8. GERMPLASM MONITORING AND REGENERATION

8.1 Germplasm monitoring

What is monitoring?

Monitoring is the regular checking of quality (viability) and quantity (number or weight) of germplasm accessions stored in a genebank. The objective of monitoring is to determine whether regeneration or multiplication of an accession is required.

Why should accessions be monitored?

Accessions are monitored for two main reasons:

- The viability of seeds stored in the genebank decreases during storage; it is important to monitor viability of accessions to ensure that they do not lose their capacity to produce viable plants when needed.
- The removal of seeds for distribution and germination testing results in a decrease of seed quantity over time.

To avoid excessive deterioration of seed quality or quantity, genebank accessions should be monitored both for viability and seed quantity during storage.

How frequently should accessions be monitored?

Seed quantity by number or weight should be monitored every time seeds are distributed from the genebank. This facilitates immediate identification of accessions with insufficient quantities of seeds for further conservation. Viability-monitoring tests should be conducted regularly. The monitoring interval depends on the species, storage environment (seed moisture content and temperature) and viability at the beginning of storage.

- The FAO/IPGRI Genebank Standards (1994) recommend that the first monitoring test should be conducted after ten years for seeds stored in base collections under preferred conditions (-18°C) with high initial viability (>90% germination).

- Seeds of species known to have poor longevity, including most oily crops and accessions with relatively low initial viability (85–90% germination) in base collections, as well as all seeds stored in active collections under preferred conditions (see Table 6.2), should be monitored for viability after five years.
- The interval between subsequent tests should be based on experience, and may be adjusted up or down depending on the extent of viability loss observed during the first monitoring test (see Table 8.1).

Remember to update the seed quantity in the inventory database, deducting the number of seeds drawn for the germination test. Also, update the germination data in the inventory database after the final result is obtained.

Viability monitoring

Viability is monitored by conducting a germination test on a fixed sample or by sequential germination (see Flowchart 8.1).

Fixed sample size germination test

For the fixed sample size germination test, it is recommended to use a minimum of 200 seeds (as two replicates of 100 seeds). If the quantity is limited, 50–100 seeds can be tested in two replications.

1. Identify and list all accessions that require testing, and schedule the tests on a weekly or monthly basis (depending on availability of space in germinators and human resources).
2. Locate the containers in storage from the inventory.
3. Remove the containers from storage and leave them overnight at room temperature to warm.
4. Open each container, draw a sample of seeds for the test and immediately close the containers.
5. Conduct the germination tests using the methods and conditions described in Chapter 5.
6. Calculate the mean percentage germination from the results of the two replicates. Repeat the germination test if the difference between the two replicates exceeds 10% or the maximum tolerance limits at 2.5% probability (see Ellis et al., 1985).
   - If the percentage germination is above 85% of the initial germination percentage, continue to store the accession. Fix the date for next test depending on the current percentage germination (see Table 8.1).
   - If the mean germination is below 85% of the initial germination percentage, schedule the accession for regeneration (see Table 8.2).

Sequential germination tests

The sequential germination test uses fewer seeds per replicate than the standard germination test. Otherwise, the methods and conditions for germination are the same as described for the fixed sample size germination test.

Flowchart 8.1. Viability monitoring.

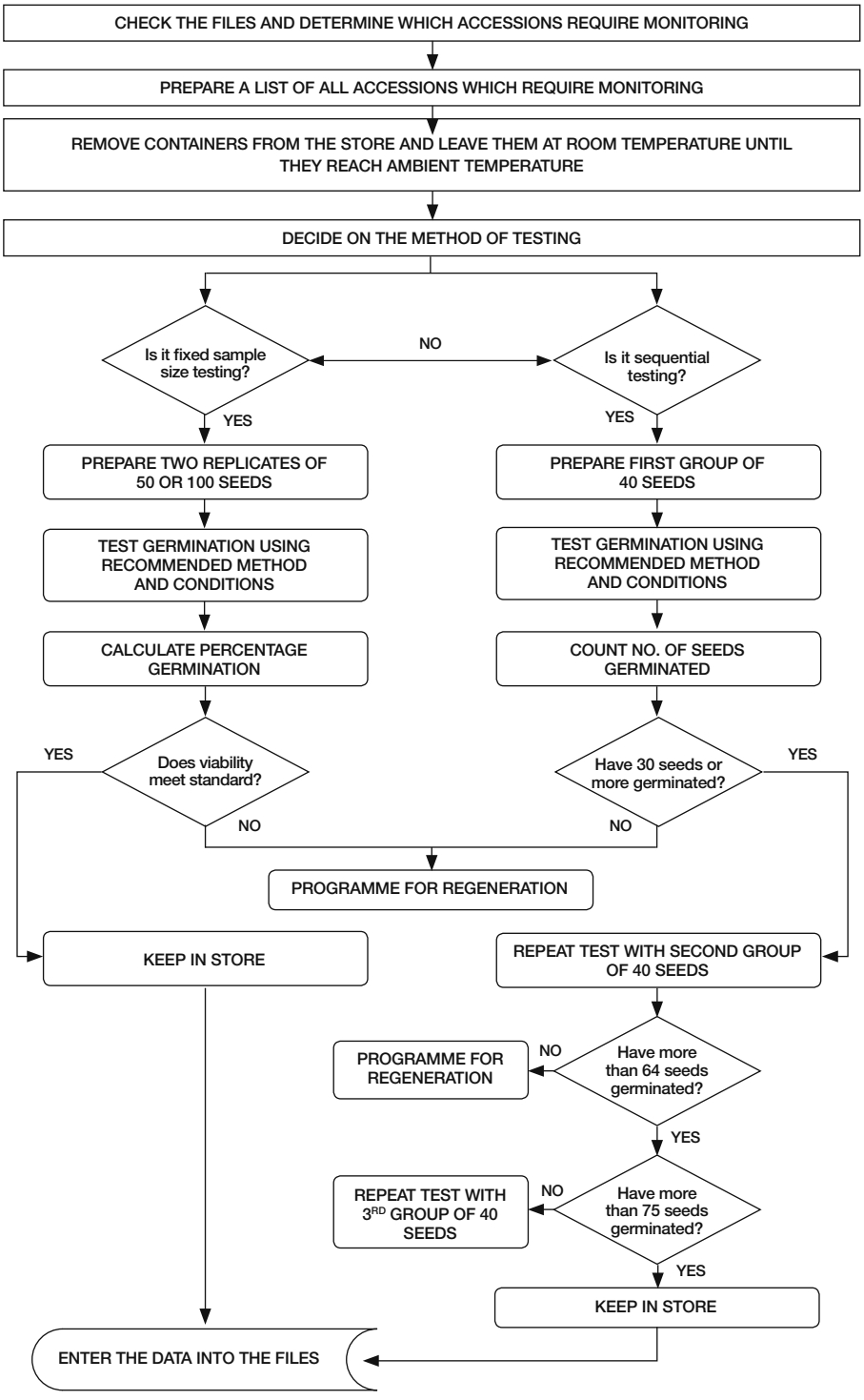

Table 8.1. Suggested interval for monitoring germination of active or base collections in oily and non-oily seeds.

Present level of germination (%)	Monitoring interval (years)			
	Active collection (4–5ºC)		Base collection (-20ºC)	
	Non-oily seeds	Oily seeds	Non-oily seeds	Oily seeds
<80	3	1	5	2
80–85	5	3	10	5
85–95	8	5	15	8
>95	12	8	20	12

Table 8.2. Threshold germination percentages for regeneration of accessions.

Initial germination	Regenerate if percentage germination after monitoring is below
100	85
99	84
98	83
97	82
96	82
95	81
94	80
93	79
92	78
91	77
90	77
89	76
88	75
87	74
86	73
85	72

The number of seeds required for each replicate may vary, but it is recommended to use at least 40 seeds per replicate.

1. Conduct the germination test according to the methods described in Chapter 5 using (for example) 40 seeds
2. Count the number of seeds germinated after the prescribed period of testing.
3. Compare the results with the number germinated in Table 8.3, paying attention to the line with the value of 40 in the first column (number of seeds tested).
   - If the number of seeds germinated is 29 or less, the accession requires regeneration.
   - If the number of seeds germinated is more than 29, then the test must be repeated with another sample of 40 seeds exactly as described above.

Table 8.3. Sequential germination test plan for 85% regeneration standard when testing seeds for germination in groups of 40.†

Number of seeds tested	Regenerate if the number of seeds germinated is less than or equal to	Repeat test if number of germinated seeds is in the range of	Store if number of seeds germinated is more than or equal to
40	29	30–40	-
80	64	65–75	76
120	100	101–110	111
160	135	136–145	146
200	170	171–180	181
240	205	206–215	216
280	240	241–250	251
320	275	276–285	286
360	310	311–320	321
400	340	-	341

† When 400 seeds have been tested, the test can be terminated because enough tests have been conducted for an informed decision to be made.

It is important to use the same number of seeds when repeating the test so that the different samples can be treated as replicates.

4. Count the number of seeds germinated in the second test and add this number to the result of the first test.
5. Compare the results of the test with the number germinated in Table 8.3, following the line with the value equal to the total number of seeds used for all tests (80 seeds) in the first column (number of seeds tested).
   - If the number of seeds germinated is 64 or less, the accession must be regenerated.
   - If the number germinated is above 75, the accession can be continued in storage.
   - If the number germinated is between 65 and 75, the accession must be tested again with another sample of 40 seeds and the results compared with the value equal to the total number of seeds used in all tests (120 seeds) in Table 8.3.
6. Continue the test in this way until a decision can be made regarding regeneration or continued storage, or until the test is repeated ten times.

For more information on test plans for other group sizes (20, 25, 50 or 100 seeds) and regeneration standards between 65% and 80%, refer to Ellis et al. (1985). The sequential test is only necessary when seed numbers are limited. Small-seeded crops like finger millet

normally have adequate numbers of seeds to use the fixed sample size method.

Monitoring seed quantity

Seed quantity can be monitored by checking the inventory data file. This is best achieved through a computer-based genebank documentation system.

1. Record the weight of the seeds initially transferred to the genebank.
2. Record all subsequent seed withdrawals for distribution, regeneration and germination-testing.
3. Update documentation of seed stocks *immediately,* adjusting the total after all seed withdrawals.
4. Prepare a list of accessions where the number of seeds in storage has fallen below the critical level (usually the number required for at least three regenerations).

Germplasm accessions identified with low viability or inadequate quantity during the course of monitoring should be regenerated as soon as possible using the method described in the following section.

Documentation

Monitoring is a crucial activity in genebank management as it helps to provide information on seed stocks that are becoming low, accessions that need viability testing and those that require regeneration. Without proper documentation of the data from preceding genebank activities, effective monitoring is difficult to carry out.

8.2 Germplasm regeneration

What is germplasm regeneration?

Regeneration is the renewal of germplasm accessions by sowing and harvesting seeds that possess the same characteristics as the original sample. Germplasm regeneration is the most critical operation in genebank management.

Why is regeneration critical in genebank management?

Germplasm regeneration involves risks to the genetic integrity of germplasm accessions due to selection pressures, out-crossing, mechanical mixtures and other factors. The risk of genetic integrity loss is usually high when regenerating genetically heterogeneous germplasm accessions. Germplasm regeneration is also very expensive.

Why should germplasm be regenerated?

Germplasm is regenerated for the following purposes:

1. Initial seed increase

In new collections or materials received as donations, the quantity of seeds received by the genebank is often insufficient for direct conservation. Seeds may also be of poor quality due to low viability or infection. All these materials require regeneration. Newly acquired germplasm of foreign origin may need to be initially regenerated under containment or in an isolation area under the supervision of the national phytosanitary authority as described in Chapter 2.

2. Replenishing seed stocks in active and base collections

Increase seed stocks of accessions that have:

- low viability identified during periodic monitoring; or
- insufficient stocks for distribution or conservation.
- Active collections should be regenerated from original seeds in a base collection; this is particularly important for out-breeding species. Using seeds from an active collection *for up to three regeneration cycles* before returning to the original seeds (base collection) is also acceptable (FAO/IPGRI 1994).
- Base collections should normally be regenerated using the residual seed from the same sample.

3. Meeting special requirements

There may be special requirements for regeneration of accessions with special traits that breeders and researchers use frequently—such as high-yielding, pest- and disease-resistant accessions and genetic stocks — or if there are insufficient seeds for safety duplication and repatriation.

Consider the following factors when regenerating germplasm accessions:

- suitability of environment to minimize natural selection;
- special requirements, if any, to break dormancy and stimulate germination (such as scarification);
- correct spacing for optimum seed set; and
- breeding system of the plant and need for controlled pollination or isolation.

Procedures for regeneration

- If possible, regenerate germplasm in the ecological region of its origin. Alternatively, seek an environment that does not select some genotypes in preference to others in a population.
- If no suitable site is found, seek collaboration with an institute that can provide a suitable site or regenerate in a controlled environment such as a growth room.
- Examine the biotic environment in the context of prior information about the plants and past experience—an inappropriate biotic

Genebanks should adopt a high regeneration standard (such as percentage germination to which an accession conserved in a genebank is allowed to reach before regenerating) to avoid genetic shifts resulting from natural selection of seeds of greater longevity in genetically heterogeneous accessions. The FAO/IPGRI Genebank Standards (1994) recommend that the initial germination value should exceed 85% for most seeds and regeneration should also be undertaken when viability falls below 85% of the initial value. Regeneration should be undertaken when the number of seeds in a base collection falls below the number required for at least three cycles of regeneration.

environment can be detrimental to plants, seed quality and the genetic integrity of an accession.

Selection of accessions

- Regeneration of accessions that have inadequate quality (low viability) should take priority over that of accessions with inadequate numbers of seeds.
- Regenerating accessions in base collections should take priority over regenerating those in active collections.

Preparation of regeneration plots

Soil

- The regeneration plot should be as uniform as possible.
- The field should have good drainage.
- Consider the need for soil analysis and apply treatments appropriate for the crop and site (fertilizers, lime, irrigation or solarization).

Solarization

Solarization consists of heating soil by covering it with polyethylene sheets during the hot summer in the tropics to control soil-borne diseases; it is conducted for at least six weeks during the hottest part of the year.

1. Thoroughly cultivate the land and level it to minimize protrusions.
2. Give 50 mm irrigation before laying the polythene sheets.
3. Use clear transparent polythene sheets, 1–2 mm thick.
4. Insert two edges of each polythene sheet in the furrows and bury the edges in the soil tightly.
5. Place weights to prevent flapping and tearing of polythene sheets in the wind.
6. When planting, leave a buffer zone of at least 0.5 m around the edges of the solarized area for dilution of heat near edges.
7. Do not allow irrigation water to flow in from other areas after solarization and during crop growth.

Weeds and soil-borne pests and diseases

- Identify weeds, pests and pathogens by inspection and prior experience.
- Consider reducing such problems during preparation of regeneration plots by applying the following treatments:
  - herbicide spray;
  - sterilizing soil;
  - ploughing to encourage germination of weeds, followed by herbicide spray; and
  - deep ploughing to kill emerging weeds.

Cleanliness

- Keep the plots absolutely clean from alien seeds and plants.
- Consider the risk of contamination with alien pollen and take appropriate measures during plot preparation, and by inter-cultivation and hand-weeding.
- Ensure that the method of plot preparation is appropriate for the chosen method of establishing plants (for example, ridges and flat beds).
- Prepare the regeneration plot considering:
  - the number of accessions to be regenerated;
  - the number of plants per accession;
  - spacing between rows and between plants; and
  - mechanical access for weeding.
- The method of preparation depends on:
  - soil structure;
  - the species to be sown or transplanted; and
  - the need for plant supports, in the case of climbers.

Preparation of seed

1. Dry, thresh and clean the seeds if the samples are newly acquired.
2. For those in storage:
   a. identify the candidate accessions that require regeneration;
   b. remove the containers from the genebank and allow them to warm up; and
   c. draw seed samples, keeping in mind the minimum sample size required for regeneration and the current level of germination.
3. Ensure absolute accuracy in identifying accessions while drawing the seeds from the genebank, packaging, and labelling seed samples. To minimize errors, it is the use of computer-based information management systems to generate labels is suggested.

The minimum number of seeds for regeneration can be estimated from the standard sample size used for regeneration and the sample viability according to the following equation:

Number of seeds required for regeneration = Desired plant population for regeneration/(Germination%[14] x Expected field establishment %[15]).

[14] Germination and field establishment percentage are expressed as decimals: 95% is expressed as 0.95.

[15] Plant establishment is generally 5% less than the germination percentage in poor conditions and 1% less in good conditions.

Example:

Desired plant population = 150
Percentage germination = 85
Expected field establishment = 80

$$\text{Number of seeds for plating} = \frac{150}{0.85 \times 0.80} = 220 \text{ seeds}$$

Seed pre-treatments

Specific pre-treatment may be necessary to improve seed germination and establishment. If the seeds are very dry (moisture content <8%), raise the moisture content by humidification as described in Chapter 5.

- Break dormancy for species or accessions (using stratification, scarification, etc.).
- Apply proprietary seed dressings to reduce disease and insect damage.
- Inoculate with appropriate symbionts (*Rhizobium* treatment for legumes).
- For accessions with limited seeds, pre-germinate in controlled conditions and transplant the seedlings into pots with sterilized soil and grow them in a screenhouse under close supervision.

Meiosis and anthesis are sensitive stages during plant development. Care must be taken to avoid any stresses such as high temperature and drought.

Sowing and crop management

Crop management for regeneration differs from normal commercial practices where interplant variation is not of primary consideration.
To avoid large losses of alleles and maximize seed yield:

- use 100 or more plants in genetically heterogeneous accessions;
- take special note of the day-length requirements for the species or they may not flower;
- provide suitable conditions for growth to trigger abundant flowering;
- eliminate competition by weeding alien plants; and
- ensure a stable source of water from irrigation if necessary.

Sowing date

- Sow at an optimum time so that maturity and harvesting coincide with the most favourable weather conditions.
- If there is much variation between accessions in flowering time, sort by early and late maturity based on previous documentation and adjust the planting dates so that all accessions mature in a uniform environment.
- Planting on the basis of maturity makes crop management and harvesting convenient.

- Sow in uniformly spaced rows and with uniform spacing between plants within rows.
- Avoid competition for light and nutrients by using wide spacing.
- Ensure complete control of pathogens and pests using standard plant-protection measures.
- Thinning should normally not be undertaken—if required, thin plants at random.
- Ensure continued absence of alien plants in the vicinity throughout the regeneration cycle by hand-weeding or using inter-cultivation.

Irrigation

- Irrigate the field when necessary.
- Never subject the crop to water stress.
- Ensure adequate drainage and no water-logging.

Regular inspection of plants is mandatory to achieve these objectives.

Verifying accession identity

- Accession identity should be verified while the plants are growing by comparing:
  - morphological data in the documentation system; or
  - reference material such as original herbarium specimens or seed.
- Roguing must be undertaken with caution and only when it is absolutely clear that the rogue plants are genuine mixtures of other accessions or varieties.
- When materials are grown in rows, plants growing off-row may be eliminated.

Pollination biology

Unless the species is an obligate inbreeder, appropriate pollination control should be implemented. A compendium of breeding mechanisms can be found at www.bioversityinternational.org/Themes/Genebanks/Species_Compendium/default.asp.

For out-breeders, elimination of alien pollen can be achieved through:

- spatial isolation (this is not practical when dealing with large number of accessions of the same species but very useful for dealing with a limited number of accessions of many species);
- temporal isolation;
- natural or artificial barriers—growing accessions in standing crops of tall-growing species like sunflower and hemp; and
- bagging selected inflorescences with pollen-proof or pollinator-proof bags made of linen or paper and erecting temporary pollen-proof or pollinator-proof nets around plots. Supplemental hand-pollination is sometimes required to improve seed set.

Insect-pollinated crops may be grown in net or nylon-screened cages with specially designed hives for insect pollinators like bees; one accession of each crop species may be planted in each cage. Insect pollinators are released inside the cage at the time of flowering. Supplemental hand-pollination may be necessary to improve seed set (such as in wild species of tomato and eggplant). Isolation cages can be expensive and shading may affect plant growth. An effective solution could be bagging and controlled pollination by hand. If plants flower during or at the end of the wet season, however, pollination bags may be damaged by rain. Excess moisture and humidity in the bags around the flowers can also lead to increased infection with bacteria and fungi. In wet or humid conditions, it is best to tag the flowers and remove the bag as soon as pollination is complete so that the fruits may develop under normal field conditions.

Harvesting and post-harvest management

- Harvest at optimum maturity (after the seeds have reached the point of physiological maturity):
  - when maximum number of seeds are ripe;
  - when seeds become tolerant to desiccation and can be threshed without mechanical injuries;
  - before deterioration sets in; and
  - before natural dispersal occurs.
- Stagger the harvest if there are differences in maturity of the accessions.
- Harvest individual plants within an accession when there are differences in flowering and maturity between plants.
- Mix an *equal proportion of seeds* from different mother plants to avoid maternal effects.
- Bags holding harvested seeds or heads should be made of porous material enabling good air circulation for drying.
- Options for harvesting depend on the crop:
  - Harvest plants individually, preferably by hand. If machine-harvesting, use purpose-built machinery because commercial machinery cannot be cleaned adequately between regeneration plots.
  - Harvest infructescences individually by hand.
- Initiate seed drying immediately after harvesting to prevent seed deterioration.
- If seeds cannot be processed quickly, they should be placed in a temporary holding area in a controlled environment such as an air-conditioned room.

Documentation

Regeneration is performed as a result of information generated by seed monitoring. As regeneration methods vary according to

species, the types of descriptors used to record information also vary. The following descriptors will help in documenting the data:

- Regeneration site
- Collaborator (where applicable)
- Plot reference
- Sowing date
- Germination in the field
- Number of plants established
- Days from sowing to flowering
- Breeding system
- Pollination control method used
- Harvest date
- Number of plants harvested
- Quantity of seeds harvested

Further reading

Ellis, R.H., Hong, T.D. and Roberts, E.H. 1985. Handbook of seed technology for genebanks. Volume 1. Principles and Methodology. Handbooks for Genebanks. No. 2, IBPGR, Rome, Italy.

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Table 8.4. Reproductive behaviour and pollination control mechanisms for regeneration of important crops.

Crop	Species	Pollination behaviour (crossing rate)	Pollination mechanism	Method of regeneration
Alfalfa (lucerne)	*Medicago sativa*	Mainly CP (84-94%)	Insects (tripping)	Isolation; screened cages with pollinators
Amaranth	*Amaranthus* spp.	CP	Wind	Isolation; bagging
Barley	*Hordeum vulgare*	SP		
Black gram	*Vigna mungo*	SP		
Bottle guard	*Lagenaria siceraria*	CP; monoecious	Insects	Bagging and hand-pollination
Brown mustard	*Brassica juncea*	Mainly SP (4-14% cross-pollination)	Insects	Bagging
Buck wheat	*Fagopyrum esculentum*	CP; self-incompatible	Wind	Bagging and hand-pollination
Buffel grass	*Cenchrus ciliaris*	CP	Wind	Isolation; bagging
Cabbage	*Brassica oleracea* var. *capitata*	CP	Insects	Screened cages with pollinators
Carrot	*Daucus carota*	CP; protandrous	Insects	Screened cages with pollinators
Castor bean	*Ricinus communis*	CP; monoecious	Wind	Bagging and hand pollination
Cauliflower	*Brassica oleracea* var. *botrytis*	Mainly CP	Insects	Bagging
Chickpea	*Cicer arietinum*	SP		
Chicory	*Cichorium intybus*	CP; strongly self-incompatible	Insects	Spatial isolation; Bagging; insect-proof cages
Common bean	*Phaseolus vulgaris*	Mainly SP; cross-pollination 8-20%	Insects	Isolation; insect-proof cages; bagging
Cotton	*Gossypium* spp.	Mainly SP; natural outcrossing 10-50%	Insects	Bagging; insect-proof cages
Cowpea	*Vigna unguiculata*	Mainly SP		
Crotalaria	*Crotalaria juncea*	Mainly SP		
Cucumber	*Cucumis sativus*	CP; monoecious	Insects	Bagging and hand pollination
Eggplant	*Solanum melongena*	Partially SP; natural outcrossing up to 48%	Insects	Isolation; bagging
Faba bean	*Vicia faba*	Mainly SP; 4-8% outcrossing	Insects	Isolation; bagging
Finger millet	*Eleusine corocana*	SP		
Foxtail millet	*Setaria italica*	SP		
Grass pea	*Lathyrus sativus*	SP; significant levels of CP can occur		Bagging
Hyacinth bean	*Lablab purpureus*			
Lentil	*Lens culinaris*	SP		
Lettuce	*Lactuca sativa*	Mainly SP; natural outcrossing 1-6%	Insects	Bagging; insect-proof cages
Lima bean	*Phaseolus lunatus*	Mainly SP (up to 18% natural crossing)	Insects	Isolation
Linseed (flax)	*Linum usitatissimum*	Normally SP; natural crossing up to 12%	Insects	Isolation; bagging
Lupin	*Lupinus* spp.	Mainly SP; some CP can occur	Insects	Isolation; insect-proof cages or bagging

Crop	Species	Pollination behaviour (crossing rate)	Pollination mechanism	Method of regeneration
Maize	*Zea mays*	CP; monoecious	Wind	Bagging ear and hand pollination with pollen pool
Mung bean	*Vigna radiata*	SP		
Oat	*Avena sativa*	SP		
Okra	*Abelmoschus esculentus*	Partially SP; natural outcrossing 4-19%	Insects	Isolation; insect-proof cages or bagging
Onion	*Allium cepa*	Mainly CP; protandrous	Insects	Screened cages with pollinators
Pea	*Pisum sativum*	Mainly SP		
Peanut	*Arachis hypogaea*	SP		
Pearl millet	*Pennisetum glaucum*	Mainly CP; protogynous	Wind	Bagging; hand-cross with pollen pool
Pepper, chilli	*Capsicum annuum*	Often CP; heterostyly	Insects	Bagging
Pigeonpea	*Cajanus cajan*	Normally SP; natural outcrossing 5-40%	Insects	Isolation; bagging; insect-proof cages
Potato	*Solanum tuberosum*	Mainly CP	Insects	Isolation; bagging
Pumpkin	*Cucurbita moschata*	CP; monoecious	Insects	Bagging and hand pollination
Radish	*Raphanus sativus*	CP; strongly self-incompatible	Insects	Screened cages with pollinators
Red clover	*Trifolium pratense*	CP; strongly self-incompatible	Insects	Screened cages with pollinators
Rice	*Oryza sativa*	SP		
Rye	*Secale cereale*	CP; strongly self-incompatible	Wind	Bagging and hand-pollination with pollen pool
Rye grass	*Lolium perenne*	CP	Wind	Bagging
Safflower	*Carthamus tinctorius*	SP		
Sesame	*Sesamum indicum*	Mainly SP; cross pollination up to 5%	Insects	
Sorghum	*Sorghum bicolor*	Mainly SP; cross-pollination up to 1-50%	Wind	Isolation; bagging
Soya bean	*Glycine max*	SP		
Spinach	*Spinacea oleracea*	CP; dioecious	Wind	Spatial isolation
Strawberry	*Fragaria ananassa*	Mainly CP	Insects	Insect-proof cages
Sugar beet	*Beta vulgaris*	CP; self-incompatible	Wind, Insects	Spatial isolation, Screened cages with pollinators
Sunflower	*Helianthus annuus*	Partially CP; protandrous	Insects	Bagging and hand pollination
Sweet clover	*Melilotus albus*	SP		
Tobacco	*Nicotiana tabacum*	SP		
Tomato	*Lycopersicon esculentum*	Normally SP; some species self-incompatible with moderate to high CP		
Triticale	*Triticosecale*	CP	Wind	Isolation; bagging
Vetch	*Vicia sativa*	SP		
Watermelon	*Citrullus lanatus*	CP; monoecious	Insects	Bagging and hand pollination
Wheat	*Triticum aestivum*	SP		

SP= Self-pollinating; CP= Cross-pollinating