Chapter 2

Literature Review

Rice is classified into Family Poaceae which is herbaceous or non-woody plant, almost annual plant, monocotyledon and adventitious root system. Rice is in genus *Oryza* and has an ability to grow in every continent except Antarctica (Chandler, 1979). There are 22 species under this genus, consisting of 20 species in wild rice and 2 species in cultivated rice. The cultivated rice are as Asia rice (*Oryza sativa* L.) that can be grown in Asia, Africa, South America, Europe and Australia and African rice (*Oryza glabberima*) group that is grown only in Southern Africa. Cultivated Asia rice are classified into types, based on both agronomic characters and distribution of growing areas, as follow:

1. Japonica type: Japonica type described as dwarf, narrow and dark green color leaves, round short grain, hard threshing cultivated rice plant. Mostly cultivated in Japan, Korea and northern China.
2. Indica type: Indica type described as tall stature, broad long - grain, cultivated area is in India, Thailand, Sri Lanka, Bangladesh and Philippines.
3. Javanica type: Javanica type described as tall stature, broad greenish leaf round large grains. It is planted only in Indonesia.

Asia produces and consumes about 90% of the world's rice (Rothschild, 1998). The global rice production must reach 800 million tons from the present 585 million tons in 2003 to meet the demand in 2025. Because irrigated rice contributes more than 75% of the total rice production, enhancing its yield potential would be a key to the global rice requirement for an additional 215 million tons (Virk *et al.*, 2004). Therefore, the average yield of irrigated rice varieties must increase in tropical rice land from 5 to 8.5 tons/hectares (Peng *et al.*, 1999). However, hybrid rice which expresses heterosis in terms of yield potential is a tool leading to yield improvement in irrigated area.

2.1 Hybrid Rice

Hybrid rice means rice crop grown from F1 seeds of a cross between two genetically-dissimilar parents (Virmani *et al.*, 1997). F1 plants will better perform the hybrid vigor or heterosis, especially higher yield, than pureline (Virmani, 1994). Hybrid rice has 20-30% yield advantage over modern semi-dwarf inbred varieties (Virmani, 1994 and Yuan and Wu, 2004). About 50% of total rice growing area in China or around 15 million hectares cultivate hybrid rice. The average yield of hybrid rice was up to 7 tons/ hectare (Yuan and Wu, 2004).

2.2 Heterosis

The term heterosis, often used synonymously with hybrid vigor, refers to the superiority of the F1 hybrid over its parents. Virmani (1994) expected that heterosis in hybrid rice can express, in terms of yield ability, higher than pure line about 20 - 30%.

However, heterosis can be used as a parameter of F1 progeny evaluation in rice hybrid program because heterosis expresses the higher or lower (positive or negative) value when compared with their respective parents (Virmani, 1994).

2.3 Genetic Basis of Heterosis

Dominance effect: According to dominance hypothesis, heterosis is due to the role of more-favorable dominant genes in a hybrid than in either parent

Overdominance effect: This hypothesis supposes that heterozygotes (Aa) are more vigorous and productive than either homozygotes (AA or aa). This has been proven in traits controlled by single or few genes. Heterozygotes perform a given function, over a range of environments, more efficiently than either homozygote.

Epistatic effect: The recent research indicated that non-allelic interaction is also a common contributor to heterosis. This model emphasizes that heterosis is a multilocus effect rather than the effect of loci acting singly (Yuan *et al.*, 2003).

2.4 Floral Biology of Hybrid

Although rice is a self-pollinating crop, outcrossing from 0 to 6.8% has been observed in some of the varieties under certain conditions. A significantly higher rate of outcrossing has been observed in wild rice such as *O. sativa f. spontanea* (50%), *O. longistaminata*, and *O. perennis* (up to 100%). The rate of natural outcrossing on cytoplasmic male sterile lines is of practical importance, as commercial seed production depends primarily upon this trait. A wide range (14-53%) of natural outcrossing has been recorded on cytoplasmic male sterile lines (Athwal and Virmani, 1972; Azzini and Rutger, 1982; Xu and Li, 1988). Generally, there are some factors concerning the spikelet opening one day after the panicle come out, low temperature delays the opening of the anther and pollen from the anthers have to reach the stigma and unite with the egg inside the ovary for the ovary to develop into a grain (Vergara, 1979).

There were relationships of floral biology to panicle emergence, sequence of blooming in a panicle, duration of blooming, opening and closing of spikelets, period of maturity of stamens, number and position of anthers, anther dehiscence, pollen load, viability of pollen, period of maturity of pistils and stigma receptivity (Ahmed and Siddiq, 1998).

Vergara (1979) described the flowering order of a panicle as follow: spikelets on the top of branches open first, the lower spikelets open last, and are usually found in some large panicles.

High daytime temperature, high solar radiation and low night temperature promote panicle production. More panicles would mean a greater number of available spikelets. It is believed that a compact panicle would lead to lower cross seed set, compared to the intermediate or open type where florets are expected to need more space for proper opening and have a wider angle of opening which, in turn, facilitates higher outcrossing. Incomplete exsertion leads to reduced seed set (Ahmed and Siddiq, 1998).

2.5 Sequence and Duration of Blooming

Blooming in a panicle is in nearly-fixed order. Spikelets on the top branches open first and those on the lower branches last. The middle spikelets open from the bottom to the top, and the uppermost-second spikelet opens last. Flowering occurs in both completely-and incompletely-emerged panicles on the day of their emergence. The floret opening is higher in fully-emerged panicles whereas it is less in panicles which are not fully exserted out of the flag leaf (Vergara, 1979). Thus, full panicle exsertion is a useful trait in increasing outcrossing. The floret opening is influenced to a great extent by the prevailing weather conditions just before flowering. The optimum temperature is 30 to 35°C, and critical temperatures are about 15 to 20°C in temperate conditions and 22 to 35°C in the tropics. Low and high temperatures cause delayed flowering. The relative humidity of 70-80% under optimum temperature conditions is congenial (Vergara, 1979; Ahmed and Siddiq, 1998).

2.6 Stamen Characteristics

The rice flower differs from the flowers of other cereals in having six stamens. About 6 days before heading, the pollen grains are mature and the flag leaf sheath swells, which is an indication of the booting stage. The filament elongates immediately after floret opening and brings the anthers to the level of the stigma. Most of the pollens are shed at the beginning of anther exsertion, and the anthers are almost empty when exserted. After about 20 to 30 min., the anther withers out and the spikelet closes, leaving the stamens sticking out from the seams of lemma and palea. Therefore, in the context of increasing pollen dispersal, the dehiscence within the floret should be minimized, while the residual pollen for anthers outside the floret should be maximized.

The total number of pollen grains per anther is reported to be directly correlated with anther size, which generally varies for 0.9 to 5.4 mm. The number of pollen grains per anther (Y) with known anther length (X) is accurately estimated using the formula ($Y = 1172 + 1277X$) (Ahmed and Siddiq, 1998) but this relation becomes biased by temperature lower than 20°C during the anther differentiation stage. However, percent residual pollen per exserted anther is independent of environmental conditions (Virmani and Kumar, 2004).

The pollen load influences outcrossing to a great extent. Maximum pollen load is found just below the panicle level, lower concentrations are found up to 25 cm below the panicle level, and it is drastically reduced above the panicle level of the pollen parent. Therefore, for maximum outcross seed set, the pollen parent should be a little taller than the seed parent. Normally, two to three pollen grains are required per stigma to fertilize one egg cell. Under natural conditions, viability of pollen grains is only for 3 to 5 min. Air-borne pollens at 1.5 or more per litre with a wind velocity of 2-3 m/s are necessary to obtain more than 50% seed set in CMS seed parents. The number of air-borne pollen is correlated with the number of flowering spikelets per unit area per day. In most of the cultivars, the pollen is shed from the anthers before they are exserted out of the spikelets. Therefore, the most desirable lines would be those in which part of the pollen is shed after they are exserted out of the spikelets. In other words, the number of residual pollen per exserted anther is most important. The number of air-borne pollen per litre is correlated significantly (r=0.87**) with wind velocity during flowering time and is also correlated directly (r=0.92**) with seed set percent of the CMS line (Ahmed and Siddiq, 1998).

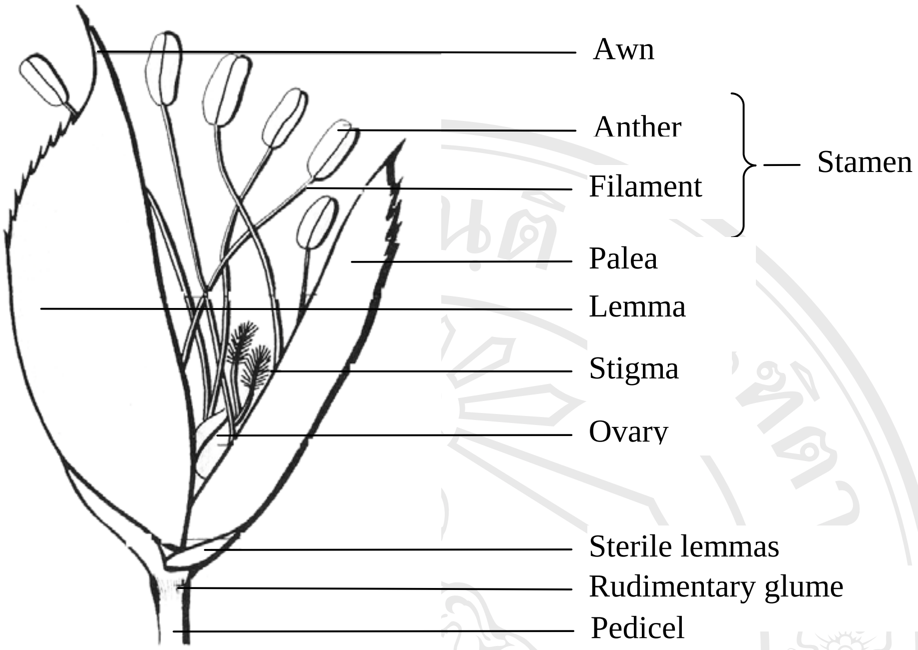

Figure 2.1 The spikelet of rice (Vergara, 1979).

2.7 Stigma Characteristics

Longer and exserted stigma, longer stigma receptivity and wider angle of floret opening promote outcrossing. Floret opening angle varies among cultivars from 25° in long, slender spikelets to 35° in short, coarse spikelets. The floret opening angles have a positive and significant correlation (0.36*) with stigma exsertion percentage.

Stigma exsertion, large stigmatic area and its receptivity all play a major role in determining high seed set in the CMS parents. The longer the pistil length, the greater will be the stigma exsertion. A high frequency of exserted stigma facilitates stigma reception of naturally-outcrossed pollen grains and enhances seed set percent. Receptivity of stigma is maximum during the first 3 days after opening of the spikelets, and then is gradually lost after 7 days (Virgara, 1979).

2.8 Extent of Heterosis

The literature of heterosis in rice has been reviewed by Kim and Rutger (1988) and Virmani (1994). Significant heterosis for the yield and yield components have been reported from several countries, which indicated significant heterosis for yield ranging from 2 to 369% in rice (Virmani *et al.*, 1981a). However, these reports on heterosis have limited scope and application as they were based on few crosses involving unadapted parents, and evaluated on a very limited scale. Besides, heterosis was often calculated on mid-parental values of unadapted parents. Standard heterosis for yield obtained over best commercial inbred line on a large scale, which is of interest to a plant breeder, was seldom reported.

Success of hybrid rice in China in the late 1970s prompted elaborate attempts to quantify the commercially-exploitable hetherosis (Byeong *et al.*, 1985; Bollich *et al.*, 1988; Yuan and Virmani, 1988).

In the majority of studies, the higher yield resulted from the incremental heterosis for more than one yield component (Subramaniam and Rathinam 1984), mainly panicle and spikelet number (Sanini *et al.*, 1974; and Devarthinam, 1984). Significant heterosis was also reported for plant height, days to flowering, dry matter production, harvest index, root characteristics, photosynthesis, respiration and several other characters. Hybrids generally were equal to or taller than the parents (Virmani *et al.*, 1982), though a few reports suggested negative heterosis for height (Singh and Singh, 1978). Hybrids were also earlier in flowering (Mallick *et al.*, 1978; Young and Virmani, 1990) and possessed longer growth duration (Deng, 1988). Although there is no unanimity on the subject, physiologically-superior parents showed significant heterosis for total dry mater (Virmani *et al.*, 1981a), which was correlated to higher yields of the hybrids. Significant heterosis for harvest index and its correlation with yield was also reported (Yoshida *et al*., 1972 and Virmani *et al*., 1981b). Attempts to explain greater biomass production of hybrids in terms of increased photosynthesis are inconclusive (Virmani, 1994), with both positive and negative reports.

2.9 Cytoplasmic Genetic Male Sterility

Athwal and Virmani (1972) developed a cytoplasmic male sterile line by substituting nuclear genes of Indica rice variety, Pankhari 203, into the cytoplasm of the semidewarf Indica variety, Taichung Native 1. However, this CMS line could not be used for breeding rice hybrids because of its instability, poor plant type and photoperiod sensitivity. The first CMS line used to develop commercial F1 rice hybrids was first developed in China in 1973 from a male sterile plant occurring naturally in a population of wild rice **(***Oryza sativa f. spontanea*) on Hainan Island in 1970. This plant was designated wild rice with aborted pollen (WA). Since then, a number of CMS lines have been developed in China and elsewhere from various wild and cultivated accessions (Lin and Yuan, 1980; Li and Zhu, 1988; Virmani and Wan, 1988). In China, about 300 strains of *O. rufipogon* were crossed as non recurrent female parents to a Japonica rice, Taichung 65, as the recurrent male parent. The cytoplasm type of every tested strain was determined according to the fertility of its progenies in BC_4 or more advanced generations. Pradhan *et al.* (1990) identified two new CMS sources among Indica cultivars, viz., “V20B” and “Sattari” in crossing with Japonica rice. It is interesting to note that “V20B” is a maintainer of CMS-WA cytoplasm but a source of cytoplasm with a Japonica rice cultivar. Obviously, it has a different source of cytoplasm.

2.10 Genetics of Fertility Restoration

Kitamura (1962) reported that high fertility in F1 hybrid of cytosterile TA 820 was controlled by recessive gene, combined with modifiers or polygenes. Shinjyo (1969) identified a single dominant fertility restoring gene (Rf) in rice cultivar Chinsurah BoroII and found that its effect was gametophytic in the male sterility-inducing cytoplasm (CMS-bo). Fertility restorers for WA cytoplasm were first identified in China during 1973. Since then several excellent restorers could be isolated for the different CMS sources in many countries of Southeast Asia. The frequency of restorer lines was found to be higher in rice germplasm originating at lower latitudes, compared to those originating at higher latitudes. Restorer frequency was much higher in indica as compared to japonica cultivars (Li and Zhu, 1988), and it ranged from 15-25% in elite indica breeding lines (Virmani and Edwards, 1983). In Asia, fertility restorers are mainly found in south-Asian countries and southern China, while maintainers are concentrated in northern China and far-eastern Asia (Li and Zhu, 1988). Restoration of fertility is not a constraint in developing commercial hybrids, particularly in indica rice. Japonica rice are, however, mostly non restorers, and the restorer genes in them need to be incorporated from indica rice through a long and tedious breeding procedure. Besides, japonica rice show weak heterosis.

Govinda Raj and Virmani (1988) reported on testing of allelism among six fertility-restorer lines (IR 26, IR36, IR54, IR 99761-19-1, IR2797-105-2.2-3 and IR 42) for CMS-WA which revealed the existence of four groups of restorers possessing different pairs of restorer genes. The existence of a large number of different genes for fertility restoration explains the greater frequency of fertility-restorer genes for the WA source among indica types.

Rice varieties of the japonica type, unlike those of the tropical indica type, do not possess genes for fertility restoration of CMS-WA. Fertility restoration for CMS-bo appeared to be simple, with a single dominant fertility-restoring gene being characterized (Shinjyo, 1969). Its effect was gametophytic in the CMS-bo cytoplasm. Shinjyo (1975) used 12 trisomic lines of rice to conduct linkage analysis for fertility-restoring gene for CMS-bo cytoplasm. The trisomic C was found to be critical for the *Rf* gene, all other 11 trisomics were found to be noncarriers for the *Rf* gene. Similar studies for CMS-WA have indicated that the two fertility restorer genes were located on chromosomes 7 and 10 (Bharaj *et al.*, 1995), the stronger (*Rf-WA-1)* being located on chromosome 10. It has also been possible to identify 6 RAPD markers, linked to another gene, for fertility restoration (*Rf-WA-3).* Three of these were mapped on chromosome 1 (Zhang *et al.*, 1997). These studies make it possible to tag fertility-restorer genes with molecular markers with the aim of practising marker-aided selection for fertility-restoring ability.

2.11 Male Sterility System

Male sterility means the abnormal function of plants that cannot generate or dispose their pollens as normal plants. Male sterility may be caused by genetical abnormality or environmental effects.

The following genetic and non-genetic male sterility systems are known for developing rice hybrids.

- Cytoplasmic- genetic male sterility
- Environmental-sensitive genetic male sterility
- Chemical-induced male sterility

2.11.1 Cytoplasmic-genetic Male Sterility

The male sterility is caused by the interaction of sterile and the recessive male sterile gene in the nucleus. But pistils of the CMS line are normal and can produce seed when pollinated by normal pollens. The male sterile line derived by:

Inter-varietal crosses derived by crossing between varieties that have different genetic background such as Indica with Japonica or crossing between traditional and exotic varieties.

Interspecific crosses derived by crossing between cultivated rice and wild rice to make the male sterility in progenies.

Yingheng (1988), studying on pollen abortion pattern, classified male sterile rice into four types:

1. Pollen-free is a level of male sterility in terms of abnormality of chromosome that causes to pollen mother cells as result of cell malformation and cannot develop to be pollens.

2. Uninucleate cells is male sterility that is found in uninucleus cell development. Being lack of protoplasm and nucleus, it is not possible to test by staining.

3. Binucleate cells is male sterility that appeares in binucleate cell development of pollen. The sterility is caused by no action of nutritive nuclei and reproductive nuclei.

4. Trinucleate cells is male sterility that appears in development of trinucleate cells of pollen. The abnormality with smaller reproduction nuclei and deformed nucleus wall, as a result of disability to development to pollen.

Horner and Palmer (1995) indicated that the mechanism of genetic male sterile can be found at every development stage of pollen cells. In sporogenous phase, the possibility to have a male sterile was about 8 to 29%. At generated microspore phase, the possibility to give male sterile was about 29%. At early–late pollen development phase, the percentage of male sterility was about 10%.

2.11.2 Environmental-sensitive Genetic Male Sterility

Environment sensitive genetic male sterile (EGMS) is an effective alternative to the CMS system. Maruyama *et al.* (1991) described in terms of thermo sensitive genetic male sterile (TGMS) and photosensitive male sterile (PGMS) that male sterility was caused by gene action which was affected by temperature and photoperiod. TGMS can be expressed when temperature reaches higher or lower than optimum.

Photoperiod-Sensitive Genetic Male Sterility: In 1973, Shi Ming Song found a male-sterile plant in the field of a Japonica rice cultivar Nong-ken 58 in Hubei province of China (Shi, 1981). It appeared to become male-sterile when plants headed under long day length and male-fertile when plants headed under short day length. The degree of male sterility was 99-100% at heading under artificial light of more than 14 h, but plants were male-fertile when grown under artificial light less than 13 h 45 min. (Lu and Wang, 1988). This male-sterile mutant was designated as Hubei-photoperiod-sensitive genetic male-sterile rice (HPG-MS). Further studies indicated that the critical stage of fertility transformation was the 1st or 2nd of September in Hunan (30-31°N, 30 m above sea level), i.e., when the plant headed from 5th of August to 1st or 2nd of September, it was male-sterile (99.5-100%), however, pollen sterility was reduced to 20% and seed setting ranged between 10-40% when plants of this mutant headed after 1st or 2nd of September. This behaviour of the mutant did not change by planting in another region. Pollen sterility during the sterile stage was stable but the degree of fertility was unstable, as it varied over locations and years (Lu and Wang, 1988). The trait was controlled by a recessive gene (Lu and Wang, 1988; Jin *et al*., 1988). Sano (1983) also identified a sterility gene (S_3) in F_1 hybrids of *Oryza glaberrima* and *O. sativa*, which was associated with photoperiod sensitivity.

The photoperiod-sensitive genetic male-sterility (PGMS) trait has been transferred to several Indica and Japonica rice cultivars in China by backcrossing. Rice hybrids developed by this male-sterility system are being evaluated in multilocation trials in China. By using PGMS in hybrid breeding, there is no need of a maintainer, therefore the cost of multiplying male-sterile line seed is reduced in hybrid seed production. Besides, any rice cultivar within a varietal group can be used as a male parent of the hybrid and there is no need to identify restorers. Thus, choice of parents in developing heterotic hybrids is broadened compared with the CMS system. The PGMS system can be used only in countries or regions where striking differences for day length exist. Chinese scientists believed that the two-line method of breeding hybrid rice, based on photoperiod-sensitive genetic male sterility, will be operational in China by 1992 (Virmani, 1994).

Thermosensitive Genetic Male Sterility: Maruyama *et al.* (1991) reported thermosensitive genetic male sterility (TGMS), a mutation induced by 20 kr of gamma rays in Japanese rice variety Remei. The male-sterile mutant, designated as H89-1, exhibited no seed set under 31/24°C, partial fertility under 28/21°C and complete fertility under25/15°C. Pollen sterility in this mutant was not changed by change in day length (viz., 15, 13.5, 12h). Lopez and Virmani (2000) created thermo-sensitive genetic male-sterile (TGMS) rice lines for developing two-line rice hybrids for the topics. The TGMS trait was transferred to a temperate Japonica TGMS mutant, Norin PL12, to indica and to tropical japonica rice varieties, using the pedigree selection procedure. Six new TGMS rice lines adapted to tropical condition were developed which showed complete pollen and spikelet sterility when maximum temperature was higher than 30^0C at 1-2 weeks after panicle initiation. However, up to 85.5% spikelet fertility was observed when these lines were exposed to 26-29^0C during the critical stage. Using the two TGMS lines, some heterotic rice hybrids showed 1-1.6 t/ha.

Behavior of this TGMS mutant has also been confirmed at IRRI. Like PGMS, TGMS can also be employed to develop rice hybrids, using two lines rather than three lines. While PGMS can be used in large countries showing striking differences in latitude but TGMS can be used in smaller countries closer to the equator where low temperature areas are available in the hills. Thus, TGMS can be utilized in tropical and subtropical areas.

The TGMS gene is being transferred to several Indica-Japonica derivative lines in order to utilize these for developing rice hybrids. Both PGMS and TGMS systems have the following advantages:

1. There is no need to develop a CMS line by backcrossing.
2. Hybrids can be developed without looking for restorer genes.
3. PGMS and TGMS lines are easier to multiply than CMS lines.

2.11.3 Chemically-Induced Male Sterility

Male sterility in crop plants is also induced by chemicals known as male gametocides, pollen suppressants and chemical emasculations. Use of male gametocides obviates the need for a genetic or cytoplasmic genetic male sterility system. It also does not require any special scheme for the development of male-sterile and restorer parents, and the maintenance and increase of parental seeds are simple (Virmani, 1985).

An ideal male gametocide should: (1) selectively induce pollen sterility without affecting female fertility, (2) be systemic or sufficiently persistent to sterilize both early and late flowers on the same plant, (3) should have no mutagenic effects, (4) have a reasonable broad “window” or target period of application to overcome the effects of adverse weather conditions and variable crop growth, to permit treatment of large hectarages, and (5) have no undue hazard either to man or plant.

A number of chemicals have been found to induce male sterility in crop plants including rice (Virmani, 1985). These include naphthalene acetic acid (NAA), RH 531 [sodium-1 (p-chlorophenyl)-1, 2-dihydro-4, 6-dimethy-2, oxonicotinate], maleic hydrazide, gibberellins, FW450 (sodium alpha beta-dichlor osisobutyrate), ethrel (2-chloroethyl phosphonic acid) and methyl arsenate salts (Zinc methyl arsenate, sodium methyl arsenate).

Perez *et al*. (1973) tested ethrel and RH 531 as foliar sprays at concentrations ranging from 500 to 8000 ppm and from 100 to 1000 ppm, respectively, on three rice varieties. The chemicals were applied at four growth stages, viz., pre-booting, early-booting, mid-booting and late-booting. The pre-booting and early-booting stages appeared to be a more suitable time for applying ethrel or RH 531 than the later stages. Ethrel-treated IR8 plants gave a maximum of 67% pollen sterility of 99% when this chemical was applied once at the pre-booting stage with 100 ppm concentration. Both chemicals, however, caused 90-100% ovule sterility.

Parmar *et al.* (1979) induced up to 90% pollen sterility by applying 6000-8000 ppm ethrel one week earlier to the boot leaf stage or by applying two sprays of 4000-6000 ppm one week before the boot leaf stage, and the other at boot leaf stage. The anomaly in the two reports with regard to the ethrel concentration inducing the highest pollen sterility could be due to the fact that the two groups of researchers might have used different amounts of ethrel solutions in their experiments. In any case, both ethrel and RH 531 were considered unsuitable because they induced only partial male sterility and tended to affect ovule fertility.

Chan and Cheah (1983) also evaluated ethrel as a selective gametocide on rice, and reported 100% spikelet sterility in bagged panicles at 3000 ppm concentration; the unbagged panicles with the same treatment showed 45.7% spikelet fertility compared to 96.1% under control.

Shao and Hu (1988) pointed out that the mechanism of chemical emasculation by MG1 and MG2 has been studied at the South China Agricultural University, using a male gametocide labeled with ^{74}AS. Within 30 min of spraying, the male gametocide in panicles amounted to 0.001% of the total sprayed. The panicles accumulated more gametocide over time and 6 h after spraying, the amount of male gametocide reached 0.01% of the total. MG1 and MG2 content, expressed by specific radioactivity in pistil, stamens and lodicules within a spikelet, was in the proportion of 2:1:1.

2.12 Rice Hybrid Production

Generally, rice hybrids can be produced under 3 systems as follow:

1. Three-line hybrid: The F1 hybrid production consists of lines/varieties, cytoplasmic genetic male sterile line or CMS line (A line), maintainer (B line) and restorer line (R line).

CMS Line (A Line): The CMS line refers to a special kind of breeding line whose anthers are abnormal. No pollen or only abortive pollen exists in the CMS anthers, so no seed can be borne by selfing. The male sterility is caused by the interaction of the sterile cytoplasm and the recessive male sterile genes in the nucleus. But pistils of the CMS line are normal and can produce seeds when pollinated by normal pollens. A desirable CMS line should have not only good agronomic characters but also the following characteristics:

Stable male sterility, the male sterility (MS) should be inherited from generation to generation without any change in pollen sterility and it should not be influenced by environmental changes, especially temperature fluctuation.

Easy to be restored, this refers to two aspects: firstly, a proposed male sterile system should have a wider restoration spectrum so that the probability of selecting superior hybrid combinations is higher; secondly, the seed set of the restored hybrids should be high and stable and less influenced by adverse environments.

Good flora structure and flowering habits: the CMS line should flower normally and the daily flowering time should synchronize well with that of the male parent. Its stigma should be well developed and exserted after flowering. The glume opening should last longer and have a wider angle.

Maintainer Line (B Line): The maintainer line is a specific pollinator variety used to pollinate the CMS line and the progenies produced still show male sterility. Therefore the role of B line is to multiply the CMS line.

The major characteristics of a CMS line are determined by its corresponding maintainer line. In fact, the CMS line and its corresponding maintainer can be considered as “twin”. They are similar to each other in appearance, differing only in some characters as shown in Table 2.1.

Table 2.1 Differences between the CMS line and its corresponding maintainer line (Yuan *et al.*, 2003)

Characteristic	CMS line	Maintainer line
Tillering ability	Stronger, with a long tillering stage	
Heading date	2- 5 days later than the maintainer line	
Panicle	Basal part of the panicle is enclosed in the flag leaf sheath	Normally extended
Flowering habit	Daily flowering time is later and lasts longer	Flowering time is short and concentrated
Anther shape	Slender, thin, milky white or yellowish in color	Plump, golden in color
Pollen	a) Irregular in shape and unstained with I-KI b) Round and unstained with I-KI c) Round and slightly stained with I-KI	Round and dark blue when stained with I-KI

Restorer Line (R line): The restorer line is a pollinator variety used for pollinating the CMS line to produce F1 hybrids that become normal in fertility and thus can produce seeds by selfing.

As an elite restorer line, it should have: a) strong restoring ability, i. e., the seed set of its F1 hybrids should be equivalent to that of a normal variety; b) good agronomic characteristics and combining ability; c) well-developed anthers with heavy pollen load, good flowering habits and normal dehiscence.

Most of the male sterile lines used in commercial production nowadays belong to CMS or three-line system. In this system, the male sterility is controlled by both cytoplasm and nucleus, i. e., the interaction between the sterile genes in the cytoplasm and recessive sterile genes in the nucleus. However, the dominant nuclear gene, R, can restore fertility in the F1 hybrids with male sterile cytoplasm. The genetic constitution and the relationships between the A, B and R-lines are shown in Figure 2.2.

The genotypes of the CMS line, maintainer line, restorer line and F_1 hybrid are S (rr), N (rr), N (RR)/S (RR) and S (Rs), respectively. Because the cytoplasm only comes from the female parent, the progenies of A/B are of S (rr) genotype, which is the same as the CMS line, and they exhibit male sterility accordingly. In the cross of A/R, their F_1 genotype is S (Rr). Since the restoring gene is dominant, fertility is recovered in F_1 plants.

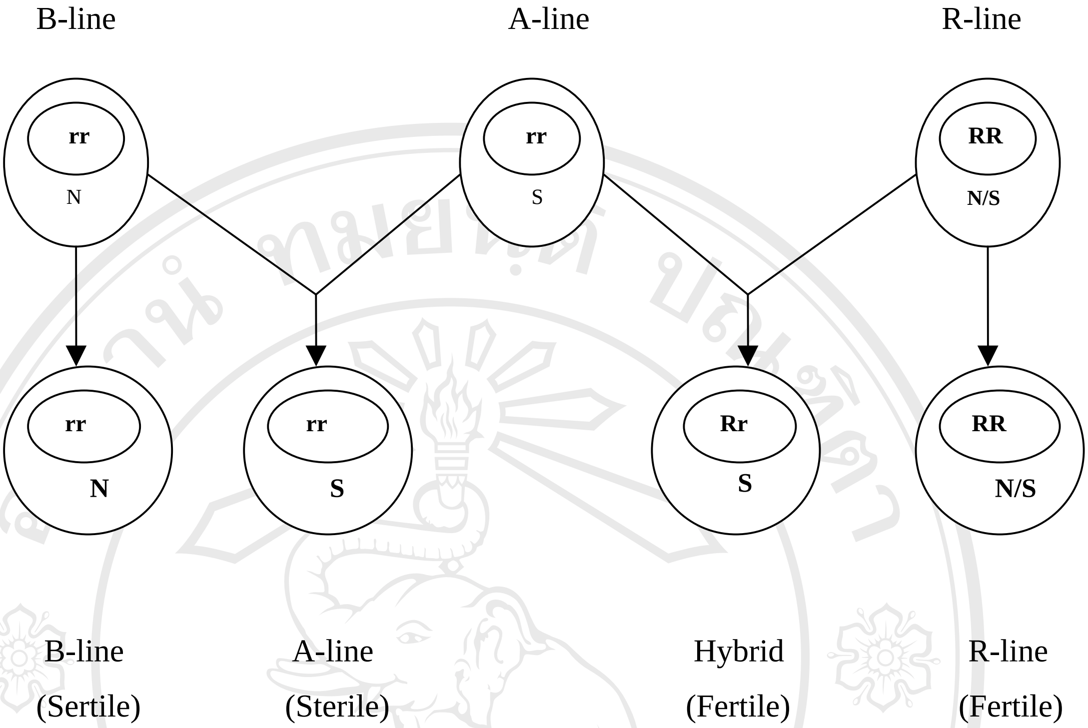

Figure 2.2 Presence of certain dominant restores gene (s) in the nucleus makes a line capable of restoring fertility in the hybrid derived from it and a CMS line (Yuan *et al.*, 2003).

2. Two-line hybrid: the F1 hybrids can be produced from crossing between (normal line or male fertile line) restorer line (R line) and male sterile line (A line), particularly, the male sterility of female parent is controlled by temperature and /or photoperiod. Diagram of hybrid produced by two-line hybrid is shown in Figure 2.3.

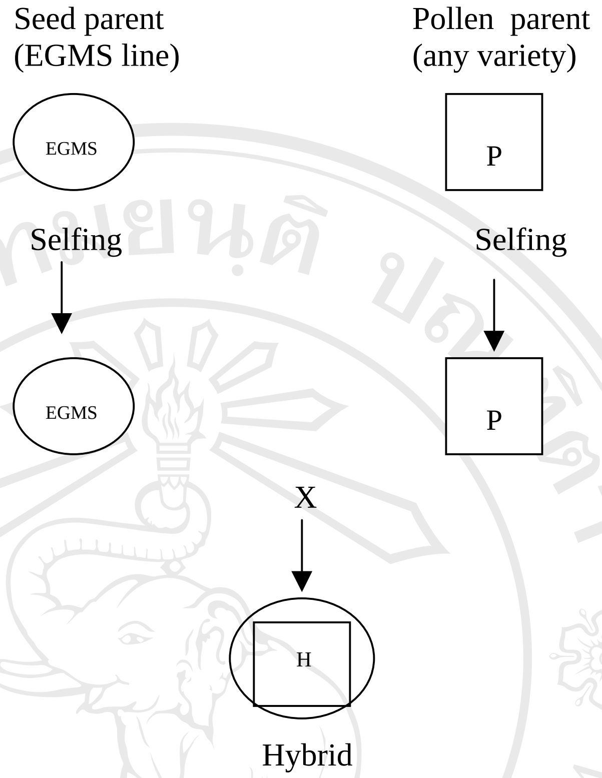

Figure 2.3 Schematic description of the use of EGMS line for developing two-line hybrids (Virmani, *et al.*, 1997)

3. One-line method: Apomixis or asexual seed production is the ultimate genetic tool for developing true breeding hybrids with permanently-fixed heterosis. Appropriate application of apomixis in rice would provide the breakthrough needed for most of the world's rice farmer to economically capture the increased yields of hybrids. Development of apomictic rice will require biotechnological research. Pathway of apomixes seed production is presented in Figure 2.4.

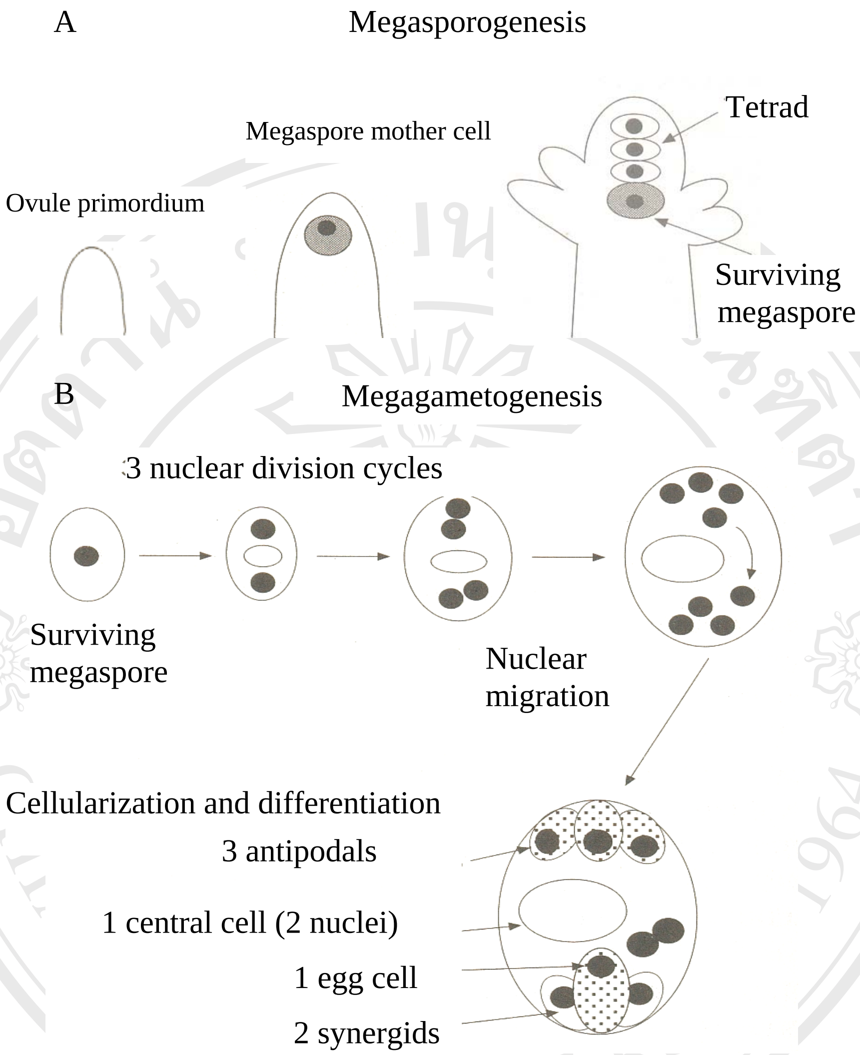

Figure 2.4 Megasporogenesis and megagametogenesis in *Arabidopsis.* (A). A single megaspore mother cell differentiates in the nucellus and undergoes meiosis to produce four megaspores. Only the most chalazal one survives and gives rise to a megagametophyte of the *Polygonum* type. (B) Development of the megagametophyte. The surrounding sporophytic tissue of the ovule is not shown. Three free synchronous nuclear division cycles produce an 8 nucleated embryo sac. Cellularization produces the seven cells of the mature female gametophyte (Grossniklaus *et al.,* 1998).

2.13 Breeding CMS Lines with a High Outcrossing Rate

Yuan *et al.* (2003) proposed this to get a high yield in hybrid rice seed production because the yield level in seed production of hybrid rice is mainly dependent on the outcrossing rate which can be improved by breeding CMS lines with improved floral traits, a better flowering behavior and some other better morphological characteristics as follow:

- Stigma with a long style which exserts and remains outside the glumes after anthesis.
- Longer and very feathery stigmas.
- Stigmas with physiological vigor for a longer duration of pollen reception.
- A wide angle (40-50°) and long duration (more than 1 hr) of glume opening.
- A loose plant type and a short, narrow and horizontal flag leaf with an angle facilitating pollen deposition on the stigmas.
- An early anthesis time and daily centralized flowering time.

Generally speaking, a CMS line is almost identical to its corresponding B line except for its male sterility. So to develop a CMS line with a high outcrossing rate, it is very important to develop a desirable maintainer with most the characteristics for a high outcrossing rate which are already mentioned before.

A few characteristics such as a wider angle and longer duration of glume opening are fully expressed only in CMS lines because of the effect of the sterile cytoplasm. The less-centralized daily flowering and higher unopened spikelets are also easily found in CMS lines. So for these characteristics, close attention should be given to selection of individual CMS plant.