytoplasms. These lines have been developed through repeated backcrossing (Tomar *et al.* 2001) and maintained in the Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi. The CMS lines are being maintained in the genetic background of traditional hexaploid wheat cultivars.

Table 3.1. CMS sources and their nuclear background in wheat

S.No.	CMS Source	Nuclear background
1.	*T. timopheevi*	HW 2022 HW 2038 HW 2041 HW 2042 HW 2046 UP 2338
2.	*T. araraticum*	HW 2022 HW 2038 HW 2041 HW 2042 HW 2046 HW 2046 HW 2099 HD 2687 PBW 343
3.	*Ae. speltoides*	HW 2022 HW 2038 HW 2042 HW 2046 HW 2643
4.	TMS 8 (source unknown)	HW 2046 HW 2687 UP 2338 PBW 343
5.	TMS 9 (source unknown)	HW 2038 HW 2041 HW 2046 HW 2099 UP 2338
6.	TMS 11 (source unknown)	HW 2019 HW 2022 HW 2046 HD 2428 UP 2338 PBW 343
7.	TMS 20 (source unknown)	HW 2041 HW 2042 HW 2046 UP 2338
8.	*Ae. caudata*	HD 2160

Three of the CMS lines carried the cytoplasm from wild species of wheat namely, *Triticum timopheevi*, *T. araraticum* (both 2n = 2 x = 28, genome AAGG), *Aegilops speltoides* (2n = 2x =14, genome SS) and *Ae. caudata* (2n=2X=14, genome CC). The rest of the CMS lines were designated TMS 8, TMS 9, TMS 11 and TMS 20 which were also maintained in the Division of Genetics, IARI, New Delhi with unknown cytoplasmic source. All the CMS lines have been maintained by hand pollination. Thirteen diverse genotypes were selected for conversion into CMS lines (Table 3.2) and six to seven backcrosses were administered to restore full nuclear genome. Most of the genotypes selected for conversion in CMS showed resistance to rusts, a major wheat disease causing severe yield losses.

Table 3.2. Pedigree of the genotypes used for nuclear background in different CMS lines

Sl.No.	Genotype	Pedigree
1	HW 2019	WH 542*6 / TR 380 –14 *7 / 3 Ag # 14
2	HW 2022	WH 147*6 // DARF*6/3 Ag #3 / Kite
3	HW 2038	HD 2285*7 //CS 2A / 2M # 4/2
4	HW 2041	Lok –1*7 // Sunstar *6/ C80-1
5	HW 2042	WH 147*7 // Sunstar*/ C80-1
6	HW 2046	HD 2329*7 // Sunstar */ C80-1
7	HW 2099	HW 2033 / Kite
8	HD 2160	M2*3 //YT54/N10B/3/CAL/4/TOB/CFTN/5/HD 1949
9	HD 2428	HD 1949 / HD 2160
10	HD 2643	VEE 'S' / HD 2407 // HD 2329
11	HD 2687	CPAN 2009 / HD 2329
12	PBW 343	ND / VG 7944 // KAL // BB/3/YACO 'S' / 4/VEE # 5 'S'
13	UP 2338	UP 368 / VL 421 // UP 262

3.1.2. Experimental methods

The various CMS lines were sown during *rabi* 1999-2000 under three environments created by different dates of sowing. *i.e.*, 26th November, 11th December

and 28th December. The observations on pollen fertility and spikelet fertility were used as indices to establish the stability of CMS lines. Observations were recorded on following parameters:

3.1.2.1. Pollen fertility

At the time of flowering, spikes from five randomly selected plants were collected for recording observation on pollen stainability. Anthers of three spikelets taken randomly from lower, middle and top portion of the main spike were smeared on a glass slide in a solution containing 0.5 per cent iodine prepared in 2 per cent potassium iodide. The pollen grains were examined under light microscope in three different microscopic fields with 10X, 25X and 40X magnification. The pollen grains were classified into four groups (Chaudhury *et al.* 1981), namely unstained withered sterile (UWS), unstained spherical sterile (USS), stained round sterile (SRS) and stained round fertile (SRF).

The number of pollen grains coming under each category was counted in different microscopic fields and those falling only under SRF category were considered as fertile and the remaining pollen grains were considered as sterile. The estimate of pollen sterility was obtained by expressing the number of sterile pollen grains in percentage of the total number of pollen grains observed. Based on the data on pollen sterility percentage the CMS lines were placed appropriately in the following groups:

Types	Pollen sterility (%)
Completely sterile	100
Highly sterile	99-99.9
Sterile	95-98.9
Partially sterile	70-94.9
Partially fertile	<70

(after Gautam *et al.* 1997)

3.1.2.2. Spikelet fertility

At the time of spike emergence, prior to anthesis the main spikes of five randomly chosen plants were bagged with butter paper bag (Plate.1). The estimate of spikelet sterility was obtained by counting the number of seeds set on the spike.

3.1.2.3. Phenotypic stability

The data on five randomly chosen plants in each of the three environments was recorded for agronomic and floral characters are as follows:

3.1.2.3.1. Floral traits

The measurements on the floral traits were made at the time of anthesis. The floral traits, *viz.*, anther size, filament length, ovary size and length of style were measured using microscope.

(a) Glume opening angle (degrees)

The angle subtended by the lemma and palea at the time of anthesis is referred to as glume opening angle. The angle was measured using a protractor graduated in degrees.

(b) Anther size (mm)

The anther size was measured in millimeter in its longest dimension *i.e.*, from the tip to the point of its attachment to the filament.

(c) Filament length (mm)

The length of filament was measured in millimeter between the point of attachment of the filament to the anther and the point of its attachment to the base of the ovary.

Plate 1. Field view of A (centre) and B (extreme left and right) lines.

(d) Ovary size (mm)

The shape of the ovary is generally peculiar and does not fall in any dimension. Therefore, the ovary size was measured at the point where it showed maximum width.

(e) Length of style (mm)

The length of style was measured in millimeter from the point of its attachment with ovary to the tip.

(f) Stigma receptivity (days)

One spike of each male sterile line was selected. The spikelets on the top and bottom of the spike were removed and not more than 11-13 spikelets were retained so as to ensure similarity in the maturity of the spikelets. The selected spikes were pollinated with the pollen from their respective maintainer lines by giving 0, 1, 2, 3, 4, 5 and 6 days starvation and covered with butter paper bags. At maturity, crossed spikes were harvested and threshed separately and seed yield recorded.

(g) Out-crossing potential (percent)

The out crossing potential of the CMS lines was estimated by two methods as follows:

Method I

The male sterile (A) line and its respective maintainer line (B) were planted randomly. The seeds of A line were dibbled at 10 cm distance keeping a row to row distance of 30 cm.

The out-crossing potential was estimated after allowing for open pollination. Natural outcrossing was allowed. Spikes from 10 randomly selected plants from each CMS line were harvested and outcrossing potential was calculated using the formula and using the number of seeds produced by each maintainer (B) line (Table 3.3).

$$\text{Outcrossing potential (\%)} = \frac{\text{No. of seeds set on the main spike of the CMS line}}{\text{Av. No. of seeds on the main spike of the corresponding B line}} \times 100$$

Table 3.3. List of maintainers / varieties (B lines) and their average number of seeds per spike

Sl.No.	Variety / maintainer (B line)	Number of seeds
1.	HW 2019	51
2.	HW 2022	70
3.	HW 2038	62
4.	HW 2041	42
5.	HW 2042	65
6.	HW 2046	60
7.	HW 2099	85
8.	HD 2428	61
9.	HD 2643	52
10.	HD 2687	56
11.	PBW 343	60
12.	UP 2338	70

Method II

During 2001-2002, A and B lines were planted in 2:1, 3:1 and 4:1 ratios as shown in Fig.1. Only three CMS lines *viz.*, *arar* 2022 A, TMS 8/2046 A and TMS 20/2338 A were considered in this method for estimation of out-crossing potential. The seeds of A and B lines were dibbled at 10 cm distance keeping a row to row distance of 30 cm.

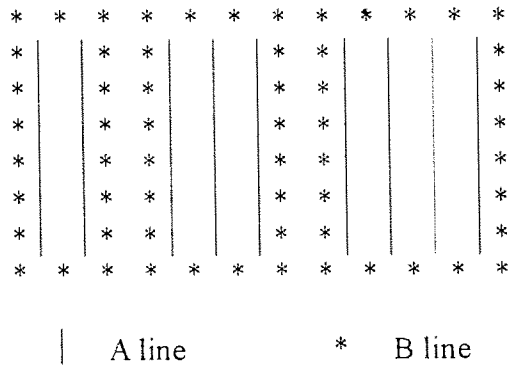


Fig. 1. The scheme of growing A and B lines to estimate outcrossing potential in wheat.

The out-crossing potential was estimated after allowing for open pollination. Neither rope pulling during anthesis nor artificial pollination was done. Spikes from 10 randomly selected plants from each of the CMS lines was harvested and out crossing potential was calculated as in method I.

3.1.2.3.2. Agronomic traits

(a) Number of tillers

The number of productive (spike bearing) tillers was counted in each randomly selected plant at the time of maturity.

(b) Plant height (cm)

The data on plant height *i.e.*, the vertical distance of the plant from the ground level to the tip of the main ear excluding awns was taken at the time of maturity.

(c) Main spike length (cm)

The data on linear extent of the main spike from the base to its tip excluding the awn was taken at the time of maturity.

(d) Number of spikelets per main spike

The total number of spikelets present on the rachis of a single main spike was counted in each selected plant.

(e) Days to 50% flowering

It was measured as the number of days taken for 50 % of the plant population to initiate anthesis.

(f) Synchrony of tillers

It is considered as the number of days taken by all the tillers of a single plant to reach anthesis.

3.1.3. Statistical Analysis

The mean values of the entries were subjected to standard statistical analysis to test whether A and B lines differed significantly for their floral and agronomic traits.

3.1.3.1. Paired *'t'* test

The A and B lines, though grown at different dates of sowing are expected to be correlated. Therefore paired 't' test was considered appropriate which is estimated as follows (Chandel, 1999):

$$t = \frac{|d|}{s/\sqrt{n}} \text{ with } (n-1) \text{ d.f.}$$

Where,

$\bar{d}$ = mean of differences

$$\bar{d} = \frac{1}{n}\sum d$$

s = estimate of the standard deviation

$$s = \sqrt{\frac{1}{n-1}\sum (d-\bar{d})^2}$$

The two sets of data in which the observations are paired, the differences of these paired observations were taken. A null hypothesis was set with an assumption that there was no difference between the observations of any of the different pairs. When the calculated 't' value at 5% level of significance for n-1 d.f. was greater than the table value, it was found to be significant and the null hypothesis was rejected.

EXPERIMENT II

3.2. Identification of maintainers and restorers

3.2.1. Experimental materials

The experimental materials consisted 521 test cross combinations involving eight cytoplasmic male sterile (CMS) sources and 294 pollen parents (Appendix I). The pollen parents composed of land races, indigenous and recently developed genotypes, synthetic wheats and inter-specific derivatives. Test crosses were made during *rabi* 1999- 2000.

3.2.2. Experimental methods

The test cross hybrids were sown during *rabi* 2000-2001 at the Experimental Farm, IARI, New Delhi. The hybrids were grown in a single row plot of 4 m length

spaced at 30 cm. Recommended agronomic practices were followed for raising the crop. The observations on spikelet fertility of the hybrids (test cross) was recorded and used to identify maintainers and restorers of different CMS lines:

3.2.2.1 Estimation of spikelet fertility

At the time of flowering, prior to anthesis, the main spike of five randomly selected plants were covered with butter paper bag. Fertility was recorded by counting the number of seeds set on the main spike. Based on the number of seed set, the pollen parents of the test cross hybrids were classified into the following categories:

Category	Number of seed set per main spike (range)
Complete maintainers	0-1
Partial maintainers	2-25
Partial restorers	26-45
Complete restorers	>45

EXPERIMENT III

3.3. Inheritance studies

3.3.1. Inheritance of fertility restoration

3.3.1.1. Experimental materials

Out of 12 fully fertile F_1 hybrids, only five hybrids were selected to study the inheritance of fertility restoration. These F_1 hybrids comprised of five cross combinations involving TMS 9 / 2041 A, TMS 20 / A 2046 A with PWR 4101, and TMS 9 / 2046 A, TMS 11 / 2019 A and TMS 20 / 2046 A with PWR 4099. Based on the seed set in F_1 hybrids, two exotic lines PWR 4101 and PWR 4099 were considered as complete restorers.

3.3.1.2. Experimental methods

The parental lines, F_1's and F_2's were grown in 6m-row plot maintaining seed to seed distance of 10 cm and row-to-row distance of 30 cm. More than 300 plants in each cross were grown to maintain sufficient F_2 population. Observations on pollen fertility and seed set in the main spike were used as indicators of fertility restoration for analyzing the segregation pattern. Following the same procedure as mentioned under, the pollen fertility was determined. For recording observation on seed set, the main spike was covered with butter paper bag (Plate.2) at the time of spike emergence prior to anthesis and the spikelet fertility was computed by counting the number of seeds set per spike. Based on the observations recorded on pollen as well as on spikelet fertility, the segregating F_2 plants were classified into different categories as follows:

Classification of spikelet fertility in F_2 generation in wheat

Category	Number of seed set on main spike	Range of pollen fertility (%)
Completely fertile	>45	95-100
Partially fertile	2-45	70-95
Completely sterile	0-1	<70

3.3.1.3. Statistical analysis

Employing chi-square analysis, the goodness of fit of null hypothesis was tested as follows:

$$\chi^2 = \frac{\Sigma(O-E)^2}{E}$$

Plate 2. Field view showing selfing of individual F_2 generation spikes derived from A X R lines

Where O is the observed frequency, and E, is the expected frequency. A null hypothesis that there is no difference between observed and expected frequencies was set when the calculated χ^2 value was less than the table value (at P = 0.05) for n-1 degrees of freedom. If the calculated χ^2 value was greater than the table value, null hypothesis was rejected and an alternate genetic ratio was tested.

3.3.2. Genetics of kernel colour of R lines

3.3.2.1. Experimental materials

Six F_1 hybrids namely, CMS 9 / 2041 X PWR 4099, CMS 8 / 2046 X PWR 4099, PBW 226 X PWR 4099, HW 2045 X PWR 4099, HW 2099 X PWR 4099, and HW 2048 X PWR 4099 were considered to work out the inheritance of seed colour.

3.3.2.2. Experimental methods

Kernel colour was recorded on visual observations of the kernels. The kernel of restorer line was red whereas the grains of A/B lines were amber. Only two categories, namely, amber and red were made to score the kernel colour of individual plants.

3.3.2.3 Statistical analysis

Chi-square test was applied to analyse the data recorded on seed harvested from each F_2 plant.

$$\chi^2 = \frac{\Sigma (O-E)^2}{E}$$

Where O is the observed frequency, and, E is the expected frequency. A null hypothesis that there is no difference between observed and expected frequencies was set when the calculated χ^2 value was less than the table value (at P = 0.05) for n-1 degrees of

freedom. If the calculated χ^2 value was greater than the table value, null hypothesis was rejected and an alternate genetic ratio was tested.

EXPERIMENT IV

3.4.1. Studies on yield and yield components

Eleven hybrids *viz.*, PHW 1, PHW 2, PHW 3, PHW 4, PHW 5, PHW 6, PHW 7, PHW8, PHW 9, PHW 10 and PHW 11 (Table 3.4.) along with their maintainer and restorer lines were grown in rows six metres with three replications following randomized block design (RBD), in 2001-2002. Row to row distance was kept as 30 cm and plant-to-plant distance was maintained at 10 cm. Data was taken on ten randomly selected plants from each replication and was subjected to statistical analysis.

Table 3.4. List of wheat hybrids along with their parentage used under study

Sl.No	Hybrid	Parentage
1	PHW 1	*arar* 343 A X PWR 4099
2	PHW 2	*arar* 2022 A X PWR 4099
3	PHW 3	*arar* 2099A X PWR 4099
4	PHW 4	TMS 8/2046 A X PWR 4099
5	PHW 5	TMS 9/2041A X PWR4099
6	PHW 6	TMS 11/2428 A X PWR 4099
7	PHW 7	TMS 9/2046 A X PWR 4099
8	PHW 8	TMS 11/2019 A X PWR 4099
9	PHW 9	TMS 20/2046 A X PWR 4099
10	PHW 10	TMS 9/2041 A X PWR 4101
11	PHW 11	TMS 20/2046 A X PWR 4101

3.4.2. Studies on yield performance of hybrids

An experiment was conducted during *rabi* 2002-2003 in randomized block design to study the comparative yield performance of hybrids and varieties. The treatments (genotypes) consisted five hybrids *viz.*, PHW 1, PHW 2, PHW 3, PHW 12 and PHW 13 (Table 3.5), six varieties namely, PBW 343, HW 2022, HW 2099, HW 2046, HD 2329, HD 2687 and a restorer PWR 4099. Each hybrid and check variety was grown in 6 rows of 5 m length with row-to-row distance of 23 cm. The experimental material was replicated three times. All the plots were harvested manually and threshed in Pullman thresher.

Table 3.5. List of wheat hybrids and their parentage under study

Sl.No.	Hybrid	Parentage
1	PHW 1	*arar* 343 A X PWR 4099
2	PHW 2	*arar* 2022 A X PWR 4099
3	PHW 3	*arar* 2099 A X PWR 4099
4	PHW 12	*arar* 2046 A X PWR 4099
5	PHW 13	*arar* 2687 A.X PWR 4099

3.4.3. Statistical analysis

Replication-wise mean values of each genotype (parents / varieties / standard check, hybrids) for yield per hectare and yield components were recorded and used for statistical analysis.

3.4.3.1. Analysis of variance

The significance of differences between genotypes for yield and yield components was tested for significances by using analysis of variance technique (Panse and Sukhatme, 1985). Analysis of variance was done on the basis of following model:

$Y_{ij} = \mu + g_i + r_j + e_{ij}$

Where

Y_{ij} = Phenotypic observation of i^{th} genotype in j^{th} replication

μ = Overall mean of the replication

g_i = Effect of the i^{th} genotype

r_j = Effect of j^{th} replication

e_{ij} = Random error associated with i^{th} genotype and j^{th} replication

The structure of analysis of variance (ANOVA) was as follows:

Source	**d.f.**	**MS**	**Expected MS**	**F value**
Replication	r-1	Mr	$\sigma^2e + g\,\sigma^2r$	
Treatment (genotype)	g-1	Mg	$\sigma^2e + r\,\sigma^2g$	Mg/Me
Error	(r-1) (g-1)	Me	σ^2e	
Total	(rg-1)			

Where

r = Number of replications

g = Number of genotypes

Mr, Mg and Me = Mean squares due to replication, genotype and error, respectively

σ^2e = Error variance (Me)

σ^2g = Genotypic variance (Mg – Me/r)

σ^2p = Phenotypic variance ($\sigma^2g + \sigma^2e$)

MS, due to genotypes were tested against the error variance using F test at P = 0.05 or P = 0.01 with V_1 and V_2 degrees of freedom, where V_1 in the degree of freedom for higher value of variance and V_2 in the degree of freedom for lower value of variance.

3.4.3.2. Estimation of standard error of mean and critical difference

Mean value for yield was worked out by dividing the total value with the corresponding number of observations. Standard error of mean and critical difference were calculated as follows:

$$\text{Standard error of mean (S.Em)} = \sqrt{\frac{EMS}{r}}$$

Critical difference of mean = S.Em x t value at 5% level of significance.

3.4.4 Estimation of heterosis

Heterosis was estimated for yield and yield characters *viz.*, number of tillers, plant height, spike length, number of spikelets, number of grains per spike, yield per spike, yield per plant and 1000-grain weight. Heterosis was estimated over mid parent (average heterosis), over better parent (heterobeltiosis), and over standard check (standard heterosis). The estimation of heterosis was calculated according to the following formulae.

1.

$$\text{Average heterosis (\%)} = \frac{F_1 - MP}{MP} \times 100$$

F_1 = Mean value of F_1
MP = Mean value of the two parents involved

2.

$$\text{Heterobeltiosis (\%)} = \frac{F_1 - BP}{BP} \times 100$$

F_1 = Mean value of F_1
BP = Mean value of better parent

3.

$$\text{Standard Heterosis (\%)} = \frac{F_1 - \text{Standard check}}{\text{Standard check}} \times 100$$

F_1 = Mean value of F_1
Standard check = Mean value of standard check

The standard check variety used was PBW 343.

3.4.4.1. Testing the significance of heterosis

The critical differences (CD) for testing the significance of heterosis were calculated as follows (Roy, 2000):

Critical difference for heterosis over mid-parent (MP)

$$CD(MP) = \sqrt{\frac{3\,Me}{2r}} \times t$$

Critical differences for heterosis over better-parent (BP) or standard check variety

$$CD\ (BP \text{ or standard check variety}) = \sqrt{\frac{2\,Me}{r}} \times t$$

Where

Me = error mean squares

r = number of replication

t = table value of t at 5 or 1 percent level of significance

RESULTS

"But in science, the credit goes to the man who convinces the world, not to the man whom the idea first occurs".

- SIR FRANCIS DARWIN

4. RESULTS

To meet the ever-growing demand of wheat, a dynamic technology for diversification of wheat production is needed. With continuous exploitation of genetic diversity, breeders are left with limited option for raising yield levels either through the restructuring of plant type or development of hybrid technology. The commercial success of hybrid rice, strictly a self-pollinated crop, has demonstrated the potential of hybrid technology to meet the global demands. Recently, the possibility to produce hybrid wheat has been met with enthusiasm. The success in production of hybrid wheat depends on the efficiency of a system to produce hybrid wheat either through two line or through three line breeding approach. The present investigation was therefore, undertaken to characterize the cytoplasmic male sterile lines, to identify fertility restorers, to carryout genetic analysis of fertility restoration and to develop hybrid seed technology in order to identify heterotic hybrids.

The experimental results of the studies conducted during the present investigation have been presented under the following heads.

4.1. Characterization of CMS lines

4.2. Stability of CMS lines

4.3. Identification of maintainers and restorers

4.4. Genetics of fertility restoration and kernel colour

4.5. Heterosis manifestation

4.1. CHARACTERIZATION OF CMS LINES

4.1.1. Floral traits

The results of paired 't' test with respect to floral traits, namely, anther size, filament length, ovary size and length of style have been presented in Table 4.11. The observations presented show the mean values of six floral traits (glume opening angle, anther size, filament length, ovary size, length of style and stigma receptivity) of CMS lines and their fertile counterparts. The genetic variation between CMS (A) and maintainer (B) lines were however observed for only four floral characters, *viz.*, anther size, filament length, ovary size and length of style. In general, the CMS lines had values less than or equal to that of their fertile counterparts with respect to all the floral traits. For example, the CMS lines produced small, shrunken and empty anthers as compared to their maintainer lines (Plate 3).

Four out of six CMS lines carrying *T. timopheevi* cytoplasm, *timo* 2038 A, *timo* 2042 A, *timo* 2046 A and *timo* 2338 A had significantly less mean values for anther size than their respective maintainer lines. The rest of the two CMS lines also had numerically less values than the B lines but were not statistically significant. Only two CMS lines (*timo* 2042 A, and *timo* 2046 A) had significantly less values for filament length while three lines had significantly less value for ovary size (*timo* 2038 A, *timo* 2042 A and *timo* 2338 A). Except for *timo* 2042 A, all the CMS lines carrying *timo* cytoplasm had significantly lower mean values for style length. None of the six CMS lines with *T. timopheevi cytoplasm* showed higher mean values than their respective B lines for any of the floral trait. Fifty per cent of the eight CMS lines carrying *T. araraticum* cytoplasm (*arar* 2022 A, *arar* 2038 A, *arar* 2042 A and *arar* 2099 A) had significantly lower mean than B lines for anther size.

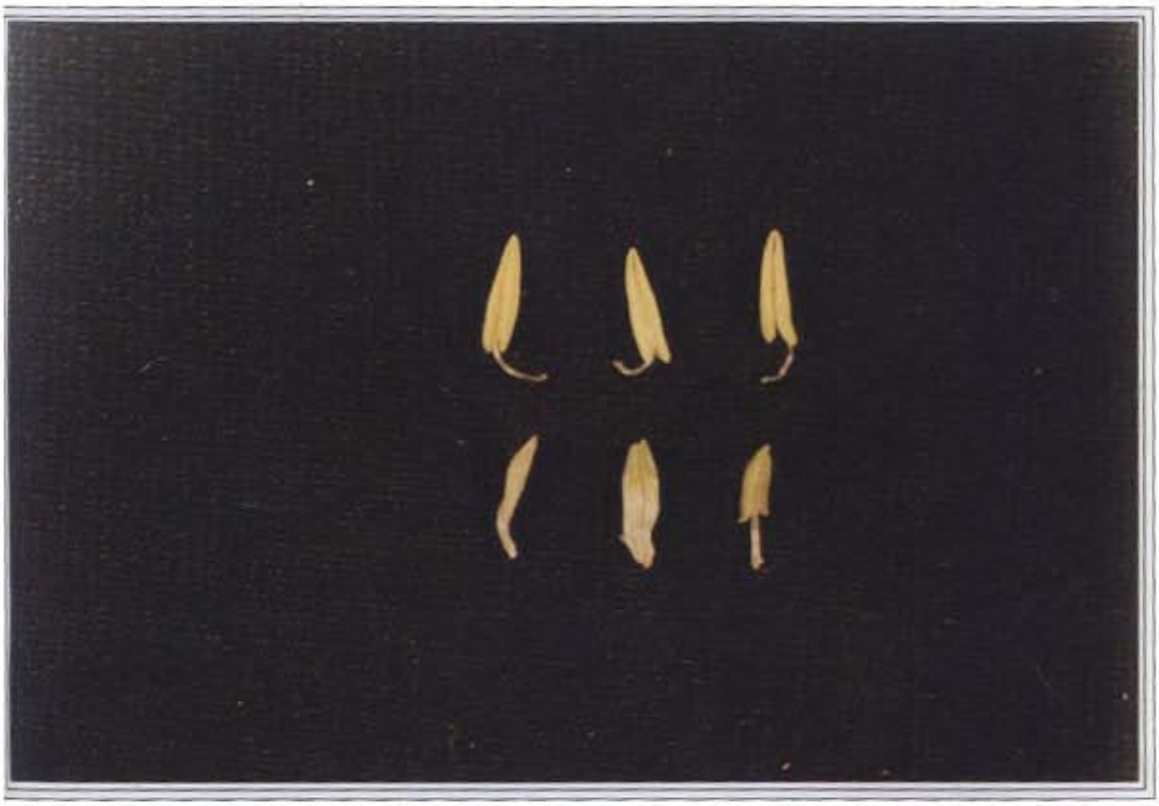


Plate 3. Normal anthers of maintainer (B) line (top) and abnormal anthers produced by CMS line

The remaining four CMS lines also had numerically lower values than respective B lines. Six CMS lines (arar 2022 A, arar 2041 A, *arar* 2042 A, *arar* 2046 A, *arar* 2687 A and *arar* 343 A) showed significantly lower mean values for filament length. Five CMS lines, namely, *arar* 2038 A, *arar* 2042 A, *arar* 2046 A, *arar* 2099 A, and *arar* 343 A exhibited significantly low mean values for ovary size, while *arar* 2687 A had higher significant value for the same character. Only two CMS lines, *arar* 2099 A and *arar* 2637 A had significantly lower values for length of style. There were two CMS lines (*arar* 2041 A and *arar* 2042 A), which exhibited marginally higher mean values as compared to their respective maintainer lines but were statistically non-significant.

It was interesting to note that majority of CMS lines possessing *Ae. speltoides* cytoplasms had significantly higher mean values for all the four floral traits. The lines *spelt* 2038 A and *spelt* 2643 A had larger anthers than their B lines and varied significantly. The CMS line *spelt* 2042 A also had larger anthers but the difference in size was not significant. The rest of the CMS lines *viz.*, *spelt* 2022 A and *spelt* 2046 A had significantly lower mean values. The filament length of the CMS lines carrying *Ae. speltoides* cytoplasm and their maintainer line exhibited significant differences except for *spelt* 2643 A. The CMS line *spelt* 2038 A possessed longer filaments than its B line. Three lines *viz.*, *spelt* 2038 A, *spelt* 2042 A and *spelt* 2643 A had smaller ovaries than their B lines while *spelt* 2046 A had larger ovaries but did not show significant difference. Out of the five, two CMS lines (*spelt* 2038 A and *spelt* 2643 A) possessed longer styles than their B lines, while two others (*spelt* 2022 A and *spelt* 2046 A) had shorter styles.

Significant differences in anther size were observed among all the four CMS lines (TMS 8 / 2046 A, TMS 8 / 2687 A, TMS 8 / 2338 A and TMS 8 / 343 A) carrying TMS 8

cytoplasms of which only TMS 8 / 2687 A had larger anthers than its B line while only two CMS lines TMS 8 / 2687 A and TMS 8 / 2338 A showed significantly different values for filament length. The CMS line TMS 8 / 2046 A had longer filament while, TMS 8 / 343 A had shorter filament but the differences in both the cases were non-significant. The CMS line TMS 8 / 2687 A had larger ovary than that its B line, while the remaining CMS lines did not show any significant difference in ovary size. All the four CMS lines with TMS 8 cytoplasm varied significantly for length of style. The line TMS 8 / 2338 A, exceeded in stylar length as compared to the B line; and the remaining lines had reduced style length in comparison to their fertile counterparts (Table 4.11).

All the CMS lines carrying TMS 9 cytoplasm, except TMS 9 / 2038 A had smaller anthers than their fertile counterparts. The differences in anther size were significant. The lines having TMS 9 cytoplasm showed poor variation for ovary size. However, TMS 9 / 343 A showed non-significant variation for ovary size. Only three CMS lines *viz.*, TMS 9 / 2038 A, TMS 9 / 2041A and TMS 9 / 2046 A showed significant difference for filament length the first two being shorter and the latter was longer than the B lines. Numerically, three A lines TMS 9 / 2041 A, TMS 9 / 2099 A and TMS 9 / 2338 A had shorter style length than the B lines.

Out of six CMS lines possessing TMS 11 cytoplasm only two, TMS 11 / 2022 A and TMS 11 / 343 A showed significant differences for anther size with their maintainer lines. Only two lines, TMS 11 / 2019 A and TMS 11 / 2338 A did not have significant differences for filament length, while the remaining lines had significantly lower values. One CMS line, TMS 11 / 2046 A had larger ovary size (1.88 mm) than its B line (1.72 mm) while two others (TMS 11 / 2428 A and TMS 11 / 343 A) had significantly smaller ovary size than their B lines.

Table 4.11. Floral traits of CMS lines and their fertile counterparts in wheat

Nuclear background	CMS source	Floral traits										
		Glume opening angle (degree)		Anther size (mm)		Filament length (mm)		Ovary size (mm)		Length of style (mm)		Stigma receptivity (days)
		Ave.	Range	A	B	A	B	A	B	A	B	
HW 2022	*T.timopheevi*	7.8	5-12	3.96	4.00	2.90	3.02	1.80	1.80	2.40**	2.70	4
HW 2038		8.3	7-10	3.40*	3.84	1.94	1.98	1.58**	1.90	2.46**	2.68	4
HW 2041		8.0	8.0	3.52	3.68	1.98	2.0	1.50	1.62	2.32**	2.98	4
HW 2042		10.0	10.0	3.54*	3.92	2.04**	2.58	1.52**	1.98	2.36	2.64	4
HW 2046		8.7	8-10	3.76*	4.08	2.00 *	2.54	1.58	1.72	2.56**	3.00	4
UP 2338		7.4	5-10	3.56*	3.92	1.92	2.12	1.52*	1.76	1.98**	2.30	4
HW 2022	*T.araraticum*	13.3	12-15	3.76*	4.00	2.58*	3.02	1.72	1.80	2.64	2.70	4-5
HW 2038		7.6	7-8	3.10**	3.84	1.84	1.98	1.42*	1.90	2.58	2.68	4
HW 2041		12.8	12-15	3.48	3.68	1.70**	2.00	1.62	1.62	3.08	2.98	5
HW 2042		8.6	8-10	3.18**	3.92	2.04**	2.58	1.64*	1.98	2.68	2.64	4
HW 2046		7.1	6-8	4.00	4.08	2.04*	2.54	1.50*	1.72	3.00	3.00	4-5
HW 2099		7.4	5-10	3.62**	4.04	1.98	2.12	1.38*	1.72	1.60**	2.60	4
HD 2687		8.8	7-13	3.52	3.70	2.00**	2.40	1.84**	1.62	1.62**	2.50	5
PBW 343		11.1	10-12	3.98	4.04	2.08**	2.90	1.58**	1.72	2.98	3.06	4
HW 2022	*Ae.speltoides*	7.2	5-10	3.76*	4.00	2.10**	3.02	1.80	1.80	2.34*	2.70	4-5
HW 2038		6.3	5-7	4.58**	3.84	2.52**	1.98	1.54*	1.90	2.90*	2.68	4
HW 2042		17.9	17-20	4.04	3.92	2.16*	2.58	1.48*	1.98	2.64	2.64	4
HW 2046		8.4	8-10	3.54**	4.08	1.98**	2.54	1.86	1.72	1.54**	3.00	4-5
HW 2643		6.2	5-8	4.44**	3.78	2.32	2.32	1.52**	1.74	2.96**	2.54	4
HW 2046	TMS 8 (Source unknown)	10.8	10-12	3.58**	4.08	2.80	2.54	1.56	1.72	1.80**	3.00	4-5
HW 2687		13.5	12-15	3.98*	3.70	2.00**	2.40	2.08*	1.62	1.82**	2.50	4
UP 2338		9.4	6-12	3.50*	3.92	1.98*	2.12	1.66	1.76	2.68**	2.30	4
PBW 343		10.0	10.0	3.64**	4.04	2.82	2.90	1.54	1.72	2.14**	3.06	4

Contd....

Nuclear background	CMS source	Floral traits										
		Glume opening angle (degree)		Anther size (mm)		Filament length (mm)		Ovary size (mm)		Length of style (mm)		Stigma receptivity (days)
		Ave.	Range	A	B	A	B	A	B	A	B	
HW 2038	TMS 9 (Source unknown)	5.4	5-7	3.58	3.84	1.70*	1.98	1.60	1.80	2.44**	2.70	4-5
HW 2041		8.7	5-12	3.16*	3.68	1.70**	2.00	1.58	1.62	2.94	2.98	4-5
HW 2046		13.3	10-15	3.22**	4.08	3.00**	2.54	1.58	1.72	1.74*	3.00	4-5
HW 2099		7.7	6-10	3.60**	4.04	1.98	2.00	1.60	1.72	2.62	2.60	4
UP 2338		6.1	5-7	3.50**	3.92	1.94	2.12	1.62	1.76	2.48	2.30	4
PBW 343		5.8	5-7	3.60*	4.04	1.78**	2.90	1.50*	1.72	2.52**	3.06	5
HW 2019	TMS 11 (Source unknown)	6.5	5-7	3.82	3.82	2.5	2.64	2.14*	2.44	3.02	3.00	5
HW 2022		8.1	7-12	3.82*	4.00	2.12**	3.02	1.68	1.80	2.50*	2.70	4
HW 2046		6.4	6-8	4.04	4.08	2.06*	2.54	1.88*	1.72	2.34*	3.00	4-5
HD 2428	.	7.5	6-14	3.70	3.94	2.20*	2.62	1.82**	2.26	2.36	2.54	4-5
UP 2338		10.6	10-12	3.98	3.92	2.06	2.12	1.64	1.76	1.68**	2.30	4-5
PBW 343		8.7	7-10	3.68*	4.04	2.74*	2.90	1.52*	1.72	2.46**	3.06	4-5
HW 2041	TMS 20 (Source unknown)	8.5	8-10	3.08*	3.68	1.74	2.00	1.66	1.62	2.62	2.98	4-5
HW 2042		10.3	10-15	3.98*	3.92	2.12*	2.58	1.46**	1.98	2.38	2.64	4-5
HW 2046		12.8	10-15	3.66**	4.08	2.02**	2.54	1.98*	1.72	2.62*	3.00	4-5
UP 2338		9.0	7-10	3.96	3.92	1.80	2.12	1.66	1.76	1.98*	2.30	4-5

A – CMS; B-maintainer. ****, * Significant at 0.01% and 0.05%, respectively**

None of the CMS lines had higher values for style length but three of them *viz.*, TMS 11 / 2022 A, TMS 11 / 2046 A and TMS 11 / 2338 A showed significant differences for style lengths than B lines (Table 4.11). The CMS line TMS 20 / 2042 A had larger anthers while TMS 20 / 2041 A and TMS 20 / 2046 A had small anthers than their B lines; Although the above mentioned CMS lines carried the same sources of cytoplasm, the CMS lines TMS 20 / 2042 A and TMS 20 / 2046 A had shorter filaments in comparison to the B lines. However, the former CMS line had smaller and the latter had bigger ovaries respectively. TMS 20 / 2046 A and TMS 20 / 2338 A had shorter styles than their fertile counterparts.

As compared to other floral traits, the glume-opening angle had a larger variation within and among different CMS sources. Glume opening in male sterile line has been depicted in Plate 4. The minimum was 6.2 degrees (*spelt* 2643 A) and the maximum 17.9 degrees (*spelt* 2042 A). Stigma receptivity of the various CMS lines had the least variation, as all of the lines were receptive for a minimum of 4 days and to a maximum of 5 days. None of the line exceeded 5 days for stigma receptivity (Table 4.11).

4.1.2. Studies on out-crossing

Data on pollen and spike fertility and out crossing potential of the CMS lines is presented in Table 4.12. The pollen fertility and seed set data in method I was in full agreement for all the CMS lines. There was no seed set upon selfing, despite the occurrence of marginal pollen fertility in a few CMS lines such as *timo* 2041 A, *arar* 2038 A, *arar* 2041 A, *arar* 2046 A, *arar* 2099 A, *arar* 343 A, *spelt* 2042 A, TMS 8 / 2687 A, TMS 9 / 2038 A and TMS 11 / 2338 A. In uncovered spikes the maximum seed set of 8.1 per spike was recorded for *arar* 2022 A which is almost equal to an out crossing potential of 13.38%. The CMS line *timo* 2022 A and *timo* 2338 A did

not set seed even on out crossing. Only six other CMS lines *viz.*, TMS 8 / 2046 A, TMS 9 / 2038 A, TMS 9 / 2041 A, TMS 9 / 2046, TMS 11 / 2428 A and TMS 11 / 343 A had an out crossing potential of over 10%. However, the supply of pollen was not guaranteed (Table 4.12).

In the first experiment for studying outcrossing potential, the supply of pollen to A line was not assured. Therefore, another method for finding out the outcross potential was designed, where A and B lines were planted in three different ratios (2:1, 3:1 and 4:1 respectively for A and B lines) to ensure supply of pollen. Only three CMS lines *viz.*, *arar* 2022 A, TMS 8 / 2046 A and TMS 20 / 2338 A were considered in method II for calculating outcrossing potential.

The data on seed set and the out crossing potential of the three selected CMS lines are presented in Table 4.13. The Table shows about 3.5 fold increase (38.36 percent) in out crossing for TMS 8 / 2046 A in method II as compared to method I (10.60 percent) whereas the increase in out crossing for TMS 20 / 2338 A was little over 2.5 times (12.98 percent and 4.86 percent for method II and I, respectively). The outcrossing in *arar* 2022 A showed more than a two-fold (30.67 percent) increase over method I (13.38 percent). The CMS lines *arar* 2022 A and TMS 8 / 2046 A could produce highest amount of seeds through outcrossing (35.56 percent and 41.95 percent respectively) in 3:1 ratio, whereas, TMS 20 / 2338 A produced the highest amount of outcrossing (19.79 percent) in 4:1 ratio. However, the 4:1 ratio followed for TMS 8 / 2046 A resulted in higher out crossing potential (41.48 percent) over 2:1 ratio (31.67 percent). The same 4:1 ratio for the other CMS line *arar* 2022 A showed a marginal reduction in out-crossing potential (27.69 percent) than 2:1 ratio (28.78 percent).

Plate 4. Glume opening in cytoplasmic male sterile lines

Table 4.12. Fertility status and seed setting of CMS lines in staggered sowing (av. of three seasons) in wheat

CMS Source	Nuclear background	Pollen fertility (%)	Seed set per main spike (number)		Out-crossing potential (%)
			On selfing	On out-crossing	
T. timopheevi	HW 2022	0.0	0	0.0	0.0
	HW 2038	0.0	0	3.4	5.48
	HW 2041	0.12	0.0	5.3	8.83
	HW 2042	0.0	0	1.0	1.54
	HW 2046	0.0	0	2.0	3.33
	UP 2338	0.0	0	0.00	0.00
T. araraticum	HW 2022	0.0	0	8.1	13.38
	HW 2038	0.37	0	4.0	6.45
	HW 2041	0.05	0	5.0	8.33
	HW 2042	0.0	0	3.2	4.93
	HW 2046	0.56	0	5.2	8.67
	HW 2099	0.46	0	3.2	3.90
	HD 2687	0.0	0	4.0	7.12
	PBW 343	0.13	0	4.2	7.0
Ae. speltoides	HW 2022	0.0	0	1.3	2.2
	HW 2038	0.0	0	5.2	8.3
	HW 2042	0.46	0	2.8	4.3
	HW 2046	0.0	0	2.0	3.4
	HW 2643	0.0	0	2.4	4.62
TMS-8 (source unknown)	HW 2046	0.0	0	6.4	10.6
	HW 2687	0.04	0	2.4	4.3
	UP 2338	0.0	0	3.8	5.43
	PBW 343	0.0	0	4.0	6.67

Contd....

CMS Source	Nuclear background	Pollen fertility (%)	Seed set per main spike (number)		Out-crossing potential (%)
			On selfing	On out-crossing	
TMS-9 (source unknown)	HW 2038	0.04	0	7.6	12.25
	HW 2041	0.0	0	6.4	10.67
	HW 2046	0.0	0	6.2	10.33
	HW 2099	0.0	0	4.6	5.61
	UP 2338	0.0	0	4.5	6.43
	PBW 343	0.0	0	2.6	4.32
TMS-11 (source unknown)	HW 2019	0.0	0	4.1	8.04
	HW 2022	0.0	0	3.6	6.00
	HW 2046	0.0	0	4.5	7.50
	HD 2428	0.0	0	6.4	10.65
	UP 2338	0.41	0	6.3	9.0
	PBW 343	0.0	0	6.2	10.34
TMS-20 (source unknown)	HW 2041	0.0	0	3.8	6.30
	HW 2042	0.0	0	1.8	2.77
	HW 2046	0.0	0	3.0	5.00
	UP 2338	0.0	0	3.4	4.86

Table 4.13. Out-crossing potential in selected CMS lines in wheat

CMS line	2:1 ratio		3:1 ratio		4:1 ratio		Grand mean	
	No. of seeds per spike	Out-crossing (%)	No. of seeds per spike	Out-crossing (%)	No. of seeds per spike	Out-crossing (%)	No. of seeds per yield	Out-crossing (%)
T. arar / 2022 A	20.15	28.78	24.89	35.36	19.38	27.69	21.47	30.67
TMS 8 / 2046 A	19.0	31.67	25.17	41.95	24.89	41.48	23.02	38.36
TMS 20 / 2338 A	7.1	10.14	6.33	9.04	13.85	19.79	9.09	12.99

4.1.3. Agronomic traits

The observations recorded on yield related traits like number of tillers, plant height, main spike length, number of spikelets per main spike, days to 50% flowering and synchrony of tillers for various male sterility inducing cytoplasms are presented in Tables 4.14 to 4.20. The general trend regarding the effect of male sterility inducing cytoplsam, derived from various sources, on the agronomic characters was that of reduction in intensity of the character as compared to B lines with few exceptions.

4.1.3.1. CMS lines with *T. timopheevi* cytoplasm

The data presented in Table 4.14 shows the effect of male sterility inducing cytoplasm, derived from *T. timopheevi* on agronomic characters. Out of six CMS lines compared with their respective B lines, only two lines *viz.*, *timo* 2022 A and *timo* 2038 A showed reduced number of tillers (10.8 and 9.6, respectively) than their fertile counterparts (12.2 and 11.6). The remaining four CMS lines had lesser number of tillers but the differences were non significant. Five CMS lines showed reduced plant height of which three (*timo* 2022 A, *timo* 2038 A and *timo* 2041 A) were significantly lower. The CMS line *timo* 2042 A had taller plants than B lines but the difference in height was non significant. Three CMS lines, namely, *timo* 2022 A, *timo* 2038 A and *timo* 2338 A had longer spikes and the differences were significant, while the CMS lines *timo* 2042 A and *timo* 2046 A had shorter main spikes than the B lines. Except for *timo* 2338 A, all the CMS lines possessing *T. timopheevi* cytoplasm had lesser number of spikelets per main spike of which only two *viz.*, *timo* 2038 A and *timo* 2042 A showed significant difference. Barring a CMS line *timo* 2042 A, none of the six CMS lines with *timopheevi* cytoplasm flowered early (50% flowering) as compared to their maintainer lines.

Table 4.14. Effect of male sterility inducing cytoplasm derived from *T. timopheevi* on various agronomic characters in wheat

Lines	No. of tillers		Plant height (cm)		Main spike length (cm)		No. of spikelets per main spike		Days to 50% flowering		Synchrony of tillers (days)	
	A	B	A	B	A	B	A	B	A	B	A	B
HW 2022	10.8*	12.2	77.3*	86.8	11.9*	10.7	21.0	21.0	95.2	93.0	7.4	7.0
HW 2038	9.6**	11.6	77.9*	74.2	12.2*	11.0	19.4**	22.8	83.2	81.6	8.2	8.2
HW 2041	9.6	10.8	78.8*	89.4	10.9	11.5	19.4	20.2	87.6**	80.8	8.0	8.2
HW 2042	9.0	10.8	85.2	82.4	9.1**	12.0	20.2*	22.8	85.0*	88.4	8.0	7.2
HW 2046	8.8	9.0	74.3	76.6	10.6**	11.2	18.2	19.2	92.6**	87.6	7.2	8.0
UP 2338	8.6	8.2	76.7	77.1	15.5**	11.7	22.6*	21.0	102.0**	93.8	8.0	7.2

**, * Significant at 0.01% and 0.05%, respectively.

There was no significant difference exhibited for synchrony of tillers between the CMS and B lines.

4.1.3.2. CMS lines with *T. araraticum* cytoplasm

The effect of male sterility inducing cytoplasm derived from *T. araraticum* on agronomic characters is presented in Table 4.15. Like *timo* 2022 A and *timo* 2038 A, the CMS lines *arar* 2022 A and *arar* 2038 A had significantly lesser number of tillers than their B lines. The rest of the six CMS lines did not show significant difference in number of tillers. Plant height showed similar trend of reduction in height in comparison to the B lines as it was observed in the lines carrying *timopheevi* cytoplasm. Five lines, namely, *arar* 2022 A, *arar* 2041 A, *arar* 2099 A, *arar* 2687 A and *arar* 343 A were significantly different for plant height. The two CMS lines, *arar* 2038 A and *arar* 2046 A were taller than their counterparts but the differences in height were not significant. Only one CMS line *i.e.*, *arar* 2041 A had longer main spike than the B line while the remaining lines being longer or shorter in spike length did not show any significant difference.

The number of spikelets also followed the same trend with five of the CMS lines *viz.*, *arar* 2038 A, *arar* 2041 A, *arar* 2042 A, *arar* 2687 A and *arar* 343 A showing significantly lower number of spikelets per spike than the maintainer lines. Similarly, five CMS lines *arar* 2038 A, *arar* 2041 A, *arar* 2042 A, *arar* 2046 A and *arar* 2099 A were late in flowering as compared to the B lines. Two CMS lines *viz.*, *arar* 2038 A and *arar* 2041 A had significant difference with respect to synchrony of tillers and were early in flowering than their respective B lines. The CMS line *arar* 2046 A fell in a different category as it showed non-significant variation for all the characters except for synchrony of tillers.

Table 4.15. Effect of male sterility inducing cytoplasm derived from *T. araraticum* on various agronomic characters in wheat

Lines	No. of tillers		Plant height (cm)		Main spike length (cm)		No. of spikelets per main spike		Days to 50% flowering		Synchrony of tillers (days)	
	A	B	A	B	A	B	A	B	A	B	A	B
HW 2022	9.8**	12.2	77.7*	86.8	11.0	10.7	20.4	21.0	95.8	93.0	8.0	7.0
HW 2038	9.8**	11.6	75.5	74.2	11.7	10.9	19.6*	22.8	96.6**	81.6	7.0*	8.2
HW 2041	12.0	10.8	75.8**	89.4	14.1**	11.5	18.0**	20.2	88.4**	80.8	6.2**	8.2
HW 2042	10.6	10.8	79.0	82.4	11.9	12.0	18.8**	22.8	92.2**	88.4	8.0	7.2
HW 2046	10.6	9.0	77.2	76.6	11.5	11.2	20.6	19.2	92.2*	87.6	8.0	8.0
HW 2099	10.0	8.6	80.0**	84.5	12.2	12.1	21.4	21.4	89.8**	95.6	8.2	7.4
HD 2687	9.4	10.0	69.3**	75.6	11.0	11.0	19.8*	21.0	92.2	92.6	7.2	8.0
PBW 343	9.0	11.2	77.3**	86.2	11.4	12.0	20.0*	23.0	90.0	90.0	8.0	8.0

****, * Significant at 0.01% and 0.05%, respectively.**

4.1.3.3. CMS lines with *Ae. speltoides* cytoplasm

Table 4.16 presents the effect of male sterility inducing cytoplasm derived from *Ae. speltoides* on various agronomic characters. Out of five, only *spelt* 2022 A CMS line had significantly lower number of tillers than the rest of the CMS lines. Significant differences in plant height were observed in four CMS lines (Table 4.16). The line *spelt* 2643 A was short in height, while the CMS lines *spelt* 2038 A, *spelt* 2042 A, and *spelt* 2046 A were taller than the B lines. The CMS line, *spelt* 2022 A did not exhibit significant variation for any other trait. Two CMS lines, *spelt* 2038 A and *spelt* 2042 A and their B lines differed significantly for main spike length and number of spikelets. No significant difference for spike length and number of spikelets per spike were observed in remaining CMS line (Table 4.16). None of the five CMS lines with *speltoides* cytoplasm flowered early in comparison to their maintainer lines. Only two lines, *spelt* 2046 A and *spelt* 2643 A having *speltoides* cytoplasm differed significantly with their maintainer line for synchrony of tillers. The remaining lines were similar to the B lines with respect to synchrony of tillers.

4.1.3.4. CMS lines with TMS 8 cytoplasm

The results on the effect of male sterility inducing cytoplasm derived from TMS 8 on agronomic characters are presented in Table 4.17. The CMS lines, TMS 8/2046 A and TMS 8/2687 A had lesser number of tillers as compared to their B lines. Two CMS lines *viz.*, TMS 8 / 2687 A and TMS 8 / 343 A showed significant differences for plant height; the former being taller and the latter being shorter than their maintainers. The CMS line TMS 8 / 2687 A also had longer spikes than its B line. None of the four CMS lines carrying TMS 8 cytoplasm (Table 4.17) showed any significant differences for number of spikelets per spike whereas they showed significant variation for days to 50% flowering.

Table 4.16. Effect of male sterility inducing cytoplasm derived from *Ae. speltoides* on various agronomic characters in wheat

Lines	No. of tillers		Plant height (cm)		Main spike length (cm)		No. of spikelets per main spike		Days to 50% flowering		Synchrony of tillers (days)	
	A	B	A	B	A	B	A	B	A	B	A	B
HW 2022	7.8**	12.2	89.6	86.8	10.4	10.7	20.2	21.0	90.4	93.0	8.0	7.0
HW 2038	9.2	11.6	77.8*	74.2	12.1**	10.9	19.8**	22.8	82.2	81.6	8.0	8.2
HW 2042	10.2	10.8	85.9*	82.4	10.4*	12.0	20.2*	22.8	94.2**	88.4	8.2	7.2
HW 2046	9.0	9.0	94.6**	76.6	10.8	11.2	19.8	19.2	89.8	87.6	6.0*	8.0
HD 2643	9.2	10.6	79.8**	87.0	10.7	11.0	19.0	19.2	95.0**	90.0	10.0*	8.0

**, * Significant at 0.01% and 0.05%, respectively.

Table 4.17. Effect of male sterility inducing cytoplasm derived from TMS 8 on various agronomic characters in wheat

Lines	No. of tillers		Plant height (cm)		Main spike length (cm)		No. of spikelets per main spike		Days to 50% flowering		Synchrony of tillers (days)	
	A	B	A	B	A	B	A	B	A	B	A	B
HW 2046	6.8*	9.0	76.5	76.6	11.0	11.2	17.8	19.2	92.0**	87.6	6.0**	8.0
HD 2687	8.4*	10.0	87.0**	75.6	11.6*	11.0	20.0	21.0	97.2*	92.6	7.0	8.0
UP 2338	8.0	8.2	80.8	77.1	12.7	11.7	20.4	21.0	97.2*	93.8	7.0	7.2
PBW 343	10.2	11.2	80.0**	86.2	12.1	12.0	20.4	23.0	96.0**	90.0	8.0	8.0

**, * Significant at 0.01% and 0.05%, respectively.

Only one CMS line *i.e.*, TMS 8 / 2046 A exhibited a reduction in number of days for synchrony of tillers.

4.1.3.5. CMS lines with TMS 9 cytoplasm

Table 4.18 indicates the effect of male sterility inducing cytoplasm derived from TMS 9 on different agronomic characters. Two out of the six CMS lines namely, TMS 9 / 2038 A and TMS 9 / 343A showed general trend for having lesser number of tillers than their B lines. Our of the remaining four CMS lines carrying TMS 9 cytoplasm, two lines had more number of tillers while the other two had less number of tillers than the B lines However, the differences with respect to number of tillers were non significant.

Two CMS lines TMS 9 / 2038 A and TMS 9 / 2046 A deviated from general trend for plant height as they were taller than the B lines. The CMS line TMS 9 / 343 A was shorter in height. None of the six CMS lines with TMS 9 cytoplasm had significant differences with the B lines for main spike length. Two CMS lines, *viz.*, TMS 9 / 2038 A and TMS 9 / 2099 A showed significant differences for number of spikelets; the former having lesser and the latter having higher number of spikelets than their maintaner lines. Of the four CMS lines that were significantly different for days to flowering, three CMS lines TMS 9 / 2046 A, TMS 9 / 2099 A and TMS 9 / 343 A were early in flowering, while TMS 9 / 2038 A was late. The variation for synchrony of tillers did not exceed two days which was the maximum (TMS 9 / 2099 A) followed by TMS 9 / 2041 A. However, the differences were statistically significant.

4.1.3.6. CMS lines with TMS 11 cytoplasm

The effects of male sterility inducing cytoplasm derived from TMS 11 on agronomic characters are described in Table 4.19.

Table 4.18. Effect of male sterility inducing cytoplasm derived from TMS 9 on various agronomic characters in wheat

Lines	No. of tillers		Plant height (cm)		Main spike length (cm)		No. of spikelets per main spike		Days to 50% flowering		Synchrony of tillers (days)	
	A	B	A	B	A	B	A	B	A	B	A	B
HW 2038	9.6**	11.6	78.4*	74.2	11.0	10.9	21.0*	22.8	91.2**	81.6	8.2	8.2
HW 2041	9.8	10.8	84.6	89.4	11.7	11.5	19.4	20.2	83.0	80.8	9.6*	8.2
HW 2046	11.0	9.0	83.0*	76.6	11.0	11.2	19.0	19.2	83.0*	87.6	8.0	8.0
HW 2099	8.8	8.6	82.6	84.5	12.0	12.1	23.6**	21.4	86.6**	95.6	9.4*	7.4
UP 2338	9.0	8.2	75.3	77.1	12.1	11.7	21.0	21.0	94.6	93.8	8.0	7.2
PBW 343	8.8**	11.2	75.2**	86.2	11.6	12.0	21.2	23.0	86.0*	90.0	9.6	8.0

**, * Significant at 0.01% and 0.05%, respectively.

Table 4.19. Effect of male sterility inducing cytoplasm derived from TMS 11 on various agronomic characters in wheat

Lines	No. of tillers		Plant height (cm)		Main spike length (cm)		No. of spikelets per main spike		Days to 50% flowering		Synchrony of tillers (days)	
	A	B	A	B	A	B	A	B	A	B	A	B
HW 2019	8.6*	11.6	65.9	68.0	9.8	10.8	20.2	21.0	96.2	94.8	7.8	7.8
HW 2022	9.6	12.2	81.2	86.8	12.0	10.7	23.0	21.0	91.4	93.0	8.2**	7.0
HW 2046	12.0**	9.0	80.4	76.6	11.8	11.2	20.0	19.2	86.6	87.6	8.0	8.0
HW 2428	10.4*	13.8	83.0*	74.0	13.0	12.0	19.8*	22.8	86.2	85.8	7.2	7.2
UP 2338	12.2*	8.2	97.0**	77.1	12.5	11.7	23.0*	21.0	87.4*	93.8	7.8	7.2
PBW 343	8.0**	11.2	75.0*	86.2	11.3	12.0	20.0	23.0	86.6*	90.0	6.8	8.0

**, * Significant at 0.01% and 0.05%, respectively.

Five of the six CMS lines showed significant variation for number of tillers. Among them, two CMS lines, TMS 11 / 2046 A and TMS 11 / 2338 A had higher number of tillers and three CMS lines such as TMS 11 / 2019 A, TMS 11 / 2428 A and TMS 11 / 343 A had lesser number of tillers as compared to the B lines. The CMS lines, TMS 11 / 2428 A and TMS 11 / 2338 A were taller than the B line, while TMS 11 / 343 A was significantly shorter in height. None of the CMS lines showed significant variation for main spike length. Two CMS lines such as TMS 11 / 2428 A and TMS 11 / 2338 A showed significant variation of number of spikelets. Four out six CMS lines flowered early but the differences were not significant. Only two CMS lines (TMS 11 / 2338 A and TMS 11 / 343 A) showed significant variation for days to 50% flowering. The CMS line TMS 11 / 2022 A differed significantly for synchrony of tillers only. However, it did not show significant difference for any of the other agronomic traits.

4.1.3.7. CMS lines with TMS 20 cytoplasm

The results observed on the effect of male sterility inducing cytoplasm, derived from TMS 20 on various agronomic characters are presented in Table 4.20. None of the four CMS lines with TMS 20 cytoplasm showed significant variation for number of tillers; of which two lines had lower number of tillers and the other two had higher number of tillers than their fertile counterparts.

Significant variation could not be observed in most of the CMS lines for various agronomic characters. However, significant variation for main spike length and days to 50% flowering was observed in TMS 20 / 2041 A, whereas TMS 20 / 2042 A showed variation for synchrony of tillers. The CMS lines, TMS 20 / 2046 A and TMS 20 / 2338 A showed significant difference in plant height as both the lines were taller than B lines.

Table 4.20. Effect of male sterility inducing cytoplasm derived from TMS 20 on various agronomic characters in wheat

Lines	No. of tillers		Plant height (cm)		Main spike length (cm)		No. of spikelets per main spike		Days to 50% flowering		Synchrony of tillers (days)	
	A	B	A	B	A	B	A	B	A	B	A	B
HW 2041	8.6	10.8	87.0	89.4	10.9*	11.5	20.4	20.2	90.8**	80.8	8.0	8.2
HW 2042	8.6	10.8	81.0	82.4	10.7	12.0	21.0	22.8	88.4	88.4	10.0**	7.2
HW 2046	9.8	9.0	80.0*	76.6	11.5	11.2	20.2	19.2	88.0	87.6	8.6	8.0
UP 2338	9.4	8.2	84.1*	77.1	11.4	11.7	21.2	21.0	94.2	93.8	8.6	7.2

**, * Significant at 0.01% and 0.05%, respectively.

None of the CMS lines with TMS 20 cytoplasm exhibited significant variation for number of tillers and number of spikelets.

4.2. STABILITY OF CMS LINES

In order to assess the stability of male sterility, 39 CMS lines were evaluated for sterility behaviour in three environments viz., E1, E2, and E3, created by planting the CMS lines at three different dates of equal intervals of 15 days (26th November, 11th December and 28th December, 1999-2000, respectively). The investigation on CMS line 2160 A carrying *Ae. caudata* cytoplasm was discontinued due to poor maintaining ability. This particular CMS line exhibited pistillody *i.e.*, modification of stamen into feathery, ovary like structures (Plate. 5).

The stability of sterility in CMS lines was judged on the basis of pollen fertility and seed setting in bagged spikes under different environments. All the CMS lines exhibited complete spikelet sterility as evidenced by the absence of seed setting in bagged spikes (Table 4.12). The data on relative frequency of different categories of pollens observed in CMS lines under different environments has been presented in Table 4.21 as percentage of total number of pollen grains examined under microscope in different fields and magnification (Plate 6).

Among the four categories of pollen grains distinguished on the basis of shape and staining pattern, *viz.*, Stained Round Fertile (SRF), Stained Round Sterile (SRS), Unstained Spherical Sterile (USS) and Unstained Withered Sterile (UWS), the relative proportion of USS pollen was found to be remarkably high in all CMS lines followed by UWS pollens in all the environments (Table 4.21). These two categories of pollen contributed to more than 97 percent of pollen grains observed in all CMS lines.

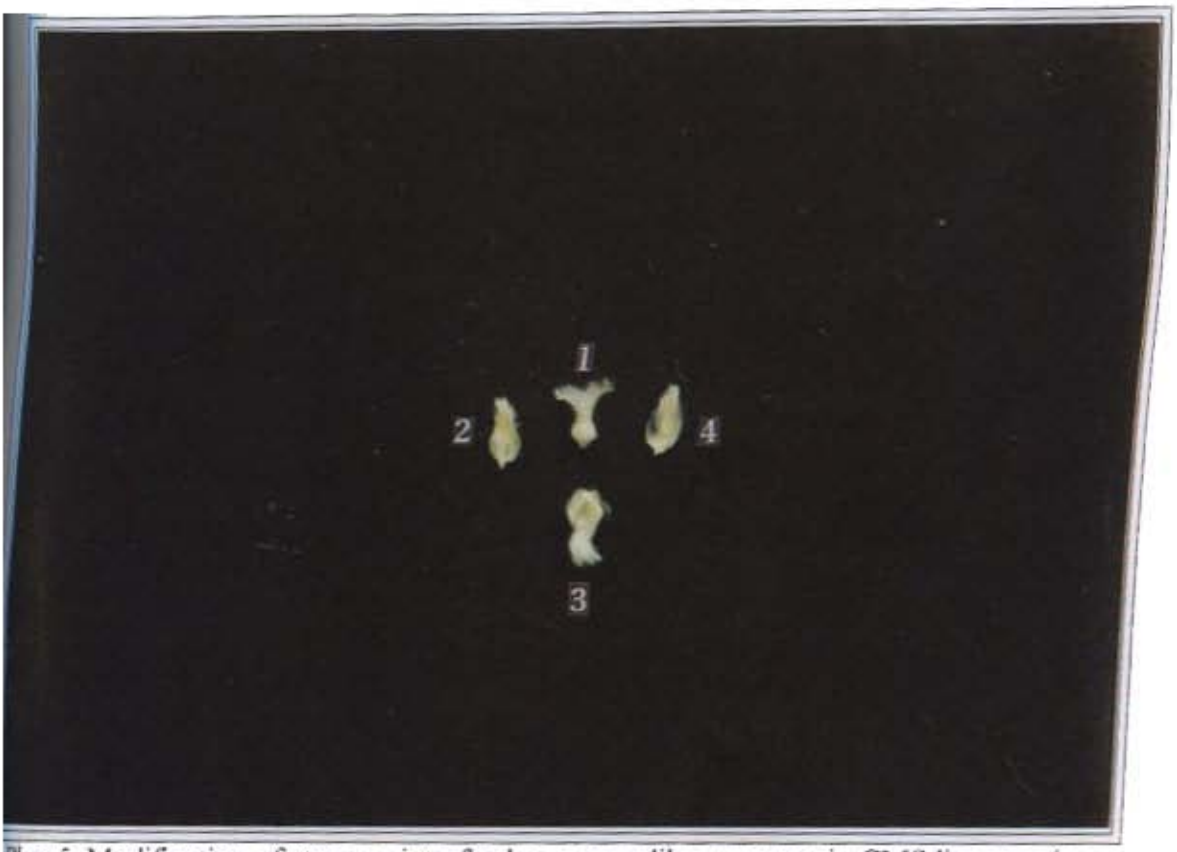
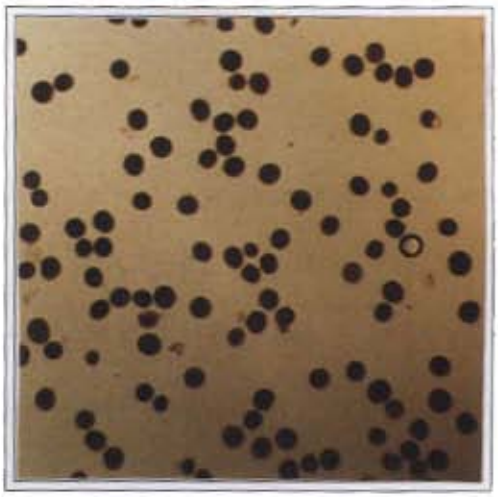
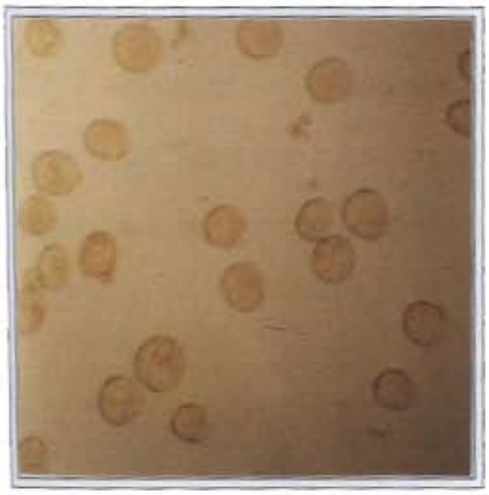


Plate 5. Modification of stamens into feathery ovary like structure in CMS line carrying cytoplasm from *Aegilops caudata*.
1 = Normal ovary; 2, 3 & 4 = modified stamens

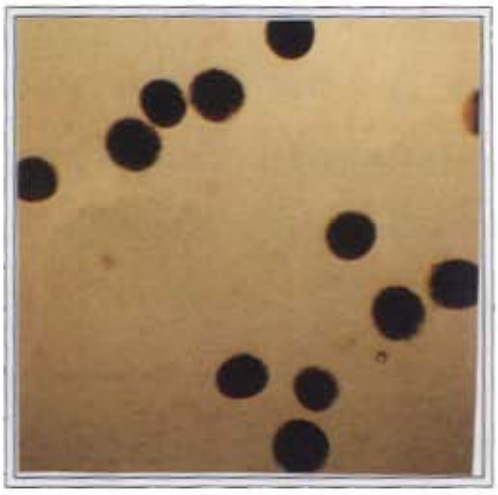
10 X



10 X



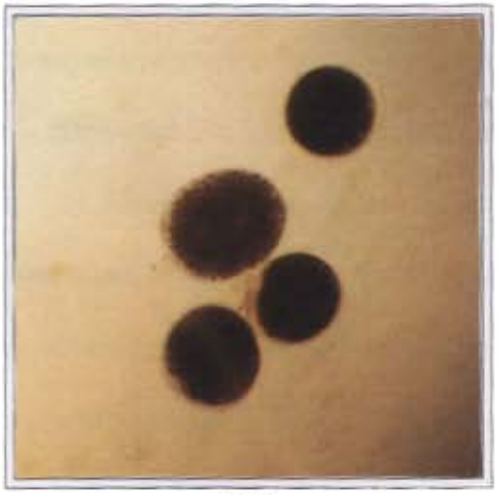
25 X



25 X

40 X

A



40 X

B

Plate 6. Sterile (A) and fertile (B) pollen grains under 10 X, 25 X and 40 X magnification

The relative frequency of USS pollen ranged from 71 percent (TMS 9 / 2046 A) in E2 to 96 percent (TMS 11 / 2046 A) in E1 and that of UWS pollens from 4 per cent (TMS 9 / 2046 A) in E1 to 26.5 per cent (TMS 9 / 2046 A) in E2. The CMS lines TMS 11 / 2046 A and TMS 9 / 2046 A in E1 had the highest (95.87 per cent and 95.70 per cent respectively) and TMS 9 / 2041 A in E2 had the lowest frequency (71.57 percent) of USS pollen grains and *vice-versa* for UWS pollens.

The CMS lines with *timopheevi* cytoplasm recorded relatively higher range (79.63 to 90.26%) of USS pollen over the environments (Table 4.22) while the CMS lines with TMS 8 cytoplasm had a narrow range of USS pollen frequency (80.45 to 85.36%). However, in TMS 8 cytoplasm, the trend of UWS pollen frequency was in opposite order of the USS pollen (9.74% to 20.37%). The CMS lines with TMS 20 cytoplasm had the lowest frequency (11.39 to 14.21%) with narrow range of UWS pollen as compared to TMS 8. Out of.39, 29 CMS lines were found to be completely male sterile and stable in all the environments as none of the pollen grains appeared as SRF. The rest of ten CMS lines produced SRF pollen though at an extremely low frequency, the highest being 0.56% for *arar* 2046 A (Table 4.22) and accordingly placed in highly sterile group. All the CMS lines with TMS 20 cytoplasm exhibited complete pollen sterility as none of the lines produced SRF pollen. It was interesting to note that all the CMS lines produced very less pollen grains than their fertile counterparts. In addition to production of low frequency of pollen grains, a few CMS lines *viz.*, *timo* 2338 A, *arar* 2022 A and TMS 20 / 2042 A had their anthers modified into feathery structures and filaments turned stub like. Such modifications could probably have been due to homeotic mutation. The results further indicated that the CMS lines belonged to Type I cytosterility group since almost all pollen grains appeared as USS and UWS (more than 99%).

Table 4.21. Relative frequency of different categories of pollens (%) in CMS lines under three environments

CMS Source	Nuclear background	Stained Round Fertile (SRF)			Stained Round Sterile (SRS)			Unstained Withered Sterile (UWS)			Unstained Spherical Sterile (USS)		
		E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3
T. timopheevi	HW 2022	0.00	0.00	0.00	0.00	0.00	0.00	10.23	9.46	10.46	89.77	90.54	89.54
	HW 2038	0.00	0.00	0.00	0.00	0.00	0.00	7.41	13.10	8.72	92.59	86.90	91.28
	HW 2041	0.27	0.00	0.10	0.58	0.13	0.87	17.02	21.31	14.38	82.13	78.56	84.65
	HW 2042	0.00	0.00	0.00	1.30	0.87	2.40	14.18	13.47	9.83	84.52	85.66	87.77
	HW 2046	0.00	0.00	0.00	1.65	0.60	0.00	15.27	12.41	10.81	83.08	86.99	89.19
	UP 2338	0.00	0.00	0.00	0.00	0.00	0.00	25.72	16.60	18.79	74.28	83.40	81.21
T. araraticum	HW 2022	0.00	0.00	0.00	0.87	1.65	0.00	12.23	7.83	13.22	86.90	90.52	86.78
	HW 2038	1.10	0.00	0.00	0.00	2.37	1.08	18.68	15.43	19.42	80.22	82.20	79.50
	HW 2041	0.15	0.00	0.00	0.00	0.00	0.75	13.28	14.78	13.87	86.57	85.22	85.38
	HW 2042	0.00	0.00	0.00	0.00	0.00	0.00	15.06	14.95	17.66	84.94	85.05	82.34
	HW 2046	0.61	1.08	0.00	0.65	0.00	0.00	7.84	12.92	20.05	90.90	86.00	79.95
	HW 2099	0.00	0.00	1.38	0.00	1.80	0.60	23.10	12.43	14.81	76.90	85.77	83.21
	HW 2687	0.00	0.00	0.00	0.00	0.00	0.00	11.42	9.83	14.95	88.58	90.17	85.05
	PBW 343	0.40	0.00	0.00	1.41	0.00	0.56	17.32	6.78	10.83	80.87	93.22	88.61

CMS Source	Nuclear background	Stained Round Fertile (SRF)			Stained Round Sterile (SRS)			Unstained Withered Sterile (UWS)			Unstained Spherical Sterile (USS)		
		E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3
Ae. speltoides	HW 2022	0.00	0.00	0.00	0.47	0.18	1.32	18.36	17.27	11.29	81.17	82.55	87.39
	HW 2038	0.00	0.00	0.00	0.00	0.00	0.00	23.32	12.72	9.79	76.68	87.28	90.21
	HW 2042	1.38	0.00	0.00	0.41	0.60	0.00	16.09	18.72	12.41	82.12	80.68	87.59
	HW 2046	0.00	0.00	0.00	0.84	0.00	0.00	17.41	4.38	9.32	81.75	95.62	90.68
	HW 2643	0.00	0.00	0.00	0.00	0.00	0.00	12.17	23.01	6.84	87.83	76.99	92.66
TMS-8 (Source unknown)	HW 2046	0.00	0.00	0.00	1.30	2.43	0.00	14.95	8.94	16.31	83.75	88.62	83.69
	HW 2687	0.10	0.00	0.00	0.00	0.00	0.00	19.42	12.10	17.27	80.48	87.90	82.73
	UP 2338	0.00	0.00	0.00	0.00	0.00	0.00	10.40	18.12	30.13	89.60	81.88	69.87
	PBW 2338	0.00	0.00	0.00	2.34	1.00	3.40	21.83	6.70	8.93	75.83	92.30	87.67
TMS-9 (Source unknown)	HW 2038	0.12	0.00	0.00	0.00	0.00	0.00	6.83	15.29	12.94	93.05	84.71	87.06
	HW 2041	0.00	0.00	0.00	2.37	0.00	0.00	7.84	23.41	14.82	89.79	76.59	85.18
	HW 2046	0.00	0.00	0.00	0.00	2.00	0.00	4.38	26.43	18.23	95.62	71.57	81.77
	HW 2099	0.00	0.00	0.00	1.38	0.00	0.00	23.14	9.79	16.39	75.48	90.21	83.61
	UP 2338	0.00	0.00	0.00	0.00	0.00	0.00	12.81	15.37	18.13	87.19	84.63	81.87
	PBW 343	0.00	0.00	0.00	1.65	0.00	0.00	8.17	12.43	21.90	90.18	87.57	78.10

CMS Source	Nuclear background	Stained Round Fertile (SRF)			Stained Round Sterile (SRS)			Unstained Withered Sterile (UWS)			Unstained Spherical Sterile (USS)		
		E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3
TMS-11 (Source unknown)	HW 2019	0.00	0.00	0.00	3.10	0.00	0.00	11.38	20.05	18.36	85.52	79.95	81.64
	HW 2022	0.00	0.00	0.00	0.00	0.16	1.65	15.30	7.53	10.41	84.70	92.31	87.94
	HW 2046	0.00	0.00	0.00	0.00	1.28	0.00	4.13	25.75	18.10	95.87	72.97	81.90
	HD 2428	0.00	0.00	0.00	0.80	0.00	0.00	12.13	11.30	21.00	87.07	88.70	79.00
	UP 2338	0.00	1.23	0.00	0.00	0.00	0.00	17.06	18.60	21.69	82.94	80.17	78.31
	PBW 343	0.00	0.00	0.00	0.00	0.00	0.00	23.10	17.35	20.03	76.90	82.65	79.97
TMS 20 : (Source unknown)	HW 2041	0.00	0.00	0.00	1.40	0.00	0.00	4.87	23.04	12.38	93.73	76.96	87.62
	HW 2042	0.00	0.00	0.00	0.00	0.00	0.00	15.27	14.83	11.58	84.73	85.17	88.42
	HW 2046	0.00	0.00	0.00	0.00	0.00	0.00	19.32	15.43	7.89	80.68	84.57	92.11
	UP 2338	0.00	0.00	0.00	3.00	0.00	0.00	21.38	7.83	4.95	75.62	92.17	95.05

Table 4.22. Pollen stainability pattern pooled over environments for CMS lines in wheat

Nuclear background	CMS Source	Proportion of pollen grains (%)				Pollen sterility (%)	Sterility groups
		SRF	SRS	UWS	USS		
HW 2022	*T. timopheevi*	0.00	0.00	10.05	89.95	100.00	CS
HW 2038	"	0.00	0.00	9.74	90.26	100.00	CS
HW 2041	"	0.12	0.53	17.57	81.78	99.88	HS
HW 2042	"	0.00	1.52	12.50	85.98	100.00	CS
HW 2046	"	0.00	0.75	12.83	86.42	100.00	CS
UP 2338	"	0.00	0.00	20.37	79.63	100.00	CS
HW 2022	*T. araraticum*	0.00	0.84	1.10	88.06	100.00	CS
HW 2038	"	0.37	1.15	17.84	80.64	99.63	HS
HW 2041	"	0.05	0.25	13.97	85.73	99.95	HS
HW 2042	"	0.00	0.00	15.89	84.11	100.00	CS
HW 2046	"	0.56	0.22	13.60	85.62	99.44	HS
HW 2099	"	0.46	0.80	16.78	81.96	99.54	HS
HD 2687	"	0.00	0.00	12.07	87.93	100.00	CS
PBW 343	"	0.13	0.66	11.65	87.56	99.87	HS
HW 2022	*Ae. speltoides*	0.00	0.66	15.64	83.70	100.00	CS
HW 2038	"	0.00	0.00	15.27	84.73	100.00	CS
HW 2042	"	0.46	0.34	15.74	83.46	99.54	HS
HW 2046	"	0.00	0.28	10.37	89.35	100.00	CS
HW 2643	"	0.00	0.17	14.01	85.82	100.00	CS
HW 2041	TMS 8 (source unknown)	0.00	1.24	13.40	85.36	100.00	CS
HW 2687	"	0.04	0.00	16.26	83.70	99.96	HS
UP 2338	"	0.00	0.00	19.55	80.45	100.00	CS
PBW 343	"	0.00	2.25	12.43	85.27	100.00	CS

Nuclear background	CMS Source	Proportion of pollen grains (%)				Pollen sterility (%)	Sterility groups
		SRF	SRS	UWS	USS		
HW 2038	TMS 9 (source unknown)	0.04	0.00	11.69	88.27	99.96	HS
HW 2041	"	0.00	0.79	15.36	83.85	100.00	CS
HW 2046	"	0.00	0.67	16.35	82.98	100.00	CS
HW 2099	"	0.00	0.46	16.44	83.10	100.00	CS
UP 2338	"	0.00	0.00	15.44	84.56	100.00	CS
PBW 343	"	0.00	0.55	14.17	85.28	100.00	CS
HW 2019	TMS 11 (source unknown)	0.00	1.03	16.60	82.37	100.00	CS
HW 2022	"	0.00	0.60	11.08	88.32	100.00	CS
HW 2046	"	0.00	0.42	16.00	83.58	100.00	CS
HD 2428	"	0.00	0.27	14.81	84.92	100.00	CS
UP 2338	"	0.41	0.00	19.12	80.47	99.59	HS
PBW 343	"	0.00	0.00	20.16	79.84	100.00	CS
HW 2041	TMS 20 (source unknown)	0.00	0.47	13.43	86.10	100.00	CS
HW 2042	"	0.00	0.00	13.90	86.10	100.00	CS
HW 2046	"	0.00	0.00	14.21	85.79	100.00	CS
UP 2338	"	0.00	1.00	11.39	87.61	100.00	CS

CS : Completely sterile

HS : Highly sterile

4.3. IDENTIFICATION OF MAINTAINERS AND RESTORERS

The experiment on identification of maintainers and restorers consisted 521 testcrosses. The cross combinations involved seven cytoplasmic sources inducing male sterility and 294 pollen parents comprising of land races, indigenous and recently developed cultivars, synthetic wheats, inter-specific derivatives and exotics. The test cross F_1 hybrids were evaluated during *Rabi*, 2000-2001. Based on spike fertility, which was used as an index for fertility restoration, the pollen parents of the test cross hybrids were categorized as complete restorer, partial restorer, partial / weak maintainer and complete maintainer (Table 4.23).

The seed set on the covered main spike in the test cross progenies ranged from 0 to 77. Among all the crosses attempted, only two exotic genotypes, PWR 4099 and PWR 4101 used as pollen parents produced seeds in covered spikes, which indicated that the exotics possess fertility restorer gene(s). The highest spike fertility was observed in two F_1 hybrids in which the number of seed set ranged from 46 to 77 (Table 4.28). However, two F_1 hybrids involving CMS lines with *T. timopheevi* and *Ae. speltoides* cytoplasm did not produce any seed upon selfing, when crossed with PWR 4099 and PWR 4101 . These two pollinators were able to restore fertility only in TMS 9 and TMS 20 sterile cytoplasm, while in the rest, only either of the pollen parents restored fertility. A very high frequency (87.14 per cent) of the testcrosses were male sterile and were accordingly classified as complete maintainers. The intermediate category (partial maintainers and partial restorers) accounted for about 10 per cent while the frequency of complete restorers was only 2.31 per cent (Tables 4.23 and 4.24).

Of the total 294 pollen parents, only 55 belonged to the intermediate category (48 partial maintainers and 7 partial restorers (Table 4.24). The pollinator T 1854 derived

from cross C-306 / T. *militinae* produced partially fertile progenies in *T. timopheevi*, TMS 9 and TMS 11 cytoplasms and a derivative T 1985 from DARF/Lok Bharti produced the same results in *T. araraticum* and TMS 9 cytoplasms. But a general trend observed in all the male sterility inducing cytoplasmic sources was that of appearance of a greater frequency of sterile test cross F_1 hybrids than partial and completely fertile F_1 hybrids. The experiment led to the identification of complete maintainers of male sterility among a large number of pollinators used.

Many of the pollen parents maintained complete sterility in various cytoplasmic sources indicating the stable sterility maintaining ability, as was evident from the higher frequency of complete maintainers classified as complete maintainers. The CMS lines with *Ae. speltoides* cytoplasm produced the highest (98.04 percent) and TMS 11 produced the lowest (78.75 percent) frequency of male sterile test cross F_1 hybrids. The highest frequency of fertility restoration was observed in TMS 11 (3.75 percent) and TMS 20 (3.77 percent) (Table 4.24). *T. timopheevi* and *Ae. speltoides* cytoplasmic sources did not produce any fertile F_1 hybrids in the test crosses effected. No partial maintainers were found for the male sterility inducing cytoplasms of *T. araraticum* and *Ae. speltoides*. Whereas in case of TMS 8 cytoplasm both partial restorers as well as partial maintainers could not be identified.

4.3.1. Identification of maintainers

The sterility maintaining behaviour of different male sterility inducing cytoplasmic sources has been presented in Tables 4.23 and 4.24. *Ae. speltoides* CMS source produced the highest frequency of complete maintainers (98.04 percent), while TMS 11, the lowest (78.75 per cent). Among the different CMS lines in which more than

15 test crosses were effected, *arar* 2338 A and *spelt* 2038 A resulted in 100 per cent complete maintainers.

It was interesting to note that the pollen parent that maintained complete sterility in one particular cytoplasm was not specific to only that cytoplasm but maintained male sterility in other cytoplasms also. The exceptions were the derivatives of C-306 / *T. militinae* and DARF / Lok Bharti crosses which apart from being perfect maintainers, also restored fertility of variable degrees in *T. timopheevi*, *T. araraticum*, TMS 9 and TMS 11 cytoplasms (Table 4.24).

4.3.2. Identification of fertility restorers

Among the cross combinations evaluated, only 2.31 per cent of the testcrosses were fertile (Table 4.24) indicating poor fertility restoration ability among the pollen parents. The pollen parents, namely, PWR 4099 and PWR 4101 were identified as effective (complete) restorers in the present investigation. Complete fertility restoration was observed in the lines carrying cytoplasm from five sources, namely, *T. araratium*, TMS 8, TMS 9, TMS 11 and TMS 20 (Table 4.23). However, PWR 4099 and PWR 4101 did not restore fertility in *T. timopheevi* and *Ae. speltoides* cytoplasms. The exotic line PWR 4099 restored complete fertility in all the sterile cytoplasms except *T. timopheevi* and *Ae. speltoides* while the other pollinator PWR 4101 effected complete restoration in only the lines having TMS 9 and TMS 20 cytoplasms (Table 4.25). Apart from the two exotic lines restoring complete fertility, there were five pollinators *viz.*, Bakhatwar, Shanghai 77, T 1854 (C306 / *T. militinae*), T 957 (*T. aestivum* / *T. dicoccum*) and WR 1044 identified as partial restorers (Table 4.25), which constitute only 1.34 per cent of test cross progenies (Table 4.24).

Table 4.23. Frequency of perfect maintainers and restorers for different alloplasmic lines (per cent) in wheat

CMS lines	Number of crosses	Complete maintainers	Partial maintainers	Partial restorers	Complete restorers
T. timopheevi 2038A	35	31(88.60)	4(11.40)	0.00	0.00
T. timopheevi 2042A	23	20(86.95)	1(4.35)	2(8.70)	0.00
T. timopheevi 2022A	1	1(100.00)	0.00	0.00	0.00
T. araraticum 2022A	54	42(79.78)	11(20.37)	0.00	1(1.85)
T. araraticum 2046A	33	30(90.90)	3(9.10)	0.00	0.00
T. araraticum Chinese spring A	15	14(93.33)	1(6.67)	0.00	0.00
T. araraticum 2338 A	24	24(100.00	0.00	0.00	0.00
T. araraticum 343 A	7	6(85.71)	0.00	0.00	1(14.29)
T. araraticum 2099 A	4	3(75.00)	0.00	0.00	1(25.00)
Ae. speltoides 2022 A	2	2(100.00)	0.00	0.00	0.00
Ae. speltoides 2038 A	20	20(100.00)	0.00	0.00	0.00
Ae. speltoides 2042 A	26	25(96.15)	1(3.85)	0.00	0.00
Ae. speltoides 2643 A	3	3(100.00)	0	0	0
TMS 8/2046A	16	15(93.75)	0	0	1(6.25)
TMS 9/2041A	78	67(85.90)	7(8.98)	2(2.56)	2(2.56)
TMS 9/2046A	25	22(88.00)	2(8.00)	0	1(4.00)
TMS 9/2099A	12	11(91.67)	1(8.33)	0	0
TMS 9/2338A	7	7(100.00)	0	0	0
TMS 9/343A	3	3(100.00)	0	0	0
TMS 11/2046A	11	9(81.8)	1(9.10)	0	1(9.10)
TMS 11/2338A	42	35(83.34)	6(11.28)	1(2.38)	0
TMS 11/2019A	8	5(62.50)	2(25.00)	0	1(12.50)
TMS 11/2428A	19	14(73.70)	3(15.78)	1(5.26)	1(5.26)
TMS 20/2042A	10	10(100.00)	0	0	0
TMS 20/2046A	32	25(78.12)	4(12.50)	1(3.13)	2(6.25)
TMS 20/2338A	11	10(90.90)	1(9.10)	0	0
Total	**521**	**454**	**48**	**7**	**12**

Note: Values within parenthesis indicate the percentage.

Table 4.24. Frequency of maintainers and restorers for different male sterility inducing cytoplasms in wheat.

Source of cytoplasm	Number of crosses	Complete maintainers	Partial maintainers	Partial restorers	Complete restorers
T. timopheevi	59	52(88.14)	5(8.47)	2(3.39)	0
T. araraticum	137	119(86.86)	15(10.94)	0	3(2.20)
Ae. speltoides	51	50(98.04)	1(1.96)	0	0
TMS 8	16	15(93.75)	0	0	1(6.25)
TMS 9	125	110(88.00)	10(8.00)	2(1.60)	3(2.40)
TMS 11	80	63(78.75)	12(15.00)	2(2.50)	3.3(3.75)
TMS 20	53	45(84.90)	5(9.43)	1(1.90)	2(3.77)
Total	**521**	**454(87.14)**	**48(9.21)**	**7(1.34)**	**12(2.31)**

Note : Values within parenthesis indicate percentage

Table. 4.25. Partial maintainers, partial and complete restorers for different male sterility inducing cytoplasms in wheat

Source of cytoplasm	Partial maintainer	Partial restorer	Complete restorer
T. timopheevi	T 226 (C-306/*T.militinae*)	Bakhatwar	
	T 1459D (Gigas derivative)	Annapurna	
	WR 196		
	Pitic 62		
	Unnat Kalyansona / BW 1109		
T. araraticum	PWR 4101		PWR 4099
	PDSN 447		
	PDSN 887		
	No. T2605 (*Militinae* derivative)		
	RNB 680 (Kalyansona type)		
	T 1985(DARF/Lok Bharti)		
	Chinese spring/*T.araraticum*/6x		
	SG 22		
	SG 88		
	Charter		
	Flavina		
	CI 12632		
	Quan Feng		
	T567		
	Synthetic elite no. 11		
Ae. speltoides	S2427 (Sonalika / *Ae. speltoides*)		
TMS 8	-	-	PWR 4099
TMS 9	T 3336 (DARF / Lok Bharti)	Shanghai 77	PWR 4099
	T 229 (Sonalika / GD charter)		PWR 4101
	NP 4		
	NP 836		
	WR 251		
	SG 175		
	DL 218-6		
	Synthetic 3		
	T 2600 (C306 / *T. militinae*)		
	Chuanmai – 18		
TMS 11	S 2427	T 1854 (C-306 / *T. militinae)*	PWR 4099
	C306 / *T. timopheevi*	T 957 (*Dicoccum* derivative)	
	T. dicoccum / GD / Sonalika	T 1985 (DARF/Lok Bharti)	
	KNS 33 / PH 127 (Lr-9)		
	T 2600 (C306 / *T. militinae*)		
	T 2605 (*Militinae* derivative)		
	WR 509		
	HW 2011 / Veerys'		
	JGB 1876		
	RNB 259 (Unnat Kalyansona / MACS 2496)		
	HW 2046		
	HD 2833		
	RNB 570 (HD 2329 / Veery's)		
	KMS 9 / R		
TMS 20	T 1638 (*T.dicoccum* / Sonalika)	WR 1044	PWR 4099
	EGS 113		PWR 4101
	PBN 3953		
	WR 1012		
	WR 1049		

4.3.2.1 Differential fertility restoration ability of restorers

Although the present study included CMS lines with various male sterility inducing cytoplasmic sources, no differential fertility restoration pattern was observed. The pollen parents identified as complete restorer of one CMS source did not behave as partial restorer or weak maintainer for other CMS sources. In general, the pollen parents PWR 4099 and PWR 4101 were found to restore complete fertility in all the CMS lines except in *T. timopheevi* and *Ae. speltoides* cytoplasms, as they did not behave as partial restorers in any cross combination. However, PWR 4101 was observed as a partial maintainer for *T. araraticum* cytoplasm (Table 4.25). Variation in the restoration ability of pollen parents identified as partial restorers was also observed in the crosses with different CMS sources. Since the B lines genetically differ in their ability to produce different number of seeds per spike, the variation in number of seeds in various F_1 hybrids was due to the inherent capacity of producing seeds in B lines. The variation in number of seeds may also be due to the differential restoring ability of fertility restorers. The highest spike fertility was observed in the cross *arar* 2099 A X PWR 4099 (77.30 seeds per spike) and the lowest in the cross TMS 9 / 2041 A X PWR 4101 (45.90 seeds per spike).

The range of variation in restoration ability of the pollen parent PWR 4101 was not high since the highest spikelet fertility recorded was only 46.90 seeds (TMS 20 / 2046 A X PWR 4101) and the lowest, 45.90 seeds (TMS 9 / 2041 A X PWR 4101). But the variation in restoration ability of the pollinator PWR 4099 was high with the highest spike fertility producing 77.30 seeds per spike (*arar* / 2099A X PWR 4099) and the lowest being 49.20 seeds per spike in TMS 11 / 2019A X PWR 4099 (Table 4.28). The differential behaviour of CMS lines with the same set of restorer line reflected nuclear-

cytoplasmic interaction, which had a profound influence on the expression of fertility in test cross hybrid progenies.

4.4.1. Inheritance of fertility restoration

All the F_1 hybrids involving CMS lines and two fertility restorer lines PWR 4101 and PWR 4099 were fully fertile. The data on segregation pattern with respect to spike fertility in five F_2 populations recorded during *Rabi* 2001-2002 have been presented in Table 4.26. The fertility restoration pattern of spikes in F_2 population derived from the cross TMS 9/2041 A X PWR 4101 segregated into 1149 fully fertile (FF) and 361 completely male sterile (CS) plants. The frequency of fertile and sterile plants was in agreement with the expected ratio 3F:1S plants with χ^2 value of 0.9616 at 1 d.f. Similarly, in the cross TMS 20/ 2046 A X PWR 4101, 227 plants were fully fertile and 68 plants were completely sterile falling in the expected segregation ratio of 3F:1S (χ^2 = 0.597). These results indicated that a single dominant gene controls fertility restoration in PWR 4101.

In the F_2 population of the cross TMS 9 / 2046 A X PWR 4099, 185 plants were observed to be fully fertile (FF), 108 plants were partially fertile (PF) while 17 segregants were completely sterile (CS). The observed frequency of plants corresponded with the expected modified Mendelian ratio of 9 FF: 6 PF: 1 CS, with χ^2 value of 1.524 at 2 d.f. In the cross TMS 11 / 2099 A X PWR 4099, the F_2 population segregated into 240 FF, 149 PF and 31 CS plants. The observed frequency of plants fitted well to a segregation ratio of 9:6:1 with the χ^2 value of 1.377 at 2 d.f. In another F_2 population of the cross TMS 20/ 2046 A X PWR 4099, the frequency of FF, PF and CS were, 209, 153 and 23 respectively. The distribution pattern of observed plants for spike fertility was in

agreement with the expected 9:6:1 ratio with an estimated χ^2 value of 0.826 at 2 d.f. (Table 4.26).

The goodness of fit of FF and CS plants into 3:1 ratio in the two F_2 populations involving PWR 4101 as restorer indicated that a single dominant gene controls fertility restoration. Similarly, the goodness of fit of FF, PF and CS plants to 9:6:1 in the three F_2 populations involving PWR 4099 as restorer, indicated that the mode of fertility restoration is controlled by two dominant genes corresponding to a polymeric gene action (epistasis). The occurance of partially fertile plants, in the F_2 population of the above crosses involving PWR 4099 further supported that the exotic line PWR 4099 carries two dominant genes for fertility restoration.

4.4.2. Genetics of kernel colour

The inheritance of kernel colour of the restorer PWR 4099 was investigated and crosses were made to a variety of female parents that included CMS lines (CMS 9 / 2041 A and CMS 8 / 2046 A) and 4 genotypes namely PBW 226, HW 2045, HW 2099 and HW 2048). The parents used as female parents had amber grains while the pollinator PWR 4099 had red grains. A total of six crosses were made and F_2 population was raised in *Rabi* 2001-2002 to elucidate the mode of inheritance of kernel colour. All the F_1 hybrids were red grained.

The data on the pattern of kernel colour segregation recorded in six F_2 populations is presented in the Table 4.27. The segregation pattern in F_2 population of the cross CMS 9 / 2041 A X PWR 4099 segregated into 222 plants with red grains and 66 with amber grains. The observed segregation pattern in this cross fitted well to expected ratio indicating monogenic inheritance for red kernal colour. The χ^2 value (0.6666 for 1 df) for 3:1 ratio was non significant.

Table 4.26. F_2 segregation for floret fertility in wheat

Cross	Total no.of plants	No.of completely fertile plants (>45 seeds)	No.of partial fertile plants (2-45 seeds)	No.of sterile plants (0-1 seed)	Expected genetic ratio	χ^2 value	P value
CMS lines							
TMS 9/ 2041 A	10	0	0	10	-	-	-
TMS 9/ 2046 A	10	0	0	10	-	-	-
TMS 11/2019 A	10	0	0	10	-	-	-
TMS 20 /2046 A	10	0	0	10	-	-	-
Restorers							
PWR 4101	10	10	0	0	-	-	-
PWR 4099	10	10	0	0	-	-	-
F_1's							
TMS 9/2041 A X PWR 4101	10	10	0	0	-	-	-
TMS 20/2046 A X PWR 4101	10	10	0	0	-	-	-
TMS 9/2046 A X PWR 4099	10	10	0	0	-	-	-
TMS 11/2019 A X PWR 4099	10	10	0	0	-	-	-
TMS 20/2046 A X PWR 4099	10	10	0	0	-	-	-
F_2's							
TMS 9/2041 A X PWR 4101	1510	1149	-	361	3:1	0.9616	0.30-0.50
TMS 20/2046 A X PWR 4101	295	227	-	68	3:1	0.597	0.30-0.50
TMS 9/2046 A X PWR 4099	310	185	108	17	9:6:1	1.524	0.30-0.50
TMS 11/2019 A X PWR 4099	420	240	149	31	9:6:1	1.3777	0.50-0.70
TMS 20/2046 A X PWR 4099	385	209	153	23	9:6:1	0.826	0.50-0.70

Table 4.27. F_2 segregation for kernel colour in wheat

S.No	Cross	Total no. of plants	Kernel colour		Expected genetic ratio	χ^2 value	P value
			Red	Amber			
1	TMS 9/2041 A X PWR 4099	288	222	66	3:1	0.6666	0.30-0.50
2	TMS 8 / 2046 A X PWR 4099	174	125	49	3:1	0.9273	0.30-0.50
3	PBW 226 X PWR 4099	377	292	85	3:1	1.2094	0.20-0.30
4	HW 2045 X PWR 4099	306	229	77	3:1	0.0044	0.90-0.95
5	HW 2099 X PWR 4099	75	54	21	3:1	0.3600	0.50-0.70
6	HW 2048 X PWR 4099	58	46	12	3:1	0.5747	0.30-0.50
	Pooled over six crosses	**1278**	**968**	**310**	**3:1**	**0.3767**	0.50-0.70

Similarly, the cross CMS 8 / 2046 A X PWR 4099 produced 125 plants with red grains and 49 plants with amber grains in F_2 generation. The observed data also fitted well into expected ratio of 3 red: 1 amber with χ^2 value (0.9273) being non-significant. In the F_2 population derived from the cross PBW 226 X PWR 4099, 292 plants were red grained and 85 plants were with amber grains. The observed frequency of both the classes fitted well into 3 red: 1 amber with χ^2 value of 1.2094. The F_2 population of the cross HW 2045 X PWR 4099 segregated into 229 plants with red grains and 77 with amber grains, which corresponded to the segregation ratio of 3red : 1 amber. The χ^2 value (0.0044) was non significant, which validated the hypothesis (Table 4.27). The observed data in F_2 population derived from the cross HW 2099 X PWR 4099 segregated into 54 plants with red and 21 plants having amber grains. The frequency of plants with red and amber grains were in agreement with the expected 3:1 ratio with a χ^2 value 0.3600. Similarly, the cross HW 2048 X PWR 4099 produced 46 and 12 plants with red and amber grains, respectively, the data fitted well in the expected segregation ratio of 3 red: 1 amber. These results described in preceding paragraphs indicated a perfect Mendelian segregation ratio of 3:1 for red kernel colour and the goodness of fit revealed that a single dominant gene controlled kernel colour in the exotic line PWR 4099.

4.5. MAGNITUDE OF HETEROSIS

The magnitude of heterosis expressed as percentage deviation in the performance regarding eleven experimental hybrids for yield and yield-attributing characters have been presented in Table 4.28. The trait-wise results are presented below. The ANOVA (Table. 4.29) shows significant differences for all the yield and yield attributing characters.

Table 4.28. Relative performance of parents and hybrids for yield and other ancillary characters in wheat

Hybrid/parent	No. of tillers	Plant height (cm)	Spike length (cm)	No. of spikelets per spike	No. of grains per spike	1000-grain weight	Grain yield (g)	
							Per spike	Per plant
PBW 343	11.5	82.3	12.0	21.5	60.3	43.0	2.2	17.8
HW 2022	12.43	88.43	9.82	19.71	60.50	31.50	2.00	22.37
HW 2099	13.24	88.29	11.79	23.41	82.24	26.00	2.23	16.35
HW 2046	11.72	75.06	10.94	19.00	54.16	35.45	1.73	25.80
HW 2041	10.53	77.74	9.47	14.63	40.26	42.84	1.41	16.45
HD 2428	14.0	72.0	12.0	23.0	59.0	37.5	1.9	18.50
HW 2019	11.0	95.0	11.9	23.0	51.0	35.0	1.8	14.10
PWR 4099	8.60	80.00	11.70	23.00	66.40	37.40	2.44	14.90
PWR 4101	9.80	85.00	9.20	20.50	47.30	38.30	1.81	12.70
PHW 1 (arar/343A x PWR 4099)	10.86	83.60	12.59	23.15	63.27	37.76	2.35	15.00
PHW 2 (arar/2022A x PWR 4099)	13.22	89.36	10.23	22.50	67.45	36.26	2.45	22.45
PHW 3 (arar/2099A x PWR 4099)	13.60	87.28	12.18	23.73	77.30	31.50	2.52	18.25
PHW 4 (TMS8/2046A x PWR 4099)	13.00	81.30	11.38	22.10	59.85	33.72	2.12	18.76
PHW 5 (TMS9/2041A x PWR4099)	12.72	87.78	10.82	20.45	58.43	37.18	2.23	17.80
PHW 6 (TMS11/2428A x PWR4099)	15.26	86.36	11.34	22.81	60.26	38.00	2.22	20.50
PHW 7 (TMS9/2046A x PWR 4099)	13.00	86.50	14.30	22.60	69.30	36.80	2.55	22.30
PHW 8 (TMS11/2019A x PWR 4099)	10.50	82.75	12.00	22.60	49.20	35.70	1.76	14.68
PHW 9 (TMS20/2046A x PWR 4099)	11.91	85.72	12.95	22.00	55.40	38.10	2.11	20.26
PHW 10 (TMS9/2041A x PWR4101)	13.50	95.80	10.60	20.00	45.90	37.21	1.71	18.64
PHW 11 (TMS20/2046A x PWR4101)	11.20	88.70	10.70	20.50	46.90	37.70	1.77	19.25
S.Ed.	0.2041	1.4263	0.1918	0.3620	0.9981	0.6120	0.0343	0.7412
C.D. (P=0.05)	0.4131	2.8874	0.3884	0.7329	2.0206	1.2390	0.0695	1.4979
C.D. (P=0.01)	0.5534	3.8677	0.5202	0.9817	2.7066	1.6596	0.0931	2.0042

PHW – Pusa Hybrid Wheat; PWR – Pusa Wheat Restorer

Table 4.29 ANOVA for yield and yield components

Sl.No.	Character	MS due to		F_{cal} value
		Genotype	Error	
1.	Number of tillers	7.7569**	0.0625	124.1722
2	Plant height	104.6738**	3.0515	34.3024
3	Spike length	4.5033**	0.0552	81.5692
4	Number of spikelets	13.5539**	0.1966	68.9438
5	Number of grains per spike	336.7942**	1.4943	225.3782
6	1000-grain weight	42.3070**	0.5618	75.3011
7	Grain yield per spike	0.4404**	0.0671	249.0433
8.	Grain yield per plant	32.8301**	0.8241	39.8375

F at 5 % = 1.79; F at 1 % = 2.29

4.5.1. Number of productive tillers per plant

The magnitude of heterosis for number of tillers has been presented in Table. 4.30. All the hybrids invariably showed heterosis over mid-parent value. The heterosis ranged from 4.08 per cent (PHW 11) to 35.04 per cent (PHW 6). However, three hybrids namely, PHW 1, PHW 8 and PHW 11 showed negative heterobeltiosis as well as standard heterosis. The value of standard heterosis of hybrid PHW 11 was statistically non-significant. (Table 4.30). Hybrid PHW 6 showed high magnitude of standard heterosis (32.7 per cent) as it produced maximum number of tillers per plant (15.26), which was significantly higher over the standard check, which could produce only 11.5 tillers per plant (Table. 4.28).

4.5.2. Plant height

The magnitude of heterosis for plant height of eleven hybrids over mid parent, better parent and standard check is presented in Table. 4.31. All the hybrids, except PHW 1 showed statistically significant differences for mid parent heterosis. The hybrid PHW 8 had a negative heterosis value of –5.43 per cent over the mid parent. The lowest and highest magnitude of mid parent heterosis was observed, respectively, in PHW 3 (3.73 per cent) and PHW 10 (17.73 per cent) (Table. 4.31). These two hybrids had an average plant height of 87.28 cm and 95.80 cm, respectively. Seven hybrids showed significant differences with respect to their better parents; PHW 8 had a negative deviation of –12.90 per cent from its better parent. Maximum heterobeltiosis was observed in PHW 10 (12.71 per cent) and minimum in PHW 11 (4.35 per cent).

Table 4.30. Magnitude of heterosis (per cent) for number of productive tillers per plant in wheat

Hybrid	Mid-parent heterosis	Heterobeltiosis	Standard heterosis
PHW 1 (*arar*/343 A X PWR 4099)	8.06 **	-5.56 **	-5.56 **
PHW 2 (*arar*/2022 A X PWR 4099)	25.73 **	6.35 **	14.96 **
PHW 3 (*arar*/2099 A X PWR 4099)	24.54 **	2.72	18.26 **
PHW 4 (TMS 8/2046 A X PWR 4099)	27.95 **	10.92 **	13.04 **
PHW 5 (TMS 9/2041 A X PWR 4099)	32.98 **	20.80 **	10.61 **
PHW 6 (TMS 11/2428 A X PWR 4099)	35.04 **	9.00 *	32.70 **
PHW 7 (TMS 9/2046 A X PWR 4099)	27.95 **	10.92 **	13.04 **
PHW 8 (TMS 11/2019 A X PWR 4099)	7.14 **	-4.55 *	-8.70 **
PHW 9 (TMS 20/2046 A X PWR 4099)	17.22 **	1.62	3.57
PHW 10 (TMS 9/2041 A X PWR 4101)	32.81 **	28.20**	17.40**
PHW 11 (TMS 20/2046 A X PWR 4101)	4.08 *	-4.44 *	-2.61
C.D. (P=0.05)	0.3572	0.4124	0.4124
C.D. (P=0.01)	0.4778	0.5518	0.5518

****, * significant at 0.01 % and 0.05%, respectively**

Table 4.31. Magnitude of heterosis (per cent) for plant height in wheat

Hybrid	Mid-parent heterosis	Heterobeltiosis	Standard heterosis
PHW 1 (*arar*/343 A X PWR 4099)	3.02	1.57	1.57
PHW 2 (*arar*/2022 A X PWR 4099)	6.11 **	1.05	8.58 **
PHW 3 (*arar*/2099 A X PWR 4099)	3.73 *	-1.14	6.05 **
PHW 4 (TMS 8/2046 A X PWR 4099)	4.86 **	1.63	-1.22
PHW 5 (TMS 9/2041 A X PWR 4099)	11.30 **	9.73 **	6.66 **
PHW 6 (TMS 11/2428 A X PWR 4099)	13.63 **	7.95 **	4.93 **
PHW 7 (TMS 9/2046 A X PWR 4099)	11.57 **	8.13 **	5.10 **
PHW 8 (TMS 11/2019 A X PWR 4099)	-5.43 **	-12.90 **	0.55
PHW 9 (TMS 20/2046 A X PWR 4099)	10.56 **	7.15 **	4.16 *
PHW 10 (TMS 9/2041 A X PWR 4101)	17.73 **	12.71 **	16.40 **
PHW 11 (TMS 20/2046 A X PWR 4101)	10.83 **	4.35 *	7.78 **
C.D. (P=0.05)	2.4963	2.8820	2.8820
C.D. (P=0.01)	3.3400	3.8567	3.8567

****, * significant at 0.01 % and 0.05%, respectively**

Of the eight hybrids that showed significantly superior performance over the standard check, PHW 10 was the best (16.40 per cent) and was 95.80 cm tall as compared to the standard check PBW 343 (82.3 cm) (Table 4.28).

4.5.3. Main spike length

The relative performance of 11 hybrids over mid parent, better parent and standard check is presented in Table 4.32. Eight hybrids showed significant mid parent heterosis while two, PHW 2 and PHW 6 were negative. The magnitude of mid parent heterosis for main spike length ranged from 3.70 per cent (PHW 3) to 26.33 per cent (PHW 7). A similar number of hybrids had significant heterosis (Table 4.31) over better parent. PHW 7 had the highest magnitude of heterobeltiosis of 22.22 per cent, while PHW 1 had the lowest magnitude of 4.92 per cent. Only three hybrids, namely, PHW 1, PHW 7 and PHW 9 had positive standard heterosis. The values for heterosis ranged from a minimum of 4.92 per cent in PHW 1 to a maximum of 19.17 per cent in PHW 7. The hybrid PHW 7 remained superior over mid parent, better parent and the standard check. Plate 7 indicates the comparable length of the spike in respect of A, B, R and A X R lines. It had a main spike length of 14.30 cm as compared to 12.0 cm of the standard check (Table. 4.28).

4.5.4. Number of spikelets per main spike

The performance of 11 hybrids in terms of percentage deviation over mid parent, better parent and standard check for number of spikelets per main spike is presented in Table. 4.33. Eight hybrids registered significant positive mid parent heterosis ranging from 3.80 per cent in PHW 11 to 13.86 per cent in PHW 10. None of the hybrids under study showed positive heterobeltiosis for number of spikelets (Table 4.33).

Plate 7: (L-R) Spikes of male sterile (A), maintainer (B), restorer (R) lines and F_1 hybrid (AxR)

Table 4.32. Magnitude of heterosis (per cent) for main spike length in wheat

Hybrid	Mid-parent heterosis	Heterobeltiosis	Standard heterosis
PHW 1 (*arar*/343 A X PWR 4099)	6.24 **	4.92 **	4.92 **
PHW 2 (*arar*/2022 A X PWR 4099)	-4.93 **	-12.56 **	-14.75 **
PHW 3 (*arar*/2099 A X PWR 4099)	3.70 *	3.31 *	1.50
PHW 4 (TMS 8/2046 A X PWR 4099)	0.53	-2.74	-5.17 **
PHW 5 (TMS 9/2041 A X PWR 4099)	2.22	-7.52 **	-9.83 **
PHW 6 (TMS 11/2428 A X PWR 4099)	-4.30 **	-5.50 **	-5.50 **
PHW 7 (TMS 9/2046 A X PWR 4099)	26.33 **	22.22 **	19.17 **
PHW 8 (TMS 11/2019 A X PWR 4099)	1.69	0.84	0.0
PHW 9 (TMS 20/2046 A X PWR 4099)	14.40 **	10.68 **	7.92 **
PHW 10 (TMS 9/2041 A X PWR 4101)	13.55 **	11.92 **	-11.67 **
PHW 11 (TMS 20/2046 A X PWR 4101)	6.26 **	--2.19	-10.83 **
C.D. (P=0.05)	0.3358	0.3877	0.3877
C.D. (P=0.01)	0.4493	0.5188	0.5188

****, * significant at 0.01 % and 0.05%, respectively**

Table 4.33. Magnitude of heterosis (per cent) for number of spikelets per main spike in wheat

Hybrid	Mid-parent heterosis	Heterobeltiosis	Standard heterosis
PHW 1 (*arar*/343 A X PWR 4099)	4.04 **	0.65	7.67 **
PHW 2 (*arar*/2022 A X PWR 4099)	5.36 **	-2.17	4.65 **
PHW 3 (*arar*/2099 A X PWR 4099)	2.26	1.37	10.37 **
PHW 4 (TMS 8/2046 A X PWR 4099)	5.23 **	-3.91 *	2.80
PHW 5 (TMS 9/2041 A X PWR 4099)	8.69 **	-11.09 **	-4.88 **
PHW 6 (TMS 11/2428 A X PWR 4099)	-0.83	-0.83	6.09 **
PHW 7 (TMS 9/2046 A X PWR 4099)	7.62 **	-1.74	5.12 **
PHW 8 (TMS 11/2019 A X PWR 4099)	-1.74	-1.74	5.12 **
PHW 9 (TMS 20/2046 A X PWR 4099)	4.76 **	-4.35 **	2.33
PHW 10 (TMS 9/2041 A X PWR 4101)	13.86 **	-2.44	-6.98 **
PHW 11 (TMS 20/2046 A X PWR 4101)	3.80 *	0	-4.65 **
C.D. (P=0.05)	0.6336	0.7317	0.7317
C.D. (P=0.01)	0.8477	0.9789	0.9789

****, * significant at 0.01 % and 0.05%, respectively**

Out of the five hybrids that showed positive standard heterosis, PHW 3 recorded the maximum deviation of 10.37 per cent (23.73 spikelets) over the standard check (21.5 spikelets) (Table. 4.28). The minimum standard heterosis was noted in the hybrids PHW 7 and PHW 8 (5.12 per cent).

4.5.5. Number of grains per spike

Table 4.34 shows the magnitude of heterosis of 11 hybrids for number of grains per spike. The deviation of mid parent heterosis was from –16.18 per cent in PHW 8 to 14.96 per cent in PHW 7. Five hybrids, namely, PHW 2, PHW 3, PHW 4, PHW 7 and PHW 10 were positively significant for mid parent heterosis. Eight out of the 11 hybrids showed negative deviation with respect to better parent heterosis (Table. 4.34); the maximum being –25.90 per cent in PHW 8. Four hybrids, *viz.*, PHW 1, PHW 2, PHW 3 and PHW 7 exhibited significant positive standard heterosis, whereas an equal number of hybrids, PHW 8, PHW 9, PHW 10 and PHW 11 showed negative standard heterosis. The highest magnitude of heterosis over the standard check was observed in PHW 3 (28.19 per cent), which produced 82.24 grains per spike as compared to 60.3 grains per spike in the standard check, PBW 343.

4.5.6. 1000-grain weight

The magnitude of heterosis with respect to 1000-grain weight in 11 hybrids expressed in percentage is presented in Table 4.35. Only two hybrids, PHW 2 and PHW 9 had significantly positive values for mid parent heterosis (5.25 per cent and 4.60 per cent, respectively). Four hybrids registered significant and negative mid-parent heterosis. It ranged from –6.07 per cent in PHW 1 to –8.28 per cent in PHW 10.

Table 4.34. Magnitude of heterosis (per cent) for number of grains per spike in wheat

Hybrid	Mid-parent heterosis	Heterobeltiosis	Standard heterosis
PHW 1 (*arar*/343 A X PWR 4099)	-0.13	-4.71 **	4.93 **
PHW 2 (*arar*/2022 A X PWR 4099)	6.30 **	1.58	11.85 **
PHW 3 (*arar*/2099 A X PWR 4099)	4.00 **	-6.01 **	28.19 **
PHW 4 (TMS 8/2046 A X PWR 4099)	-0.71	-9.86 **	-0.75
PHW 5 (TMS 9/2041 A X PWR 4099)	9.56 **	-12.00 **	-3.13
PHW 6 (TMS 11/2428 A X PWR 4099)	-3.89 **	-9.24 **	-0.06
PHW 7 (TMS 9/2046 A X PWR 4099)	14.96 **	4.37 **	14.93 **
PHW 8 (TMS 11/2019 A X PWR 4099)	-16.18 **	-25.90 **	-18.41 **
PHW 9 (TMS 20/2046 A X PWR 4099)	-8.09 **	-16.56 **	-8.13 **
PHW 10 (TMS 9/2041 A X PWR 4101)	4.84 *	-2.96	-23.88 **
PHW 11 (TMS 20/2046 A X PWR 4101)	-7.55 **	-13.40 **	-22.22 **
C.D. (P=0.05)	1.7469	2.0172	2.0172
C.D. (P=0.01)	2.3373	2.6989	2.6989

****, * significant at 0.01 % and 0.05%, respectively**

Table 4.35. Magnitude of heterosis (per cent) for 1000-grain weight in wheat

Hybrid	Mid-parent heterosis	Heterobeltiosis	Standard heterosis
PHW 1 (*arar*/343 A X PWR 4099)	-6.07 **	-12.19 **	-12.19 **
PHW 2 (*arar*/2022 A X PWR 4099)	5.25 **	-3.05	-15.67 **
PHW 3 (*arar*/2099 A X PWR 4099)	-0.63	-15.78 **	-26.74 **
PHW 4 (TMS 8/2046 A X PWR 4099)	-7.43 **	-9.84 **	-21.58 **
PHW 5 (TMS 9/2041 A X PWR 4099)	-7.33 **	-13.21 **	-13.53 **
PHW 6 (TMS 11/2428 A X PWR 4099)	1.47	1.33	-11.63 **
PHW 7 (TMS 9/2046 A X PWR 4099)	1.03	-1.60	-14.42 **
PHW 8 (TMS 11/2019 A X PWR 4099)	-1.38	-4.55 **	-16.96 **
PHW 9 (TMS 20/2046 A X PWR 4099)	4.60 **	1.87	-11.40 **
PHW 10 (TMS 9/2041 A X PWR 4101)	-8.28 **	-13.14 **	-13.49 **
PHW 11 (TMS 20/2046 A X PWR 4101)	2.24	-1.56	-12.33 **
C.D. (P=0.05)	1.0712	1.2369	1.2369
C.D. (P=0.01)	1.4332	1.6549	1.6549

**, * significant at 0.01 % and 0.05%, respectively

The remaining were non-significant. Six hybrids showed significant deviation in magnitude of heterosis over better parent. All of them had negative deviation (Table. 4.35) ranging from –4.55 per cent in PHW 8 to –15.78 per cent in PHW 3. None of the 11 hybrids showed significant positive standard heterosis for 1000-grain weight indicating the superiority of the standard check for this character. The maximum deviation was observed in PHW 3 (-26.74 per cent) and the minimum in PHW 9 (-11.40 per cent).

4.5.7. Grain yield per spike

The performance of 11 hybrids expressed in terms of per cent deviation over mid parent, better parent and standard check for yield per spike is presented in Table. 4.36. Six hybrids had mid parent heterosis significant and positive. The values ranged from 6.21 per cent (PHW 10) to 22.30 per cent (PHW 7). The hybrid PHW 11 did not show mid parental heterosis at all. Only two hybrids, PHW 3 and PHW 7 exhibited significant heterosis of 3.27 per cent and 4.51 per cent, respectively over their better parents. Seven hybrids were significantly inferior to their better parents. Four hybrids, namely, PHW 1, PHW 2, PHW 3 and PHW 7 showed significant and positive differences over the standard check (Table 4.36). The maximum yield per spike was observed in PHW 7 (2.55 g per spike) as compared to the yield of 2.2 g per spike in the standard check, PBW 343 (Table. 4.28).

4.5.8. Yield per plant

The magnitude of heterosis for yield per plant is presented in Table. 4.37. Six hybrids, *viz.*, PHW 2, PHW 3, PHW 5, PHW 6, PHW 7 and PHW 10 registered significant mid parent heterosis. The heterosis ranged from 9.58 per cent in PHW 7 to 27.89 per cent in PHW 10. The hybrid PHW 11 did not exhibit mid parent heterosis.

Table 4.36. Magnitude of heterosis (per cent) for grain yield per spike in wheat

Hybrid	Mid-parent heterosis	Heterobeltiosis	Standard heterosis
PHW 1 (*arar*/343 A X PWR 4099)	1.29	-3.69 *	6.82 **
PHW 2 (*arar*/2022 A X PWR 4099)	10.36 **	0.41	11.36 **
PHW 3 (*arar*/2099 A X PWR 4099)	7.92 **	3.27 *	14.55 **
PHW 4 (TMS 8/2046 A X PWR 4099)	1.68	-13.11 **	-3.64 *
PHW 5 (TMS 9/2041 A X PWR 4099)	15.84 **	-8.61 **	1.36
PHW 6 (TMS 11/2428 A X PWR 4099)	2.30	-9.02 **	0.91
PHW 7 (TMS 9/2046 A X PWR 4099)	22.30 **	4.51 **	15.91 **
PHW 8 (TMS 11/2019 A X PWR 4099)	-16.98 **	-27.86 **	-20.00 **
PHW 9 (TMS 20/2046 A X PWR 4099)	1.20	-13.52 **	-4.10 **
PHW 10 (TMS 9/2041 A X PWR 4101)	6.21 **	-5.52 **	-22.27 **
PHW 11 (TMS 20/2046 A X PWR 4101)	0.0	-2.21	-19.55 **
C.D. (P=0.05)	0.0601	0.0694	0.0694
C.D. (P=0.01)	0.0804	0.0928	0.0928

**, * significant at 0.01 % and 0.05%, respectively

Table 4.37. Magnitude of heterosis (per cent) for grain yield per plant in wheat

Hybrid	Mid-parent heterosis	Heterobeltiosis	Standard heterosis
PHW 1 (*arar*/343 A X PWR 4099)	-8.26 *	-15.73 **	-15.73 **
PHW 2 (*arar*/2022 A X PWR 4099)	20.47 **	0.36	26.12 **
PHW 3 (*arar*/2099 A X PWR 4099)	16.80 **	11.62 *	2.53
PHW 4 (TMS 8/2046 A X PWR 4099)	-7.81 *	-27.29 **	5.39
PHW 5 (TMS 9/2041 A X PWR 4099)	13.56 **	8.21	0
PHW 6 (TMS 11/2428 A X PWR 4099)	22.75 **	10.81 **	15.17 **
PHW 7 (TMS 9/2046 A X PWR 4099)	9.58 **	-13.57 **	25.28 **
PHW 8 (TMS 11/2019 A X PWR 4099)	1.24	-1.48	-17.53 **
PHW 9 (TMS 20/2046 A X PWR 4099)	-0.44	-21.47 **	13.82 **
PHW 10 (TMS 9/2041 A X PWR 4101)	27.89 **	13.31 **	4.71
PHW 11 (TMS 20/2046 A X PWR 4101)	0	-25.38 **	8.15
C.D. (P=0.05)	1.2973	1.4979	1.4979
C.D. (P=0.01)	1.7357	2.0042	2.0042

****, * significant at 0.01 % and 0.05%, respectively**

Out of the remaining four hybrids two, *viz.*, PHW 1 and PHW 4 showed significantly negative and the other two *viz.*, PHW 8 and PHW 9 exhibited non-significant mid-parent heterosis (Table. 4.37). Three hybrids namely, PHW 3, PHW6 and PHW 10 showed significant positive heterobeltiosis. Four hybrids, *viz.*, PHW 2, PHW 6, PHW 7 and PHW 8 registered better performances than the standard check. The maximum yield per plant over the standard check was observed in PHW 2 (22.45 g per plant; 26.12 per cent) and the minimum standard heterosis was observed in PHW 9 (20.26 g per plant; 13.82 per cent). The standard check produced an yield of 17.8 g per plant (Table 4.28).

4.6. RELATIVE YIELD PERFORMANCE OF HYBRIDS

A total of five hybrids namely PHW1 (*arar* 343 A X PWR 4099), PHW 2 (*arar* 2022 A X PWR 4099), PHW 3 (*arar* 2099 A X PWR 4099), PHW 12 (*arar* 2046 A X PWR 4099) and PHW 13 (*arar* 3687 A X PWR 4099) were included in the study to assess their yield performance on larger plot area (Plate 8). The results on grain yield of the five hybrids, six cultivars *viz.*, PBW 343, HW 2022, HW 2099, HW 2046, HD 2329, and HD 2687 and one restorer line, PWR 4099 obtained in the trial conducted in randomised block design with three replications are presented in Table 4.38. The ANOVA revealed the statistical significance for grain yield performance of hybrids.

Among the parents, the lowest and highest grain yield was recorded in PWR 4099 (45.58 q ha^{-1}) and HD 2687 (56.85 q ha^{-1}), respectively, whereas among the hybrids, PHW 3 recorded the lowest grain yield (50.96 q ha^{-1}) while PHW 13 recorded the highest (59.45 q ha^{-1}). Table 4.38 indicates that the hybrid PHW 13 significantly outyielded other hybrids and cultivars. The exotic line PWR 4099 was significantly inferior to all

Plate 8. Visual assessment of F_1 hybrids in replicated trial

the hybrids and genotypes included in the study. The performance of all the hybrids and the check varieties has been represented in a bar chart.

4.6.1. Magnitude of heterosis (per cent) for grain yield in wheat

The magnitude of heterosis expressed as percentage deviation in the performance of five hybrids for grain yield has been presented in Table 4.40. All the five hybrids, *viz.*, PHW 1, PHW 2, PHW 3, PHW 12 and PHW 13 showed statistically significant differences over mid-parent heterosis. The differences were however, positive and ranged from 6.92 in PHW 3 to 16.08 per cent in PHW 13. None of the hybrids exhibited superiority over better parent. Standard heterosis was calculated with respect to three standard checks *viz.*, PHW 343, HD 2329 and HD 2687 and the results are presented in Table 4.40. Only PHW 13 showed statistical superiority over PBW 343 (10.13 per cent) and HD 2329 (5.05 per cent). PHW 13 also showed superiority over the standard check HD 2687 but the differences were non-significant. None of the remaining four hybrids showed superiority in yield performances over the three standard checks.

Table 4.38. Yield performance of hybrids in wheat

Genotypes	Mean yield (q ha^{-1})
HW 2022	52.62
HW 2046	53.30
HW 2099	49.74
HD 2329	56.59
HD 2686	56.85
PBW 343	53.98
PWR 4099	45.58
PHW 1 (*arar* 343 A X PWR 4099)	54.61
PHW 2 (*arar* 2022 A X PWR 4099)	53.03
PHW 3 (*arar* 2099 A X PWR 4099	50.96
PHW 12 (*arar* 2046 A X PWR 4099)	53.01
PHW 13 (*arar* 2687 A X PWR 4099)	59.45

S.Ed = 1.3128

C.D. (0.05) = 2.7227

C.D. (0.01) = 3.7007

CV(%) = 3.016

Table 4.39. Analysis of Variance (ANOVA) for hybrid wheat trial

Source of variation	d.f.	SS	MS	F	F	
					5%	1%
Replication	2	13.9975	6.9988			
Treatment (genotype)	11	425.7147	38.7013	14.9709 **	2.23	3.12
Error	22	56.8727	2.5851			
Total	35	496.5849				

****, * significant at 0.01 % and 0.05%, respectively**

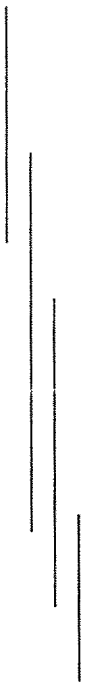
Table 4.40. Magnitude of heterosis (per cent) in different hybrids in wheat

Hybrid	Mid-parent heterosis	Hetero-beltiosis	Standard heterosis		
			PBW 343	HD 2329	HD 2687
PHW 1 (*arar* 343 A X PWR 4099)	9.70 **	1.17	1.17	-3.50	-3.94
PHW 2 (*arar* 2022 A X PWR 4099)	8.00 **	0.78	-1.76	-6.29 *	-6.72 **
PHW 3 (*arar* 2099 A X PWR 4099)	6.92 **	2.45	-5.59 *	-9.95 **	-10.36 **
PHW 12 (*arar* 2046 A X PWR 4099)	7.22 **	-0.54	-1.80	-6.33 *	-6.75 **
PHW 13 (*arar* 2687 A X PWR 4099)	16.08 **	4.57	10.13 **	5.05 *	4.57
C.D. (P=0.05)	2.36	2.72	2.72	2.72	2.72
C.D. (P=0.01)	3.21	3.70	3.70	3.70	3.70

****, * significant at 0.01 % and 0.05%, respectively**

Bar chart 1. Bar chart representing the performance of hybrids and check varieties

Genotypes	Mean Yield ($q\ ha^{-1}$)
PHW 13	59.45
HD 2687	56.85
HD 2329	56.59
PHW 1	54.61
PBW 343	53.98
HW 2046	53.30
PHW 2	53.03
PHW 12	53.01
HW 2022	52.62
PHW 3	50.96
HW 2099	49.74
PWR 4099	45.58



DISCUSSION

"The philosophies of one age have become the absurdities of the next, and the foolishness of yesterday has become the wisdom of tomorrow".

- SIR WILLIAM OSLER

5. DISCUSSION

The annual production of wheat (*Triticum aestivum*) in India has been quite impressive as it moved from 68.76 million tonnes in 1994-95 to 71.45 million tonnes in 2001-2002. The production records of the year 1999-2000 witnessed an all-time record of 76.37 million tonnes, harvested from almost the same area of 25.5 m.ha. The cultivated area under wheat during the last one decade has been more or less static, which illustrates that extensive agriculture is no longer possible in India. The yield potential of present day varieties may not be sufficient to meet the targets projected for the year 2020 and beyond as the recently developed varieties through conventional breeding methods may provide very less yield gains. Therefore, the main target of wheat breeding is to employ alternate approaches for enhancing its genetic yield potential in a sustainable manner. The best viable option could be effective exploitation of heterosis in genetic up gradation of the yield potential of wheat, an important staple food crop of the world.

Exploitation of heterosis, the benefits of which have been amply demonstrated in several crops, has also been suggested as an important genetical approach for raising the productivity levels of crop plants, which are agriculturally important. After the discovery of cytoplasmic male sterility (Kihara, 1951) and its use in hybrid wheat breeding, the possibility of developing hybrids in wheat has attracted the attention of many breeders for quite long time. Since then, extensive efforts have been made to exploit heterosis in wheat improvement. Exploitation of heterosis at commercial level depends on the availability of either cytoplasmic genetic male sterility (CGMS) or through certain chemicals known as chemical hybridizing agents (CHAs). The success of hybrid wheat

programme other than CHA approach lies on two key elements *viz.*, development of stable CGMS lines and perfect restorer system. However, this methodology is offset by many obstacles and limitations such as non-availability of breeding stocks containing CGMS and fertility restorers, their instability and the laborious method of heterosis breeding. Besides being tedious and time consuming, this technique becomes sometimes untenable due to the lack of consistent restorer system for genetic restoration of fertility (Miri, 1968; Stroike, 1987; Singh and Rathore, 1992; Chakraborty *et al.* 2000).

In India, at present, commercially usable and widely adaptable CMS lines with good grain quality are not readily available. Divergent perfect restorers are also not readily available. The level of heterosis in wheat is also not high (Sun *et al.* 1997) due to lack of divergent heterotic parents. Substantial heterosis in grain yield can be realized if floret fertility is increased in the hybrids by breeding better restorers, which have more than one gene for restoring fertility. The strategic approach to solve this problem is to analyse genetically the basis of fertility restoration and formulate appropriate breeding methodology to ensure satisfactory level of restoration in the hybrids. During the present investigation, information has been generated on the above-mentioned aspects. An attempt has been made to interpret critically the results of the present study under the following heads.

5.1. Stability of sterility in CMS lines

The stable expression of sterility is the most important consideration in the selection of CMS lines to be used in heterosis breeding; hence, their evaluation for stable expression of sterility assumes importance. Keeping this in view, 39 CMS lines with

seven diverse male sterile cytoplasms were evaluated in three environments created by different dates of sowing.

The CMS lines included in the study did not have fertile pollen grains in any of the environments. The failure of seed setting in bagged spikes conclusively suggested that all CMS lines exhibited stable expression for spikelet sterility over the environments. Twenty-nine CMS lines did not produce Stained Round Fertile (SRF) pollen in any of the environments (Table 4.22). Among the different categories of pollen grains, SRF pollen grains are considered as viable (Chaudhury *et al.* 1981) and their appearance is regarded as an indication of instability of sterility. All the CMS lines studied, on a account of the failure of production of SRF pollens and due to the production of a remarkably high proportion of Unstained Withered Sterile (UWS) and Unstained Spherical Sterile (USS) pollens, can be stated to have recorded stable expression of sterility across different dates of sowing.

The pollen stainability pattern indicated traces of fertility in 10 CMS lines namely, *timo* 2041 A, *arar* 2038 A, *arar* 2041 A, *arar* 2046 A, *arar* 2099 A, *arar* 343 A, *spelt* 2042 A, TMS 8 / 2687 A, TMS 9 / 2038 A and TMS 11 / 2338 A. These CMS lines produced SRF pollens though at an extremely low frequency in one or more environments. Pickett (1993 b) could also observe unstable expression of sterility of *timopheevi* cytoplasm. It was interesting to note that *timo* 2041 A and *arar* 2046 A produced SRF pollens in two of the three environments (E1 & E3; E1 & E2, respectively) while the rest of them in any of the three environments (Table 4.21). Virmani *et al.* (1986) also observed that the pollen sterility of certain CMS lines is affected by the date of seeding while other CMS lines are stable and unaffected by environmental influence.

The sterility in cytosterile lines of rice has been observed to be significantly affected by temperature (Ali *et al.* 1998) although photoperiod and relative humidity may also affect male sterility. Similarly, in wheat, male sterile line with *Ae. crassa* cytoplasm was found to be affected by genotypic background (Murai, 2001). The stage at which temperature affects pollen mother cells are not precisely known, however, at that period, pre-meiotic stage is considered to be the most vulnerable stage in CMS lines. In some cases fertility is enhanced at high temperature while in some other cases, lower temperature favours male fertility. The dehiscence of anthers is dependent on the amount of viable pollen they contain and the minimal amount of pollen required to cause dehiscence, which varies according to the relative humidity in the air. Higher temperature and low relative humidity can convert the anthers of CMS lines to partial fertility while lower temperature and high relative humidity ensures consistent expression of male sterility. Instability of sterility in a CMS line may be found in common wheats of hybrid origin (Powers, 1932 a, 1932b), and due to the genetic distance between the cytoplasmic donor and recipient parents (Chaudhury *et al.* 1981; Virmani and Edwards, 1983). Genetical, cytological and histochemical studies are, therefore, necessary to establish the exact cause of unstable expression of sterility in these CMS lines. Moreover, unstable expression of sterility may also be due to occasional out-crossing, however, this can be verified by further studies under controlled conditions. The sensitivity of these CMS lines may be a limiting factor in certain cultivars. The problems associated with sensitivity in male sterile lines caused by environmental factors can be avoided by adjusting the planting dates.

In the present study, in addition to the CMS line 2160 A possessing *Ae. caudata* cytoplasm, *timo* 2338 A, *arar* 2022 A and TMS 20 / 2042 A also produced modified stamens. The occurrence of pistillody suggests that this could have been caused by the effect of genetic differences between the alien and wheat cytoplasms (Mukai *et al.* 1978; Suemoto, 1978; Sasaki *et al.* 1978). Series of investigations by Murai *et al.* (1991 a) on the use of D^2 cytoplasm of *Ae. crassa* also confirmed the occurrence of pistillody of all three stamens. But it seems to have been caused due to long day conditions. Male sterility is also caused by the modification of anthers into fully fertile ovaries, which takes place in about 85 percent of the florets per spike (Anonymous, 2001). It seems that the anthers had homeotically transformed into fertile ovaries.

From the reference of data on different categories of pollen presented in Table 4.22, it is apparent that the relative proportion of USS pollens was remarkably high in all the CMS lines under all environments. Since almost all pollen grains (more than 97 per cent) appeared as UWS and USS, the 29 CMS lines belonged to type I cytosterility group in which pollen abortion mostly takes place early at uni-nuclear stage. Choudhury *et al.* (1981) also classified CMS lines into various cytosterility groups based on the appearance of various kinds of pollen grains. This is in agreement with the findings of Maan and Mc. Cracken (1968) who are of the view that external environment does not appear to influence meiotic instability after the onset of meiosis. All the 29 CMS lines were classified accordingly into completely sterile and highly sterile groups. Remaining 10 CMS lines were grouped under highly male sterile category (Table 4.22).

The studies on out-crossing showed that when pollen supply was not guaranteed, the seed set data for all the CMS lines was in full agreement with their respective pollen

fertility level, *i.e.*, there was no seed set in bagged spikes while in uncovered spikes, the maximum out-crossing was 13.38 percent (*arar* 2022 A). Only six other CMS lines *viz.*, TMS 8 / 2046 A, TMS 9 / 2038 A, TMS 9 / 2041 A, TMS 9 / 2046 A, TMS 11 / 2428 A and TMS 11 / 343 A had an out-crossing potential of over 10 percent. The very low seed setting observed on the male sterile plants (in method I) despite allowing open pollination might be due to poor availability of pollen during anthesis indicating the need for synchronous flowering of A and B lines.

5.1.1. Stability of CMS lines with respect to agronomic characters

Stability is a temporal concept referring to the performance of a genotype with respect to changing environmental factors over time within a given location. Phenotypic stability in the CMS lines for important agronomic traits influencing hybrid seed yield is highly desirable over a wide range of sowing dates to ensure commercially feasible and economically viable hybrid seed production. Several studies have been conducted to know the role of cytoplasm in deciding the ultimate phenotype along with nuclear genome in the crops, where cytoplasmic male sterility has been used for commercial seed production. Therefore, study of phenotypic stability of CMS lines becomes an important aspect. Reports regarding both favourable and unfavourable influence of cytoplasm across the locations are available in self-pollinated crop like rice. Keeping this in view, 39 CMS lines in seven different cytoplasmic backgrounds were tested for six agronomic characters *viz.*, number of tillers, plant height, main spike length, number of spikelets, days to 50% flowering and synchrony of tillers in three environments created by ranging the dates of sowing.

Variation with respect to the male sterility adjudged by seed set in covered spike was not observed, when the CMS lines were planted at three different dates of sowing. Relative reduction in plant height, spike length, spikelet number per spike, was however, observed when the CMS lines were planted late. Reports on reduction in chlorophyll content, leaf area and yield per plant were given by Ashraf and Bhatti (1998) and Panelia *et al.* (1993) in late planted wheat varieties. Variation with regard to the male sterility was not observed across the locations. The days to 50% flowering were less by 7 days at Lahaul as compared to Delhi because of the longer day length at higher hills. Though stability analysis was not carried out, the observation recorded at two locations indicated that all the CMS lines were stable with regard to all the agronomic traits studied.

5.1.2. Differential interaction of cytoplasm in diverse nuclear backgrounds

Differential response of diverse cytoplasms on various floral and agronomic traits having same or different nuclear genetic background was also studied. The summarized observation presented in Tables 5.1 to 5.5. indicated, in general, a depression in the performance of floral traits depicting an appreciable interaction between nuclear and cytoplasmic genes. However, it was interesting to note that *T. araraticum* cytoplasm with three different nuclear genomes produced a wide angle of glume opening. The cytoplasm of TMS 9 also influenced the glume opening in positive direction but only in the background of HW 2046. Wide glume opening is a desirable feature of any A line, since it plays a crucial role in attracting more pollen for fertilization in A X B and A X R crosses. Except in a few cases, the anther size was small irrespective of the nuclear genetic backgrounds. Empty and small anther is a desirable characteristic feature of male sterile lines.

With respect to agronomic traits, characters like spike length and spikelet number per spike did not show any variation either with common or different nuclear genomes. However, all the A lines except the nuclear backgrounds of HW 2038 and PBW 343 showed numerical superiority for both the above traits. In some cases, *e.g.*, T. *timopheevi* cytoplasm with the interaction of UP 2338 nucleus produced even longer spikes. Okocha (1999) has observed that interaction between nucleus and cytoplasm in F_1 hybrids varied depending on the nuclear genotype. He also found that the cytoplasms of *Aegilops squarrosa*, *Ae. speltoides*, *Triticum timopheevi* and *Haynaldia villosum* depressed plant height while positively influencing main ear length, grains per plant and fresh weight. Simultaneously, he also pointed out that extra nuclear DNA was largely under the control of the nuclear genotype.

Variation for tiller number and plant height was also observed among the different lines possessing common as well as different nuclear genome. Most of the CMS lines were late in flowering irrespective of the nuclear background indicating the influence of cytoplasm on days to 50 % heading. Earlier studies conducted by Sasakuma *et al.* (1979) also indicated that cytoplasm of *Aegilops ovata* and *Ae. umbellulata* delayed heading and significantly affected number of tillers per plant as well as plant height. Jost *et al.* (1976) observed in A lines with *T. timopheevi* cytoplasm, shorter plants than B lines. It was also observed in the present study, and increase in number of tillers in a majority of CMS lines in the nuclear background of HW 2046 and UP 2338.

Table 5.1. Effect of different cytoplasms on various floral and agronomic characters in wheat in the nuclear background of HW 2046.

CMS source	Floral traits						Agronomic traits					
	Glume opening angle (degree)	Anther size (mm)	Filament length (mm)	Ovary size (mm)	Length of style (mm)	Stigma recepti-vity (days)	No. of tillers	Plant height (cm)	Main spike length (cm)	No. of spikelets per main spike	Days to 50% flowering	Synchrony of tillers (days)
T. timopheevi	8.7	3.76	2.00	1.58	2.56	4	8.8	74.3	10.6	18.2	92.6	7.2
T. araraticum	7.1	4.00	2.04	1.50	3.00	4-5	10.6	77.2	11.5	20.6	92.2	8.0
Ae. speltoides	8.4	3.54	1.98	1.86	1.54	4-5	9.0	94.6	10.8	19.8	89.8	6.0
TMS 8	10.8	3.58	2.80	1.56	1.80	4-5	6.8	76.5	11.0	17.8	92.0	6.0
TMS 9	13.3	3.22	3.00	1.58	1.74	4-5	11.0	83.0	11.0	19.0	83.0	8.0
TMS 11	6.4	4.04	2.06	1.88	2.34	4-5	12.0	80.4	11.8	20.0	86.6	8.0
TMS 20	12.8	3.66	2.02	1.98	2.62	4-5	9.8	80.0	11.5	20.2	87.6	8.6
HW 2046		**4.08**	**2.54**	**1.72**	**3.00**	**4-5**	**9.0**	**76.6**	**11.2**	**19.2**	**87.6**	**8.0**

Table 5.2. Effect of different cytoplasms on various floral and agronomic characters in wheat in the nuclear background of UP 2338.

CMS source	Floral traits						Agronomic traits					
	Glume opening angle (degree)	Anther size (mm)	Filament length (mm)	Ovary size (mm)	Length of style (mm)	Stigma receptivity (days)	No. of tillers	Plant height (cm)	Main spike length (cm)	No. of spikelets per main spike	Days to 50% flowering	Synchrony of tillers (days)
T. timopheevi	7.4	3.56	1.92	1.52	1.98	4	8.6	76.7	15.5	22.6	102.0	8.0
TMS 8	9.4	3.5	1.98	1.66	2.68	4	8.0	80.8	12.7	20.4	97.2	7.0
TMS 9	6.1	3.5	1.94	1.62	2.48	4	9.0	75.3	12.1	21.0	94.6	8.0
TMS 11	10.6	3.98	2.06	1.64	1.68	4-5	12.2	97.0	12.5	23.0	87.4	7.8
TMS 20	9.0	3.96	1.80	1.66	1.98	4-5	9.4	84.1	11.4	21.2	94.2	8.6
UP 2338		**3.92**	**2.12**	**1.76**	**2.30**	**4**	**8.2**	**77.1**	**11.7**	**21.0**	**93.8**	**7.2**

Table 5.3. Effect of different cytoplasms on various floral and agronomic characters in wheat in the nuclear background of HW 2022.

CMS source	Floral traits						Agronomic traits					
	Glume opening angle (degree)	Anther size (mm)	Filament length (mm)	Ovary size (mm)	Length of style (mm)	Stigma receptivity (days)	No. of tillers	Plant height (cm)	Main spike length (cm)	No. of spikelets per main spike	Days to 50% flowering	Synchrony of tillers (days)
T. timopheevi	7.8	3.96	2.90	1.80	2.40	4	10.8	77.3	11.9	21.0	95.2	7.4
T. araraticum	13.3	3.76	2.58	1.72	2.64	4-5	9.8	77.7	11.0	20.4	95.8	8.0
Ae. speltoides	7.2	3.76	2.10	1.80	2.34	4-5	7.8	89.6	10.4	20.2	90.4	8.0
TMS 11	8.1	3.82	2.12	1.68	2.50	4	9.6	81.2	12.0	23.0	91.4	8.2
HW 2022		**4.00**	**3.02**	**1.8**	**2.70**	**4**	**12.2**	**86.8**	**10.7**	**21.0**	**93.0**	**7.0**

Table 5.4. Effect of different cytoplasms on various floral and agronomic characters in wheat in the nuclear background of HW 2038.

CMS source	Floral traits						Agronomic traits					
	Glume opening angle (degree)	Anther size (mm)	Filament length (mm)	Ovary size (mm)	Length of style (mm)	Stigma receptivity (days)	No. of tillers	Plant height (cm)	Main spike length (cm)	No. of spikelets per main spike	Days to 50% flowering	Synchrony of tillers (days)
T. timopheevi	8.3	3.40	1.94	1.58	2.46	4	9.6	77.9	12.2	19.4	83.2	8.2
T. araraticum	7.6	3.10	1.84	1.42	2.58	4	9.8	77.5	11.7	19.6	96.6	7.0
Ae. speltoides	6.3	4.58	1.54	1.54	2.90	4	9.2	77.8	12.1	19.8	82.2	8.0
TMS 9	5.4	3.58	1.60	1.60	2.44	4-5	9.6	78.4	11.0	21.0	91.2	8.2
HW 2038		**3.84**	**1.98**	**1.80**	**2.70**	**4**	**11.6**	**74.2**	**10.9**	**22.8**	**81.6**	**8.2**

Table 5.5. Effect of different cytoplasms on various floral and agronomic characters in wheat in the nuclear background of PBW 343.

CMS source	Floral traits						Agronomic traits					
	Glume opening angle (degree)	Anther size (mm)	Filament length (mm)	Ovary size (mm)	Length of style (mm)	Stigma receptivity (days)	No. of tillers	Plant height (cm)	Main spike length (cm)	No. of spikelets per main spike	Days to 50% flowering	Synchrony of tillers (days
T. araraticum	11.1	3.98	2.08	1.58	2.98	4	9.0	77.3	11.4	20.0	90.0	8.0
TMS 8	10.0	3.64	2.82	1.54	2.14	4	10.2	80.0	12.1	20.4	96.0	8.0
TMS 9	5.8	3.6	1.78	1.50	2.52	5	8.8	75.2	11.6	21.2	86.0	9.6
TMS 11	8.7	3.68	2.74	1.52	2.46	4-5	8.0	75.0	11.3	20.0	86.6	6.8
PBW 343		**4.04**	**2.90**	**1.72**	**3.06**	**4-5**	**11.2**	**86.2**	**12.0**	**23.0**	**90.0**	**8.0**

5.2. Identification of maintainers and restorers

Identification of maintainers and restorers, their improvement through hybridization between maintainers X maintainers, maintainers X restorers and restorers X restorers (Yang, 1997; Xie *et al.* 1997; Bharaj and Virmani, 1997) and finally their utilisation in the development of three-line hybrids appear to be a long term breeding approach for the exploitation of heterosis in rice. In the short term, screening of locally adopted advanced breeding lines for isolation of maintainers and restorers, conversion of maintainers into CMS lines, evaluation of component lines for combining ability and heterosis and their utilization in the development of three-line hybrids are the principal steps commonly followed in hybrid rice breeding programme. Undoubtedly, making a substantial contribution to research and development has fostered the release of hybrids in rice.

Though cytoplasmic male sterility and fertility restorer systems are well known in wheat (Worland *et al.* 1987), precise information on the exploitable level of useful heterosis over the standard varieties has been often lacking. However, tremendous success achieved in rice has stimulated the interest in development of hybrid wheat. It can be seen from data presented in Tables 4.23 and 4.34 that a considerable number of segregants belonged to the category of complete and partial maintainers while only a few plants fell into partial and complete restorer category. The highest frequency of sterility maintainers identified in *timopheevi*, *speltoides*, TMS 8, TMS 9 and cytoplasms suggested that fertility in these CMS lines was difficult to restore than other CMS lines. On the other hand, the frequency of pollen parents behaving as fertility restorers was found to be maximum in TMS 11 and TMS 20 cytoplasm (3.75 and 3.77 per cent,

respectively), indicating that fertility restoration is not easy in these cases too, though partial restoration has been observed. A bulk of the pollen parents (87.14%) (Table 4.24) belong to the maintainer category while only about 10 per cent are categorized as partial restorer and partial maintainers. A study by Zong *et al.* (1996) also revealed a similar trend where a high percentage of segregants (45%) were highly sterile and 55 % restored fertility to different degrees. Miri *et al.* (1970 a) grouped the wheat varieties into five classes on the basis of their fertility restoring ability. Therefore a bulk of these pollen parents appeared to be suitable and promising for their conversion into CMS lines. The occurrence of fertility restoration to different degrees also suggests the importance and need for selection for satisfactory restorer lines for hybrid seed production.

Although several genes for fertility restoration have been identified and transferred in wheat (Nonaka *et al.* 1993; Mukai and Tsunewaki, 1979; Curtis and Lukaszewski, 1993; Bahl and Maan, 1973; Ma *et al.* 1991; Ikeguchi *et al.* 1994, 1999), the performance of restorers seems to be CMS line and location dependent (Kumari and Mahadevappa, 1998). Lack of satisfactory restoration is the major limiting factor to the development of wheat hybrids, which emphasizes the need to evaluate wheat germplasm in order to identify maintainers and restorers.

In the present study, floret fertility in terms of seed set was considered as an index to classify pollen parents among test crosses, into different categories. The pollen fertility in test cross progenies was found positively and significantly correlated with spikelet fertility indicating thereby that either pollen or spikelet fertility could be effectively used to identify pollen parents as maintainers or restorers as also suggested by Huang *et al.* (1987). However, in some cases, the effective restorers identified on the basis of pollen

fertility are likely to show low spikelet fertility (Bobby and Nadarajan, 1994). According to Maan (1985), all grades of fertility, ranging from 0 to 100 % fertile segregants occur in hybrid progenies; and estimates of gene number (controlling fertility restoration) are biased by the method employed to classify especially the partially fertile plants into fertility groups. This kind of data also leads to subjective interpretations about the number and relative importance of the genes controlling fertility restoration. High yield of the hybrids depends largely on high floret fertility. Also, recording observations on pollen fertility is more tedious and time consuming than determining spikelet/floret fertility. Therefore, floret/spikelet fertility could alone be used as the basis for classification of pollen parents into maintainers and restorers.

The results obtained in the present study revealed further that only very few test cross progenies under investigation (2.31%) were fertile indicating poor fertility restoration ability among the pollen parents across the different sterile cytoplasmic sources. Only two pollen parents *viz.*, PWR 4099 and PWR 4101 were found to have restored complete fertility in the *araraticum,* TMS 8, TMS 9, TMS 11 and TMS 20 cytoplasms. It was interesting to note that the pollen parents identified as complete restorer in one CMS line did not behave as partial restorer or a weak maintainer for other CMS lines, which is contrary to the observations of Kumari and Mahadevappa (1998) who stated that the performance of restorers seems to be CMS line and location dependent.

The results in the present study further revealed that the maintainer line of a particular cytoplasm was not specific to only that cytoplasm but could maintain in diverse cytoplasmic sources as well. These findings are also in contrary to that of the results

obtained by Kumari and Mahadevappa (1998) who observed that the maintainer line of a particular cytoplasm was specific to that cytoplasm only and restored variable degree of fertility when crossed with CMS lines carrying diverse cytoplasm.

5.3. Differential expression of fertility restoration

Cytoplasmic male sterility in wheat results from the interaction between the nucleus and cytoplasm of different genomes involved (Kihara, 1951; Mukai and Tsunewaki, 1979; Nonaka *et al.* 1994). Presence or absence of dominant fertility restorer nuclear gene(s) is/are known to confer fertility restoring or sterility maintaining ability of a genotype for a specific male sterility inducing cytoplasm. The genotype can serve as a maintainer or restorer of that particular cytoplasm depending on its capability of maintaining complete sterility or restoration of normal fertility. In the present study, the pollen parents PWR 4099 and PWR 4101 identified as complete restorers of one cytoplasm did not show partial restoration or weak maintaining ability in other cytoplasms, with the sole exception of PWR 4101 which besides restoring fertility also functioned as maintainer for *araratium* cytoplasm (Table 4.25). However, the variation in the restoration ability of pollen parents identified as partial restorers was observed when such pollen parents were used in crosses with different cytoplasms.

It is also evident from the data presented in Table 4.28 on the number of seeds set per spike, that fertility restoration by the two restorer lines *viz.*, PWR 4101 and PWR 4099 differ in individual CMS lines. The differential fertility restoration behaviour of the two restorers may either be due to the influence of the genetic background of the varieties or due to the modifiers present in them (Miri *et al.* 1970 b; Singh and Rathore, 1992). The results obtained by Tahir (1969) also support the observation that restorers were

effective only for specific varieties and there was also a high degree of fluctuation in their effectiveness under different climatic zones. Miri *et al.* (1970 b) suggested that there could be evolutionary reasons for variability in fertility restoration in different cytoplasms. They proposed that in *T. dicoccoides*, cytoplasmic differentiation might have also progressed during the course of evolution. With the gradual change in cytoplasm, from normal cytoplasm to male-sterility inducing cytoplasm in a particular species, there might have occurred corresponding genetic differentiation by successive mutations for pollen fertility restoration of varying strengths, resulting in modifiers or partial restoring genes, which subsequently introgressed into emmer and dinkel wheats over a long period of time. The cytoplasm of varying strengths of male sterility might have been eliminated from the populations due to selective advantage of the major fertility-restoring genes by the time the speciation process was complete.

It is known that high yield of the hybrids depends largely upon high spikelet/floret fertility which is determined by the number and kind of restorer gene(s) present and mode of gene action prevalent in the restorer lines of the hybrids (Bharaj *et al.* 1991; Shen *et al.* 1996). Therefore, substantial heterosis for grain yield could be realized in the hybrids if fertility restoration is enhanced by improving the restorer lines (Ramesha *et al.* 1998; Singh and Maurya, 1999). Singh and Rathore (1992) suggested that the existence of modifier gene(s) in addition to major gene(s) in the pollen parents could have significant influence on fertility restoration ability of restorers. Thus, in such a complex mode of inheritance of fertility restoration, the development of new and diverse restorer lines by transferring fertility restorer gene(s) to promising breeding lines (Bharaj *et al.* 1991) has to be undertaken vigorously.

Therefore, in order to improve the efficiency of hybrid wheat breeding, it is essential to augment it with a parental line improvement activity to broaden the genetic base of the parental lines (Liao *et al.* 1998) so that a large number of usable lines are available for developing good hybrids. The emphasis should be placed on developing genotypes, to be used as A or R lines, possessing relatively high frequencies of favourable alleles in the hybrid parents.

5.4. Inheritance of fertility restoration in CMS lines

Although genetic basis of fertility restoration in wheat and rice CMS lines have been investigated extensively, the subject still remains to be addressed. In the present study, therefore, inheritance of fertility restoration was investigated in the crosses involving TMS 9, TMS 11 and TMS 20 cytoplasms and two different restorers PWR 4099 and PWR 4101 (Table 4.26).

The classification of F_2 segregating plants into sterile and fertile groups was based on the absence or presence of seed set in spike of each F_2 segregant which is as follows- complete maintainers, 0-1 seed; partial maintainer, 2-25 seeds; partial restorers, 26-45 seeds and complete restorers, > 45 seeds. Almost a continuous variation of fertility ranging from one seed to more than 45 seeds per spike was observed in the segregating population. At one end of the distribution, there were plants with low floret/spikelet fertility and at the other end; those plants with high floret/spikelet fertility were observed indicating an almost continuous distribution of fertility in F_2 population. A maximum of three classes of plants *viz.*, fully fertile (FF), partially fertile (PF) and completely sterile (CS) and a minimum of two classes, FF and CS were identified in the segregating populations. The segregation of fertility and sterility into just two or three distinct classes

(Table 4.26) suggested that only few major genes were involved in fertility restoration in the CMS lines crossed with two restorers under study.

The segregation pattern for fertility restoration differed depending on the restorer parent involved in the cross combinations. The F_1 plants derived from both the restorers were completely fertile. However, the fertility restoration pattern in F_2 generation of the crosses TMS 9 / 2041 A X PWR 4101 and TMS 20 / 2046 A X PWR 4101 fitted well in the expected ratio of 3 FF : 1 CS plants indicating that a single gene controlled fertility restoration in the above crosses involving PWR 4101 as pollen parent. On the other hand, the F_2 population derived from the fertile F_1 hybrids *viz.*, TMS 9 / 2046 A X PWR 4099, TMS 11 / 2019 A X PWR 4099 and TMS 20 / 2046 X PWR 4099 exhibited a segregation ratio that corresponded to an expected ratio of 9FF : 6PF : 1CS. The data indicated that two dominant genes are involved in fertility restoration in these crosses involving PWR 4099 as pollen parent and that one out of two genes restore fertility with greater degree than the other. Studies by Ganesan and Rangaswamy (1997) also indicated that fertility restoration in rice lines was governed by two independent dominant genes and segregation pattern showed a semi-epistatic (additive 9:6:1 ratio) interaction.

Earlier studies conducted by Wilson (1962) on the inheritance of fertility restoration in wheat regarding the number of nuclear genes conditioning male-fertility restoration to hybrid wheat revealed that one major factor and some minor factors are necessary for full fertility restoration. Schmidt and Johnson (1963) have reported that two dominant genes are necessary for pollen restoration. Wilson (1968 b) considered that pollen fertility restoration is possibly due to either 2 genes or 3 genes. Nonaka *et al.* (1993) observed

that one dose of *Rfv*1 gene was enough to restore complete fertility. More recently, Ikaguchi *et al.* (1994, 1999) stated that a single dose of *Rfv*1 was insufficient to restore a high level of fertility. Assuming that Rf_1Rf_1 and Rf_2Rf_2 are the dominant alleles of the two restorer genes, the segregation pattern in these cross combinations indicated that the plants homozygous (rf_1rf_1 rf_2rf_2) for the recessive alleles of both genes will be completely sterile (CS) while the plants homozygous for the recessive alleles of any one of the two genes but homozygous or heterozygous for the dominant alleles of the other gene ($Rf_1\_rf_2$ rf_2 or rf_1 rf_1 $Rf_2\_$) will be partially fertile and the plants possessing the dominant alleles of both genes in homozygous or heterozygous condition ($Rf_1\_Rf_2\_$) will be fully fertile (FF). The presence of both the dominant genes is required for restoring complete fertility in the segregating plants.

5.5. Inheritance of kernel colour

Inheritance of kernel colour was investigated in six F_2 populations derived from CMS line X restorer crosses, *viz.*, TMS 9 / 2041 A X PWR 4099, TMS 8 / 2046 A X PWR 4099, PBW 226 X PWR 4099, HW 2045 X PWR 4099, HW 2099 X PWR 4099 and HW 2048 X PWR 4099 in which the restorer PWR 4099 was involved in all cross combinations. The classification of F_2 segregants producing red and amber grains was made by visual observations. The cultivars and maintainer line of the CMS used as female parents had amber grains while the pollinator PWR 4099 had red kernels. The observed segregation pattern of kernel colour in all the six F_2 populations that fitted well into only two categories either red or amber suggested that there was only one major gene governing red kernel colour. The observed frequency of both the classes fitted well into

3 red: 1 amber indicating that kernal colour in the exotic line PWR 4099 is controlled by a single dominant gene.

The F_2 population derived from crosses involving CMS lines TMS 9 / 2041 A and TMS 8 / 2046 A and the restorer PWR 4099 also segregated into two classes *i.e.*, red and amber in 3:1 ratio despite the occurrence of sterility among F_2 segregants. All the three categories of segregants in F2 population *viz.*, homozygous dominant (RR), heterozygous (Rr), and homozygous recessive (rr) were equally affected by sterility. The independent segregation pattern for inheritance of kernel colour and fertility restoration indicated the involvement of a single dominant gene and two incompletely dominant genes respectively. This further confirms that the gene (s) for fertility restoration and kernel colour are not linked.

5.6. Evaluation of hybrids for heterosis

The availability of cytoplasmic male sterility and genetic mechanisms for restoring fertility has made hybrid wheat production possible. The commercial success of hybrid wheat will depend largely on the magnitude of the heterosis manifested in grain yield. However, major considerations in commercial exploitation of heterosis are towards obtaining sufficient heterosis for characters of economic importance and search for the possibility of fixing such heterosis in pure breeding lines.

Genetically, best hybrids will be obtained from crosses of parents having a high proportion of additive, dominant or complementary epistatic genes for the favourable expression of the main components of yield. The degree of heterosis will depend on the dispersion of reasonable dominant and epistatic genes amongst the parents. Expression of heterosis varies from one character to another. In the present study, the magnitude of

heterosis expressed as percentage deviation in the performance of hybrids for yield and yield-attributing characters was estimated.

Depending on the cross, heterosis was observed either in a single component of yield or in more components. In general, only a few of the hybrids exhibited heterosis for one or more individual traits simultaneously. For instance, PHW 7 exhibited significant standard heterosis for all yield components except 1000-grain weight. Similarly, PHW 2 showed significant standard heterosis for all characters except main spike length and 1000-grain weight. Significant standard heterosis for four characters was observed in PHW 1 (main spike length, number of spikelets, number of grains per spike and grain yield per spike), PHW 3 (number of tillers, plant height, number of spikelets, grain yield per spike) and PHW 6 (number of tillers, plant height, number of spikelets and grain yield per plant). PHW 9 recorded standard heterosis for three characters *viz.*, plant height, spike length and grain yield per plant. PHW 5 and PHW 10 recorded significant standard heterosis for two characters only *viz.*, number of tillers and plant height.

Three hybrids *viz.*, PHW 4, PHW 8 and PHW 11 displayed standard heterosis for one character only (number of tillers, number of spikelets and plant height, respectively). Significant better parent heterosis for one character was observed in four hybrids namely, PHW 1 for main spike length, PHW 2 and PHW 4 for number of tillers, PHW 11 for plant height. The hybrid PHW 7 showed heterobeltiosis for five traits *viz.*, number of tillers, plant height, spike length, number of grains per spike and grain yield per spike.

Unlike for standard heterosis, many hybrids exhibited mid parent heterosis for more than one character simultaneously. Three hybrids showed heterosis for seven traits (PHW 2-except for main spike length, PHW 7 and PHW 10- except for 1000-grain

weight). The hybrids PHW 3 and PHW 5 exhibited mid parent heterosis for six traits. The former did not show heterosis for number of spikelets and 1000-grain weight while the latter did not show heterosis for number of spikelets and 1000-grain weight. PHW 9 and PHW 11 showed mid parent heterosis for number of tillers, plant height, spike length and number of spikelets; while PHW 9, in addition exhibited significant mid parent heterosis for 1000-grain weight also. The following hybrids showed mid parent heterosis for three traits *viz.*, PHW 1 (number of tillers, spike length and number of spikelets), PHW 4 (number of tillers, plant height and number of spikelets) and PHW 6 (number of tillers, plant height and grain yield per plant). The hybrid PHW 8 showed mid parent heterosis only for plant height.

Increase or reduction in the value of any yield and yield components has direct influence on the manifestation of heterosis. For instance, the hybrid PHW 2 had a significant and positive standard heterosis for number of grains per spike, grain yield per spike and grain yield per plant, while PHW 4 showed significant, negative heterosis for number of grains per spike (Table 4.34) resulting into negative heterosis for grain yield per spike as well as per plant and 1000-grain weight. Also, a similar relationship existed for PHW 8 where negative heterosis for number of grains per spike resulted in heterosis with negative direction for the characters 1000-grain weight, grain yield per spike and yield per plant (Tables 4.34 to 4.37).

It is interesting to note that eight out of 11 hybrids exhibited significant standard heterosis for plant height and was in accordance with the results of Li *et al.* (1997) who were of the view that heterosis for plant height also resulted in increase in grain weight per spike. The hybrids PHW 2 and PHW 7, which showed heterosis for plant height, also

had positive, significant standard heterosis for number of grains per spike, grain yield per spike as well as per plant. But the same was not true in case of the hybrid PHW 8, which showed negative heterotic values for number of grains per spike, grain yield per spike and per plant and 1000-grain weight, also had non significant heterosis for plant height. The hybrids PHW 3, PHW 5, PHW 10 and PHW 11 displayed negative standard heterosis for grain yield despite exhibiting positive heterosis for plant height. Only two of the hybrids namely, PHW 2 and PHW 9 exhibited positive mid-parent heterosis for 1000-grain weight. Seetharamaiah *et al.* (1999) observed that negative heterosis for test grain weight in the hybrids may be due to high test weight of the restorer lines and also due to smaller grain size of the hybrids.

5.6.1. Performance of hybrids with respect to grain yield

Yield data of hybrids and cultivars recorded in the replicated trial conducted during 2002-2003 indicated relatively low levels of heterosis as compared to the yield recorded on single plant basis. Table 4.38 indicates that only one hybrid; PHW 13 significantly out-yielded other hybrids and cultivars and had a maximum standard heterosis of 10.13 per cen, whereas, the hybrid, PHW 2, which showed 26.12 per cent standard heterosis for grain yield (Table 4.37) on single plant basis was not able to record positive standard heterosis at all (Table 4.40). Therefore, it can be concluded that heterosis calculated on the data of single plants as reported by several workers (Borghi *et al.* 1986; Zehr *et al.* 1997) is misleading. It is necessary, therefore, to estimate heterosis on the yield data recorded in replicated trials on larger plot size. The above conclusion drawn from the present study is in accordance with the observations made by Nettevich (1968), who stated that the degree of manifestation of heterosis depends upon the climatic

and agronomic conditions. He further stated that in order to obtain reliable data on hybrids it is necessary to gather information for a period of at least three years, at multilocations. The best way to assess the performance is to test their hybrid combination on larger plot size and at different locations before commercialization.

Relatively low levels of heterosis may be sufficient to make hybrid production profitable for crops producing a large number of seeds per pollination or which have a high multiplication rate. However, in self-pollinating crop like wheat, having low rate of multiplication, the amount of heterosis will have to be of considerable magnitude to cover the increased cost of the seed. There is a view that a consistent level of heterosis of up to 30 percent increased grain yield over the pureline varieties will be necessary with regard to commercial exploitation of heterosis in wheat.

SUMMARY

"And now kind friends, what I have wrote
I hope you will pass o'er,
And not criticize as some have done
Hitherto here before".

- JULIA A MOORE

6. SUMMARY

Increase in yield is an important objective of most of the breeding programmes in crops. Several strategies are adopted to enhance the levels of productivity. Among the genetic approaches to raise the crop yield potential, tailoring of physiologically more productive plant type and exploitation of hybrid vigour are two major research strategies being contemplated for raising the ceiling to yield in wheat. Amongst the two technological options, exploitation of hybrid vigour has been found to be more practical for raising the yield threshold. Hybrid wheat, like rice, has the potential to provide not only increased yields, but also stability across a wide range of environments. Also hybrid technology has proved to be the most practical and rewarding in several crops, such as sorghum, maize, cotton, sunflower, tomato, brinjal, chillies, onion, and sugar beets. The discovery of the biological system comprising cytoplasmic male sterility and the restoration of pollen fertility in wheat opens up the possibilities of commercial production of hybrid seed.

Diverse and commercially usable CMS lines with superior grain quality in wheat are not readily available at present in the country. Besides several other factors, lack of satisfactory restoration is also a major limitation to the development of wheat hybrids. Keeping above aspects into consideration, the present study was undertaken to (i) characterize the CMS lines, (ii) assess the stability of CMS lines, (iii) identify maintainers and restorers, (iv) elucidate the genetic basis of fertility restoration and kernel colour, and (v) study the manifestation of heterosis.

6.1. Characterisation of CMS lines

Six floral traits in 39 CMS lines with seven different male sterile cytoplasmic sources and their fertile counterparts were studied. Variation between the CMS lines and B lines were clearly observed for four floral characters *viz.*, anther size, filament length, ovary size and length of style. The general trend observed was that all the floral traits studied had values less than or equal to that of their fertile counterparts in all the CMS lines. Glume opening angle had the largest variation, the minimum being 5-7 degrees and maximum of 17-20 degrees, within the CMS lines carrying same cytoplasm and among the CMS lines carrying diverse cytoplasm. The CMS lines having wider glume opening angle are the most desirable ones as it may attract more pollen. The stigma receptivity had the least variation which ranged between 4 and 5 days among the various CMS lines with *arar* 2022 A having the highest out-crossing potential (13.38 per cent).

6.2. Stability of CMS lines

A set of 39 CMS lines possessing seven different male sterile cytoplasms was evaluated to study the stability for maintenance of male sterility and agronomic performance for the traits influencing hybrid seed yield. The study was carried out across three environments created by varying the dates of sowing. All the CMS lines included in the study exhibited complete floret sterility in all environments. The relative proportion of USS pollen was found to be remarkably high in all CMS lines. Both UWS and USS pollens contributed to more than 97 per cent of pollen grains. Out of the 39, 29 CMS lines were found to be completely sterile and stable in all environments as none of the lines produced SRF pollen. The remaining 10 CMS lines produced SRF pollen though at extremely low frequency. Traces of fertility in these CMS lines might be due

to nucleo-cytoplasmic interaction under different temperature and humidity regimes, which may affect pollen mother cells. It is likely that the presence of minor gene(s) for fertility linked with major sterility gene(s) existing in the CMS lines, might also affect the expression of pollen sterility.

6.3. Identification of maintainers and restorers

Using 521 test cross progenies derived from cross combinations involving seven male sterile cytoplasmic sources and 294 pollen parents, genotypes were categorized as complete restorer, partial restorer, partial/weak maintainer or complete maintainer. The classification was done using floret fertility as an index. Only two exotic pollen parents, PWR 4099 and PWR 4101 produced test cross progenies with highest floret fertility, with more than 45 seeds per spike in all the male sterility cytoplasms, except *T. timopheevi* and *Ae. speltoides*. Both these pollinators were able to restore fertility in TMS 9 and TMS 20 cytoplasms. However, differential behaviour in restoring fertility was noticed in combinations involving other sources of cytoplasm. PWR 4101 restored fertility in TMS 9 and TMS 20, while PWR 4099 restored fertility in *araraticum*, TMS 8, TMS 9, TMS 11 and TMS 20 cytoplasms. TMS 11 and TMS 20 cytoplasm produced the highest frequency of restorers (3.75 and 3.77 per cent, respectively). *T. timopheevi* and *Ae. speltoides* cytoplasmic sources did not produce any fertile progenies at all. The pollen parents identified as effective restorers of one CMS line did not behave as partial restorer or weak maintainer for other CMS lines.

A very high proportion (87.13 per cent) of the test cross progenies were sterile and accordingly classified as complete maintainers and the intermediate category accounted for about 10 per cent. The *speltoides* cytoplasm produced the highest (98.04

per cent) and TMS 11 produced the lowest (78.75 per cent) frequency of sterile test cross progenies. Many of the pollen parents maintained complete sterility in various cytoplasmic sources indicating the stable sterility maintaining ability as is evident from the higher frequency of complete maintainers.

6.4. Inheritance studies

6.4.1. Genetics of fertility restoration

The cross combination involving three CMS sources, namely, TMS 9,. TMS 11 and TMS 20 and two restorers *viz.*, PWR 4099 and PWR 4101 constituted the basic experimental materials for studying the mode of inheritance. Spikelet (floret) fertility in terms of seed set of each plant was recorded in F_2 generation. The results indicated that two independent and dominant genes governed the fertility restortion in all the crosses involving the restorer PWR 4099. The segregation pattern of fully fertile (FF), partially fertile (PF) and completely sterile (CS) plants corresponded to an epistasis with incomplete dominance (9:6:1). The fertility restoration in the cross combinations involving the pollinator PWR 4101 and the CMS lines TMS 9 and TMS 20 was under monogenic dominant control as the observed values fitted very well with the expected segregation ratio of 3(FF) : 1 (CS).

6.4.2. Genetics of kernel colour

The mode of inheritance of kernel colour was studied in F_2 generations derived from six crosses involving red grained restorer line PWR 4099 as male parent with two A lines *viz.*, TMS 9 / 2041 A and TMS 8 / 2046 A and four other amber grained genotypes, namely, PBW 226, HW 2045, HW 2099 and HW 2048. The segregation pattern in all the

six F_2 populations corresponded to a Mendelian segregation ratio of 3 red :1 amber grain, indicating that red kernel colour in the exotic line PWR 4099 (Table 4.27) was governed by a single dominant gene.

6.5. Magnitude of heterosis

Depending upon the cross combinations, heterosis was observed for one or more components of yield. Out of the 11 hybrid combinations only a few hybrids displayed significant and positive heterosis for various agronomic characters. Hybrid PHW 6 showed maximum standard heterosis for number of tillers (32.7 per cent) while eight hybrids showed significantly superior performance over the standard check. Six hybrids, namely, PHW 5, PHW 6, PHW 7, PHW 9, PHW 10 and PHW 11 were better than their better parents and all hybrids except PHW 1 and PHW 8 showed positive, significant mid-parent heterosis for plant height. The hybrid PHW 7 was superior over mid-parent, better parent and the standard check for main spike length. Six hybrids, *viz.*, PHW 1, PHW 2, PHW 3, PHW 6, PHW 7, and PHW 8 showed positive standard heterosis for number of spikelets per main spike of which PHW 3 recorded the highest magnitude of 10.37 per cent. Four hybrids, PHW 1, PHW 2, PHW 3 and PHW 7 exhibited significant standard heterosis for number of grains per spike, the highest magnitude being observed in PHW 3 (28.19 per cent).

None of the 11 hybrids showed significant positive heterosis for 1000-grain weight indicating the superiority of the standard check for this character. Only two hybrids, PHW 2 and PHW 9 could exhibit mid-parent heterosis for this trait. Four hybrids, namely, PHW 1, PHW 2, PHW 3 and PHW 7 showed significant positive standard heterosis for grain yield per spike. PHW 3 and PHW 7 exhibited significant

heterosis over their better parents while as many as six hybrids showed mid-parent heterosis. Only two hybrids, PHW 2 and PHW 7 out of the four that exhibited standard heterosis for grain yield per spike, were able to show significant positive heterosis for grain yield per plant. Two other hybrids, *viz.*, PHW 3 and PHW 8 also registered better performance over the standard check.

6.5.1. Performance of hybrids with respect to grain yield

In order to evaluate the performance of hybrids on larger plot area, five wheat hybrids namely, PHW 1, PHW 2, PHW 3, PHW 12 and PHW 13 were evaluated. A maximum of 10.13 per cent of standard heterosis was obtained in PHW 13 as against the maximum standard heterosis of 26.12 per cent by PHW 2 on single plant basis. Moreover, the hybrid PHW 2, which showed 26.12 per cent standard heterosis on single plant basis, was not able to record positive standard heterosis at all on a larger plot evaluation. It is, therefore, suggested that proper estimate of heterosis should be made under appropriate plot size.

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"Literature flourishes best when it is half a trade and half an art".

-WILLIAM RALPH INGE

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* Originals not seen.

APPENDIX I

LIST OF WHEAT STOCKS/CULTIVARS/DERIVATIVES USED AS POLLINATORS FOR DEVELOPING TEST CROSS PROGENIES

Sl.No	Stocks/Cultivars/ Derivatives	Pedigree
1.	Agent	
2.	Akbar	
3.	Annapurna	
4.	A x Minster	
5.	Bakhatawar	
6.	Benno	
7.	BH 1146	
8.	CNU 70 / Parulla	CNU 70 / Parulla
9.	C273	
10.	C 591	
11.	CIGM 295	
12.	CIGM 295-2	
13.	CIGM 295-5	
14.	CIMMYT 470	
15.	CIMMYT 478	
16.	CIMMYT 480	
17.	CIMMYT 482	
18.	Charter	GD/Charter
19.	Charter/ GD / *T. araraticum*	Charter/ GD / *T. araraticum*
20.	Chinese 84	
21.	Chinese spring / *T. araraticum* / HD 2012	Chinese spring / *T. araraticum* / HD 2012
22.	Chiriyu-3	EGS 1/3 VCS
23.	Chotti Lerma	
24.	Chuanmai-18	
25.	CM 107	
26.	CPAN 4141 / HD 2329	CPAN 4141 / HD 2329
27.	CPAN 4149 / PBW 343	CPAN 4149 / PBW 343
28.	CR 7 / UWH 147	CR 7 / UWH 147
29.	DI-105	
30.	DL 86	
31.	EGSN 113	
32.	Faislabad 83	
33.	Faislabad 85	
34.	Flake	
35.	Flavina	
36.	Glennson 81	
37.	GW 273	
38.	HD 2012	
39.	HD 2285 derivative	
40.	HD 2646*2/ HW 3007	HD 2646*2/ HW 3007
41.	HD 2687 / WR 1082*1	HD 2687 / WR 1082*1
42.	HD 2808	
43.	HS 277	
44.	HUW 234	
45.	HW 741 / HW 1042	HW 741 / HW 1042
46.	HW 741 / Unnat HD 2285	HW 741 / Unnat HD 2285

Contd...

47.	HW 1085	
48.	HW 1093	
49.	HW 2002 / CPAN 2044	HW 2002 / CPAN 2044
50.	HW 2012 / HW 1062	HW 2012 / HW 1062
51.	HW 2016	
52.	HW 2025	Kalyansona Sr – 27 / Lr 24
53.	HW 2032	NI 5439 ^{*4} / Lr32
54.	HW 2042	WH 147 ^{*6}/Sunstar ^{*6}/C 80-1
55.	HW 2046	HD 2329 ^{*6}/ Sunstar ^{*6}/C 80-1
56.	HW 2062	WH 542 ^{*6}/CS 2A/CM # 4/2
57.	HW 3018	
58.	HW 3041	
59.	HW 3042	
60.	ID 568	*T. durum* derivative
61.	Inquilab	
62.	IRSN 102	
63.	IRSN 855	
64.	IRSN 866	
65.	IRSN 876	
66.	IRSN 877	
67.	IRSN 881	
68.	IRSN 883	
69.	IRSN 886 (1999-2000)	
70.	IRSN 888	
71.	IRSN 890	
72.	IRSN 891	
73.	IRSN 899	
74.	IRSN 902	
75.	IRSN 904	
76.	IRSN 906 (1999-2000)	
77.	IRSN 920	
78.	IRSN 927	
79.	IRSN 934	
80.	IRSN 942	
81.	IRSN 947	
82.	IRSN 950 (*Rabi* 1999-2000)	
83.	IRSN 954	
84.	ISD 8	
85.	J 24	
86.	JGB 1876	C306 ^{*2}/*T.timopheevi*
87.	JHS 117	
88.	K 9606	
89.	Kalyansona	
90.	KB 81	ISWRN-198
91.	KBSN 60	BJ 67-NDAK 481
92.	KBSN 67	NC-55583
93.	KBSN 78	BAV-1267
94.	KBSN 95	ANB "S"
95.	KBSN 240 (1997-98)	
96.	Kharchia local	
97.	KMS 9-R	
98.	KNS 33 / PH 127	KNS 33 / PH 127

Contd...

99.	Kohsar	
100.	Lerma Rojo	
101.	Lok Bharti	
102.K	MACS 2494	
103.	Maris/ ALD 5	Maris/ ALD 5
104.	Mayur 3	
105.	MC 3	Lal Bahadur / *Ae.squarrosa* derivative (undesignated- 90.3943)
106.	MC 5	Amigo / Agent
107.	MC 10	Sunstar [*6] / C 80-1
108.	MC 12	Harrier
109.	MC 13	Chinese Spring / Hope 7 D
110.	MREN/BW 15011	MREN/BW 15011
111.	NP 4	
112.	NP 710	
113.	NP 718	
114.	NP 824	
115.	NP 836	
116.	NP 852	
117.	P 118	
118.	Pak 81	
119.	Parulla	
120.	PB type 9	
121.	PBN 3953	
122.	PBN 4055	
123.	PBN 5137	
124.	PBW 226	
125.	PBW 343	
126.	PDSN 289 (1999-00)	
127.	PDSN 447 (1999-00)	
128.	PDSN 887(1999-00)	
129.	PDSN 1068 (1999-00)	
130.	PH 171	
131.	PH 172	
132.	PH 173	
133.	PI 182113	
134.	Pitic 62	
135.	PM 2	Fenman
136.	Punjab 85	
137.	Pusa 5-3	
138.	PWR 4099	CBHW-R CHN Q RR 425-OCHN S-4 BV 97 (EC 414149)
139.	PWR 4101	
140.	Quan Feng	
141.	Raj 3077	
142.	RNB 30	HW 741 / HD 2285
143.	RNB 32	Unnat Kalyansona / BW 1109
144.	RNB 58	HW 2002 / CPAN 2044
145.	RNB 89	MACS 2496 / HW 2001 A
146.	RNB 237	PBW 343 / PH 137
147.	RNB 238	HW 1085 / HW 3017
148.	RNB 250	Veery's / HW 2031

Contd...

149.	RNB 259	Unnat Kalyansona / MACS 2496
150.	RNB 267	HD 2285 / PH 137
151.	RNB 268	Lok 1 / PH 167
152.	RNB 275	Unnat HD 2285 / HW 1042
153.	RNB 282	Unnat Kalyansona / CPAN 2044
154.	RNB 287	Faislabad / Unnat Kalyansona
155.	RNB 295	Unnat Kalyansona / MACS 2496
156.	RNB 297	MACS 2496 / Unnat Kalyanasona
157.	RNB 306	HD 2329 / Veery`s
158.	RNB 307	Veery`s / PH 127
159.	RNB 342	CLRP –6 / Unnat HD 2329
160.	RNB 343	CLD – 49 / Unnat HD 2329
161.	RNB 350	HD 2280 / HW 2007
162.	RNB 357	KNS 33 / PH 127
163.	RNB 363	Veerys / MC 10
164.	RNB 548	HD 2329 / Sunstar $*^{6}$ / C 80 –1
165.	RNB 570	HD 2329 $*^{2}$ / Veery`s
166.	RNB 3722	Unnat HD 2285 / HW 1042
167.	RNB 3813	Unnat HD 2329 / Veery`s
168.	RNB-W 116	HW 2008 / HW 1042
169.	RNB-W 197	HW 741 / HW 2008
170.	RNB-W 240	HD 2012 / HW 1042
171.	RNB-W 245	HW 2011 / Veery's
172.	RNB-W 549	HD 2329 / Sunstar $*^{6}$ / C 80-1
173.	RNB-W 3922	DW 880 / UWH 147
174.	RNB-W 3999	DW 876 / UWH 147
175.	RNB-WTN 19	Unnat HD 2285 / HW 1042
176.	S 1448	Sonalika / *Ae.speltoides*
177.	S 1824	Sonalika / *T. dicoccoides*
178.	S 1857	Sonalika / *Ae. speltoides*
179.	S 2427	*Ae.speltoides* derivative
180.	S 2748	Chinese Spring /*T.araratium* / 6X
181.	Sanghai 4-1	
182.	Sanghai 77	
183.	SDN DL 218 –6	
184.	Selection 111/Lr 24	Selection 111/Lr 24
185.	Setokomoogi	
186.	Sonalika / *T. militinae*	Sonalika / *T. militinae*
187.	Sonalika / *T.timopheevi* / C 306	Sonalika / *T.timopheevi* / C 306
188.	SG 15	
189.	SG 22	
190.	SG 51	
191.	SG 79	
192.	SG 88	
193.	SG 105	
194.	SG 175	
195.	SG 8809	
196.	SG 8811	
197.	Sozhoe	
198.	Spica	
199.	Sunbird	

Contd...

200.	Synthetic 2	*T. turgidum* var. *durum* X *Ae. squarrosa* amphidiploid
201.	Synthetic 3	"
202.	Synthetic elite 7	"
203.	Synthetic 8	"
204.	Synthetic 14	"
205.	Synthetic 15	"
206.	Synthetic 19	"
207.	Synthetic 29	"
208.	Synthetic 32	"
209.	Synthetic 37	"
210.	Synthetic 38	"
211.	Synthetic 39	"
212.	Synthetic 40	"
213.	Synthetic 42	"
214.	Synthetic 61	"
215.	Synthetic 62	"
216.	Synthetic 63	*T. turgidum* var. *durum* X *Ae. squarrosa* amphidiploid
217.	Synthetic 64	"
218.	Synthetic 71	"
219.	Synthetic 72	"
220.	Synthetic 74	"
221.	Synthetic 81	"
222.	Synthetic 87	"
223.	Synthetic 90	"
224.	Synthetic 91	"
225.	Synthetic 97	"
226.	Synthetic elite 11	
227.	Synthetic elite 54	
228.	T 226	C 306 *2 / *T. militinae*
229.	T 229	GD charter / Sonalika
230.	T 230	T 216 – 1WL 711 / WL 711
231.	T 360	NI 5439 derivative
232.	T 513	*T. dicoccum* / GD charter / Sonalika
233.	T 565	Selection 111 *2/HW1042
234.	T567	
235.	T 572	VL 616 *2 / MACS 2496
236.	T 575	
237.	T 1455	HW 3006 x Lok 1
238.	T 1459 D	*Gigas* derivative
239.	T 1599	NI 5439 / Lr 32
240.	T 1638	Sonalika / *T. dicoccum*
241.	T 1837	*T. dicoccoides* / GD / Sonalika
242.	T 1867	Sonalika / GD charter
243.	T 1985	DARF / Lok Bharti
244.	T 2600	*T.militinae* derivative
245.	T 2890	Chinese Spring / *Ae. scorpium*
246.	T 3972	DW 876 / UHW 147
247.	T 2968-D	EGS 106 – Mango VCS
248.	T 585 K 96	
249.	TDL 1092	

Contd...

250.	*T. dicoccum* derivative	
251.	*T. sphaerococcum*	
252.	*Turaco*	
253.	Unnat HD 2329 / Veery`s	Unnat HD 2329 / Veery`s
254.	V 725-1-8	
255.	VA 92-10/ HW 2007	VA 92-10/ HW 2007
256.	VL 421	
257.	Volcan	
258.	WR 95	(Kalyansona / *T. turgidum*// HD 1999) X Sonalika / *T. carthlicum* // Sonalika
259.	WR 195	Selection 79-219 X (Kalyansona X *T.dicoccum* // *T. turgidum*) X HD 2009
260.	WR 196	Kalyansona / *T. turgidum* // HD 1999 / 3 / Sonalika / *T. carthlicum* // Sonalika
261.	WR 251	*T. turgidum* / Sel. 215 // Kalyansona / H-38
262.	WR 368	Vee [*8] 'S' / 3 / R 37 / Ghl 12 / Kal / Bb
263.	WR 509	
264.	WR 717	CHIL / PRL
265.	WR 764	Lal Bahadur / Transac / Lal Bahadur[3]
266.	WR 881	HW 2002 Unnat Kalyansona X WR 366
267.	WR 882	"
268.	WR 952	
269.	WR 956	CPAN 3004 X WR 424 / HW 2007 Unnat HD 2329
270.	WR 958	(C 306 X *T. absinicum*) X C 306
271.	WR 965	HD 2329 X WR 402 // HW 2002 Unnat Kalyansona X WR 389
272.	WR 966	"
273.	WR 970	"
274.	WR 1012	(Lal Bahadur X *T. isphanicum*) X Lal Bahadur
275.	WR 1013	(Sujata X WR 389) (HW 2004 Unnat C 306 X WR 389)
276.	WR 1044	(CPAN 2004 X WR 4261) X HW 2002 Unnat HD 2329
277.	WR 1052	29 IBWSN-229
278.	WR 1049	RL-LR-YR-45
279.	WR 1053	
280.	WR 1068	Sonalika X *T.sphaerococcum* / HW 2004 X Unnat C 306
281.	WTN 48	NI 5439 / MC 10
282.	WTN 55	Veery`s / HW 2011
283.	WTN 79	
284.	WTN 175	Faislabad 85 / Unnat Kalyansona
285.	WTN 184	WH 542[*6] / MC 6
286.	WTN 373	HW 741 / HW 2008
287.	WTN 554	JGB/C306
288.	WTN 758	Faislabad 85 / HW 2002
289.	WTN 1871	
290.	WTN 3757	
291.	WTN 3952	
292.	WTN 3987	HW 2002 / CPAN 2041
293.	WTN 4001	HD 2012 / HW 1042
294.	WTN 4008	

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