Chapter-2

REVIEW OF LITERATURE
Hybrid cotton revolution was successful in India not only because of the release of superior hybrids like H-4, Varalaxmi, etc. but also due to the bold and imaginative scientifically oriented approach adopted to produce seeds by cumbersome technique of hand emasculation and pollination. The higher expenditure on manual labour and material required for hybrid seed production increases the cost of hybrid seed production. The development of stable cytoplasmic male sterile lines and good fertility restorers is helpful in the production of low cost first generation hybrid seeds. It has also been observed that cytoplasmic factor or cytoplasmic genetic interactions have a definite effect on hybrid performance. Limited work has been done in this field where development of restorers from diverse genetic base is almost untouched. The literature available in this area has been reviewed under the following headings.

1. Cytoplasmic genetic male sterility
2. Heterosis
3. Combining ability
4. Stability analysis
5. \textit{In vitro} pollen germination
6. DNA finger printing.
2.1 Cytoplasmic genetic male sterility

Although, many workers in cotton have reported a desirable heterosis, hybrid vigor has not been exploited on large scale in cotton as compared to other major crops like maize, sorghum and pearl millet. The major reason of not widely utilizing heterosis in cotton is that the seed production techniques used in cotton i.e. hand emasculation and genetic male sterility approaches were not technically easy and economically desirable. The economics and ease of hybrid seed production are important considerations in the utilization of hybrid vigor in crop plants. Use of CGMS line approach is perhaps the best method of hybrid seed production in cotton. The use of CGMS system can reduce cost of hybrid seed production considerably by almost half (Basu and Paroda, 1995).

The best-known sterile cytoplasmic source available for heterosis breeding in cotton is from *G. harknessii* developed by Meyer (1965 and 1975). Development of CGMS lines with the adopted agronomic bases has been taken up in hirsutum cottons in India by many workers (Weaver, 1978 and Shroff, 1980). At present it is the only stable and dependable CGMS source under varied environments on which hybrids like PKVHy-3, PKVHy-4, Suguna and MECH-4 have been released officially and a few private company hybrids may represent this category (Narayanan et al., 1992).

Any normal *G. hirsutum* pollen cannot restore fertility in A line, unless the Rf gene is present. The dominant Rf factor was improved for higher fertility restoration by superimposing an enhancer factor 'E' or 'Rf' as 'RfE' and E was from *G. barbadense* source. Enhancer present in homozygous
Dominant condition (FFPP) strongly restores the fertility. Combination of FfPp, Ffpp do restore fertility but not effective in enhancing hybrid vigor.

The studies performed on the effect of \textit{G. harknessii} cytoplasm on the number of fertility of cotton anthers by Meyer (1971), however revealed that in a reciprocal cross involving \textit{G. hirsutum} and the wild diploid \textit{G. harknessii} anther number was significantly higher with \textit{G. harknessii} cytoplasm. Meyer (1972) further studied the effect of \textit{G. longicalyx} cytoplasm on anther numbers in inter specific hybrids of cotton. He observed significant difference in anther number in F\textsubscript{1} hybrids with \textit{G. longicalyx} compared to reciprocal hybrids with cytoplasm from diploid \textit{G. harknessii} and tetraploid \textit{G. barbadense} and \textit{G. tomentosum}. However, differences between reciprocal hybrids in anther numbers were far less for the Asian and African diploid species, \textit{G. herbaceum}, \textit{G. arboreum} and \textit{G. anomalum}.

Meyer (1973) studied reciprocal hybrids between upland cotton and experimental lines with cytoplasm from seven other species. Back crosses with cytoplasm from two tetraploid species, \textit{G. barbadense} and \textit{G. tomentosum} showed no significant differences in their reciprocal hybrids with upland cytoplasm, but similar basic crosses with cytoplasm from each of the five diploid species viz., \textit{G. herbaceum}, \textit{G. arboreum}, \textit{G. anomalum}, \textit{G. harknessii} and \textit{G. longicalyx} from their reciprocal hybrids showed significant differences in plant height and boll size. The reports of Murthy and Weaver (1974) revealed that CGMS lines with \textit{G. harknessii} cytoplasm flower were smaller in size with rudimentary anthers compared to fertile flowers.
Rosales and Davis (1976) studied the comparative performance of cytoplasmic genetic male sterile lines (*G. harknessii* x *G. hirsutum* L.) versus similar male fertile lines for yield and lint percentage under the conditions of natural pollination. For yield between sterile and fertile 8 out of 12 comparisons were statistically significant. The male sterile lines produced less seed cotton and lint when they are grown away from B lines.

Weaver and Weaver (1977) observed significantly higher lint percentage for fertile plants and higher micronaire value for sterile plants in cytoplasmic sterile upland cotton. Thombre and Mehetre (1979) observed large number of bolls in okra leaf cytoplasmic genetic male sterile in American cotton, the boll and seed setting was about 70 per cent.

The effect of cytoplasm and interaction between cytoplasmic genetic factors on petal size and anther number was studied (Shroff *et al.*, 1982). They observed that petal size of A line JCMSK-2 was significantly smaller than Khandwa-2 maintainer line (B line) and the number of anthers in A line was enhanced to 120.50 as compared to B line which has only 77.5 anthers. The anther number and petal size in intra *hirsutum* F₁ hybrid between JCMSK-2 x JDFSHAL-1613 was significantly decreased than inter-specific hybrid.

Shroff (1985) again observed significant difference with respect to petal size and anther number between CGMS and fertile genotypes of cotton. Patel *et al.* (1986) compared the hybrids developed by utilizing cytoplasmic male sterility restorer system with that of hybrid developed by conventional method and found that both the hybrids were similar in most of the morphological
characters except percentage hair on stem and leaves, plant height and number of sympodia. Hence, it was reported that the use of male sterility did not have any substantial deleterious effect on the performance of the commercial hybrids.

Sethi et al. (1988) observed reduced inter nodal length in cytoplasmic male sterile “H-777” but whereas plant height was significantly increased and number of monopodia were to be non significant. Soddi (1995) in comparative study of CGMS (G. harknessii cytoplasm), GMS and their respective male fertile counter lines of G. hirsutum, revealed that significant reduction in flower morphological characters in CGMS genotypes compared to maintainer lines. But plant height was similar to their normal counter part.

Kowsalya and Raveendran (1996) studied the comparative influence of G. harknessii and G. hirsutum cytoplasm on vegetative traits in cotton hybrids. Three cytoplasmic genetic male sterile (CGMS) lines possessing G. harknessii cytoplasm and their respective maintainer (B) lines possessing G.hirsutum cytoplasm was used as female parents. In case of inter-specific hybrids, G. barbadense as pollinator to G. hirsutum and G. harknessii female revealed absence of difference between hybrids of two female bases for plant height.

Shroff (1982) reported that use of cytoplasmic sterile lines appears to be advantages in several ways. Maintenance of male sterile population for seed production is easier as compared to genetic male sterile lines. Moreover, sterility source under reference is stable. Low ginning in male sterile line is not inherited in F₁ hybrids is also advantageous in obtaining
higher proportion of hybrid seed. Cytoplasmic nuclear interactions affect petal size and anther numbers. Such markers can be used in establishing identity of parental lines and also for ascertaining genetic purity.

Some studies indicated that *G. harknessii* cytoplasm has detrimental effect on yield (Weaver, 1986, Wei et al., 1995). On the other hand some other reports like Davis (1979) who studied the A x R and R x B combinations to clearly determine the effect of *G. harknessii* upon the performance of hybrids found no obvious differences between performances of A x R and R x B hybrids.

The varied performance between sterile and fertile lines might be the result of differential behavior of the same genome in the sterile and fertile cytoplasm's (Meyer, 1973) or sterile cytoplasm provides an unstable substrate(s) for the parental genes. In addition, the presence of the cytoplasmic factors *per se* may influence the expression of the phenotype.

Morphological traits did not differ significantly in GMS and CGMS systems compared to their fertile versions (Richmond and Kohel, 1961). Sterile lines (GMS/CGMS) had significantly lower values for various flower related traits except anther number compared to the fertile lines. However, there was no significant difference between GMS and fertile plant for characters like calyx area, ovary length and width or style length. Shroff (1985) made similar observations.

Weaver (1986) reported that hybrids using cultivars as the female parent (not sterile cytoplasm) consistently produced the highest yields in 1984.
and 1985. However, hybrids produced by using restorers with *G. harknessii* cytoplasm as the female parent showed about 8 per cent reduction in yield.

Sethi *et al.* (1988) observed that locule/boll, seeds/locule, boll weight were found non significant in H-777 having the genome of *G. hirsutum* cytoplasm of wild species *G. harknessii*. Waller and Mamood (1992) studied upland and pima cottons as pollen donors for male sterile upland seed parent. The seeds/boll produced were more when upland pollen was used. Thus more pollen grains/stigma is needed to set an equal number of seeds using Pima pollen compared with using upland pollen.

Cook and Namken (1994) evaluated 42 cytoplasmic male sterile restorer cotton F₁ hybrids for yield components, lint yield and fiber quality where deltapine-50 and Stoneville-132 used as commercial control, 23 hybrids produced more lint yield than control. The hybrids had greater lint percentage and total lint yield and fewer days to crop maturity. This appears to be a superior and stable fertility-restoring parent for use in hybrid cotton production system.

Meti (1995) showed that majority of the CGMS based hybrids possessed higher boll weight, and lint index than conventional hybrids. CGMS based hybrids proved better than conventional hybrids by showing superior yield and fiber. Soddi (1995) studied the performance of CGMS and GMS cotton genotypes for yield and yield related traits, which were on par with their respective counterparts. However, two B lines had significantly higher boll weight, lint weight per boll, ginning outturn, lint index compared to A lines.
Kowsalya and Raveendran (1996) studied the comparative influence of G. harknessii and G. hirsutum cytoplasms on yield and yield components in cotton hybrids. Three CGMS lines possessing G. harknessii cytoplasm and their respective maintainer lines possessing G. hirsutum cytoplasm were used as female parents. The intra-specific hybrids involving B lines of all the three CGMS lines were superior in ginning outturn compared to hybrids with A line. In case of inter-specific hybrids, G. barbadense as pollinator to G. hirsutum and G. harknessii female revealed that there was no difference between hybrids of two female bases for sympodia per plant, number of bolls per plant, boll weight, seed cotton yield per plant and ginning outturn.

According to Gunaseelan and Rao (1997) better fiber properties were observed in inter-specific CGMS hybrids G. hirsutum x G. barbadense than CGMS intra-specific hybrids G. hirsutum x R-line and conventional hybrids. Assessment of cytoplasmic effect in cytoplasmic genetic male sterile lines of upland cotton revealed that there was a detrimental effect of CGMS cytoplasm on yield and yield components (Zhu et al., 1998). The detrimental effect was closely related to an increased number of immature seeds per boll.

The performance of CGMS hybrids with DESHAF-277 restorer concluded that, lower restorability of DESHAF-277 was the major reason of lower yield in its hybrids (Wang et al., 1998 and Yu and Wang, 1999). The lower restorability makes hybrid lower in pollen viability, boll setting percentage, lint yield and higher in aborted seed percentage.

Soddi and Khadi (1999) reported that the interaction of G. harknessii cytoplasm with nuclear genome certainly influences the fiber properties like
2.5% span length towards fiber length in sterile lines. But there was no difference between sterile and fertile lines for fiber uniformity ratio and tenacity.

Shroff (2004) observed in his experiments that *G. barbadense* enhancer in R line produces favorable cytoplasmic genetic interaction even in over coming the deleterious effect of *G. harknessii* cytoplasm. Superiority of CGMS hybrids like MPRHC-404 and MPRHC-403 in comparison to conventional upland cotton hybrids disproved adverse effect of *G. harknessii* cytoplasm on yield and quality parameters. He also reported that commercial utilization of CGMS seed production technique requires very rigorous supervision and maintenance of homozygous restorer lines with enhancer factor is inevitable.

Tuteja *et al.* (2004a) observed seed fertility percentage had no significant association with seed cotton yield and its related parameters except with ginning outturn, it showed significant negative association. These findings are contrary to earlier reports of Sarkar *et al.* (2002) that, in rice exploitation of heterosis from the potential crosses the level of fertility restoration would likely to be the key for yield advantage. However, in cotton fertility restoration and its impact on seed cotton yield and its related parameters has been a complex phenomenon.

Dutt *et al.* (2004) showed that, sterile cytoplasm has detrimental effect on yield traits in hybrid cotton. But this detrimental effect could be avoided or alleviated by using better CGMS line, restorer with high restorability which allow hybrid to produce higher viable pollen per cent and higher boll setting
per cent, reduce the number of aborted seeds per boll, and thereby increasing
the yield per plant. They also observed that the mean lint yield and boll per
plant of B x R hybrids was higher than either A x R or R x B hybrids. This
revealed that, sterile cytoplasm has negative effect for these traits. Tuteja et al. (2005b and 2007) noticed the detrimental effects of harknessii cytoplasm
on yield traits in CGMS hybrids and also reported some of the hybrid
combinations superior to check hybrid for seed cotton yield and fibre
properties. These findings indicate that, it is possible to overcome detrimental
effects of harknessii cytoplasm by using better CMS lines and restorers with
high restorability.

2.2 Heterosis

Manifestation of hybrid vigour, which results in the phenomenon of
heterosis, expresses superiority of the progeny over the parental mean or
over the better parent (Hayes et al., 1955). Heterosis in F₁ for growth and
vigour of hybrids mainly from an accumulation of maximum number of
dominant favorable alleles contributed or complimented by either of the
constituent parents. Heterosis breeding has been very successful in several
crop plants and cotton is one such crop in India.

Development and release of hybrids H-4 (Patel, 1971) and Varalaxmi
(Katarki et al., 1972) was the most significant milestones in the history of
cotton breeding in India. Many workers reviewed the work on heterosis and
references relevant to the present study are briefly reviewed character wise
and presented below.
2.2.1 Days to 50 per cent flowering

Patil (1975) observed significant heterosis in *G. hirsutum* cotton for days to first flowering. In inter specific crosses *G. hirsutum* and *G. barbadense* Krishnaswamy and Kothandaraman (1977) observed negative heterotic effects in respect of days to flowering. Kajjidoni (1982) in desi cotton observed negative significant heterosis for days to 50 per cent flowering over the mid, better parent and commercial check Jayadhar.

Kenchanagoudar (1983) in *G. hirsutum* noticed negative significant heterosis over the mid parent and both negative and positive significant heterosis over the better parent for days to first flowering. Kadapa and Prajapati (1990) observed significant heterosis over mid, better parent and commercial check for days to flowering. Khadi *et al.*, (1993) reported that the hybrid DHH-25 showed highly significant and negative heterosis for days to 50% flowering over the commercial check.

Meshram *et al.* (1998) in their study observed standard heterosis in two hybrid combinations with *G. aridum* cytoplasm and one hybrid combination with *G. harknessii* cytoplasm over check hybrid PKV Hy-2. Dheva *et al.* (2002a) in a study of line x tester analysis reported that seven hybrids exhibited significant negative heterobeltiosis. Kajjidoni and Patil (2003) reported significant heterosis in desirable direction for days to 50% flowering in both (*G. arboreum* x *G. herbaceum*) GMS based inter-specific and conventional hybrids. Punitha and Ravikesavan (2004) reported significant negative heterobeltiosis in cytoplasmic genetic male sterile based cotton
hybrids. In upland cotton Potdukhe and Parmar (2005) observed significant negative and positive heterobeltiosis for day to 50 per cent flowering.

2.2.2 Number of Monopodia per plant

Patil (1973a) in a half-diallel analysis of *G. herbaceum* reported varying degree of heterosis over mid parent and better parent and none of the cross was statistically significant for this character. Kajjidoni (1982) reported that the heterotic effects for monopodia per plant were smaller over the respective mid-parental values, but positive in most of the crosses. The magnitude of useful heterosis ranged from -21.75 to -2.78 percent, which indicated earliness. Kaushik *et al.* (1984) also reported significant heterosis over mid and better parent.

Meshram *et al.* (1998) reported heterotic hybrids based on *G. harknessii* and *G. aridum* cytoplasm over check PKV Hy-3 for number of monopodia per plant. In an evaluation of *G. hirsutum* crosses, Tuteja *et al.* (2004b) observed significant positive heterosis for monopodia per plant over best check. Similarly, Kharde *et al.* (2004), Katnalli (2004b) and Potdukhe and Parmar (2005) reported high degree of heterosis over better parent.

2.2.3 Number of Sympodia per plant

Bhatade *et al.* (1980) reported moderate heterosis over better parent for number of sympodia. Similarly, Soomre *et al.* (1982) also indicated superiority of hybrids over mid parents. In *G. arboreum* x *G. herbaceum* crosses Kajjidoni (1982) reported that, the extent of heterosis for sympodia was superior over the mid parental values, Whereas, Kolte and Thombre (1984) reported high degree of heterosis for sympodia.
Rao and Reddy (2001) in a study of upland cotton recorded significant and positive heterosis over best parent and none of the hybrids recorded significant and positive standard heterosis. Kajjidoni and Patil (2003) in a study of GMS and conventional crosses of diploid cotton (*G. arboreum* x *G. herbaceum*) observed significant heterosis in desirable direction in both group of crosses. Tuteja *et al.* (2004b) reported the significant positive heterosis in *G. hirsutum* for number of sympodia per plant over best check. Range of heterosis over standard check was 22.06 to 113.24 per cent. Punitha and Ravikesavan (2004) reported the range of per cent heterosis over better parent from -36.22 to 47.03 for this trait in cytoplasmic genetic male sterile based cotton hybrids. High degree of heterosis over better parental value in PKVHy-2, NHH-44, H-6 and H-8 is reported by Kharde *et al.* (2004). Similar observations were made by Katnalli *et al.* (2004b) and Potdukhe and Parmar (2005) in upland cotton.

### 2.2.4 Number of bolls per plant

Bhale and Bhat (1990) while evaluating CGMS based hybrids during 1985 reported that only two out of 15 hybrids were at par with H-4 for boll number and during 1986 evaluation none of the hybrids were superior to H-4. In contrast to this, Shroff *et al.* (1983) reported that inter-specific hybrids involving cytoplasmic genetic male sterility recorded higher number of bolls and were at par with traditional hybrids Varalaxmi and JKHY-11. While, the intra specific hybrids involving cytoplasmic genetic male sterility recorded lower number of bolls per plant as compared to inter-specific hybrids, but were at par with respective traditional hybrids JKHY-1 and Hybrid 4 used as
checks. Raveendran et al. (1992) observed considerable degree of heterosis in CGMS hybrids over MCU-5, a check variety.

Gunaseelan et al. (1996) in CGMS hybrids observed up to 25 percent heterosis for boll number. Meshram et al. (1998) in a study of different cytoplasmic based hybrids reported that *G. harknessii* cytoplasm based hybrid showed significant positive heterosis ranging from 104.69 to 130.61 per cent over three check hybrids for number of bolls per plant. Punitha and Ravikesavan (2004) observed significant positive heterobeltiosis in cytoplasmic genetic male sterile based cotton hybrids.

Many workers reported considerable amount of heterosis in intra-*hirsutum* crosses, Singh and Narayanan (1990), Patil et al. (1991), Katageri et al. (1992) and Baloch et al. (1993). Maximum heterobeltiosis of 64 per cent was observed for this trait by Pavasia et al. (1999a) in crosses of *G. hirsutum*. Dheva et al. (2002a) reported in a study of line x tester analysis in *G. hirsutum* that almost all the hybrids exhibited significant positive heterobeltiosis for number of bolls per plant and the magnitude of heterosis ranged between 5.15 to 125.13 per cent.

The heterosis for number of bolls per plant was found positive and significant over best check in crosses of *G. hirsutum* was reported by Tuteja et al. (2004b). Potdukhe and Parmar (2005) observed high degree of heterobeltiosis in *G. hirsutum* crosses.
2.2.5 Boll weight

Next to boll number, boll weight is the main contributing factors of seed cotton yield per plant, which in turn is governed by number of seeds per locule, seed index and lint index. Shroff et al. (1983) while evaluating CGMS based cotton hybrids noticed significant heterosis for boll weight and reported that the boll weight values in first picking were low as compared to second and third picking.

Silva et al. (1985) in their study involving 12 male sterile lines (A-lines), two fertility restorer lines and their F₁ population reported heterosis for boll weight. Bhale and Bhat (1990) in their two years evaluations of CGMS based hybrids reported that 2 out of 15 hybrids were at par with H-4 for boll weight in one year (1985), while in the next year (1986) none of the hybrids were superior to H-4.

Jagtap and Kolhe (1987) in a line x tester analysis of G. hirsutum observed high magnitude of heterobeltiosis for boll weight. Kadapa and Prajapati (1990) in intra G. barbadense hybrids observed significant heterosis over mid, better parent and commercial check.

Patil et al. (1991) and Lancon et al. (1993) recorded heterosis to the extent of 51.6 per cent and 9.0 per cent respectively for boll weight. Khadi et al. (1993) reported that intra-hirsutum hybrids showed significantly higher boll weight than commercial variety Sharada. Gunaseelan et al. (1996) studied 56 CGMS hybrids and reported up to 25 percent heterosis for boll weight.
Meshram et al. (1998) in a study of *G. harknessii* and *G. aridum* cytoplasmic based hybrids reported that, none of the hybrids exhibited significant positive heterosis for boll weight. Similar observation were made by Khadi et al. (2003a) and Kajjidoni and Patil (2003) in a study of GMS based intra and inter specific diploid cotton hybrids.

In GMS based hybrids of *G. hirsutum* Khadi et al. (2003b) reported maximum heterosis of 9.52 per cent over conventional hybrid DHH-11. Punitha and Ravikesavan (2004) reported significant positive heterobeltiosis in cytoplasmic genetic male sterile based cotton hybrids for boll weight.

2.2.6 Number of seeds per boll

Shroff et al. (1983) in their study of CGMS hybrids reported that inter-specific hybrids had lower number of seed per boll in different pickings and the number of seeds ranged from 17.88 to 21.88. Whereas, in intra hirsutum hybrids it ranged from 23.55 to 29.58, except three hybrids remaining had lower number of seed in first picking as compared to second and third pickings. Hybrid JCMSBN x JDESHAF-16/3 recorded the highest number of seeds in all the pickings.

Kolte and Thombre (1984) recorded significant positive heterosis for number of seeds per boll however, non-significant mid parent heterosis was reported by Gunaseelan and Krishnaswamy (1988). Meshram et al. (1998) reported non-significant heterosis for number of seeds in their study of cytoplasmic based hybrids of *G. harknessii* and *G. aridum*. Rao and Reddy (2001) in a study of 9 x 9 diallel crosses reported that, significant positive
heterosis in seven crosses over L-389 and the range of heterosis was from 8.58 to 24.25 per cent.

2.2.7 Plant height

Gunaseelan et al. (1996) noticed heterosis for plant height was up to 73 per cent in CGMS hybrids. Kajjidoni and Patil (2003) observed significant positive heterobeltiosis for plant height in both GMS and conventional based hybrids of diploid cotton (*G. arboreum* x *G. herbaceum*).

The plant height showed significant positive heterotic effect in crosses of *G. hirsutum* was observed by Tuteja et al. (2004b). Kharde et al. (2004) also observed high degree of heterosis over better parental value in four *G. hirsutum* hybrids viz., PKV Hy-2, NHH-44, H-6 and H-8.

Punitha and Ravikesavan (2004) reported in cytoplasmic genetic male sterile based cotton hybrids that, the heterobeltiosis range from -41.14 to 79.41 per cent. Katnalli et al. (2004b) in a study of intra plant type (robust x robust) and line x tester crosses of inter plant type (robust x compact) of *G. hirsutum* observed significant positive heterosis in majority of the intra plant type crosses over better parent. Similar observation was also made by Potdukhe and Parmar (2005) in the study of upland cotton.

2.2.8 Seed index

In CGMS hybrids, seed index values were low in first picking as compared to second picking with few exceptions. The inter specific hybrids recorded higher seed index values in second picking, while the intra hirsutum hybrids recorded higher seed index in third picking (Shroff *et al.*, 1983). In
conventional hybrids positive and significant heterosis for seed index was reported by Bhatade et al. (1980) and Mehta et al. (1986). Gupta and Singh (1987) reported -10.29 to 11.20 per cent heterosis for seed index.

In a line x tester analysis of intra G. barbadense Kadapa and Prajapati (1990) noticed significant heterosis over mid parent, better parent and commercial check for thousand seed weight. Khadi et al. (1993) reported positive significant seed index in intra hirsutum hybrids over commercial variety Sharada indicating their contribution for higher lint production.

Dheva et al. (2002a) reported significant positive and negative heterosis and the range of heterobeltiosis was -14.99 to 28.99 per cent. Punitha and Ravikesavan (2004) observed heterobeltiosis ranging from -34.28 to 28.02 per cent in cytoplasmic genetic male sterile based G. hirsutum hybrids for this trait. High degree of heterobeltiosis for seed index reported by Kharde et al. (2004), Katnalli et al. (2004b) and Potdukhe and Parmar (2005) in a study of upland cotton.

2.2.9 Lint index

Significant positive heterosis for the lint index was reported by Sarsar (1986), Gupta and Singh (1987) and Singh et al. (1988). While, non-significant heterosis was reported by Katageri and Kadapa (1989). Khadi et al. (1993) in intra specific crosses of G. hirsutum reported positive significant heterosis for lint index over check (Sharada) in majority of the crosses.

Dheva et al. (2002a) observed significant positive heterobeltiosis in G. hirsutum crosses. On the contrary, Kajjidoni and Patil (2003) reported none
of the heterotic hybrids in conventional and GMS based diploid cotton for this trait. However, the GMS based hybrids exhibited narrow range of heterobeltiosis compared to conventional crosses.

Khadi et al. (2003a) reported maximum significant heterosis of 22.18 per cent in GMS based intra-arboreum hybrid (DDaa-1). Similarly, Singh et al. (2003) observed useful heterosis of 28.92 to 58.3 per cent in intra hirsutum crosses. Punitha and Ravikesavan (2004) in a study of cytoplasmic genetic male sterile based G. hirsutum hybrids reported range of heterosis from -25.34 to 38.90 per cent over better parent for lint index. Kharde et al. (2004) observed highly significant heterosis over better parent in crosses of upland cotton.

2.2.10 Ginning outturn

Shroff et al. (1983) in their study on CGMS hybrids reported significant heterosis over the conventional hybrid checks for ginning outturn. In CGMS based intra hirsutum hybrids evaluation, hybrids NHHM-302 (37.0%) and NHHM-33 (36.5%) were found most promising for ginning outturn (Anonymous, 1993b).

Katageri and Kadapa (1989) in inter specific crosses of G. hirsutum and G. barbadense observed negative significant heterosis. However, non-significant heterosis recorded by many workers (Thombre et al., 1982, Kolte and Thombre, 1984, Mehta et al., 1986 and Hapase et al., 1987). Gunaseelan et al. (1996) reported 25 per cent heterosis for ginning percentage in CGMS hybrids.
Rao and Reddy (2001) reported significant positive heterosis over both L-389 and LAHH-4 for ginning percentage in crosses of upland cotton. Similarly Khadi et al. (2003a) observed significant heterosis in GMS based intra arboreum hybrids.

Singh et al. (2003) observed useful heterosis range of 4.3 to 16.9 per cent for ginng outturn in intra-hirsutum crosses. Punitha and Ravikesavan (2004) reported heterosis range of -25.65 to 33.59 per cent over better parent in cytoplasmic genetic male sterile based cotton hybrids for ginning outturn. Low magnitude of heterosis and heterobeltiosis for ginning outturn reported by Kharde et al. (2004), Tuteja et al. (2004b) and Potdukhe and Parmar (2005) in G. hirsutum crosses.

Katnalli et al. (2004b) observed significant negative heterosis over better parent in majority of the crosses in intra plant type crosses of G. hirsutum for this trait. On the contrary Tuteja et al. (2005a) reported significant positive heterosis over commercial check.

2.2.11 Seed cotton yield

Shroff et al. (1983) reported that, the inter specific hybrids from cytoplasmic genetic male sterility namely JCMSRB-50 X JPR-6 and JCMSK-2 X JPR-9 were at par in seed cotton yield to the traditional hybrids Varalaxmi, JKH-11, H-4 and JKH-1. The intra specific hybrids JCMSRB-50 x JDESHAF-16/3 and JCMSK x JDESHAF-16/3 have recorded low seed cotton yield.

Shroff (1985) reported that inter specific hybrid developed on CGMS background gave very encouraging results. He also found that single cross
hybrids out yielded three way cross hybrids, while the latter were more stable in performance over environments. However, Bhale and Bhat (1990) in CGMS based hybrids observed non-significant heterosis for seed cotton yield over the commercial check H-4.

CGMS restorer based intra hirsutum hybrids were found promising for seed cotton yield and at par with conventional hybrid check NHH-44 (Anonymous, 1993b). Out of 22 CGMS based hybrids evaluated five hybrids were found to be promising for seed cotton yield with an increase ranging from 28 per cent to 74 per cent (Anonymous, 1993c).

Gunaseelan et al. (1996) studied CGMS hybrids and reported maximum heterosis for seed cotton yield in inter specific hybrid is 120.1 per cent and in intra specific hybrid 145.1 per cent. In a study of GMS based G. hirsutum cotton hybrids Khadi et al. (2003b) observed the top yielding GMS based hybrid DGMSHH-201 exhibited significant standard heterosis of 54.99 per cent for seed cotton yield over conventional hybrid DHH-11.

Khadi et al. (2003a) observed more number of inter specific GMS hybrids were found to be highly heterotic for seed cotton yield than intra specific diploid GMS hybrids. Meshram et al. (1998) reported significant positive heterosis in cross combinations based on G. harknessii cytoplasm over check PKVHy-3 for seed cotton yield. Punitha and Ravikesavan (2004) reported significant positive heterobeltiosis in cytoplasmic male sterile based cotton hybrids for seed cotton yield.

The first commercial intra hirsutum hybrid, Hybrid-4, exhibited 138 per cent heterosis (Patel, 1971) and inter specific hybrid Varalaxmi recorded 53 per cent (Katarki et al., 1970). Gunaseelan and Krishnaswamy (1988) recorded 200 per cent heterosis for yield, while, Katageri and Kadapa (1989) noticed heterosis between 100 to 200 per cent.

Pavasia et al. (1999a) observed maximum heterobeltiosis of 74.2 per cent in G. hirsutum. On the contrary, Rao and Reddy (2001) observed negative non-significant standard heterosis. Dheva et al. (2002a) in a line x tester analysis of G. hirsutum, observed significant heterobeltiosis for seed cotton yield per plant and it was ranged from -11.5 to 191.08 per cent.

Patnaik et al. (2003) in a study of 12 x 12 diallel mating of G. hirsutum, out of 66 combinations eight crosses out yielded the commercial check DHH-11 with the economic heterosis, mid parent heterosis and heterobeltiosis ranged from 4.08 to 13.58 per cent, 70.32 to 95.04 per cent and 52.04 to 73.93 per cent respectively.

Significant positive standard heterosis observed by Tuteja et al. (2004b) in evaluation of G. hirsutum crosses. Similarly, Khadi et al. (2004) also reported two hybrids (DHH-281 & DHH-815) exhibited significant and positive heterosis over all the four checks.
2.2.12 Lint yield

El-kadi (1974) compared the heterotic performance of hybrids produced on individual male sterile plants from three genetic sources at two localities and reported that some of the hybrids were superior for lint yield. Sheetz (1985) in CGMS based intra specific crosses of G. hirsutum reported 100 to 133 per cent heterosis over best parent for lint yield.

Significant heterosis for lint yield was reported by many workers (Singh et al., 1980, Kadapa and Prajapati, 1990, Wang and Pan, 1991). Patil et al. (1991) reported heterosis to the extent of 169.4 per cent in a half diallel cross of G. hirsutum cotton.

KhadI et al. (2003b) observed significant standard heterosis of 60.42 per cent in GMS based intra hirsutum hybrid DGMSHH-201 over DHH-11 for lint yield. Similarly Tuteja et al. (2005a) reported significant standard heterosis in G. hirsutum crosses.

2.3 Combining ability

Selection of parents for hybridization programme is an important aspect in crop improvement programme. Knowledge of the relative importance of general and specific combining ability for quantitative characters influencing yield and its components is very useful in selection of parents which can produce superior hybrids. Sprague and Tatum (1942) were the first to develop the concept of combining ability in terms of genetic variation in corn. They described general combining ability as the average performance of lines in a series of hybrid combinations and specific combining ability is the deviation of certain crosses from the average performance of
lines. Their results confirmed that general combining ability was due to additive effects of genes, whereas specific combining ability was the result of dominance and epistatic interactions. Review pertaining to combining ability is presented below:

2.3.1 Days to 50 per cent flowering

In a diallel analysis of *G. hirsutum* Chinnadorai *et al.* (1973) observed significant GCA variance compared to SCA variance thus indicating the importance of additive gene action for this character. In a similar study involving *G. herbaceum* Patil (1973b) reported significant estimates of GCA and SCA, where GCA variance was greater than SCA variance. Kajjidoni *et al.* (2002) in a comparative study of *G. arboreum* crosses reported that predominance of GCA variance for days to 50 per cent flowering in both GMS and conventional crosses.

The contradictory results were also reported by Patil (1975) in Line x tester analysis where in estimates of SCA variance were greater than GCA variance for days to 50 per cent flowering. Similar observations was also made by Laxman and Ganesh (2003) and Hanamaratti *et al.* (2004).

Kenchanagoudar (1983) noticed significant positive and negative gca effects and significant negative sca effects. Jagtap and Kolhe (1987) and Sivaprasad *et al.* (2004) observed both additive and non-additive gene action for days to 50 per cent flowering. Potdukhe and Parmar (2006) reported predominant role of non-additive component for this trait.
2.3.2 Number of Monopodia per plant

Singh and Gupta (1970) reported significant GCA and SCA variance indicating the importance of both additive and non-additive gene action. They also noticed significant GCA effects for number of Monopodia per plant. Kajjidoni (1982) observed significant gca effect and non-significant sca effects for number of monopodia per plant in a line x tester analysis of desi cotton. Hapase (1987) also reported significant gca effects for number of monopodia per plant in desi cotton.

Katnalli et al. (2004a) observed GCA variance was significant and higher in magnitude than SCA variance in a study of intra-plant type (robust x robust) indicating the importance of additive gene effects and additive x additive gene interactions. They also observed the similar trend in interplant type (robust x compact). Singh et al. (2005) also reported higher magnitude of additive genetic component in upland cotton.

On the contrary, Dheva et al. (2002b) observed highly significant SCA variance for this trait in a study of line x tester analysis of G. hirsutum. Similarly, Laxman and Ganesh (2003) and Verma et al. (2004) in G. hirsutum crosses reported that predominance for SCA variance. Sivaprasad et al. (2004) and Potdukhe and Parmar (2006) also observed non-additive gene action of this trait.

2.3.3 Number of Sympodia per plant

Singh and Gupta (1970) in a diallel analysis of G. hirsutum noticed significant GCA and SCA variance for number of sympodia per plant. They also reported significant gca effects for this trait. Kenchanagoudar (1983) and
Rao and Reddy (2002) reported that gca effects were significant for number of sympodia per plant but sca effects were non-significant. Kaushik et al. (1984) also observed significant gca effects for number of sympodia per plant in a line x tester analysis of G. hirsutum. Kolte and Thombre (1984) reported that gca and sca effects were significant with greater magnitude of gca effects for number of sympodia per plant.

Verma et al. (2004) and Sivaprasad et al. (2004) reported significant SCA variance, whereas, Potdukhe and Parmar (2006) also reported non-additive components were more pronounced for this character. Kajjidoni et al. (2002) in a study of both GMS and conventional G. arboreum crosses reported that significant GCA and SCA variance for this character in conventional cross combinations.

2.3.4 Number of bolls per plant

Thomson (1971) and Mirza (1974) reported that variation in boll number was predominantly due to gca effects and the SCA component of variance though significant was much lesser than GCA. Silva et al. (1983) reported predominant additive gene action for number of bolls per plant in CGMS hybrids.

Krishnaswamy and Gunaseelan (1985) and Siddiqui and Patil (1992) observed dominance and additive effects conditioned boll number per plant. Khan et al. (1987) observed highly significant GCA for this trait. Sivaprasad et al. (2004) observed highly significant GCA and SCA variance with higher magnitude of GCA variance. On the contrary, Singh et al. (1989) observed
significant SCA effect. Significant GCA and SCA effects for bolls per plant was observed by Wilson (1991).

Rao and Reddy (2002) reported significant GCA and SCA variance with higher magnitude of SCA variance. Similar result were obtained by other workers Muthuswamy et al. (2003), Verma et al. (2004) and Ahuja and Dhayal (2004) in G. hirsutum.

2.3.5 Boll weight

In a half diallel cross Abul-nass et al. (1983) observed significant GCA variance for boll weight. Kolte and Thombre (1984) indicated predominant additive gene effect over dominant and epistatic effects and reported that SCA effects although of lesser magnitude were significant. Katnalli et al. (2004) reported significant GCA and SCA variance and components of GCA variance was higher in magnitude indicating importance of additive gene effects, in intra-plant type (robust x robust) and inter-plant type (robust x compact) crosses in G. hirsutum genotypes. Similar observation was also made by Sivaprasad et al. (2004) and Singh et al. (2005).

2.3.6 Number of seeds per boll

Patil (1973b) observed significant GCA and SCA component of variance in respect of number of seeds per boll and predominance of additive gene action was reported by Amalraj (1989). The relative importance of both additive and dominance effects was indicated by Wilson (1991), but Singh et al. (1980) indicated the relative importance of dominance effects for this trait.


2.3.7 Plant height

Bhatade et al. (1980), Singh and Singh (1980) and Virk and Kalsy (1982) reported additive and non-additive gene action for plant height. Singh and Singh (1981) observed additive gene action. While, Katnalli et al. (2004a) reported significant GCA and SCA variance with higher magnitude of GCA variance in inter-plant type (robust x robust) and inter-plant type (robust x compact) crosses indicating the importance of additive gene effects and additive x additive gene interactions.

On the contrary, Shanti and Selvaraj (1995) and Dheva et al. (2002b) reported highly significant SCA variance in G. hirsutum crosses. Similarly non-additive gene action for plant height was reported by Laxman and Ganesh (2003), Ahuja and Dhayal (2004) Singh et al. (2005) and Potdukhe and Parmar (2006).
2.3.8 Seed index


2.3.9 Lint index

Gururajarao et al. (1977) and Singh and Singh (1984) reported additive gene action for lint index. Mehta et al. (1987) and Dani (1989) reported that gca effects were more important for lint index. Predominance of GCA variance was reported by Rao and Reddy (2002) and Singh et al. (2005).

Additive and non-additive gene action was observed by Hapase et al. (1987), Singh et al. (1987) and Singh and Chhabra (1991) for this trait. Kajjidoni et al. (2002) reported that both GCA and SCA variances were significant in a comparative study of GMS based and conventional crosses of G. arboreum genotypes. Sarsar (1986), Dheva et al. (2002b) and Manickam and Gururajan (2004) noticed predominance of non-additive gene action for lint index.

2.3.10 Ginning outturn

Silva and Alves (1983) in a study of CGMS hybrids reported that fiber percentage was affected significantly by epistasis. Kolte and Thombre (1984),
Katnalli et al. (2004) reported that gca effects was significant and of greater magnitude indicating predominance of additive gene effect over dominance and epistasis effects. Similar observations made by Patel et al. (2004) in a study of GMS hybrids.

Sarsar et al. (1986) observed predominance of non-additive type of gene action for ginning outturn. Similar observations was made by several workers, Rao and Reddy (2002), Deshpande and Baig (2003), Manickam and Gururajan (2004), Singh et al. (2005) and Potdukhe and Parmar (2006). Additive and non-additive gene action was reported by Murthy and Ranganathacharyulu (1998), Verma et al. (2004) and Sivaprasad et al. (2004) for ginning out turn.

2.3.11 Seed cotton yield

Several workers have studied combining ability analysis with respect to seed cotton yield. In a study of Thomson (1971) and Amalraj (1989) GCA variance was predominant compared to SCA variance even though both were significant. The contrary results were also obtained by Singh et al. (1971), Kajjidoni et al. (1984) and Kaushik et al. (1984), where in SCA variance was larger than GCA variance.

Significant sca effects for seed cotton yield was reported by Khan and Khan (1985) and Mehta et al. (1987). Ahuja and Tuteja (2003), Patel et al. (2004) and Ahuja and Dhayal (2007) observed predominant non-additive gene effects for seed cotton yield in Upland Cotton. While, Green and Culp (1990) observed non-significant gca effects for seed cotton yield. Both additive and non-additive gene actions for seed cotton yield was reported by Murthy and Ranganathacharyulu (1998) and Pavasia et al. (1998).

2.3.12 Lint yield

Cano-Rios and Davis (1981) in a diallel analysis observed highly significant GCA and SCA for lint yield. Wilson (1991) observed significant gca and sca effects for lint yield. Sadykhova (1986) in a diallel cross of G. hirsutum observed over dominance for fiber yield. Tang et al. (1993) reported that sca effects were smaller and less significant than gca effects. Whereas, predominance of non-additive gene action was observed by Laxman and Ganesh (2003) and Deshpande and Baig (2003) in G. hirsutum crosses.

2.4 Stability analysis

2.4.1 Genotype and environment interactions and stability analysis

Genotype and environment interaction is of universal occurrence for any quantitative character. A specific genotype does not exhibit the same phenotypic characteristics under all environments and different genotypes (G) respond differently to a specified environment (E). To overcome this situation Allard and Bradshaw (1964) have suggested the significance of G x E interaction on the basis of the relative magnitude of different variances estimated from multilocation tests conducted over years.
A dynamic approach to the interaction of varietal adaptation to varying environments was developed by Finley and Wilkinson (1963). It led to the discovery that the components of a genotype and environmental interactions were linear in relation to environmental effects. The above technique was improved further by Eberhart and Russell (1966) by adding another stability parameter i.e. deviation from regression. They defined stable varieties as those having high mean yield, a regression coefficient $b_i=1$ and deviation from regression as small as possible ($S^2_{\text{di}}=0$). This method is used to select stable genotypes that interact less with the environment in which they are to be grown.

Eberhart and Russell (1969) compared the stability of single cross and double cross hybrids of maize. Few single crosses were as stable as any of the double crosses were identified. Although single crosses differed in their ability to respond to more favorable environments, the most important stability parameter appear to be the deviation means square. Since all types of gene action appear to be involved in this stability, potentially useful single crosses must be evaluated over a wide range of environments to identify stable, high yielding single crosses for commercial cultivation.

Lynch et al. (1973) studied the relative stability performance of single cross, three way cross and double cross corn hybrids recommended in Ontario between 1968-72. Yield stability of three hybrids types is described by two parameters, a regression coefficient and standard error for this regression. There were no differences in yield stability between three types of hybrids.
Nizama and Patel (1989) studied the stability of 21 three-way, 9 single cross hybrids of cotton along with four checks grown in different environments. The pooled analysis of variance showed that there were highly significant differences among the 34 cotton hybrids for lint yield indicating the experimental hybrids were significantly diverse with respect to lint yield. The two three-way hybrids (ICHB-7 and ICHB-4) and one single cross check hybrid (JKHy-1) possessed comparatively higher phenotypic stability.

Shroff et al. (1989) compared the phenotypic stability of 30 male sterile based single and three way hybrids of cotton. Stability analysis revealed that mean squares for genotype x environment (linear) and pooled deviations were significant for seed cotton yield, number of bolls per plant and boll weight. Three hybrids ICHB-3, ICHB-9 and ICH-24 were characterized as stable hybrids.

Basu (1996) reviewed the current genetic research on cotton in India. In his review highlighted the importance of three way crosses in producing more stable hybrids. Pavasia et al. (1999b) evaluated 28 hybrids along with their parents in three different environments. The pooled analysis of variance showed there were highly significant differences among the 36 genotypes for all the characters. Environmental variances and G x E interactions were found to be significant for all the characters.

Patel et al. (2000) studied the stability of 9 genotypes of cotton developed through multi spices crosses for yield in four distinct environments. Highly significant mean squares due to environment (linear) revealed that the genotypes were differed significantly. Mean squares due to pooled deviation
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were significant suggesting that variation in performance of genotypes over four locations was due to unpredictable factors. GISV-69 appeared to be most stable genotype as it showed high mean, unit regression and least deviation from regression. GISV-33 and GISV-85 recorded high mean, but significant non-linear components indicating unstable and unpredictable behavior.

Pavasia et al. (2000) conducted experiments on eight parental lines and 28 hybrids in replicated trial in three environments. Mean squares due to genotype and environments were significant when tested against pooled error for seed cotton yield, ginning per cent and fiber fineness, suggesting the presence of variation among genotypes as well as environments. G x E interactions were significant for all characters. Pooled deviation (non-linear component) variances were significant for all the characters except ginning percentage, suggesting importance of both linear and non-linear components for seed cotton yield per plant. Eleven hybrids were found to have wide adaptability for seed cotton yield per plant. Among them hybrid G.COT-10 x B.N. showed stability for ginning percentage and seed cotton yield.

Studies made on phenotypic stability of six G. arboreum genotypes for five consecutive seasons by Singh et al. (2004) indicated significant varietal differences over all the years and also in the pooled analysis. None of these varieties could give stable performance over the seasonal variations.

Siwach and Lather (2004) evaluated 24 genotypes in four environments. The pooled analysis of variance revealed that the mean differences between the genotypes and environments were highly significant.
indicating genotypes and environments were different from each other. Genotype GC-182 was stable for all the characters.

Campbell and Jones (2005) evaluated eight cultivars in 12 location year environments. G x E interactions were significant for all the agronomic traits measured, but clearly largest in effect for lint yield as the G x E sums of squares larger than the genotype component for lint yield. Genotype stability analysis revealed that genotypes performed dissimilar across the gradient of environmental indices for lint yield.

Tuteja (2006) studied the stability analysis of 12 intra-hirsutum hybrids for seed cotton yield, number of bolls per plant and boll weight. Based on the linear (bi), non-linear response (s²di) and high mean performance (x), CSHH-198, CSHH-238, CSHH-825 and Omshankar were found stable for seed cotton yield.

2.5 **In vitro pollen germination**

2.5.1 **Development of suitable in vitro pollen germination media (PGM)**

It has been realized recently that pollen/gamete play an important role in crop improvement (Shivanna, 1998; Ravikumar and Patil, 2002). *in vitro* pollen germination medium is essential for pollen selection and biotechnology.

Cotton pollen has proved to be recalcitrant to traditional *in vitro* germination and pollen tube growth protocols. Kearney and Harrison (1932) described the failure of *in vitro* techniques and went so far as to use the percentage of pollen grains that burst when placed in weak sugar solutions as
a measure of viability. Bronkers (1961) first described the reliable technique for \textit{in vitro} cotton pollen germination. Miravalle (1965) has reported that the pollen tubes grown in this media were short, the cytoplasm was cloudy and granular, and the process required 24 hours or longer. In 1972, Tailor described a medium that overcame many of the limitations outlined by Miravalle (1965). Taylor reported rapid pollen germination (2 to 3 h.), more normal appearing in cytoplasm and longer pollen tubes. Wauford (1979) further improved upon Taylor's medium and averaged 47 percent germination and 2.6 mm pollen tube lengths. Although Wauford's protocol was an improvement upon Taylor's medium, the 2.6 mm pollen tube length achieved \textit{in vitro} does not compare with the 20 to 40 mm tube lengths reported \textit{in vivo}.

A number of culture media and methods have been tried for the \textit{in vitro} germination and growth (Kearney and Harrison, 1932; Iyengar, 1938; Bronkers, 1961; Miravalle, 1965; Taylor, 1972 and Wauford, 1979). However, the media reported earlier were highly complex requiring a large number of chemicals and hormones. Further, none of the techniques reported uniformly high levels of germination in different genotypes of cotton. Recently, Burke \textit{et al.} (2004) modified the media described by Taylor (1972) and Wauford (1979) and achieved very high levels of pollen germination (71%). Although, they have simplified the media requirement by eliminating the need for some salts and hormones, the stringent micro environment (temperature, humidity, pH and Carbon source) was recommended to enhance pollen tube growth. Kakani \textit{et al.} (2005) reported cotton pollen germination of 20 to 60 per cent in improved pollen growth medium of Taylor (1972).
2.5.2 Variability in terms of germination percentage and tube length

Kumar and Sarkar (1980) reported significant correlation between pollen diameter and pollen tube length after three hours of \textit{in vitro} growth. This effect was seen only beyond the initial period of germination. However, there was a non-significant correlation between pollen tube growth rate and pollen diameter \textit{in vivo}. These observations indicate that, the influence of stylar genotype on pollen tube growth rate overwhelms any effect of pollen diameter. Once within the style, pollen tubes are subjected to a variety of influences other pollen tubes, inherent qualities of the style and stylar qualities developed in response to these and other pollen tubes. The result also stress on importance of \textit{in vivo} study even though \textit{in vitro} pollen germination studies are valuable in elucidating mechanisms (germination capacity and rate, tube growth rate) associated with genetic differences in male transmission.

Pfahler \textit{et al.} (1997) determined percentage germination and tube length of five diverse sesame (\textit{Sesamum indicum}) genotypes, at different time interval after inoculation on a semisolid medium adjusted to pH-7. Overall, five per cent germination was reached with 30 minutes after inoculation and 37 per cent after 120 minutes with no significant increase thereafter. As indicated by the highly significant genotype x time after inoculation interaction, genotypes differed in the time at which germination was initiated and maximum germination attained. Average tube length was 91 \textmu m after 30 minutes of inoculation and reaching maximum of 1000 \textmu m after 300 minutes of inoculation. There was no significant relationship between percentage germination and tube length among the genotypes. These results indicated that genetic differences among genotypes are present for \textit{in vitro} germination.
capacity, germination rate and tube growth rate and these factors singly or in combination can alter male transmission of genetic elements.

Varghese et al. (1997) observed significant variation among the five Dendrobium hybrids with respect to pollen germination and pollen tube growth. Tejaswini (1999) reported that Dianthus chinensis had higher germination percentage compared to Dianthus caryophyllus. However, D. caryophyllus had longer tube length compared to that of Dianthus chinensis. Kakani et al. (2002) observed significant variation for in vitro pollen germination and pollen tube growth of groundnut genotypes to temperature.

Burke et al. (2004) recorded high pollen germination tube elongation in G. hirsutum under stringent microenvironment conditions. Whereas, Pandey and Dagaonkar (2004) observed pollen germination of 10 to 40 per cent with tube length range of 5 to 8 mm and Polysiphonous pollens in G. arboreum genotypes. In similar in vitro studies Kakani et al. (2005) and Liu et al. (2006) reported genotypic variations for pollen germination and tube growth in G. hirsutum genotypes for cardinal temperatures.

2.6 DNA finger printing

The verification of varietal identity is an important element in seed quality control and rigorous genetic purity tests have to be applied to maintain supply of genetically pure seeds. Presently grow out test has been followed to verify the genetic purity of varieties and hybrids that involves time, money and effort (Smith and Smith, 1992). The morphological characters have been used for varietal identification and verify the crosses. But, their ability to provide reliable discriminating identification is more cumbersome (Patterson
and Whatherup, 1984 and Smith et al., 1991). To have an accurate and reliable estimate of genetic relationships, there is a need of polymorphic molecular markers. Therefore, RAPD markers have been successfully used for the estimation of genetic similarities and cultivar's analysis of various plant spices.

In cotton Multani and Lyon (1995) showed that RAPD markers could distinguish very closely related varieties. The classification of cotton varieties based on RAPD markers and morphological characters produced similar results (Tatineni et al., 1996). RAPD marker analysis of G. hirsutum and G. arboreum varieties revealed that the inter-varietal genetic relationships of several varieties were related to their center of origin (Iqbal et al., 1997). Patil et al. (2000) showed that RAPD marker could be used as a reliable indicator as compared to morphological marker for identification of restorer lines of cotton. RAPD markers were also utilized for hybrid seed genotyping in cotton (Ranade et al., 2000, Vamadevaiah et al., 2004, Hanchinal et al., 2004 and Mehetre et al., 2005).

The review on development of male sterile based hybrids emphasis the fact that G. harknessii source of cytoplasm used in CGMS hybrid has not yielded potential hybrids. There are indications of lack of compatibility between cytoplasm and nucleus and problems associated with improper restoration of male sterility. Though some instances of CGMS based hybrids being more potential than checks are reported such studies have not led to development and release of potential CGMS based hybrids. Visualizing the present situation, the defect of harknessii cytoplasm, the lack of diversity in R
lines, emphasis was laid on developing new potential restorer lines based on sterile cytoplasm. This helps in correctly assessing the restoration status and in developing potential A x R crosses. Realizing the need for developing more potential male sterile lines, attempt was made to utilize lines derived from maintainer populations with the help of such maintainer lines new A lines were developed and used in the study. With the help of potential varieties involved in multiple crosses new maintainer lines were derived and these were utilized in present study as B lines.

The potentiality of these new A lines, B lines and R (restorer) lines were assessed by developing groups of A x R and B x R crosses. These hybrids (A x R and B x R) were assessed for their performance across different environments to determine their stability of performance.

Genotypic differences exist for different gametophytic traits, however the available literature shows that, these differences have not been studied. This may be because of lack of standard media and protocol for gametophytic studies. In the present study attempt was made to develop new media compositions by assessing pollen germination and growth on these media compositions for cotton pollen studies was identified.

In the recent years great emphasis is laid on developing markers for identifying genotypic differences. The review indicates that among different approaches RAPD analysis is ideal. Hence in this study an attempt was made to distinguish hybrids and their parents based on RAPD analysis.
Encompassing these aspects of research, objectives were framed in this study entitled as “Genetic Studies on New Sterile (A), Maintainer (B) and Restorer (R) Lines Developed Through Recombination Breeding in Cotton Gossypium hirsutum (L.)”. 