Regeneration guidelines

Maize

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Introduction

Maize (*Zea mays* L. subsp. *mays*) is an outcrossing, monoecious annual crop that evolved in southern Mexico, possibly from the close relative teosinte. The seed bearing ears are borne laterally at the mid-nodes of the plan, while the male flowers (tassel) are on top of the plant. There are more than 250 races and local cultivars of maize in Latin America. Some of the mid-altitude races in Latin America have growing seasons of more than 10 months while some early maturing races take less than 3 months from planting to harvest. Some races are 4–5 metres tall, making artificial pollination difficult. Traditionally, maize breeders classified maize ecotypes by their adaptation to growing environments: tropical (<1200 m), mid-altitude (1200–1900 m) and highland (1900–2600 m) for those growing between 26° north and 26° south; and temperate for those cultivars growing in latitudes above 26° north and below 26° south.

The diverse phenotypes and widely differing adaptations of the races of maize and local cultivars are often constraints to regeneration. The germplasm accessions are either genetically heterozygous (panmictic populations) or homozygous (inbreds). The recommended practices and procedures for maize germplasm regeneration are compiled from experience and consultation of theoretical studies on sample size and mating system.

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Choice of environment and planting season

Climatic conditions

- If possible, choose an environment corresponding to the original collection site conditions.
- Under rainfed conditions, 500–700mm of rainfall is considered optimal (depending on the germplasm accessions and soil texture); at lower rainfall supplementary irrigation is needed.
- Temperate maize germplasm is adapted to long-day conditions of ≥13.4 hours of light. Tropical maize usually needs shorter day lengths for floral initiation in temperate latitudes.
- Regenerating maize landraces adapted to cool environments with growing seasons of >10 months, such as those found in the Andean mid-highlands, Central American mid-highlands and southern Mexico, will require collaboration with national genebanks.
- Maize can grow in a temperature range of 5–45ºC, but generally does best at 25–35ºC. Extreme high temperatures, especially combined with low humidity, may reduce pollen viability and cause poor seed set.

Preparation for regeneration

When to regenerate

- When the number of viable seeds per accession is <1500 in active or base collections of panmictic populations and <250 seeds in inbred lines.
- When seed viability falls below 85% of the initial germination percentage in active collections, determined by viability monitoring (see FAO/IPGRI 1994 and ISTA 2008 for more details).

Pre-treatments

- It is recommended to apply fungicide and insecticide to seeds to help protect seedling emergence and growth in the field.

Precautions

- For panmictic populations, maintain an equal and large effective population size (>100 ears or more than four times the initial sample size, whichever is smaller) throughout regeneration cycles, to avoid genetic drift, inbreeding, and subsequent loss of alleles (Crossa 1987; Crossa et al. 1994; Wang et al. 2004).
- Inspect and screen seed-producing plants for pests and disease under quarantine regulations, before and after regeneration to provide quality seeds for seed exchange and use (Mezzalama et al. 2001).
- Take extra precautions if there is a risk of GMO contamination. Screen seed lots for the presence of GMOs after regeneration and eliminate contaminated lots (Mezzalama et al. 2008).

Method of regeneration

Regenerate maize using controlled pollination.

Artificial pollination

This method is the most commonly used for germplasm accession regeneration and multiplication. It can be done either by plant-to-plant or chain crossing. Chain crossing is recommended for regenerating large numbers of accessions.

- Plant-to-plant cross (dioecious mode) — uses each plant either as male or female. It requires twice as much land as chain crosses to produce the same number of ears and doubles the effective population size (if 100 ears are harvested, the effective population is 200).
- Chain cross (monoecious mode) — uses each plant as a male and female.

Procedure

1. Cover the ear shoot of each plant with a shoot bag (glassine envelope) before emergence of silks (photo 2).
2. Place a tassel bag (pollination bag) to collect pollen on the male flower (tassel) the day before pollination (photo 3).
3. The next morning, bend and shake the plant slightly to collect the pollen in the tassel bag (photo 4).
4. Remove the glassine cover from the silks of the female plant and pollinate the silks with the pollen from the tassel bag.
5. Immediately cover the silks with the glassine bag and the tassels with the tassel bag again until harvesting.

- Good synchronization between silking and tasseling is needed.
- Carry out pollination before the temperature reaches 36°C.

Natural or open pollination

- Natural pollination (i.e. open pollinated mode of seed fertilization) may be used if regeneration is carried out on-farm based on a contract with farmers who cultivate local maize races specially adapted to on-farm growing conditions. Under these circumstances, use isolated plots in the farmer's fields.
- Collect a large seed sample (3–5 kg) for the active and base storage banks from the open-pollinated regeneration field plots.

Prevention of GMO contamination

- When carrying out artificial pollination, prevent GMO contamination by pollen migration from outside the regeneration plot. Cover silks with air-tight, glassine envelopes and tassels with pollen bags, followed by swift and accurate pollination.
- Plant sentinel border plants (well adapted materials, hybrids or varieties) for detection of unintentional GMO contamination from outside and inside regeneration fields if any risk of contamination is expected. Such plants should be detasseled and open-pollinated by the mixtures of the pollen sources migrating into the regeneration plots. Test the bulked seeds from the sentinel rows for unintentional presence of GMOs (Mezzalama et al. 2008).

Floral induction

- Under temperate conditions, shading of day-length-sensitive tropical maize germplasm accessions for 8 hours a day for 6–8 weeks after sowing prompts floral initiation, allowing limited numbers of such accessions to be pollinated and harvested (Mark Millard, pers. comm.). Use this technique for regenerating a few long-season germplasm accessions per year.

Planting layout, density and distance

- Lay out regeneration plots as a non-replicated experiment, separated from the breeding plots or production fields.
- To the extent possible, group the accessions by maturity, plant height and pollination type (selfing or sibbing) in different blocks to facilitate field management and operations.
- Alternate grain colours to facilitate detection of unwanted cross-pollination.
- Adjust plot size and plant density with the germplasm accessions under regeneration. For example, to establish 256 plants per plot (60m^2), with a harvest of more than 100 ears (panmictic populations), use 16 rows, 5 m long, separated by 75 cm between the rows per accession. Plant two seeds per hill to establish 16 plants per row after thinning.
- Proper density and plot size can be used according to the maturity and plant height of the accessions.
- Plant inbred lines in 8–10 rows per accession (21 plants per 5-m-long row) to have 168 plants to produce enough seeds. Maintain purity of the lines by planting the same original seed parents (8–10 self-pollinated ears) in the subsequent regenerations instead of planting the bulked seeds of the previous regeneration. Harvest selfed ears, which have uniformity in plant, ear and grain type.
- In the case of natural pollination, plant accessions 200–300 m apart, with more than 200 plants per accession in the field plots, to achieve 100 well-filled half-sib ears (the effective population is 100), and harvest 100 ears from the centre of the plot to represent the accession.
- If regeneration fails to produce 100 ears (or any other required number of seeds), carry out a second regeneration of the same accession, using the same seed origin. Combine the ears of the first and second regeneration to represent the regeneration cycle.

Crop management

Maize is generally grown under rainfed conditions but can also be grown under irrigation.

Irrigation

- Apply supplementary irrigation during drought spells.
- If regeneration is being done under irrigation, provide moisture stress 2 weeks before and after flowering as this is critical for good seed set and ear development.

Fertilization

- Apply sufficient soil mineral nutrients for normal plant growth.
- Apply recommended pre-emergence applications of N-P-K and then N at the time of cultivation.

- In the tropics, a minimum fertilizer application of 80-40-0 of N-P-K is often used in on-farm trials.

Common pests and diseases

Contact plant health experts to identify the symptoms of the likely pests and diseases and the appropriate control measures. The following are common pests and diseases for maize:

- Root worms, cutworm, thrips, *Dalbulus maidis*, *Cicadulina* spp., *Spodoptera frugiperda*, and other insects attack roots, leaves and stalks in tropical regions (Ortega 1987).
- Diseases affecting leaves, stalk and kernels are downy mildews, maize rusts, *Turcicum* and *Maydis* leaf blights, gray leaf spot, *Pythium* stalk rot, *Fusarium* and *Gibberella* stalk rots, *Stenocarpella (*syn. *Diplodia maydis)* stalk rot, anthracnose (*Collectotrichum graminicola*) stalk rot, *Penicillium* ear rots, *Aspergillus* ear rots, *Fusarium* and *Gibberella* ear rots, *Cephalosporium* kernel rot, *Stenocarpella* ear rot, common smut (*Ustilago maydis*), maize dwarf mosaic virus, maize streak virus, maize fine stripe virus, maize bushy stunt and corn stunt (The CIMMYT Maize Program 2004).

Pest and disease control

Consult a plant health expert for guidance.

- Reduce insect damage by the timely application of the correct insecticide. However, leaf and stalk diseases and ear rots are difficult to control.
- Be aware of local pest and disease incidence in each region and avoid hot spots of damaging pests and diseases.
- Excessive moisture and drought stress aggravate the problem.
- Coordinate periodic field inspection by pathologists and virologists during the growing season.

Roguing off-types

- Roguing of off-types within the accession is carried out in the regeneration plots at seedling and flowering stages as seed accessions may be contaminated with other genotypes or accessions from the previous regeneration or from pollen contamination at the time of pollination.

Others

- Avoid contamination from foreign pollen, including transgenes.
- Follow rotational practices that are appropriate for the cropping systems of the area.

Harvesting

1. Before harvest, record all relevant agronomic traits (see 'documentation' below).
2. Immediately before harvest, record the number of plants lodged and number of plants pollinated.
3. At harvest, the black layer of the seed is formed and most leaves, especially husk leaves, have dried. Remove the pollinated ears from the plant and place the ear either under the plant or in front of the row for inspection (photos 5 a, b).
4. Further inspect the ears individually and remove diseased, contaminated or abnormal kernels on the cob before and after shelling.

5. Include clean ears with good grain quality to represent the regeneration cycle and record the number of ears forming the seed accession in the regeneration field book.
6. Treat the harvested ears with insecticide to protect them from insect damage during seed processing.

Number of seeds harvested per pollinated ear

- Collect 10 seeds from 100 maternal plants or 50 seeds from 20 maternal plants or take an equal number of seeds from the largest possible number of maternal plants to maintain a high effective population size (N_e) (Crossa et al. 1994; Vencovsky and Crossa 1999).

Post-harvest management

1. Pre-dry harvested ears in a chamber with heated air (not more than 35ºC) blown through the piles of ears to reduce the seed moisture to about 13–15%. If the maize is quite damp at harvest, keep drying temperatures below 30ºC. Where special drying facilities are not available, dry ears in the shade with good air circulation.
2. Shell the ears to the individual seed envelope and balance seed samples prepared from all ears to represent the regeneration cycle, normally by taking the same number of kernels per ear. Further cool-dry the seed bulks of the accessions. It is ideal to make several regeneration packets of two seeds each from the individual ears (for long-term preservation) for subsequent regeneration cycles (Crossa 1987).
3. Perform secondary seed drying by placing the seed in cloth or paper bags and putting these in a cool dry room at low temperature and humidity (10–15ºC and 15–20% relative humidity) for at least 4 weeks, until seed moisture reaches 6–8% in equilibrium. This is normally done using special driers which combine cooling and dehumidifying functions. If such equipment is not available, dry seeds to a moisture content of 7–8% with silica gel or another appropriate desiccant.
4. Prepare several sets of the balanced bulks for preservation in active, base and safety duplicate collections. Send a sample of each accession to a seed health laboratory for quarantine requirements.
5. Register seed test weight (1000 seed weight) and germination percentage before storage.
6. Record other regeneration data (see documentation below) into the genebank management system. Check the original passport data to see if the seed characteristics are the same as described in the original records in order to replace, if necessary (see 8 below) the old seeds with the regenerated seeds.
7. Store seed samples at the respective storage locations according to genebank norms (active, base and safety backup collections).
8. Replace old seeds in the active and/or base collections with new regenerated seeds to facilitate management and save space. A small sample of original seed may need to be kept as reference material.

Monitoring accession identity

- Confirm the regenerated seed accession by characterization data on kernel colour and texture for the accession identity.
- At harvest, recheck the seed colour and texture, ear and grain types, maturity and race classification against the original records (recorded during the original introductions)

of the accession from the genebank passport database. The plant type can be used to monitor the accession identity, but it may not be stable across the regeneration cycles, especially in different regeneration/collection environments. Race classification can be reconfirmed by plant phenotype and the ear and kernel characteristics.

- After shelling ears and during seed processing, check the seed lot against permanent reference original samples of the accession. Attach labels with the genebank accession identification number and the field plot number of the accession inside and outside the seed envelope and cloth bags.

Documentation of information during regeneration

A field book of the regeneration nursery is recommended to document identification, characterization, seed origin, number of plants pollinated and harvested and agronomic traits of the accession and introduction. The field book can contain the following detailed information:

- Regeneration site name and map/GPS reference
- Name of collaborator
- Field/plot/nursery/greenhouse reference number
- Accession number; population identification
- Source of seed
- Data, location and plot number of previous regeneration site
- Sowing date and density
- Field layout used
- Field management details (irrigation, fertilizer, control of weeds, pests, and diseases, and others)
- Environmental conditions of regeneration site (altitude, day length, temperature, precipitation, soil type, others)
- Emergence in the field or screenhouse (number of plants germinated)
- Number of plants established
- Days from sowing to silking and tassling (male flower)
- Pollination control method used: plant to plant, chain cross, open pollinated
- Number of plants pollinated
- Harvest date
- Number of plants (pollinated ears or ears) harvested
- Field weight of the harvested ears
- Seed moisture percentage at harvest
- Agronomic performance rating of the accession by considering field weight, seed quality, uniformity and standability
- Agro-morphological plant and ear traits (ear length, ear diameter, kernel row number, kernel length, kernel width, kernel thickness, plant height, ear height, number of leaves above ear leaf, days to silking, days to male flowering, ear rot rating) are recorded for characterization data and are used for multivariate analysis for grouping the accessions (Franco et al. 2005)
- Approval or repeat of the regeneration based on the effective population size and/or possible inconsistency of the seed accession with passport data and reference seed samples
- Photo of ears and kernels
- Date of seed storage

- Initial germination percentage of stored seeds
- Seed moisture percentage at seed storage
- Documentation of quarantine clearance by seed health unit

References and further reading

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1 Maize field, San Jose de Minas.
Suketoshi Taba

2 Maize ear shoots covered with glassine envelopes.
Suketoshi Taba

3 Pollination bag placed to collect pollen.
Suketoshi Taba

4 Pollen collection by shaking pollination bags.
Suketoshi Taba

5a, 5b Harvested ears for inspection in the field.
Suketoshi Taba

Notes