A. Introduction:
1. Definitions
   (a) General - a hybrid is the first generation progeny of a cross between two genetically different plants.
   (b) Legal - the first generation seed of a cross produced by controlling the pollination and by combining (1) two or more inbred lines (2) one inbred line and/or a single cross with an open pollinated variety (3) two varieties or species, except open pollinated varieties of corn.
   (c) Inbred line (pure line) - a genetically homozygous genotype of a species which is developed as the result of self-pollination (inbreeding) and selection for specific characteristics.
   (d) Isogenic lines - any two or more inbred or pure lines which are for practical purposes genetically identical except for one characteristic.
   (e) Single-cross hybrid - the first generation progeny \( (F_1) \) of a cross between two genetically different inbred or pure lines; not crosses between isogenic lines.
   (f) Three-way hybrid - the first generation of a cross between an inbred or pure line and a single cross hybrid.
   (g) Double-cross hybrid - the first generation progeny of a cross between two single cross hybrids.

2. Hybrid varieties have certain potential not possible to obtain with pure line or open-pollinated varieties.

B. Requisites for hybrid seed production
1. Breeders responsibilities
   (a) Develop inbred lines
   (b) Identification of specific parental lines
   (c) Develop system for pollen control

2. Major problems for breeders & producers
   (a) Maintenance of parental lines
   (b) Separation of male and female reproductive organs
   (c) Pollen exchange
3. Genetically it makes no difference which parental line is used as the male or female; but seed producers must consider the following characteristics of parental lines.

<table>
<thead>
<tr>
<th>Female Parent</th>
<th>Male Parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>High seed yield</td>
<td>Good pollen production</td>
</tr>
<tr>
<td>Good seed characteristics</td>
<td>Long shedding period</td>
</tr>
<tr>
<td>Pest resistance</td>
<td>Plant height</td>
</tr>
<tr>
<td>Male sterility</td>
<td>Fertility restoration</td>
</tr>
<tr>
<td>Lodging resistant</td>
<td></td>
</tr>
</tbody>
</table>

C. Basic procedures for hybrid seed production
1. Development and identification for parental lines
2. Multiplication of parental lines
3. Production of single crosses (maize, Figure 1)
   (a) Planting ratios
   (b) Planting date(s)—“nick”
4. Production of double cross hybrids (maize only, Figure 2)

D. Use of male sterile and restoration factors
1. Male sterility does not occur frequently in natural populations because an isolated male sterile plant can not reproduce itself i.e. male sterility is a dead-end in nature.
2. Male sterility is very useful in both the development of superior genotypes (plant breeding) and seed production of hybrid varieties.
3. Control of male sterility
   (a) Genetic
      (1) Usually determined by a single gene "Ms" where the homozygous recessive "msms" results in male sterility.
      (2) Stability often influenced by environmental conditions and/or modifier genes.

\[
\begin{align*}
\text{ms ms sterile} & \times \text{Ms ms fertile} & \text{Offspring} \\
50\% \text{ MS ms fertile} : 50\% \text{ sterile} \\
\text{ms ms sterile} & \times \text{MS MS fertile} & \text{All MS ms fertile} \\
\end{align*}
\]

(b) Cytoplasmic
   (1) Sterility is determined by the cytoplasm of the female parent. Remember the cytoplasm is derived almost entirely from the female gamete.

\[
\begin{align*}
\text{♀} & \times \text{♂} \\
100\% \text{ ○ S} \\
\end{align*}
\]
Figure 1. Single-cross hybrid seed (A x B) production of maize.
Figure 2. Production of double-cross hybrid seed of maize.
(2) Usually more stable under varied environments than genetic sterility.

(3) Cytoplasmic male sterility is most useful when fruit or seed are not desired i.e. flowers, onion, potato. Non-fruiting plants bloom over a longer period of time and the flowers remain "fresh" longer.

(4) Useful in hybrid seed production only when a source of pollen can be supplied.

(c) Cytoplasmic - genetic

(1) Offspring from male sterile plants are not necessarily sterile although the cytoplasm is sterile. This is due to the presence of genetically controlled restorer factor, "Rf".

(2) C-G male sterility permits the same genotype to exist in three different forms:

\[
\begin{array}{ccc}
\text{sterile} & \text{fertile} & \text{fertile} \\
\text{RFrf} & \text{RFrf} & \text{RF} \\
\text{S} & \text{F} & \text{or} \\
\end{array}
\]

(d) Problems encountered with use of male sterility in hybrid seed production.

(1) Modifier genes influence the precision of genetic male sterility systems and these modifiers are sometimes lost during the transfer of the "Ms" gene into other lines.

(2) May maintain unwanted genes or cytoplasmic factors. Example Tms and susceptibility to southern corn leaf blight.

(3) Failure to properly identify and/or utilize sterile, normal, restorer or maintainer lines.

E. Use of male sterility in hybrid seed production

1. Keep in mind (1) that cytoplasmic male sterility is carried only through the female parent, therefore, the state of the cytoplasm is immaterial in the immediate cross since it will appear normal (fertile) in the presence of the Rf gene, (2) genetic fertility genes are dominant over all sterility controlling factors.

2. Maize

(a) Plant breeders develop inbreds but seed technologists often must maintain normal, genetic sterile, cytoplasmic sterile, restorer lines.

(b) Normal and restorer lines can be maintained by planting in isolation or by hand pollination.
(c) Sterile lines must be maintained by crossing isogenic male sterile and male fertile lines in isolated crossing blocks.

(1) Seeds from the female rows of field using cytoplasmic sterility will all produce male sterile plants.

(2) Seeds from the female rows of fields using genetic sterility will produce offspring that are all fertile or 1/2 fertile and 1/2 sterile, unless the fertile plants are removed. For this reason genetic sterility is not of great practical importance in the production of hybrid maize.

(d) Production of single crosses for double-cross hybrid and production.

(1) Single crosses to be used as the female parent of a double cross (Figure 3).
   - female rows should be male sterile.
   - male rows should be male fertile but without restorer.

(2) Single crosses to be used as the male parent of a double cross.
   - female rows should be male sterile.
   - male rows should be male fertile with restorer.

(e) Double cross production (Maize Figure 4)

(1) Restorers available
   (a) Female rows will be - rf rf  S - sterile
   (b) Male rows will be Rf rf  S - fertile
   (c) Seed produced will be
       \[ \frac{1}{2} \text{[rf rf] S} - \text{fertile} \]
       \[ \frac{1}{2} \text{[Rf rf] S} - \text{sterile} \]

(2) Restorers not available
   1/2 of the production must be made without the use of sterility factors and then the fertile seeds blended with the sterile seeds prior to the time they are distributed.

3. Hybrid seeds of crops with perfect flowers

(a) Most crops species have perfect flowers, therefore, the availability of parental lines - male sterile, maintainers and fertility restorers - are absolutely necessary for hybrid seed production on a commercial scale.

(b) Sorghum (Figure 5)

(1) Breeder develops "A" lines (Cytoplasmic male sterile) "B" lines (maintainer) and "R" lines (restorer).

(2) Increase "A" line by planting in isolation with "B" line the same as in maize; 1:2 or 2:4 ratio. "R" lines maintained in isolation.

(3) Single cross fields are planted in isolation; 2:6, 2:8 or 4:12, 220 yards from other grain, 320 from forage, 440 from grass sorghums.
Figure 3. Production of double-cross hybrid seed of maize using male-sterility in female parent seed production.
FIRST YEAR

Multiplication of inbred lines

SECOND YEAR

Female Parent Seed

inbred B  male-fertile
non-pollen-restoring

inbred A  male-sterile

Pollen from B

SECOND YEAR

Male Parent Seed

inbred C  male-sterile

inbred D  male-fertile

Pollen from

THIRD YEAR

single cross seed
(A × B), produced
in isolated field

single cross seed
(C × D), produced
in isolated field

single cross plant
A × B  male-sterile

single cross plant
C × D  male-fertile

pollen from

Double Cross Hybrid Seed
(A × B) × (C × D),
produced in isolated field
(sold to farmer)
Pollen fertility is dependent
upon restorer genes

Figure 4. Production of parental single crosses and
double cross hybrid seed of maize utilizing
cytoplasmic male sterility and restorer factors.
Figure 5. Steps in the maintenance of parental lines and production of hybrid sorghum seed utilizing cytoplasmic male-sterility and restorer systems.
(c) Wheat, rice, pearl millet, sunflower, cotton.

(1) The development and testing of the "A" "B" and "R" lines are breeder responsibilities with each species having its unique techniques, although, they are similar to those for maize and sorghum.

(2) Maintenance of parental lines follows the same pattern as that for hybrid sorghum for example pearl millet (Figure 6).

(3) Specific seed production difficulties

**Wheat** - low percentage seed set because of difficulties in pollen transfer and lesser expression of hybrid vigor.

**Pearl Millet** - parental lines have very low seed production potential but millet hybrids have been widely used in India since 1970.

**Rice & Cotton** - transfer of pollen from male to female parent is essentially a "hand" operation. Hybrid cotton seed in India is all produced by hand pollination. Hybrid rice produced in China utilizes mechanical shaking of pollen onto rows of female parent.

**Sunflower** - no particular problems. Hybrid seed are widely used in Argentina, Russia and U.S.A.

**Horticultural crops** - hybrid seeds of tomatoes, onions and many ornamentals are produced but except for onions essentially all this crops require hand pollination and some hand emasculation. Thus, the seed may be very expensive; $200-$1,000/lb.
Figure 6. Parental line multiplication and production of hybrid pearl millet.