Vegetable Seed Regeneration and Quality Preservation – Seed Saving and Seed Production Technologies

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AVRDC – The World Vegetable Center

34th International Vegetable Training Course

Module 1: Vegetables: From Seed to Table and Beyond

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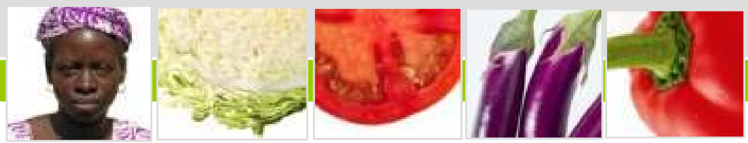

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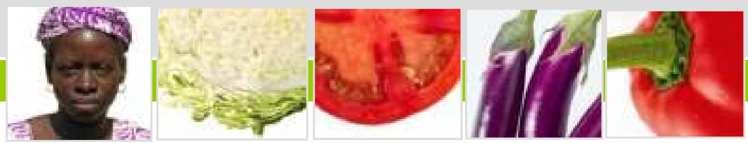
Outline

- Seed quality in germplasm maintenance
- Seed quality during seed development and harvesting
- Seed quality during seed processing, storage and priming



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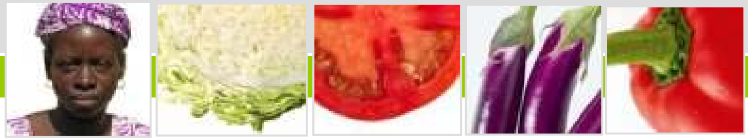
High seed quality

High seed quality can be measured in terms of viability and vigor and is essential for high crop yield and high quality of produce.

Farmers may loose their crop if they sow poor quality seed.

- Normal seed germination:
  - developed root system
  - straight and long hypocotyl
  - 2 cotyledons
  - terminal bud



Poor seed quality

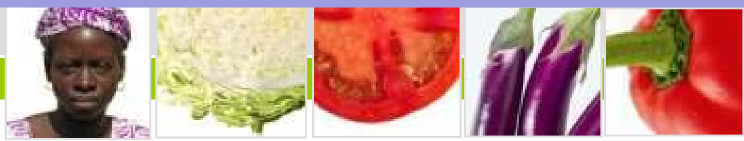
Abnormal germination:
- Damage to the primary root: stunted growth, missing, broken or decayed by primary infection
- Damage to hypocotyl: short, missing, glassy, decayed by primary infection
- Cotyledons/primary leaves: deformed, missing, or decayed due to primary infection

Hard seed:

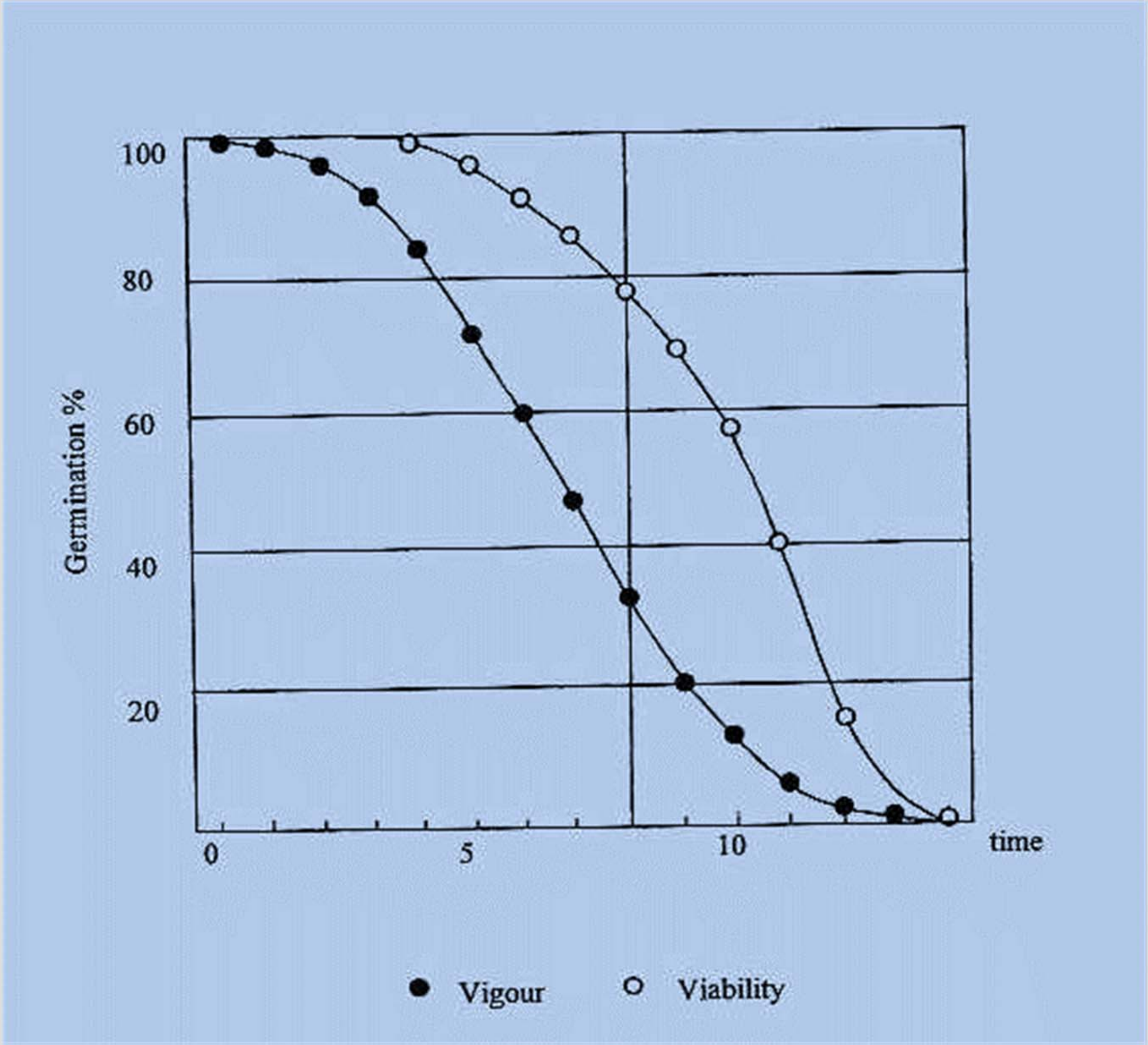
Not germinated; seed coat remains hard (dormant)

state of dormancy broken by:

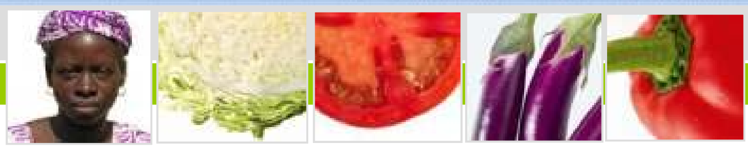
pre-chilling, preheating, gibberellic acid



Relation between vigor and viability



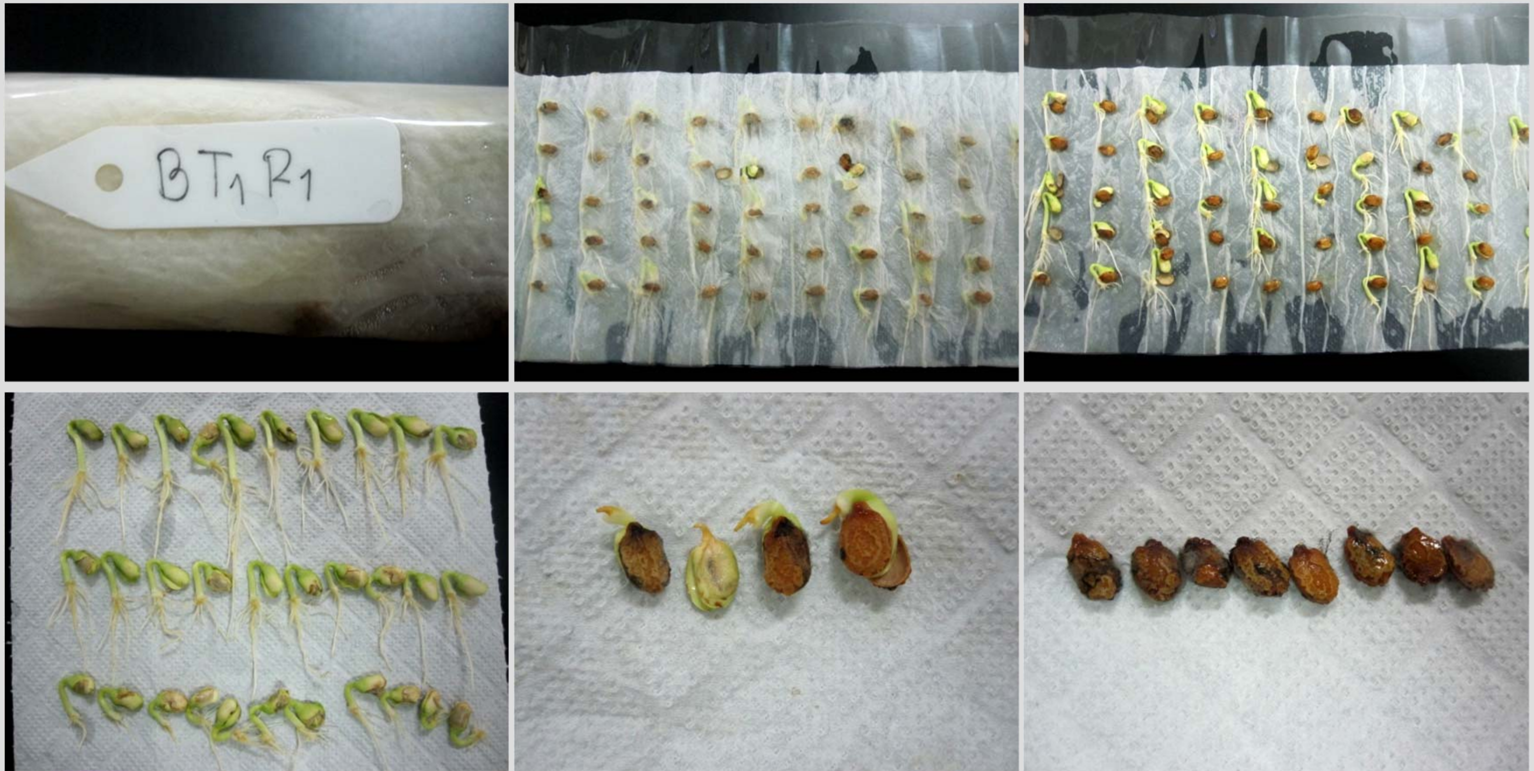
Accelerated aging of seed to test seed vigor:
expose seed to 40 ^{o}C at 10% RH for 48-96 hours

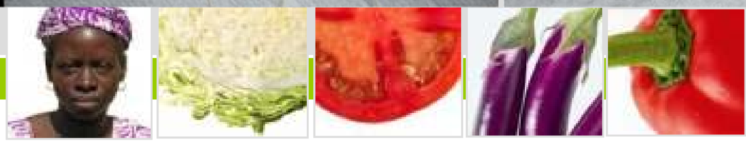


Bitter gourd germination

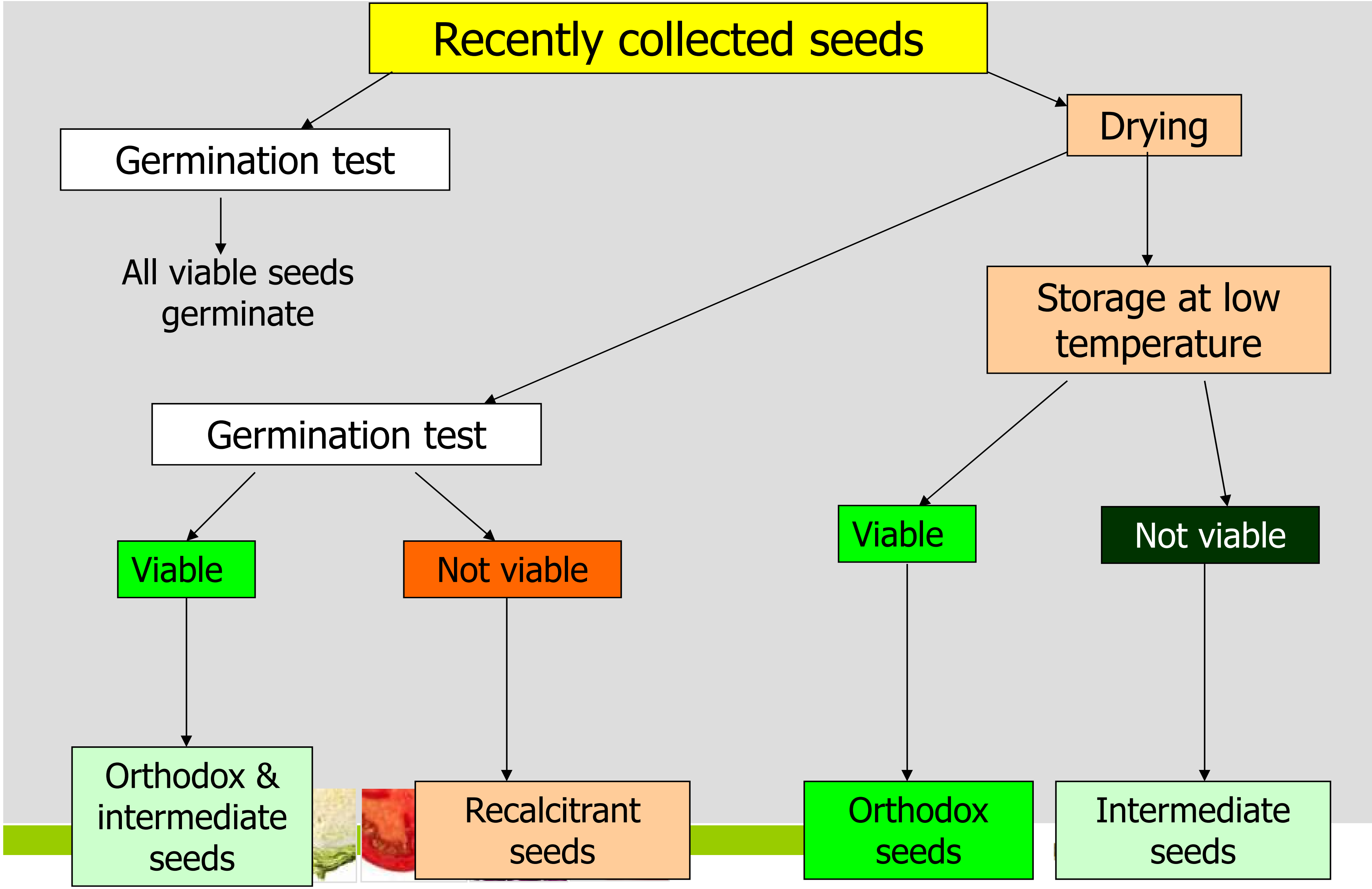
Treatment 1

Normal Seedling (%)	Ab-normal Seedling (%)	Dead Seed (%)	Hard Seed (%)
68.67	3.33	25.33	2.67



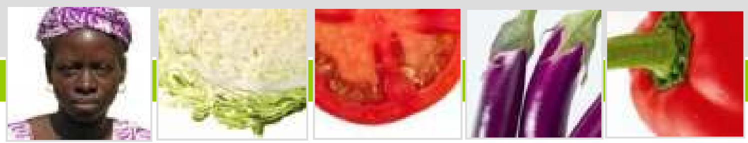


Determination of Seed Storage Behaviour



Seed Classification

	Orthodox seeds	Intermediate seeds	Recalcitrant seeds from temperate zones	Recalcitrant seeds from tropical climates
Seed moisture content for storage (%)	Very low (2-5%)	Low (7-10%) (equilibrium with HR of 30-50%)	High >30-50% at maturity (12-30% after drying)	High >30-50% at maturity (12-30% after drying)
Storage temperature	Very low -18 ^{o}C	Medium to high ~ 20 ^{o}C	Low > 0 ^{o}C	High ~ 25 ^{o}C



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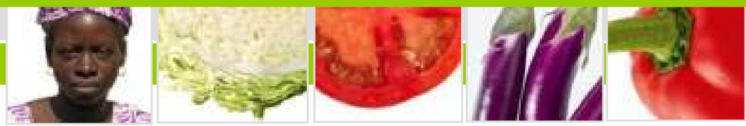
Seed quality of (vegetable) crops

Commercial vegetable seed	Vegetable seed in genebanks
✓ Highly viable and vigorous	✓ Highly viable and vigorous
✓ Genetically pure (true to type)	✓ Genetically pure (true to type)?
✓ Physically purity (percentage of pure seed versus seeds from other crops, weeds, and foreign matter)	✓ Physically purity (percentage of pure seed versus seeds from other crops, weeds, and foreign matter)
✓ Seed health: free from disease, insect pests	✓ Seed health: free from disease, insect pests
✓ Uniform size of desired type	✓ Uniform size of desired type
✓ Fairly priced	✓ Fairly priced
✓ Good yielding ability	✓ Good yielding ability?
✓ Wide adaptability	✓ Wide adaptability?

"Good seed on good land yields abundant produce"
(Hindu proverb)



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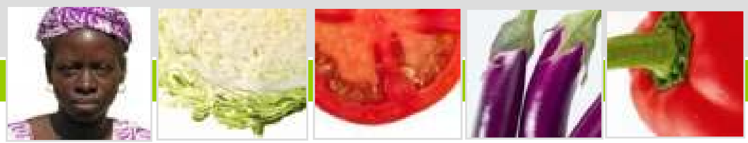
Categories of seed production

The seed category determines how stringent the procedure is that has to be followed in seed production.

The different seed categories are:

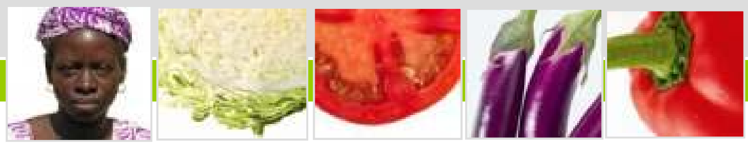
- Breeder's seed
- Foundation seed
- Certified seed
- Commercial seed

Genebank / school/community garden situation:
How are traditional varieties, landraces to be treated?



Breeder's Seed

- This seed category is produced and maintained by the plant breeder or the organization which originally developed or introduced the cultivar.
- Carefully maintained with a high level of selection by a qualified plant breeder
- Produced in small quantities from plants which have been individually selected and seeded under very strict conditions to maintain purity



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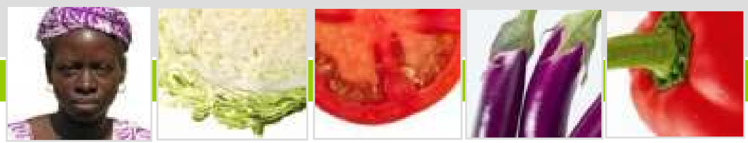
Foundation Seed

- This seed category is the progeny of breeder seed and is used for direct production of certified, commercial or standard seed.
- Foundation seed is usually produced and maintained by the organization which developed the new cultivar or by a seed producer in close collaboration with the plant breeder who developed the cultivar. A certification agency is usually supervising and approving the production of foundation seed.



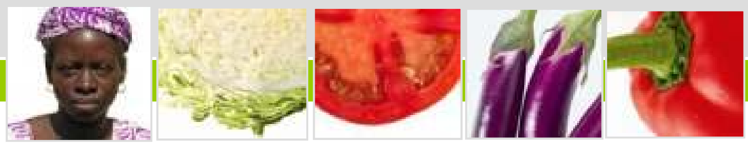
Certified Seed

"Certified seed shall be the progeny of Breeder, Foundation, or Registered seed so handled as to maintain satisfactory genetic purity and identity, and which has been acceptable to the certifying agency." (AOSCA)

➢ Certified seed has met specific production and purity requirements set by law. The fields are inspected by a third party organization that verifies the seed source and stand purity. After the seed is harvested and cleaned, a sample is sent to a certified seed lab for purity and germination analysis.

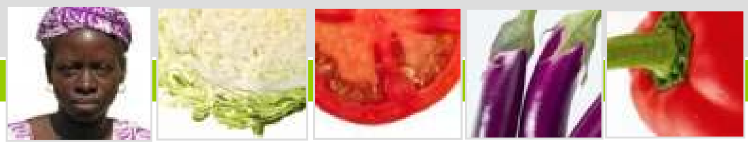
Breeder Seed → Foundation Seed → Registered Seed → Certified Seed → Uncertified Seed

1st Generation — 2nd Generation — 3rd Generation — 4th Generation



Commercial Seed

- This is the stock of seed produced from foundation seed, but not within the framework of a seed certification scheme.
- The majority of vegetable seeds used by farmers are in this category.



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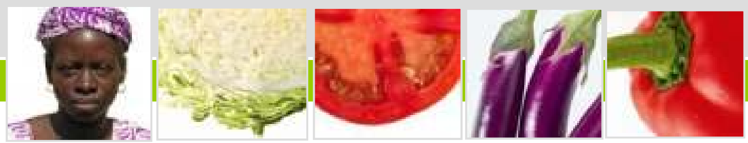
Botanical classification of vegetables

Monocotyledonae

- Alliaceae
  Allium cepa (onion)
  Allium porrum (leek)
  Allium sativum var. *sativum* (garlic)
  Allium cepa var. *aggregatum* (shallot)

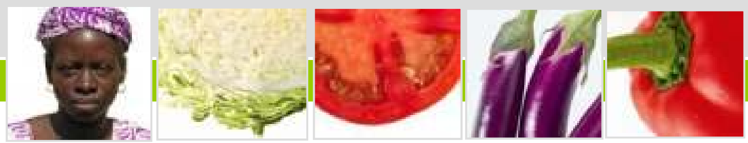
Dicotyledonae

- Solanaceae
  Solanum tuberosum (potato)
  Solanum melongena (eggplant)
  Solanum lycopersicum (tomato)
  Capsicum annuum (bell pepper, chili)
  Capsicum frutescens (tabasco)
  Capsicum chinense (habanero)
  Physalis peruviana (cape gooseberry)
  Physalis philadelphica (Mexican husk tomato)
  Cyphomandra betacea (tree tomato)
  Solanum quitoense (naranjilla)
  Solanum sessiliflorum (cocona)



Classification of crops based on nature of pollination

Highly self-pollinated **90-100% selfing**	**Often cross-pollinated** **Cross poll. > 4%**	**Highly cross-pollinated** **Up to 100% CP**
Yard-long bean (*V. unguiculata subsp. sesquipedalis*) Cluster bean (*Cyamopsis tetragonoloba*) French bean (*Phaseolus vulgaris*) Hyacinth bean (*Lablab purpureus*) Cowpea (*Vigna unguiculata* subsp. *unguiculata*) Garden pea *(Pisum sativum)* Lettuce *(Lactuca sativa)* Tomato *(S. lycopersicum)*	Lima bean (*P. lunatus*) Eggplant (*Solanum melongena*) Okra (*Abelmoschus esculentus*) Chili, sweet pepper (*Capsicum* spp.)	1. **Wind-pollinated**: amaranths, spinach (*Spinacia oleracea*), leaf, garden, sugar beet (*Beta vulgaris*) 2. **Insect-pollinated:** all cucurbits, all cole crops (*Brassica oleracea*), radish (*Raphanus sativus*), carrot (*Daucus carota)* onion (*Allium cepa*)



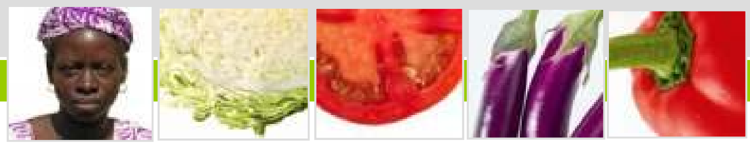
Grouping based on planting response

- **<u>Survive transplanting easily</u>**:
  all cole crops, Chinese cabbage, lettuce, tomato, eggplant, *Capsicum* spp.

- **<u>Need care in transplanting</u>**:
  onion, leek, celery, parsley, amaranths, cardamom

- **<u>Not suited for transplanting</u>**:
  garden pea, radish, carrot, spinach, gourds, garden beet

- **<u>Suits both transplanting and direct seeding</u>**:
  Chinese cabbage, chili, amaranths



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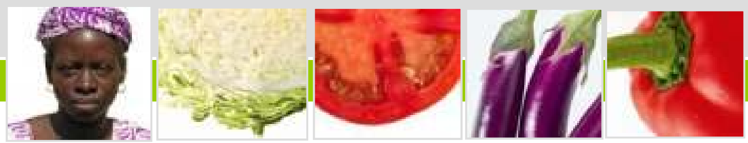
Grouping based on growth cycle

- **<u>Annual</u>**:
  tomato, eggplant, *Capsicum* spp., garden pea, amaranth, all cucurbits, celery, parsley, taro, cocoyam (*Colocasia*)

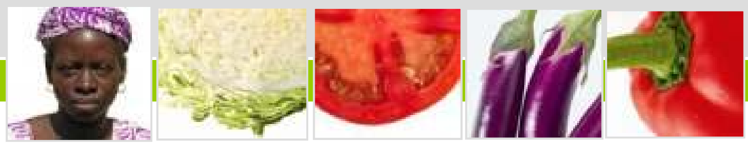
- **<u>Biennial</u>**:
  Cole crops, Chinese cabbage, onion, carrot garden beet, leek, radish, spinach

- **<u>Perennial</u>**:
  Asparagus, chayote (*Sechium edule*), ginger, cardamom, turmeric (*Curcuma longa*)



Grouping based on climatic conditions

- **<u>Temperate</u>**:
  Cabbage, kale, Brussels sprout, broccoli, sugar beet, chicory, potato, amaranths, temperate varieties of radish, carrot, garden pea, tomato
- **<u>Sub-temperate/sub-tropical</u>**:
  late cauliflower, spinach, celery, parsley, asparagus, chayote, sub-temperate varieties of radish, carrot
- **<u>Tropical</u>**:
  eggplant, okra, *Capsicum* spp., all cucurbits, ginger, turmeric, cardamom, sweet potato, Colocasia, tomato

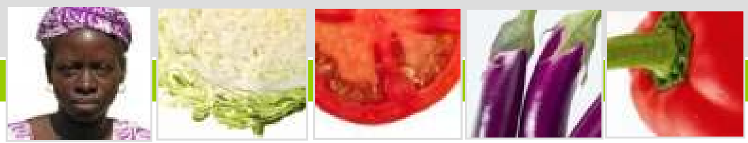


Seed quality in germplasm maintenance

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.

During regeneration, the genetic integrity of the germplasm accessions is at risk due to:

- **selection pressures (soil, climate, cultural management practices, harvesting)**
- **out-crossing and accidental mechanical mixtures**
- **small population size (viability; labor cost, etc.)**



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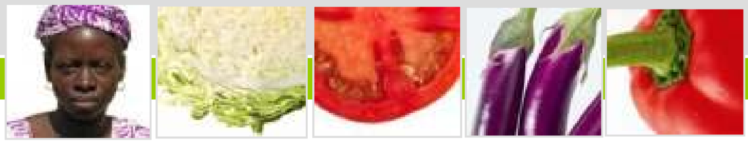
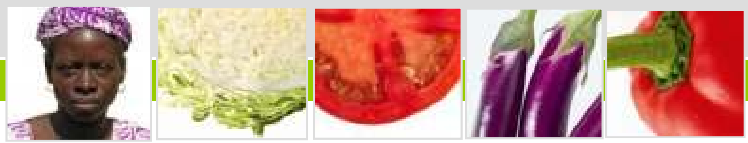
Why is regeneration necessary?

1. Initial seed increase or multiplication
   - Newly collected or donated seed samples usually contain only few seeds;
   - donated/collected seeds may be of poor quality due to low viability or infections
2. Replenish seed stocks in active and/or base collections
   - low viability observed during monitoring
   - insufficient stocks for distribution
3. To meet special requirements
   - accessions in high demand
   - accessions for safety duplication or repatriation



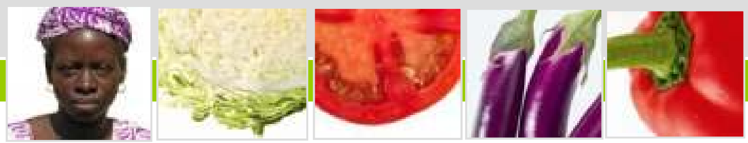
Aims of seed regeneration

- Maximize seed production
- Minimize regeneration cycles
- Maintain the genetic integrity of the population
- Maximize seed quality



Factors to be considered for regeneration

- Suitability of environment to minimize natural selection
- Special requirements to break dormancy and stimulate germination (scarification)
- Correct spacing for optimum fruit and seed set
- Effective population size
- Appropriate cultural management practices
- Breeding system and need for controlled pollination



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Crop Genebank Knowledge Base

Strengthening capacity to manage genebanks

SGRP

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Home

Improving genebank management

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Genetic diversity allows crops to evolve and adapt. It is a major resource for plant breeders to use and meet the challenges in maintaining food security and environmental stability.

The first genebanks were established over 50 years ago to conserve threatened landraces and wild species. Since those early days, research from genetics, plant and seed physiology, *in vitro* culture, cryopreservation and information technology have altered our understanding of how best to conserve and manage crop diversity.

Yet one thing remains as the basis for all our work – the need to constantly improve and use the best practices available to ensure the survival of the genetic diversity in the world's crop genebanks, so that this essential resource remains available for use by current and future generations.

We invite you to discover and learn more about some of the ways to better manage crop genebanks and to share with us information on how you manage your genebank.
Click here to contribute your best practices for germplasm management.

- Genebank blog
- Genebank news
- Discussion forum
- Pictures
- Editor's corner
- CGKB wiki

- Visit SGRP site
- Visit SINGER site

Latest updates

- Banana
- Barley
- Cassava
- Chickpea
- Forage grasses
- Forage legumes
- Maize
- Radish
- Rice
- Wheat
- Other crops (Regeneration guidelines)

Crops

Welcome to the "Crops" area of the Crop Genebank Knowledge Base.

In this area you will find information on how to set up and manage a genebank for different crops.

A range of best practices describing conservation, characterization, regeneration and safety duplication for these different crops have been compiled by crop experts. However, this list of best practices is not exhaustive. If you feel you have useful information to add to this area, please contact us or send us a comment here.

Please continue by clicking on the crop of your interest in the left menu.

- Banana
- Barley
- Cassava
- Chickpea
- Forage grasses
- Forage legumes
- Maize
- Radish
  - Conservation
  - Characterization
  - Regeneration
  - Safety duplication
- Rice
- Wheat
- Other crops (Regeneration guidelines)

Radish genetic resources

Contact person for Radish: Qiu Yang, CAAS, China

Contributors to this page: CAAS, China (Qiu Yang, Li Xixiang); Bioversity International, Italy (Imke Thormann, Ehsan Dulloo); CGN, Netherlands (Noortje Bas); IPK, Germany (Andreas Börner, Ulrike Lohwasser); AVRDC, Taiwan (Andreas Ebert); USDA, USA (Larry Robertson); NBPGR, India (Chitra Pandey); SASA, UK (George Campbell); University of Warwick, UK (Charlotte Allender).

Compilation of best practices

Information on current practices for genebank management of radish genetic resources was first gathered from literature and then valid and updated in collaboration with the following genebanks: CGN - Wageningen, IPK - Gatersleben; AVRDC-Taiwan, USDA Plant Geneti Resources Unit - USA, NGB - NBPGR - India, SASA - UK, HRIGRU Warwick - UK.

Importance and origin

Radish (*Raphanus sativus* L.) is an anciently annual or biennial cultivated vegetable. It most likely originated in the area between the Mediterranean and the Caspian Sea (Crisp 1995). It may come from the wild radish in southwest China (Cheo *et al.* 1987). It is possible that radishes were domesticated in both Asia and Europe. According to Herodotus (c. 484-424 BC), radish was one of the important crops in ancient Egypt, as radish was depicted on the walls of the Pyramids about 4000 years ago. Cultivated radish and its uses were reported in China nearly 2000 years ago (Li 1989) and in Japan radishes were known some 1000 years ago (Crisp 1995).
Based on recent studies using chloroplast single sequence repeats (cpSSRs), Yamane et al. (2009) postulate three independent domestication events which include black Spanish radish and two distinct cpSSR haplotype groups. One of the haplotype groups is geographically restricted to Asia, presenting higher cpSSR

- Maize
- Radish
  - Conservation
  - Characterization
  - Regeneration
  - Safety duplication
- Rice
- Wheat
- Other crops (Regeneration guidelines)

Introduction

Radish (*Raphanus sativus* L.) is a cross-pollinated, cool-season and annual or biennial vegetable crop. Some cultivars, especially thos developed for spring production and of tropical origin have an annual habit. In the first development year biennials develop a fleshy root. require vernalization to bolt and flower in the second year. Radishes have numerous varieties, varying in size, color and duration of requ cultivation time. The period of cold treatment needed to bolt and flower is different among varieties. Temperatures above 32 °C may not have a negative effect on seed set as the stigma dries out and the pollen may not germinate but also on seed size and percentage of se germination as the development of the embryo and endosperm will be negatively affected.

Choice of environment and planting season

Climatic conditions

- If possible, choose an environment corresponding to the original collection site conditions.
- For vegetative growth
  - Radish is a cool-weather, short-season vegetable crop. Grows best in cool and moist weather (15-22 °C for leaf, 13-18 °C for root).
- For reproductive growth
  - Radish flowers initiate (bolt) after vernalization. It is important to expose plants to cool outside temperatures below 15°C for at least 20 days (usually 30~60 days in CAAS).
  - Another option is the storage of germinating seeds or radish seedlings with two leaves in a cool room at 2-5°C for 14 to 40 days. In some specific case, the time should be up to 50~60 days.
  - Temperature at flowering and seed set is optimal at 25± 3 °C.

Preparation for regeneration

When to regenerate

- When seed stocks drop to 1,500 seeds the accession should be scheduled for regeneration. Distribution can continue until seed stc drops to less than 1000 seeds.
- When germination is reduced to 80% or to 85% of initial germination (FAO/IPGRI 1994 and ISTA 2008).
- Newly introduced, collected or received materials might require regeneration to meet international standards if seed amount and qua are insufficient.

Pre-treatments

- Treat seed with fungicides and insecticides or sterilize the seeds using 1% solution of sodium hypochlorite for 10 minutes or hot wa at 50°C for 20 minutes, if required.
- Wild types are not pre-treated as they are not sown directly into the field.
- Use local recommendations as a guide to types and rates of chemical application.

Field selection and preparation

- Banana
- Barley
- Cassava
- Chickpea
- Forage grasses
- Forage legumes
- Maize
- Radish
- Rice
- Wheat
- Other crops (Regeneration guidelines)
  - Bean
  - Breadfruit
  - Coconut
  - Cowpea
  - Faba bean
  - Finger millet
  - Grasspea
  - Lentil
  - Major aroids
  - Oats
  - Pearl millet
  - Pigeonpea
  - Sorghum
  - Sweet potato
  - Wild potato
  - Yam

Regeneration guidelines for bean

The information on this page was extracted from:
Salcedo J.M. 2008. Regeneration guidelines: common bean. In: Dulloo M.E., Thormann I., Jorge M.A. and Hanson J., editors. Crop specific regeneration guidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme, Rome, Italy. 9 pp.

Before reading the regeneration details for this crop, read the general introduction that gives general guidelines to follow by clicking here.

View regeneration guidelines in full (in PDF) by clicking on the picture above (0.2MB)

Also available in the following languages:
Arabic - French - Portuguese - Russian - Spanisl

Introduction

Common bean (*Phaseolus vulgaris* L.) belongs to the legume family (*Leguminosae*). It is widely cultivated and represents one of the largest food components in Latin America and Africa valued for its high content of protein and micronutrients such as iron and folic acid. It is one of the most economically important crops in Latin America and provides an income source for small farmers (Pachico 1989).

The common bean is mainly self-pollinated; however, many authors have reported outcrossing or natural hybridization in both wild and cultivated populations. Ibarra-Perez et al. (1997) reported cross-pollination rates between 0 and 85%.

Although outcrossing is sporadic, it is necessary to take some precautions during regeneration, mainly in wild accessions grown in the greenhouse or field in order to maintain genetic integrity.

Choice of environment and planting season

Climatic conditions

- Common bean grows well at temperatures ranging from 15 to 27°C and will withstand temperatures up to 29.5°C.
- High temperature (close to or higher than 35°C) and moisture stress during flower and pod setting results in abortion of large numbers of blossoms and developing pods.
- The ideal growing conditions are rainfall of 350–500 mm rainfall during the growing season combined with low relative humidity to minimise risk of bacterial and fungal disease.

Planting season

- Beans are considered a warm-season crop, sensitive to temperature extremes. Low temperatures slow down plant growth while high temperatures accelerate it. Generally, plants are more adaptive during short

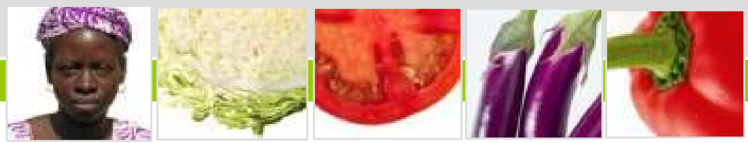
Factors affecting seed quality

<u>During seed production</u>

- Climate
- Cultural practices
- Isolation distance and rogueing
- Seed maturity
- Fruit and seed position on mother plant
- Insect pests and diseases

<u>During seed processing & storage</u>

- Seed moisture content
- Seed drying
- Storage temperature
- Relative Humidity
- Oxygen
- Storage insect pests and diseases



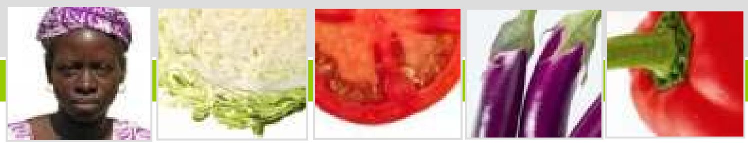
Effect of climate on seed quality

- **Temperature**
- **Relative humidity**
- **Rainfall**
- **Sunshine hours**
- **Wind velocity**

Determine growth, development and health of plants

Excessive rain or drought during flowering:
→ affect fruit and seed set → low seed yields

Seed quality also affected by environmental stress during seed filling stages

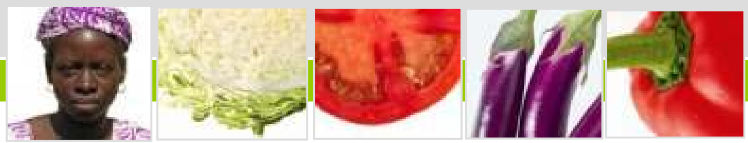


Effect of climate on seed quality…..cont.

- **Cucumber, melon and gourds prefer hot and dry or humid climate for growth; muskmelon seeds produced in humid conditions are of inferior quality.**

- **Onions need cool weather during initial stages of crop growth, but require warm and dry conditions for seed maturation and harvesting. Plants grown at 15-16 $^{\circ}$C yielded more high quality seed than those grown at 22-23 $^{\circ}$C.**

- **Tomato plants grow well at temperatures of 18-27 $^{\circ}$C; fruit and seed setting is affected by high temperature and low humidity. Temp. above 33 $^{\circ}$C adversely affect fruit set.**



Effect of heat on tomato seed production

Heat: mean temp. of 33/25°C (day/night)

normal flower

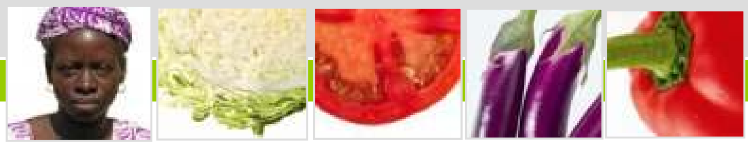
damaged flowers of CA4

Parthenocarpic fruits of CLN2498E

No fruit set in CA4

AVRDC HQ Tomato Crop Seasons

Season	Period	Environment	Seed production
Fall	Sept/Oct- Jan/Feb.	Low rainfall, favorable T° in flowering/fruit-set	Optimal for high quality seed & crossing
Spring	Feb./March- May/June	Low rainfall, usually favorable T° in flowering/fruit-set	Good if planted early and harvested by mid May
Summer	June/July- Sept./Oct	High rainfall and T°	Poor. Shows low germination, many abnormal seedlings Breeding only



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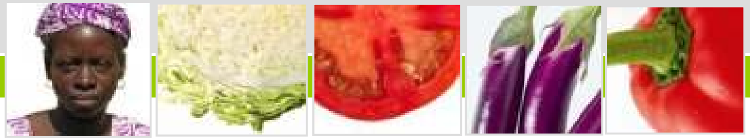
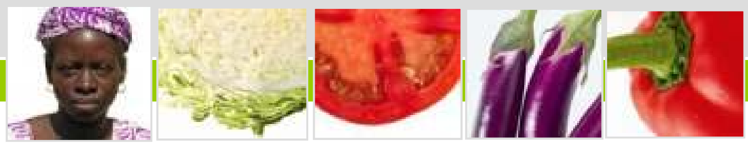
AVRDC Tomato Seed Practices

- ❖ Open field under net cages for bird avoidance
- ❖ Seed multiplication in Fall, Spring seasons
- ❖ Furrow irrigation
- ❖ Disease, insect, weed control
- ❖ Breeder inspection of plants but alert pathologists in case of unusual symptoms
- ❖ Harvest fruit at full ripe maturity for seed extraction



Common diseases and prevalence in AVRDC tomato trials

Disease	Pathogen	Fall	Spr	Sum	Seedborne
TYLCVD	Begomviruses	**	***	*	N
Tobacco mosaic virus					Y
Early blight	*Alternaria solani*	**	***	*	N
Bacterial spot	*Xanthomonas euvesicatoria* *X. vesicatoria*		*	***	Y
Southern blight	*Sclerotium rolfsii*	*	**	***	N
Fusarium wilt	*F. oxysporum* f.sp. *lycopersici*	*	**		N
Gray leaf spot	*Stemphyllium* sp.	*	**		N



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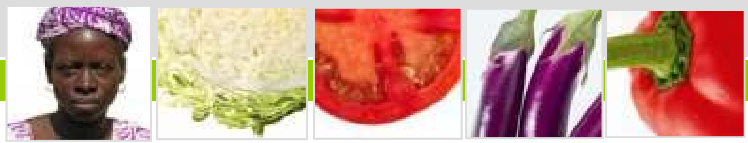
Minimum number of seeds

Population
- Desired plant population = 150

% Germination
- Percentage of germination = 85

Field
- Expected field establishment = 80

 # Seeds
- # of seeds for planting $= \dfrac{150}{0.85 \times 0.80} = 220$ seeds



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Seed pre-treatments

Specific pre-treatments to improve seed germination:

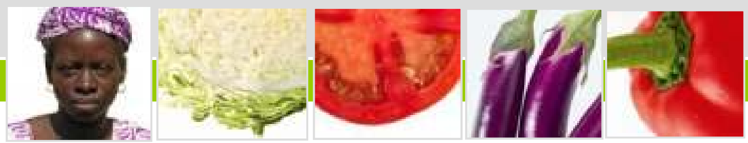
➢ **Humidification of very dry seeds (< 7% SMC to 18-20%)**

Small seeds:

- **spread small seeds on a petri dish;**
- **place 3 moist paper towels inside polythene box**
- **place petri dishes (no cover) on top of moist paper**
- **place box in incubator at 20°C for 24 hours**

Large seeds:

- **place seeds in porous nylon bags (mosquito netting); seed layer should only be 1 seed deep**
- **place bags on top of a gauge above water in a desiccator**
- **place desiccator at 20°C for 48 hours**



Seed pre-treatments (2)

Specific pre-treatments to improve seed germination:

➢ **Breaking dormancy of accessions**

Dormancy is common in freshly harvested seeds and in CWR

After-ripening (post-harvest maturation) in dry storage:

- **dormancy release and promotion of germination during prolonged dry storage (months) at room temperature**
- **Pre-drying treatment: pre-soak or pre-wash seeds before exposing seeds to drying regime**

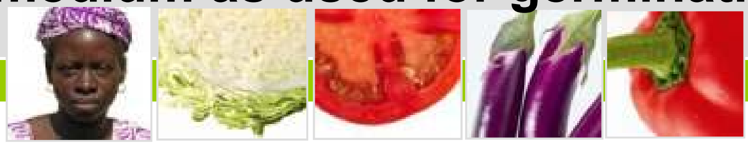
  caution: loss of viability; apply only to seed lot to be sown!

Seed coat imposed dormancy (mech. barrier; limits oxygen diff.):

- **removal, puncture or scratching (scarification) of seed coats may enable imbibed, dormant seed to germinate**

Pre-chilling or stratification:

- **pre-treatment of imbibed seeds at low temperature ($<7^{o}C$ for a minimum of 7 days before transfer to higher temp. (same medium as used for germination)**



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Seed pre-treatments (3)

Specific pre-treatments to improve seed germination:

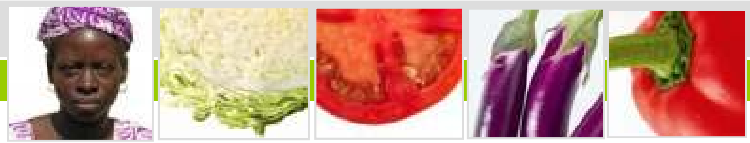
- **Breaking dormancy of accessions**

Rinsing or pre-wash treatments:

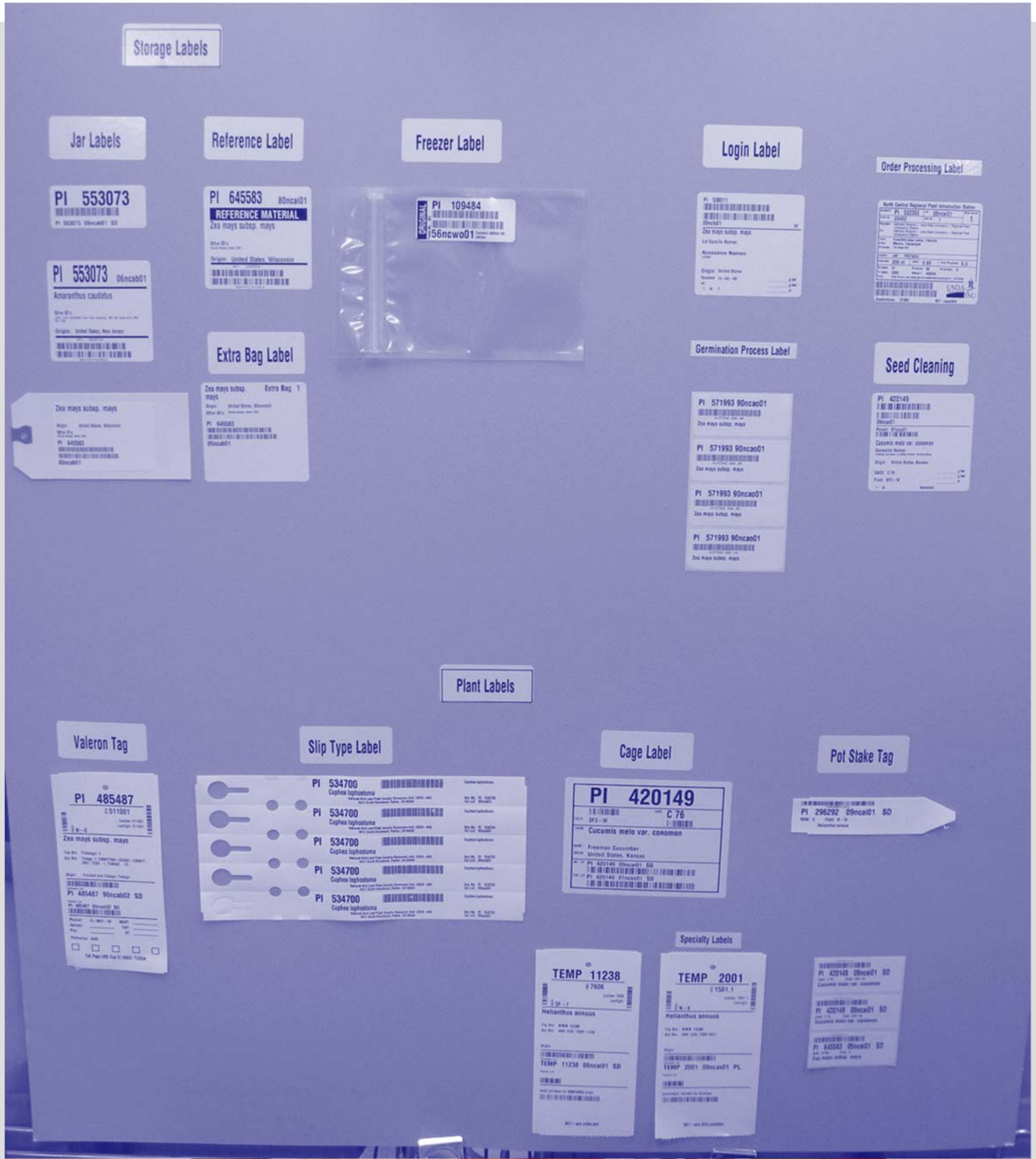
- rinsing of seed to remove inhibitors to germination from within the seed (*Vitis* spp., *Beta vulgaris*); will also soften seed coat and promote germination;
- pre-soak or pre-wash treatment is often followed by pre-dry treatments

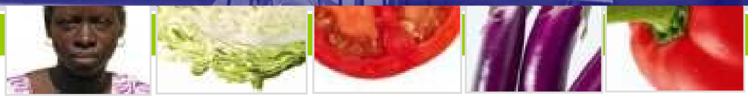
Pre– and co-applied compounds:

- pre-application: immersing seeds in a solution prior to transfer to germination chamber
- co-application: compound added to germination substrate
- used compounds: potassium nitrate, nitrites, hydrogen ions (low pH), cyanide, thiourea, gibberellins, cytokinins



Use of barcode labels in different stages





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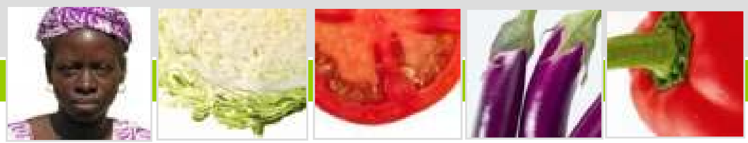
Preparation of regeneration plots

Soil

- The regeneration plot should be uniform
- The field should have good drainage
- Undertake soil analysis and apply corrective treatments (fertilizers, lime, irrigation or solarization)

Crop rotation ensures the maintenance of good soil structure and fertility.
It affects the incidence of soil-borne pests and diseases and the occurrence of volunteer plants which reduce the quality of the seeds produced.



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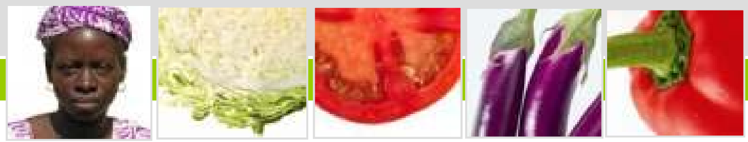
Land preparation for snow pea regeneration

- Drill hole, 75- 80 cm deep, for the installation of the iron support pipes
- Prepare raised transplanting beds
- The bed is covered with 1.5 m wide black polyethylene mulch







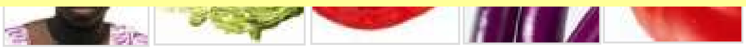
Sowing and crop management

To avoid losses of alleles and maximize seed yield:

- Use ≥100 plants in heterogeneous accessions
- Observe day-length requirements
- Promote growth to trigger abundant flowering
- Eliminate competition by weeds
- Provide stable water supply for irrigation

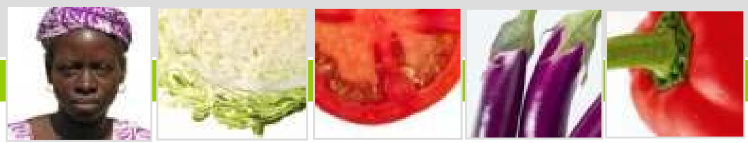
Sowing date:

- Sow at optimum time to favor maturity and harvesting
- Sort accessions by early and late maturity and adjust planting date to facilitate crop management and harvesting
- Use uniform spacing between and within rows
- Avoid competition for light and nutrients (wide spacing)
- Complete control of pathogens and pests
- Avoid thinning or do it at random
- Maintain plot free from alien plants (entire regeneration cycle)



Effect of isolation (by distance, time, barriers) & rogueing

- **Genetic purity is an important parameter of seed quality. It is affected by genetic contaminations and genetic drift in the field.**
  Pollen contamination is prevented by removing weeds or related plants and by providing isolation distance.
- **The isolation distance is about 50 m for self-pollinated crops (tomato, garden peas, French bean) and 1,000 to 1,600 m for cross-pollinated crops (cole crops, onion, cucurbits).**
- **Contamination is relevant during flowering: frequent inspection and removal of off-types can help maintain genetic purity.**
- **Rogueing can be done at vegetative, flowering and maturity stages of plants.**



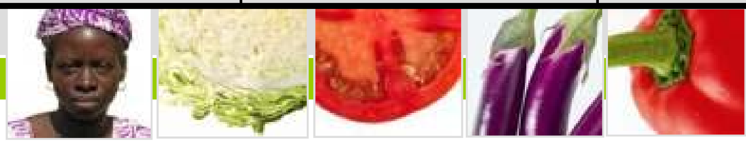
Pollination biology

For outbreeders, eliminate alien pollen through:

- Spatial isolation (limited number of acc. of different species)
- Time isolation (subsequent planting dates)
- Natural or artificial barriers (growing species between tall-growing species like sunflower and hemp)
- Bagging selected inflorescences with pollen-proof or pollinator-proof bags (linen or paper) and erecting pollen- or pollinator-proof nets around plots;
  Caution: rain may destroy pollination bags; excess moisture may increase infections with bacteria and fungi; tag flowers and remove bag soon after pollination is completed.
- Supplemental hand-pollination to improve fruit set or bee hives for cross-pollinated crops in net cages (1 accession per cage)

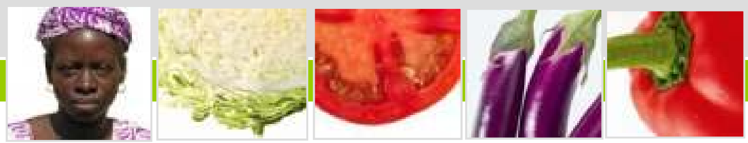
Pollination behavior of some vegetable crops

Crop	Species	Pollinat. type	Pollination mechanism	Method used
Amaranth	*Amaranthus* spp.	CP	Wind	Isolation 1000 m; bagging; net cage
Beet	*Beta vulgaris*	CP	Wind	Isolation 2000 m
Black gram	*Vigna mungo*	SP		
Bottle gourd	*Lagenaria siceraria*	CP, monoecious	Insects	Bagging & hand pollination; net c.
Brown mustard	*Brassica juncea*	Mainly SP; 4-14% CP	Insects	Isolation 1000 m; bagging; net cage
Cabbage	*Brassica oleracea* var. *capitata*	CP	Insects	Isolation 1000 m; Net cage w/ pollinator
Carrot	*Daucus carota*	CP; protandrous	Insects	Isolation 1000 m; Net cage w/ pollinator
Cauliflower	*Brassica oleracea* var. *botrytis*	Mainly CP	Insects	Isolation 1000 m; bagging; net cage
Chickpea	*Cicer arietinum*	SP		
Common bean	*Phaseolus vulgaris*	Mainly SP	Insects	Isolation 100 m; bagging; net cage



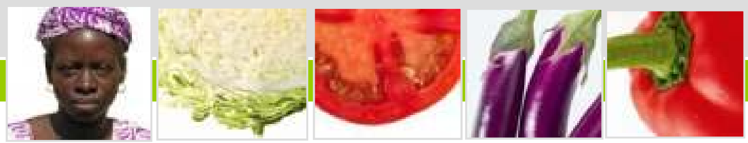
Pollination behavior of some vegetable crops (2)

Crop	Species	Pollinat. type	Pollination mechanism	Method used
Cowpea	*Vigna unguiculata*	Mainly SP		
Cucumber	*Cucumis sativus*	CP; monoecious	Insects	Isolation 1000 m; bagging & hand pollination; net cage
Eggplant	*Solanum melongena*	Partial SP; 0-8% nat. outcrossing (AVRDC)	Insects	Net cage; supple-mentary hand pollination
Endive	*Cichorium endiva*	SP		Isolation 600 m
Faba bean	*Vicia faba*	Mainly SP; 4-8% outcrossing	Insects	Isolation 1000 m; bagging; net cage
Grass pea	*Lathyrus sativus*	SP; significant levels of CP		Bagging; Net cage
Hyacinth bean	*Dolichos lablab*	Partially CP;	Insects	Isolation 500 m;
Lentil	*Lens culinaris*	SP		
Lettuce	*Lactuca sativa*	Mainly SP; 1-6% outcrossing	Insects	Isolation 100 m; bagging; net cage



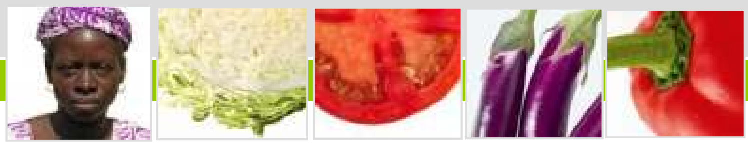
Pollination behavior of some vegetable crops (3)

Crop	Species	Pollinat. type	Pollination mechanism	Method used
Lima bean	*Phaseolus lunatus*	Mainly SP; up to 18% outcrossing	Insects	Isolation; net cage
Melon	*Cucumis melo*	CP	Insects	Isolation 1000 m
Mungbean	*Vigna radiata*	SP		
Okra	*Abelmoschus esculentus*	Partial SP; out-crossing 4-19%	Insects	Isolation 500 m; bagging; net cage
Onion	*Allium cepa*	Mainly CP; protandrous	Insects	Isolation 600 m; net cage w/ pollinator
Garden pea	*Pisum sativum*	Mainly SP		Isolation 100 m
Chili, sweet pepper	*Capsicum annuum*	Often CP	Insects	Isolation 500 m; bagging; net cage
Pigeonpea	*Cajanus cajan*	Normally SP; nat. outcrossing 5-40%	Insects	Isolation 500 m; bagging, net cage
Pumpkin	*Cucurbita moschata*	CP; monoecious	Insects	Isolation 1000 m; bagging & hand pollination; net cage



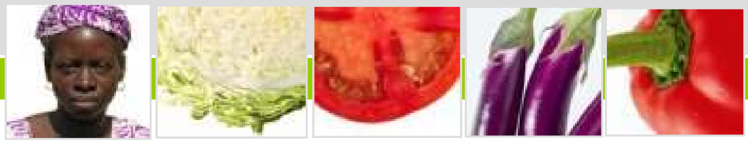
Pollination behavior of some vegetable crops (4)

Crop	Species	Pollinat. type	Pollination mechanism	Method used
Radish	*Raphanus sativus*	CP; self-incompatible	Insects	Isolation 600 m; net cage w/ pollinator
Safflower	*Carthamus tinctorius*	SP		
Sesame	*Sesamum indicum*	Mainly SP; up to 5% CP	Insects	
Soybean	*Glycine max*	SP		
Spinach	*Spinacea oleracea*	CP; dioecious	Wind	Isolation 2000 m; net cage
Sword bean	*Canavalia gladiata*	Mainly SP		Isolation 100 m
Tomato	*Solanum lycopersicum*	Normally SP; some species self-incompatible		Isolation 50 m; net cage w/ suppl. pollination, if necessary
Watermelon	*Citrullus lanatus*	CP; monoecious	Insects	Isolation 1000 m; bagging & hand pollination; net cage

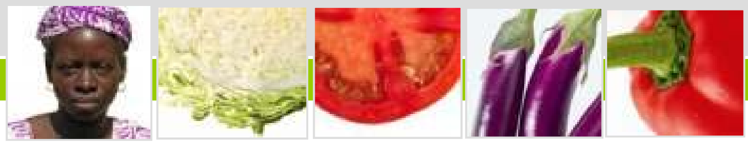


Bee hive for *Brassica* pollination in net cage

Transplanting of pepper



Covering with nylon net



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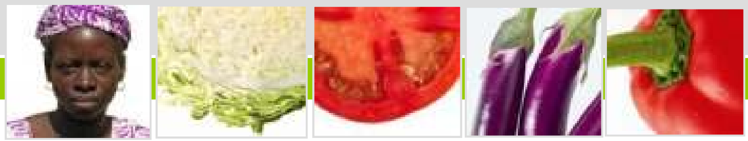
Irrigation / Accession identity

Irrigation:

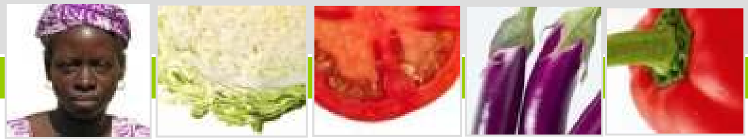
- Irrigate field when necessary
- Avoid water stress at all means
- Ensure adequate drainage
- Eliminate competition by weeds
- Provide stable water supply for irrigation

Accession identity verified during plant growth:

- Compare morphological data
- Compare with reference material (herbarium specimens or reference seeds)
- Roguing to be done with caution (i.e. plants growing off-row)

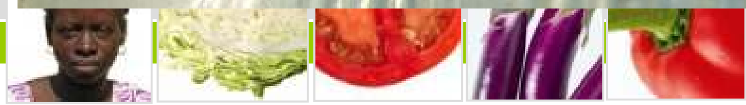


Removing nylon net



Harvesting of fruits

Regeneration of amaranth in net cages



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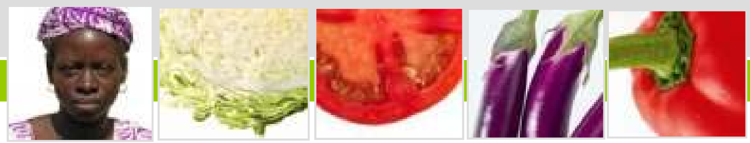
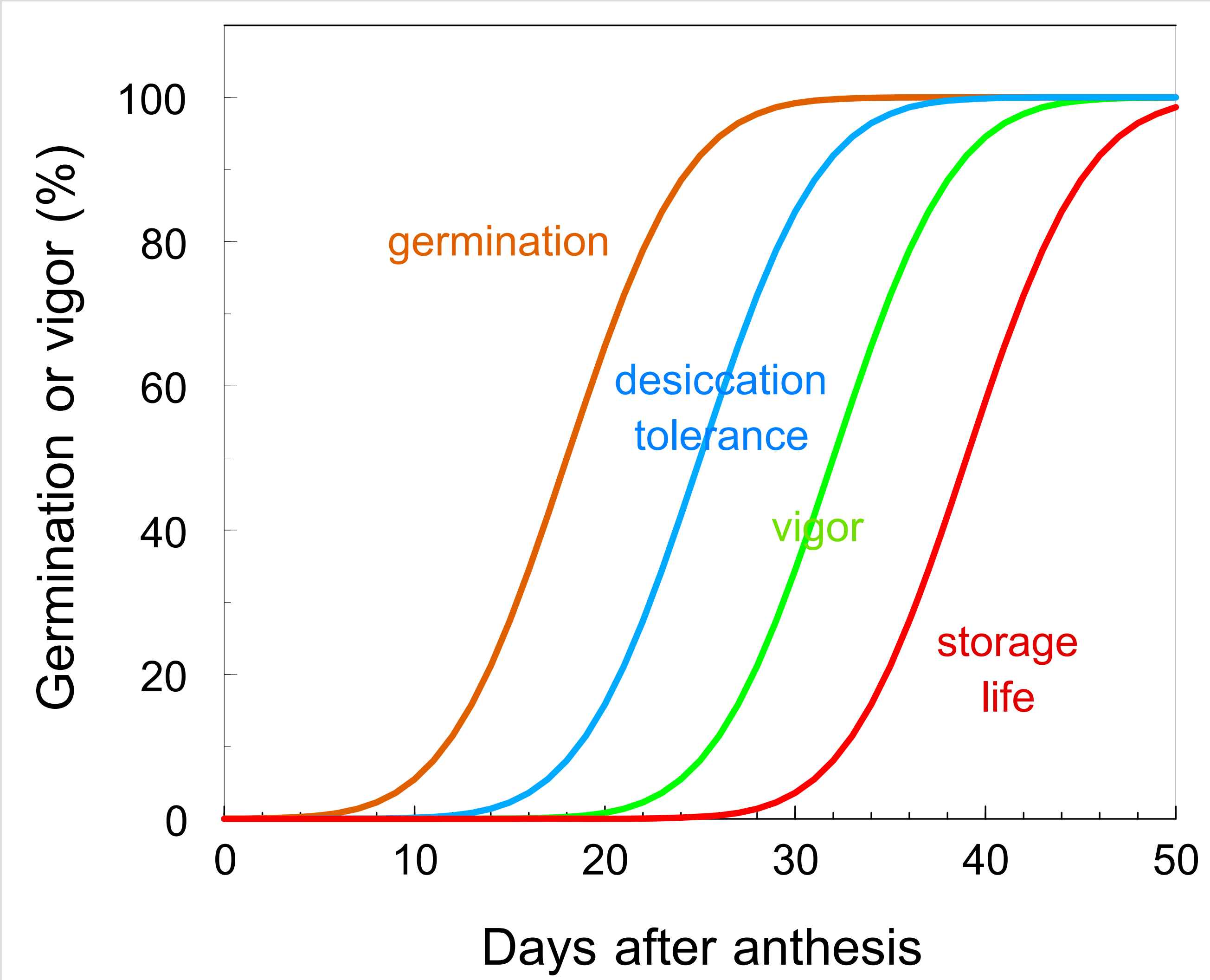
Seed regeneration of onion in netcages

Caging of shallot before flowering

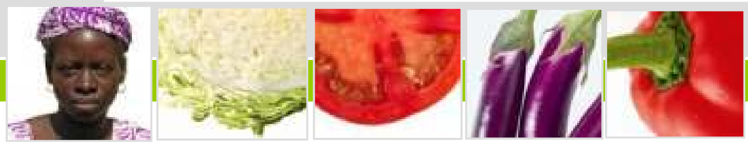
Seed quality during seed development and harvesting



Development of seed quality attributes during seed maturation



Source: Bradford et al., 2012



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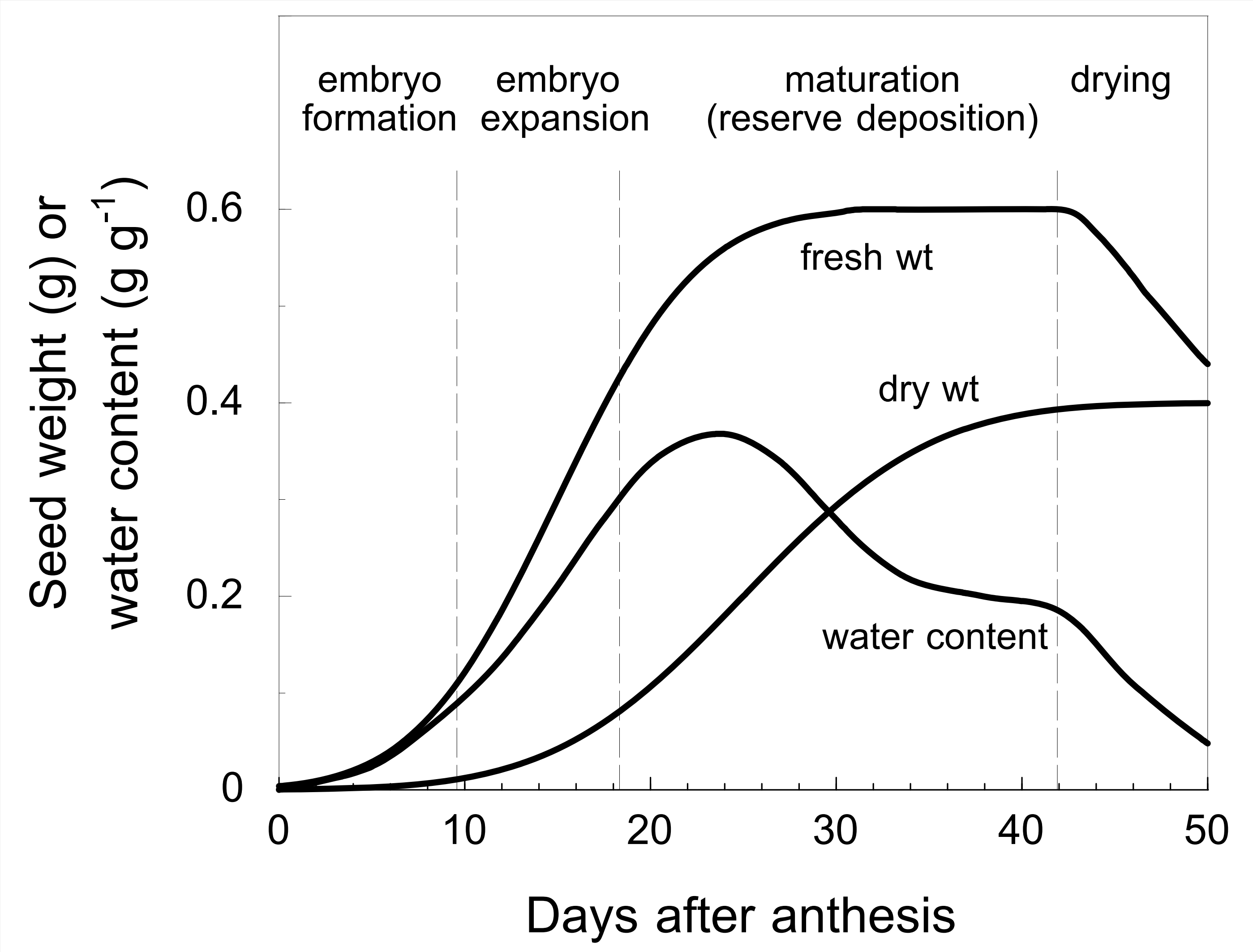
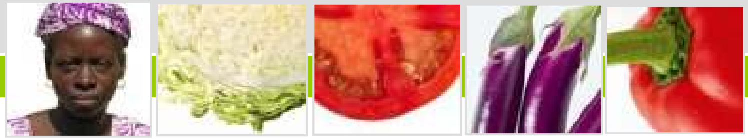
Development and maturation of orthodox seeds



Source: Bradford et al., 2012



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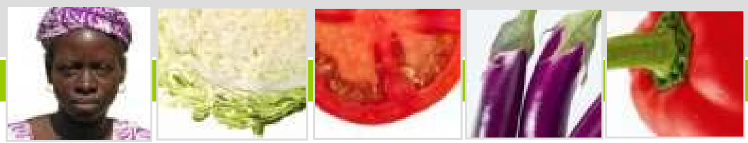
Seed maturity affects seed quality; indicators

- Seed viability and vigor are highest at the mature fruit stage and are lower with early or late harvest of the crop
- In onion, seed maturity associated with rapid reduction in chlorophyll and water content, increase in dry weight.
- Pepper seeds allowed to mature for up to one week within red fruits after harvest, for maximum germination
- Fruit cracks in okra indicate maximum seed germination and vigor
- Tomato and pepper: fruits must be fully red
- In cucurbits (watermelon), fruits are kept on the vine for some time or in storage to allow for complete physiological maturity of seeds
- In some crops, chlorophyll fluorescence of fruits is a good indicator of seed maturity (measures degreening process)



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Fruit position on plant and seed position within fruit affect seed quality

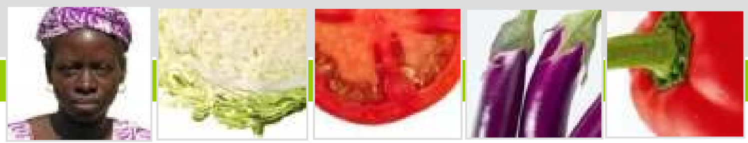
- In general, with increasing fruit numbers per plant, there is a reduction in fruit weight, seed weight and, consequently, viability

- Seeds from early pickings of tomatoes show higher seed quality

- Pepper seeds from fruits on main stem, first and sec. branches have high seed weight and germination; plants from these seeds are more productive and have better disease resistance than plants from seeds on 3rd or 4th order branches appearing later.

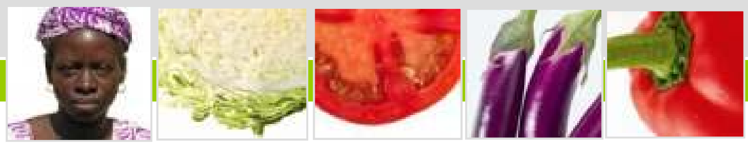
- Okra, eggplant: best quality seeds obtained from fruits borne at lower nodes

- Seed position in fruit: pepper and eggplant: seed from basal portion of fruit showed higher viability and vigor than those from middle and tip portion of fruit (**source-sink relationship**)



Insect pests and diseases

- Seed carry pathogens internally or externally; these multiply on sowing, affecting crop productivity and seed quality
- Insects and pathogens on seed cause shriveling, discoloration, decay, low germination and low vigor
- Pathogens thrive on injured seed, with high seed moisture content, high storage temperature
- Insects are often found in seeds of pod vegetables such as cowpea, French bean, and pea
- Seeds extracted from infected, rotten fruits are of inferior quality
- The microorganisms produce heat and toxins during storage, and induce biochemical changes
- Fungal pathogens (*Alternaria, Aspergillus, Penicellium, Cladosporium*) are mainly responsible for seed deterioration and reduced viability.



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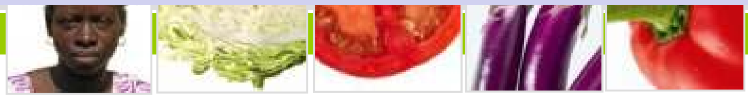
Harvesting and post-harvest management

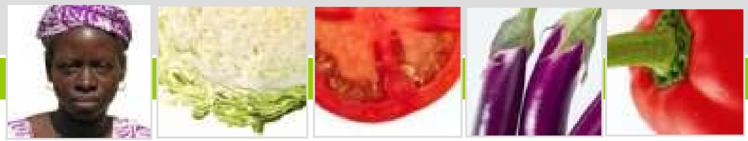
- Harvest at optimum maturity
  - when maximum # seeds are ripe
  - when seeds become tolerant to desiccation
  - before deterioration sets in
  - at or slightly before natural dispersal occurs
- Harvest repeatedly, if maturity is not uniform
- Harvest plants/infructescences individually, if maturity differs within same plot/plant
- Mix equal portions of seeds from each mother plant to avoid maternal effects
- Bags holding seeds/pods should enable good air circulation for drying
- Initiate seed drying immediately after harvesting



Seed harvesting of yard-long bean in net cage

Harvesting mature pods

Pre-drying of mature pods in screenhouse



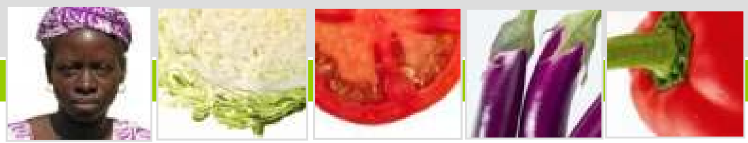
Factors affecting seed quality during seed processing, storage and priming

<u>During seed production</u>

- Climate
- Cultural practices
- Isolation distance and rogueing
- Seed maturity
- Fruit and seed position on mother plant
- Insect pests and diseases

<u>During seed processing and storage</u>

- Seed moisture
- Seed drying
- Storage temperature
- Relative Humidity
- Oxygen
- Storage insect pests and diseases

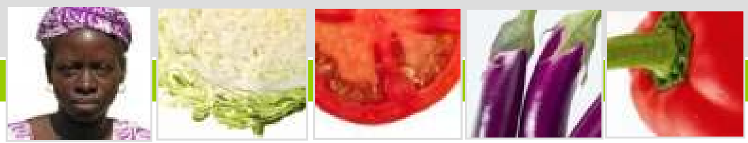


Goal of seed preservation in genebanks

- Maximize longevity of the stored seed at minimal cost

Longevity is affected by three major factors:

- Initial seed quality
- Water/moisture content of seed before entry into storage
- Conditions surrounding the seeds during storage (temperature, %RH)



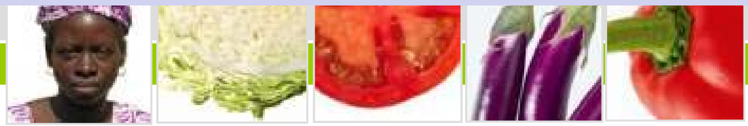
Conditions surrounding the seeds during storage: temperature and seed moisture

Longevity

Within certain limits: 5 - 14% SMC and 0 - 50 ^{o}C storage temp. $\Rightarrow$ reduction of 1% SMC or 5.6 ^{o}C (10 ^{o}F) of storage temp. doubles longevity of seeds (Harrington 1960; Harrington's rule of thumb).

General recommendations to maximize seed longevity:

- Store seeds at low temperatures (-18 ^{o}C)
- Store seeds at lowest possible SMC
- Make sure seeds are pathogen-free before storage
- Protect seeds from pathogens during storage
- Store seeds in dark
- Store orthodox and intermediate seeds with low SMC in vapor-proof storage containers
- Store recalcitrant seeds with sufficient moisture.



Conditions surrounding the seeds during storage: seed moisture content (SMC)

SMC: Amount of water in a seed

SMC is the most critical factor for seed longevity; small changes have large effects on storage life

It is recommended to **keep SMC low (5-7%) and constant.** Use hermetically sealed storage containers to avoid rehydration.

- $>$ 35% SMC: Seed germination may occur;
- 13 to 35% SMC $\Rightarrow$ seed respiration generates heat and facilitates pathogen activity;
- $<$ 10% SMC and $<$ 10 ^{o}C: metabolic activity and activity of pathogens is quite low.
- 5 $\pm$ 1% SMC: ideal for long-term conservation.

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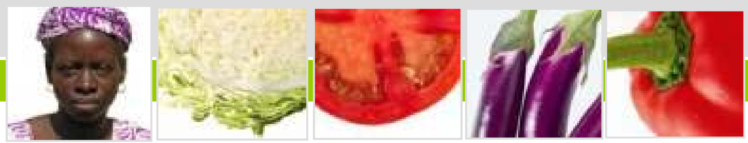
Seed moisture content (SMC)

SMC: Expressed as percentage weight of total seed weight before drying (wet-weight (wb) or fresh-weight basis:

$$\text{SMC (\% wb)} = \frac{\text{wet weight} - \text{dry weight x 100}}{\text{wet weight}}$$

SMC: Can also be expressed on a dry weight basis (db) i.e. loss in weight as a percentage of the dry weight of seeds:

$$\text{SMC (\% db)} = \frac{\text{wet weight} - \text{dry weight x 100}}{\text{dry weight}}$$



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Determination of seed moisture content (SMC)

Non-destructive methods:

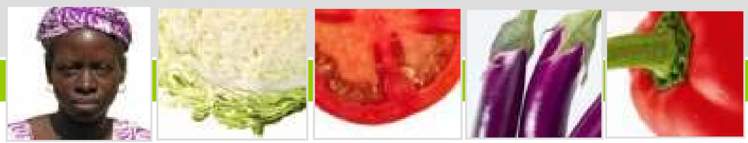
(a) <u>Quick moisture meters</u>:

Measure electric properties of seed moisture either by conductivity (electrical resistance of seed) or capacitance (ability of seeds to store electrical charge)

Need to be calibrated for each crop by standard oven-drying method; reliable in the range of 6-25%.

(b) <u>Digital humidity sensors</u>:

Measure the amount of water vapour in the air at equilibrium with a sample of seeds enclosed in a sealed chamber. Reading is expressed as equilibrium relative humidity (eRH). Using a calibration curve, it can be converted to conventional SMC.



Seed moisture content (SMC) determination

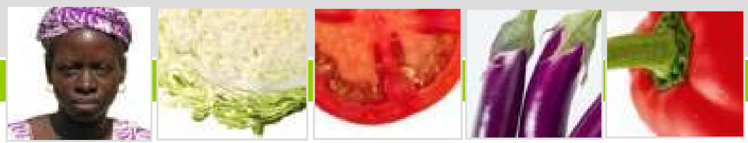
Methods for SMC determination:

- Determination of seed equilibrium RH (non-destructive)
- Determination of SMC using oven-drying method (ISTA) (destructive)
- Determination of SMC using infrared balance (destructive)

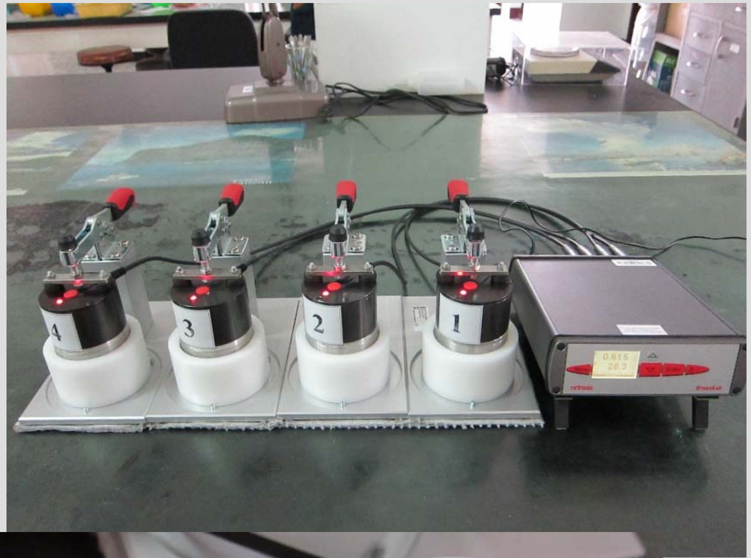
HygroLab C1

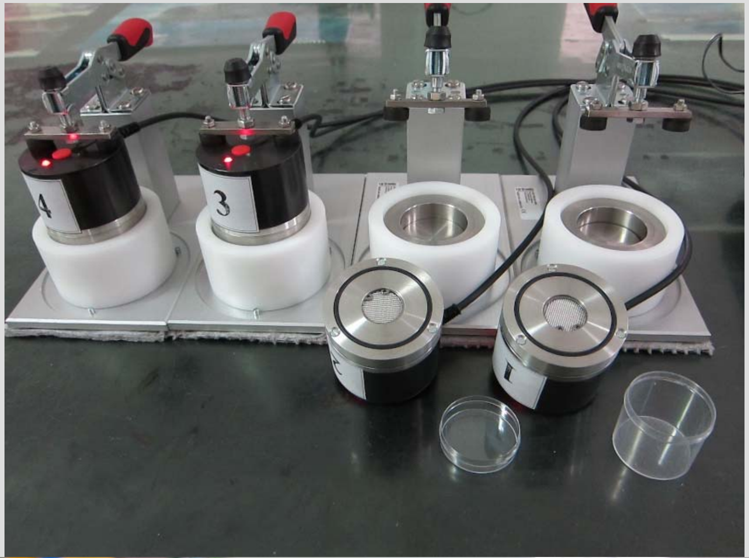
Hot oven method

Infrared balance

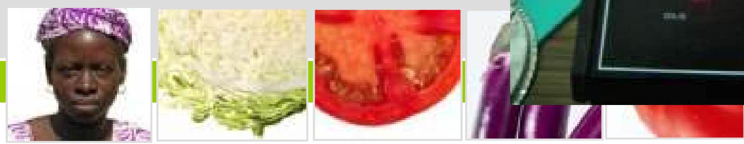


Non-destructive SMC determination with Hygro-Lab C1





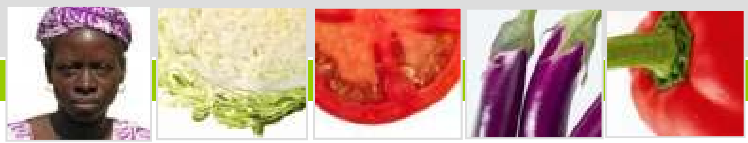
Infrared moisture balance (destructive)



The salt test

Fill a glass jar half-way with a mix of common salt and the seeds you want to test. Leave for 20 min, then shake the jar gently.

If the seeds are dry, the salt will fall to the bottom (right). If the seeds are still wet, the salt will stick to the sides of the jar.



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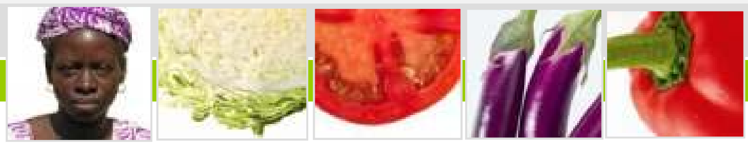
Equilibrium Moisture Content and Moisture Isotherms

Principles of seed drying and EMC

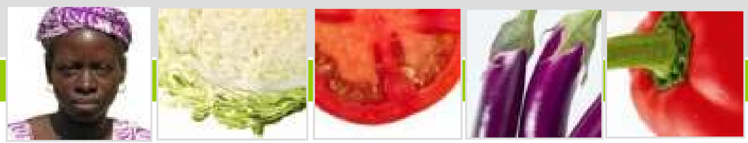
- Seeds are hygroscopic and absorb or give off moisture (depending on RH in surrounding air and gradient in water potential).
- Absorption or desorption occurs until water vapor pressure in the seed and surrounding air are balanced.
- For a given <u>species</u>: clear relationship between RH and SMC. Seeds loose or absorb water until balance is reached between SMC and RH of surrounding air at a specific temperature (moisture isotherm - MI).
- MIs depend on chemical composition of seeds and differ between species and accessions of the same species.



Equilibrium moisture content of crop seeds at 25 °C

Species	30% RH	45% RH	60% RH	75% RH
Bean, Lima	7.7	9.2	11.0	13.8
Beet	5.8	7.6	9.4	11.2
Cabbage	5.4	6.4	7.6	9.6
Carrot	6.8	7.9	9.2	11.6
Cucumber	5.6	7.1	8.4	10.1
Eggplant	6.3	8.0	9.8	11.9
Lettuce	5.1	5.9	7.1	9.6
Mustard	4.6	6.3	7.8	9.4
Okra	8.3	10.0	11.2	13.1
Soya bean	6.5	7.4	9.3	13.1
Tomato	6.3	7.8	9.2	11.1

Source: Rao et al., 2006



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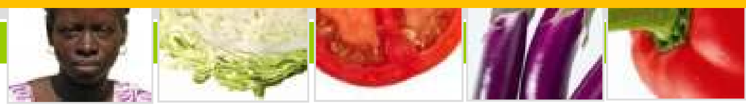
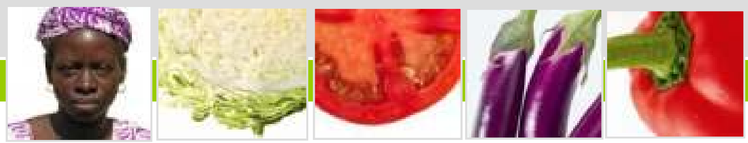
Recommendations for effective post-harvest handling of seeds

Seed maturity stage	Seed moisture status	Ambient conditions	
		“dry” (daytime RH <50%)	“humid” (daytime RH >50%)
Immature	85-100% equilibrium RH	Hold intact fruits under shaded, ambient conditions for 1-2 weeks*	
At natural dispersal	“Dry” <50% eRH	Hold in loosely packed bags in shade; minimize moisture adsorption at night	Transfer to seed bank ASAP or dry with desiccant or place in air-conditioned room
	“Wet” >50% eRH	Dry in thin layer, in well ventilated location; minimize moisture absorption at night	

*** Remove seeds from fleshy fruits as soon as morphological signs (e.g. fruit color) indicate that they are fully ripe. Allow to dry slowly under ambient conditions before transferring to a cool dry-room**

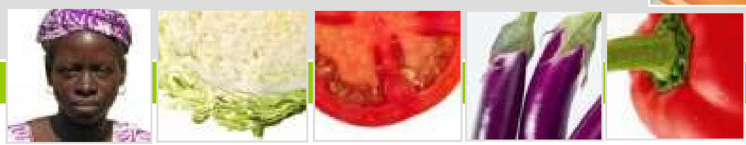


Seed extraction of tomato (*Solanum lycopersicum*)



Seed extraction of tomato (1)

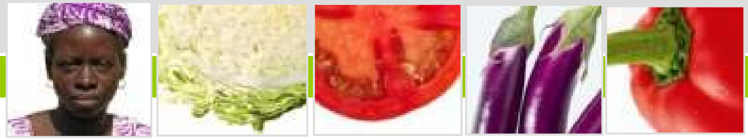
For extraction of tomato seeds, fruits are allowed to ferment for 3-5 days (depending on the temperature)



Seed extraction of tomato (2)

Rinsing the fruit flesh repeatedly to separate the seeds from the flesh with a sieve until only the seeds remain





Tomato seed extraction and packing (3)

Cleaning and washing the extracted seeds

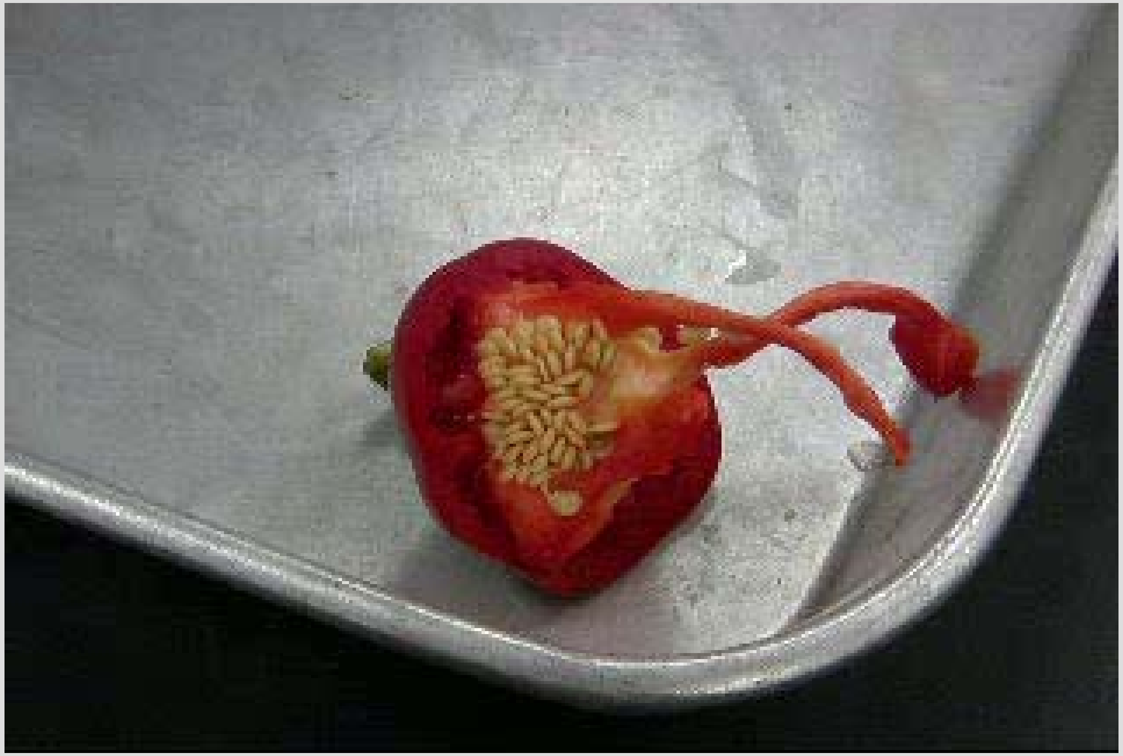
Pepper seed extraction

Manual seed extraction

Pepper seed extraction using a grinder

Video - Bulk Seed Extraction

Video - Sweet pepper seed extraction



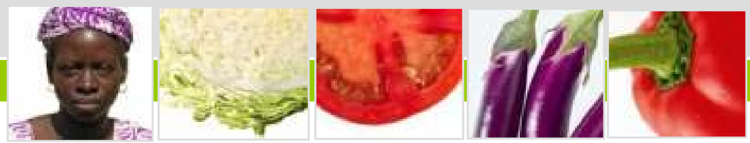
TSP seed treatment - TMV control

- Half fill a cloth or 32-mesh nylon bag with freshly harvested seeds

- Hang it in a 10% (w/v) solution of TSP for 30 min, making sure the seeds are always covered by the solution

- Transfer the bag to a fresh solution of TSP for two hours, again making sure the seeds are always covered

- Rinse the bag in running water for 45 minutes, shaking it occasionally.



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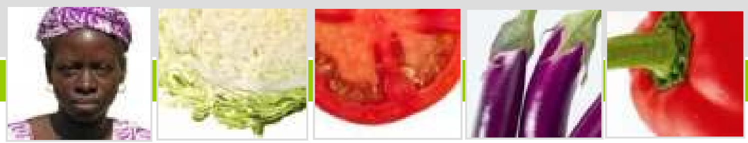
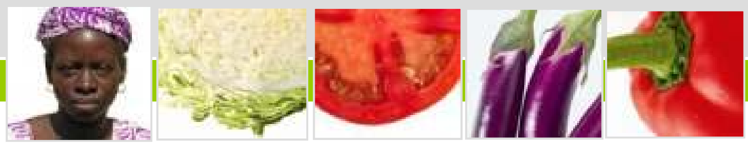
Video - TSP seed treatment - TMV control



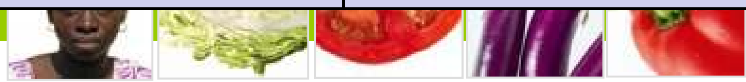
Clorox treatment for Bacterial spot control

- **Soak seeds in 13 ml of acetic acid per 1000 ml of water**
- **Shake the seeds in the solution for four hours and rinse with water three times**
- **Then soak seeds in 12.5 ml Clorox bleach per 1000 ml of water for 5 min**
- **Finally rinse under running water for 15 minutes (may decrease germination rate of seeds)**

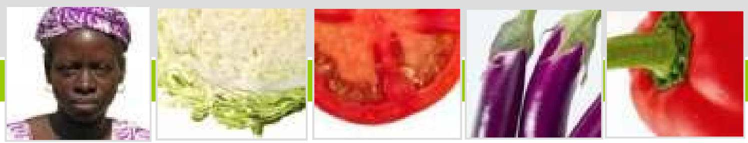


Principal Diseases of Pepper

Disease	Symptom	Control	Seed transmitted
Phytophthora blight: *Phytophthora capsici*	Damping off of young seedlings; root and crown rot; blight on leaves, stems and fruits	Resistant varieties; raised beds; crop rotation. Fungicides (RidomiMZ [metalaxyl + mancozeb] and fixed copper	No
Anthracnose: *Colletotrichum capsici; C. acutatum; C. gloeosporioides*	Mainly affects fruits, Initially, water-soaked spots, Later, spots expand, becojme dark and depressed, often with concentric rings	Pathogen-free seed; crop rotation; fungicides (Daconil/Bravo 500 [chlorothalonil], Maneb [mancozeb], Zineb	Yes?
Bacterial wilt: *Ralstonia solanacearum*	In late stage leaves turn yellow, wilt and drop. Vascular bundles in lower stem trun brown. Cut stems placed in water ooze milky streams of bacteria from vascular bundles	Resistant varieties; good soil drainage; optimum soil pH 5.5-7.0; rotate with non-Solanaceous crops	No
Insect-transmitted viruses: CMV, CVMV, PVY (aphid); Begomoviruses (whitefly); Tospovirus (Thrips)	Symptoms are variable	Resistant varieties, weed control, protect young seedlings with nets; IPM	No
Mechanically-transmitted viruses: TMV,ToMV, PMMV	Leaf mosaic, plant stunting, systemic chlorosis (bleaching of stems)	Resistant varieties.If not available, use tobamovirus-free seed ande dip tools and hands in skim milk before handling plants	Yes



Video - Sieving and packetizing pepper seed



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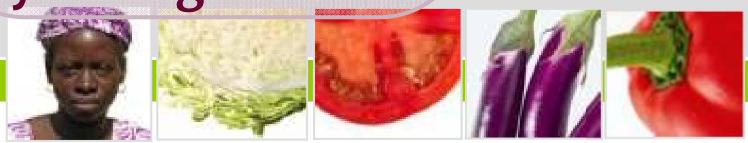
Extraction of common bean seeds

For natural drying, the pods are placed in a screen/plastic house for several days.

Thereafter, the seeds are separated from the pods through hitting the nylon bag.



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Seed extraction of yard-long bean

Drying of pods in screenhouse

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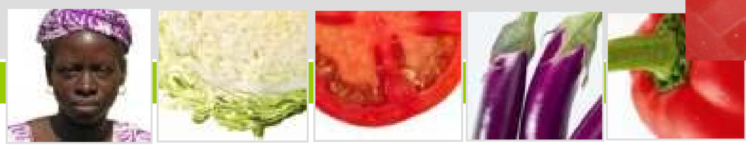
Seed extraction of yard-long bean (2)

Seed extraction

Seed extraction of yard-long bean (3)

Seed cleaning



Seed extraction of eggplant

The fruits are harvested at full maturity. The harvested fruits are kept for one week under shade, to allow the seeds to reach physiological maturity and high seed quality.

Seed extraction of eggplant (2)

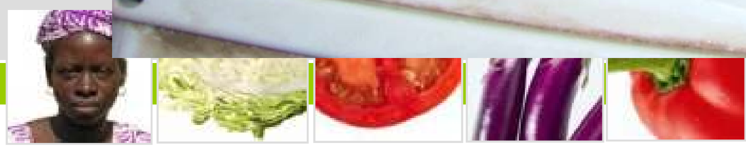
The fruits are cut into small pieces, then the seeds are extracted with the help of mincer.

Seed extraction of eggplant (3)

Rinsing of seed with water for cleaning.

Seed extraction of eggplant (4)

The extracted seed is placed in a tumbler for surface drying to remove excess water



Seed extraction of eggplant (5)

Step-by-step procedure for eggplant seed extraction

Step 1 →

Step 2 →

Step 3 →

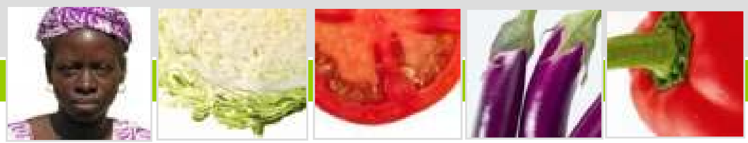
Step 4

Step 5 →

Step 6 →

Step 7 →

Step 8



Seed extraction of amaranth

The inflorescences are cut and placed into large nylon bags, with labels.

The nylon bags are kept for 10 days in a screenhouse for pre-drying.

Seed extraction of amaranth (2)

Manual seed extraction.

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Seed extraction of amaranth (3)

Seed cleaning process

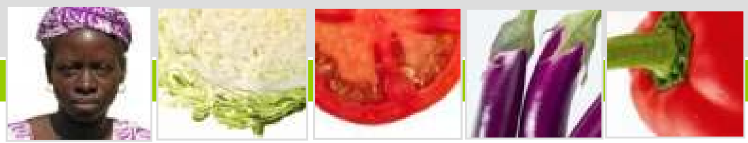
Seed drying methods

Dehumidified drying (seed bank use):

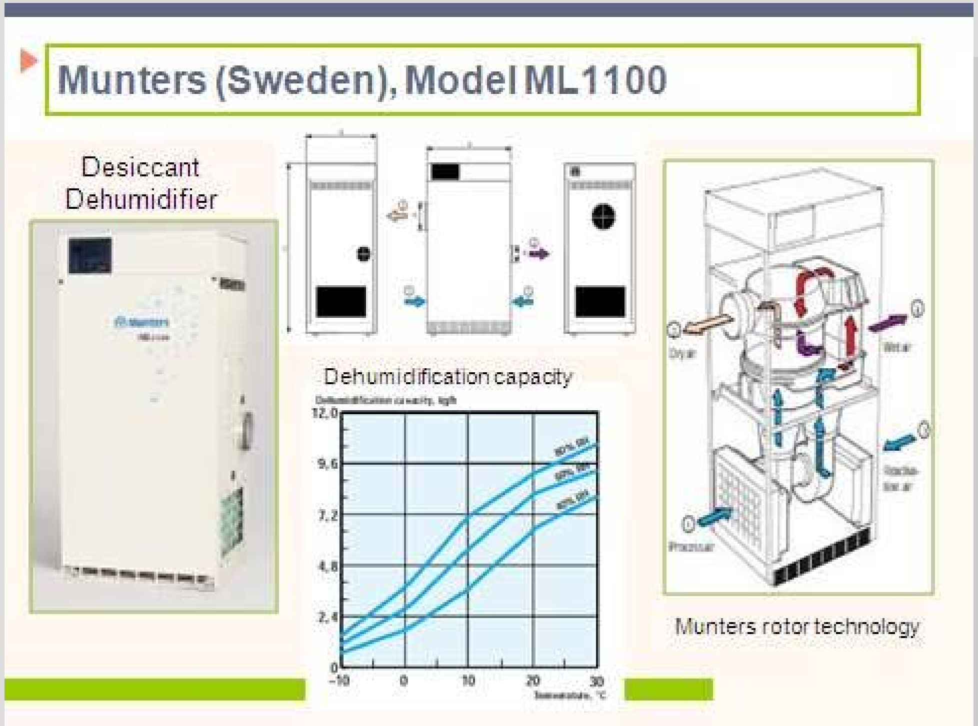
FAO Genebank standards (2011):

10-25% RH & 5-20 ^{0}C

Important: prevent overheating in the event of failure

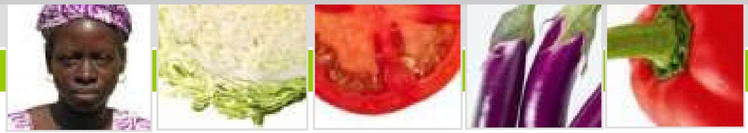


Dehumidified seed drying



Desiccant Dehumidifier, Model ML 1100, manufactured by Munters, Sweden

- 15-18°C temperature
- RH of about 10 - 15%



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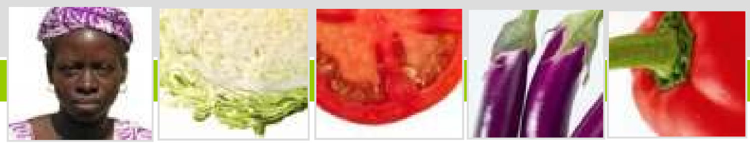
Low-tech seed drying methods

Silica gel drying:
Use of desiccators or glass jars in a cool place (20 ^{0}C) with self-indicating blue silica gel; seed/silica gel weight ratio - 1:1; for faster drying 1:3.

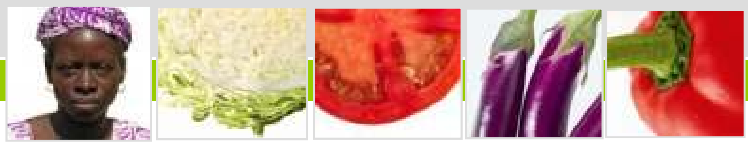
Other desiccants:
Molecular sieves, lithium chloride, calcium chloride and sulfate ($CaSO_4$); clays (bentonite); charcoal; toasted rice.



Drying with silica gel for use in field

Blue-colored silica gel at full capacity

Silica gel turns pink after water adsorption from seed

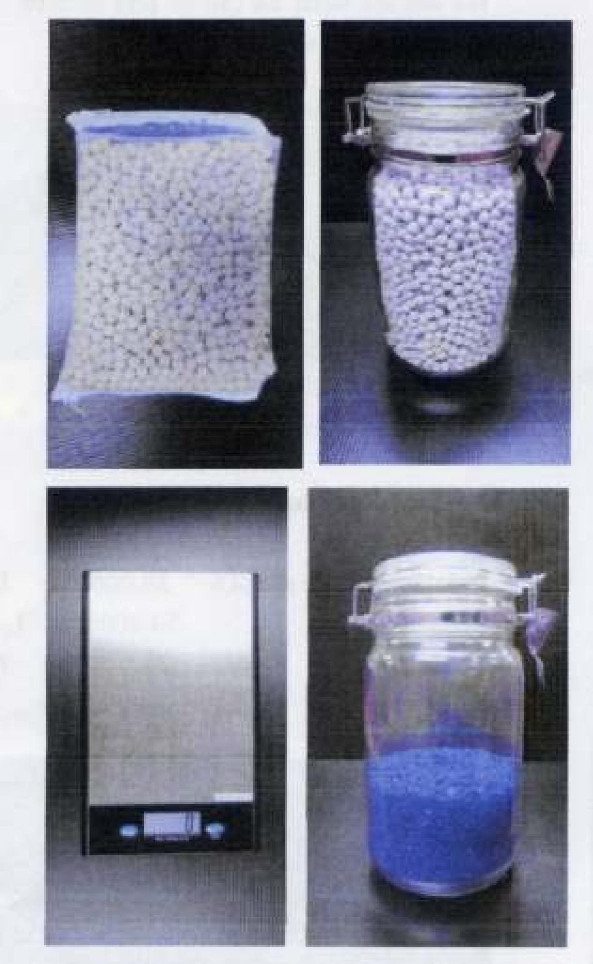


Low-tech seed drying drum

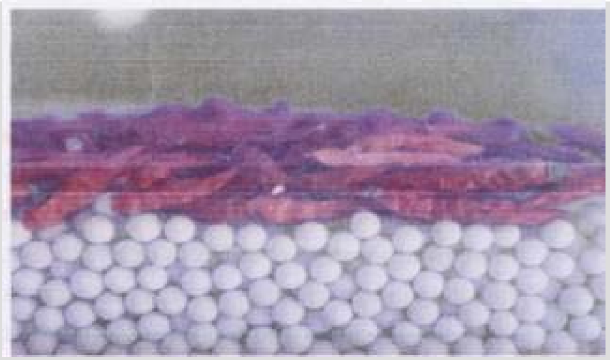
Rolled up mesh is buried in the silica gel and acts as support for seed bags (cloth or paper)

The blue drums are filled 2-tenths with a mix of clear and indicating silica beads (dry: yellow; wet (green).

Drying beads (Rhino Research, USA)

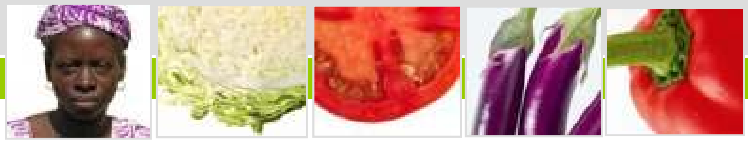


- Drying beads are effective desiccants
- On a ratio basis, silica gel might be better (rice)
- Beads do not work to 100% capacity; necessary to know starting SMC
- Useful when drying to low moisture contents in humid climates
- to maintain breeders' working collections



Drying chili with drying beads

Drying rice seeds with drying beads



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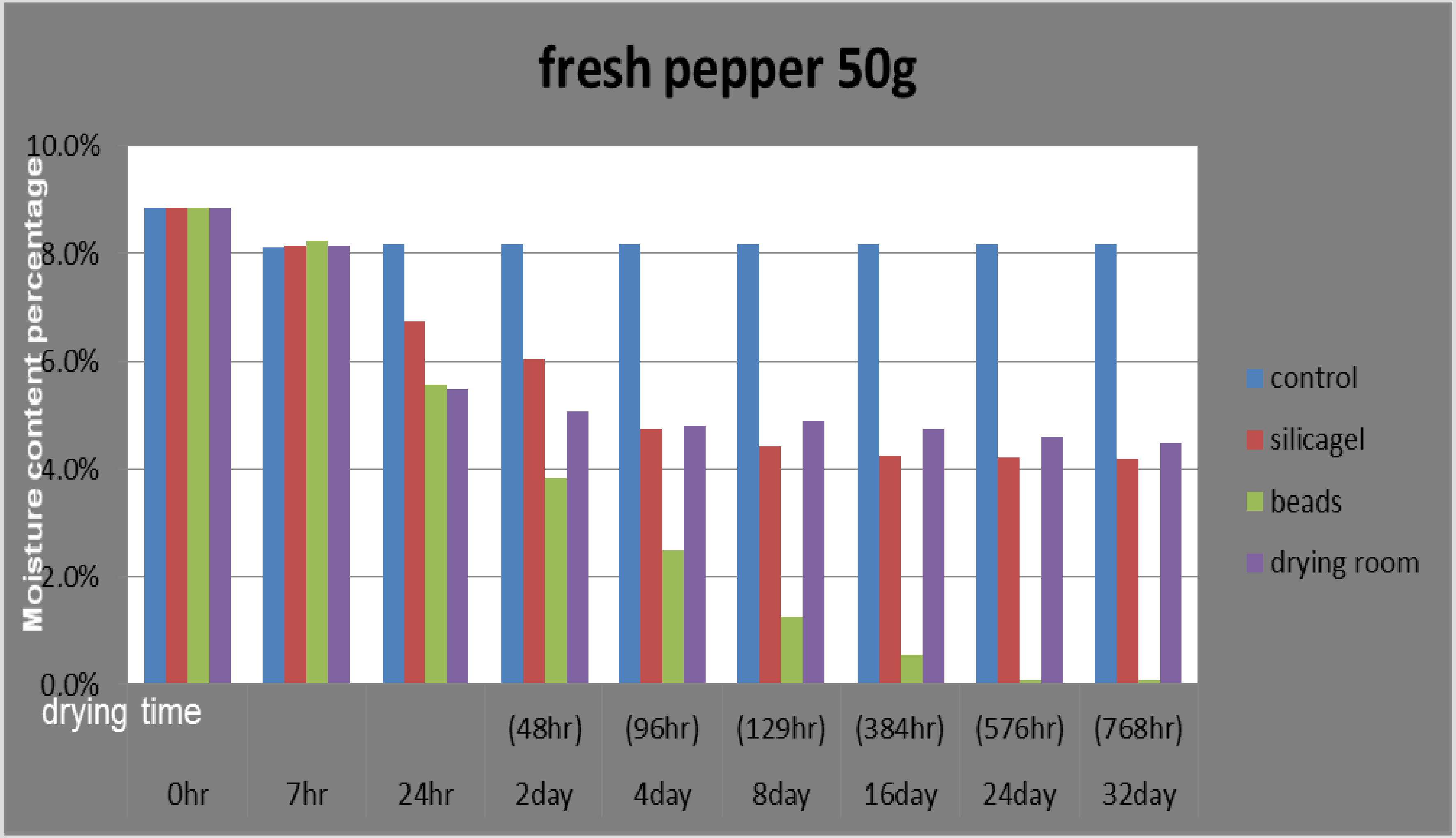
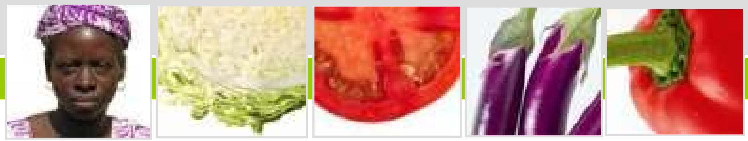
Comparison of seed drying methods





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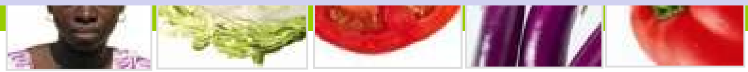
Comparison of seed drying methods

Initial seed moisture content of fresh pepper seeds: 8.9%
Target seed moisture content: 5% (blue line); highest acceptable level: 7% (red line).

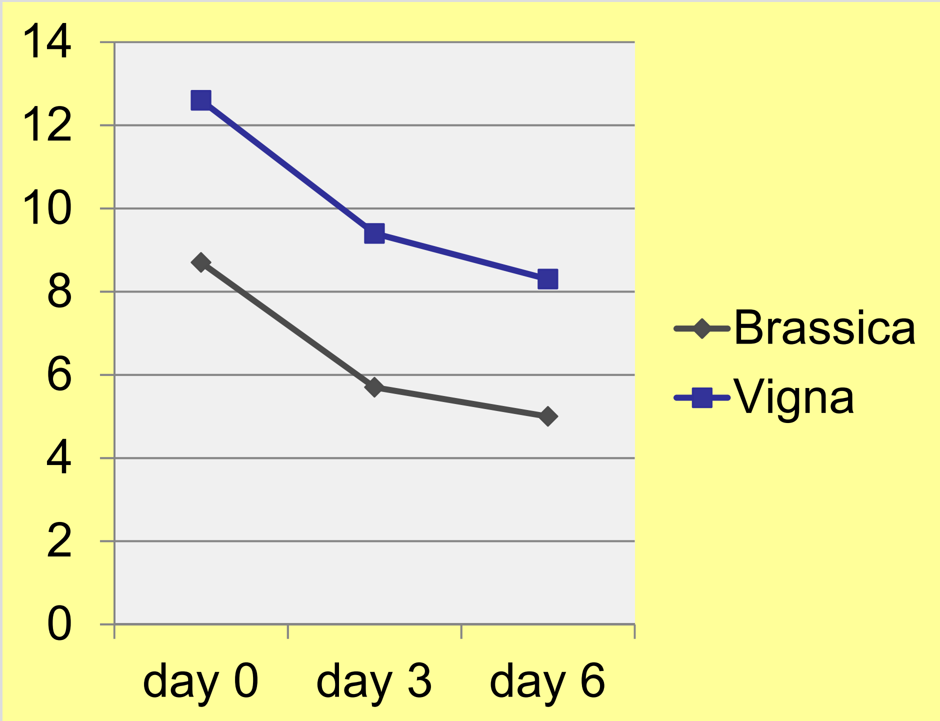
Drying room is standard condition for seed drying in genebanks and other drying methods should get close to the drying room seed moisture level to ensure high seed viability for an extended period of time.

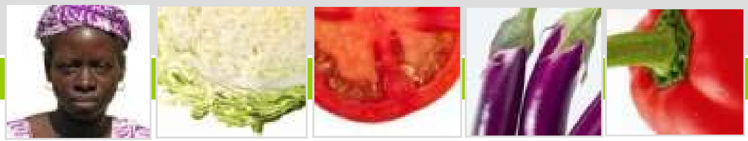
Ratio of seed to drying material used - 1 : 1 (50 g seed and 50 g of silica gel or drying beads respectively).

Results: Seeds kept in drying room and dried with silica gel reach target moisture content of just below 5% at four days and stay stable at this level thereafter. Seeds stored with drying beads show declining seed moisture content to dangerously low levels which compromise seed viability.



Drying *Brassica* and *Vigna* seeds in a refrigerator





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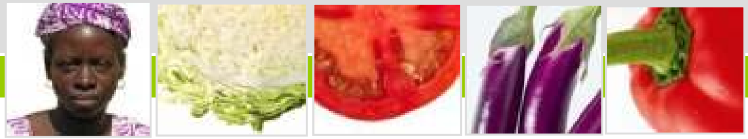
Seed drying methods at community level – baked charcoal; dried rice

Bake charcoal at low heat in an oven, allow it to cool and use it in a sealed container in a ratio of 3:1 to seed weight.

Dried rice has a similar effect.



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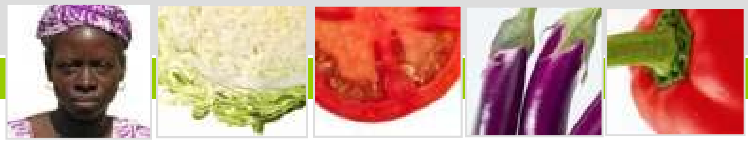
Seed storage

Seed need to be stored in air-tight containers to prevent moisture reaching the seed. Seed can be stored in small jars and the same can be placed in bigger jars. This is to maximize storage space and reduces the chance of moist air reaching the seed.

Small sachets of silica gel inside the containers are a safe method of assessing the tightness of the containers.



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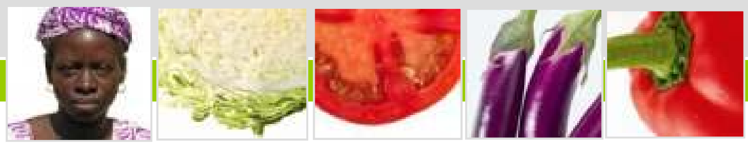
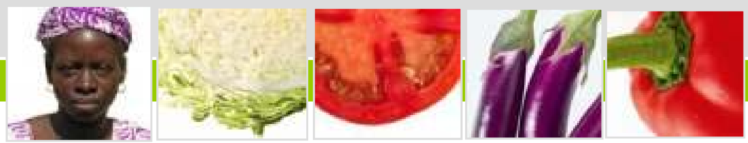
Seed Storage

- **Maintain RH + T < 60**
  - **(RH = 50% at 10 ºC)**
- **Keep good records and organization**

Aluminum foil packets



Evaluation of seed germination



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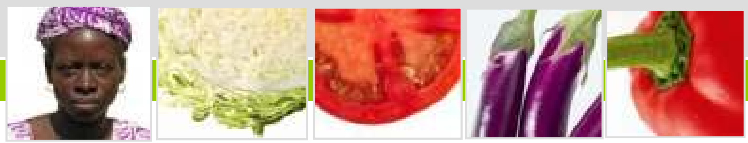
Monitoring seed viability during storage

- **Viability testing before seeds are packaged and stored**
- Intervals during storage: every 5 years in active collection (MT; 5 ^{o}C); every 10 years in base collection (LT; -18 ^{o}C)*
- Sample size: 200 seeds in four replicates (50 seeds each)
- Top-of-paper method (TP) for small seeds (most vegetables and forage grasses) in Petri dishes
- Between-paper method (BP) for medium and large seeds (cereals, grain legumes, vegetables); seeds are germinated between 2 layers of moist paper towels
- Germination in sterile moist sand for large seeds



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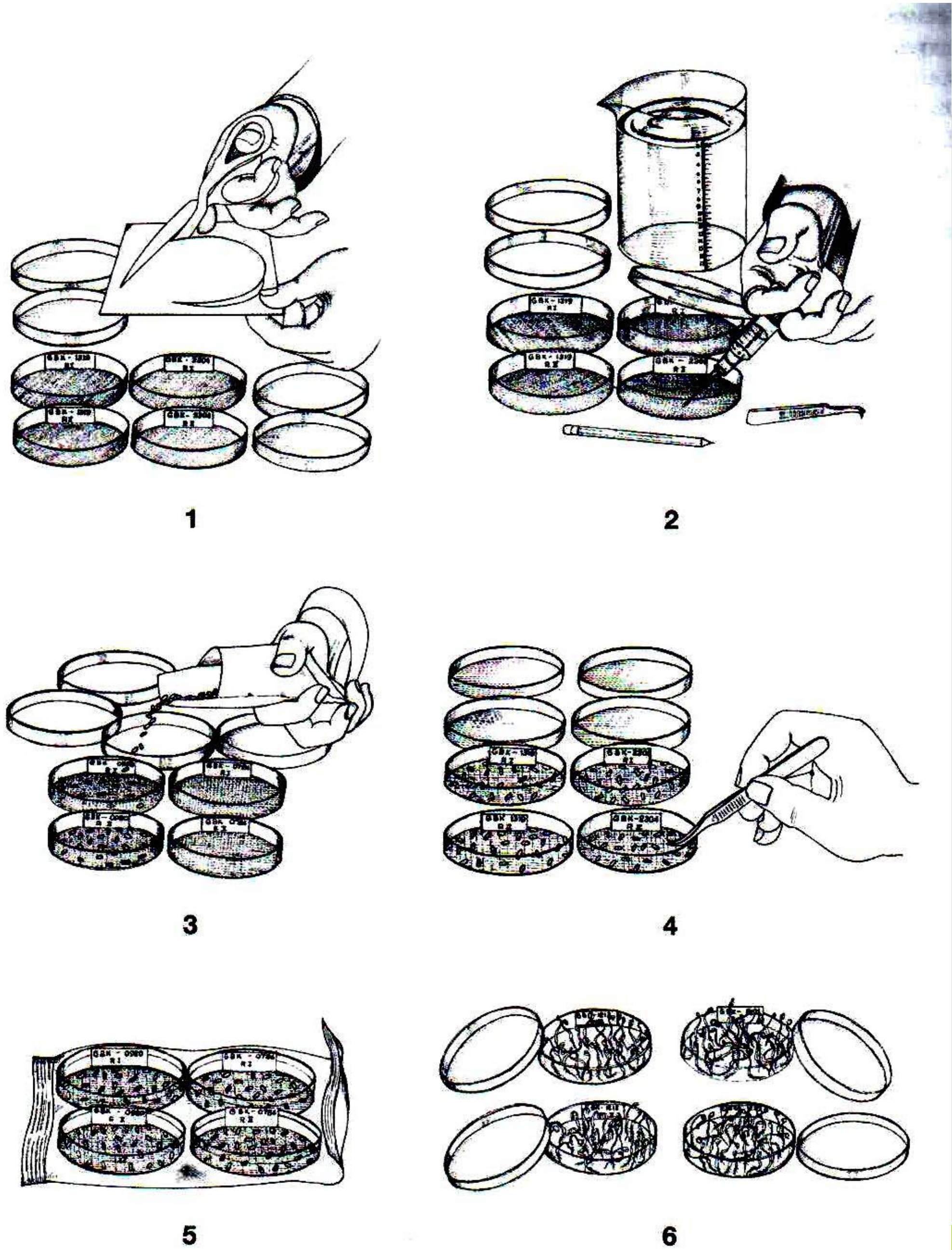
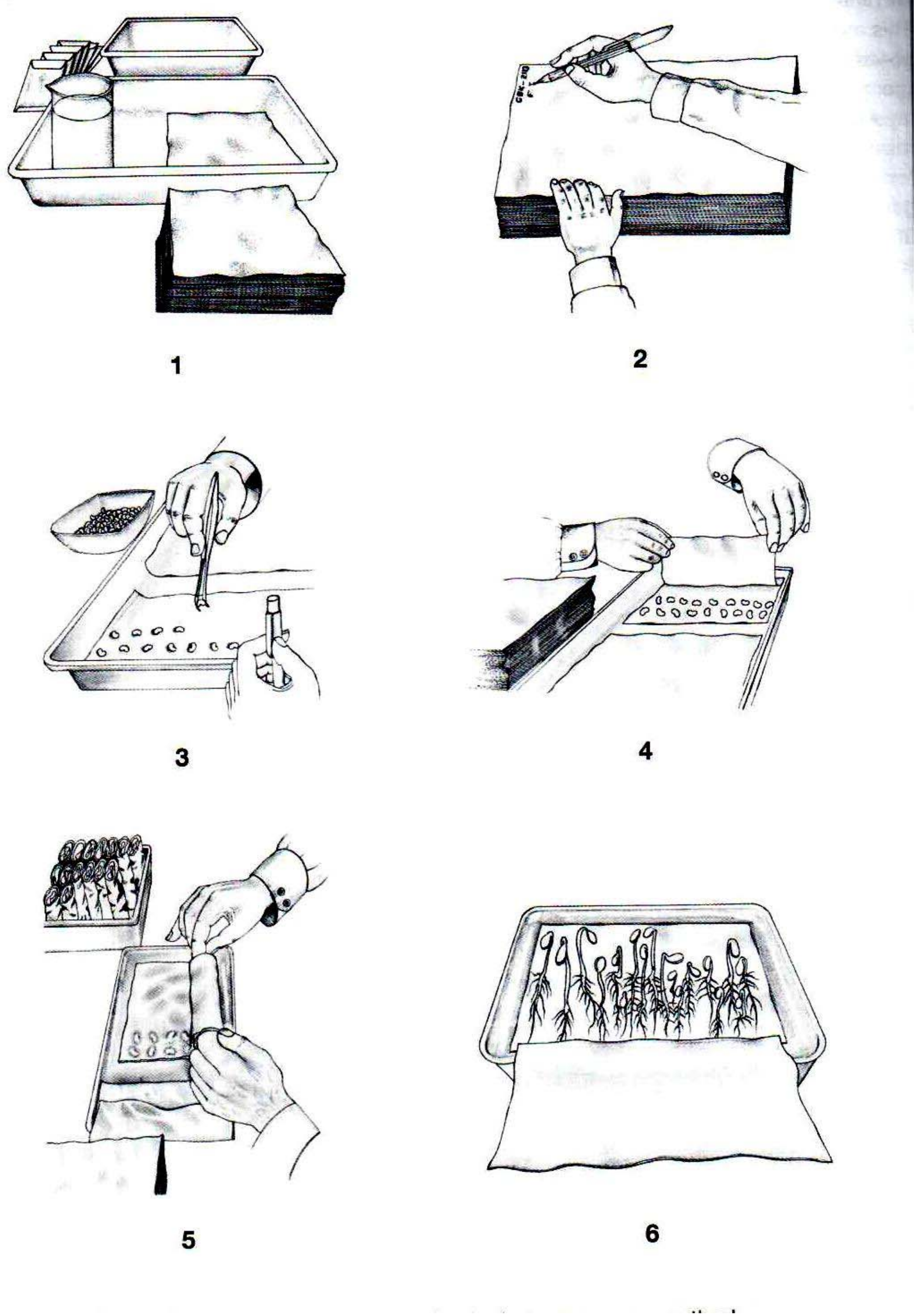
TP method



1. **Cutting absorbent paper or use round filter paper**
2. **Place paper at bottom of Petri dish and label containers with acc. #, replicate # and date**
3. **Moisten paper with distilled/boiled water**
4. **Cover the containers, place them in incubator and check moisture regularly**

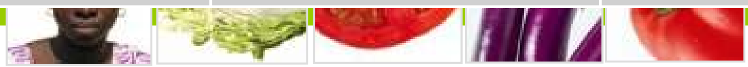
BP method

1. **Cut paper to convenient size**
2. **Label paper with acc. #, replicate # and date**
3. **Moisten paper with distilled/boiled water**
4. **Arrange seeds in rows at regular intervals**
5. **Cover the seeds with another moist paper towel**
6. **Roll the paper loosely und use rubber band to hold paper in place**
7. **Keep rolls upright in plastic tray**



Guidelines for testing germination rate

Crop	Species	Substrate	Temp. (°C)	First/final count (days)
Amaranth	*Amaranthus sp*	TP	20/30; 20	7, 14
Black gram	*Vigna mungo*	BP	20/30; 25; 20	3, 7
Bottle gourd	*Lag. siceraria*	BP; S	20/30; 20	14
Cowpea	*Vigna unguic.*	BP; S	20/30; 25	5, 8
Cucumber	*Cucumis sativ.*	TP; BP	20/30	3, 7
Eggplant	*S. melongena*	TP; BP; S	20/30	7, 14
Field bean	*Phas. vulgaris*	BP; S	20/30; 25; 20	5, 8
Mungbean	*Vigna radiata*	BP; S	20/30; 25	3,7
Okra	*A. esculentus*	BP; TP	20/30	4, 14
Pigeonpea	*Cajanus cajan*	BP	25	5, 10
Pumpkin	*C. moschata*	BP; S	20/30; 25	4, 7
Soybean	*Glycine max*	BP; S	20/30; 25	5, 8
Tomato	*S. lycopersicum*	TP; BP	20/30	5, 14

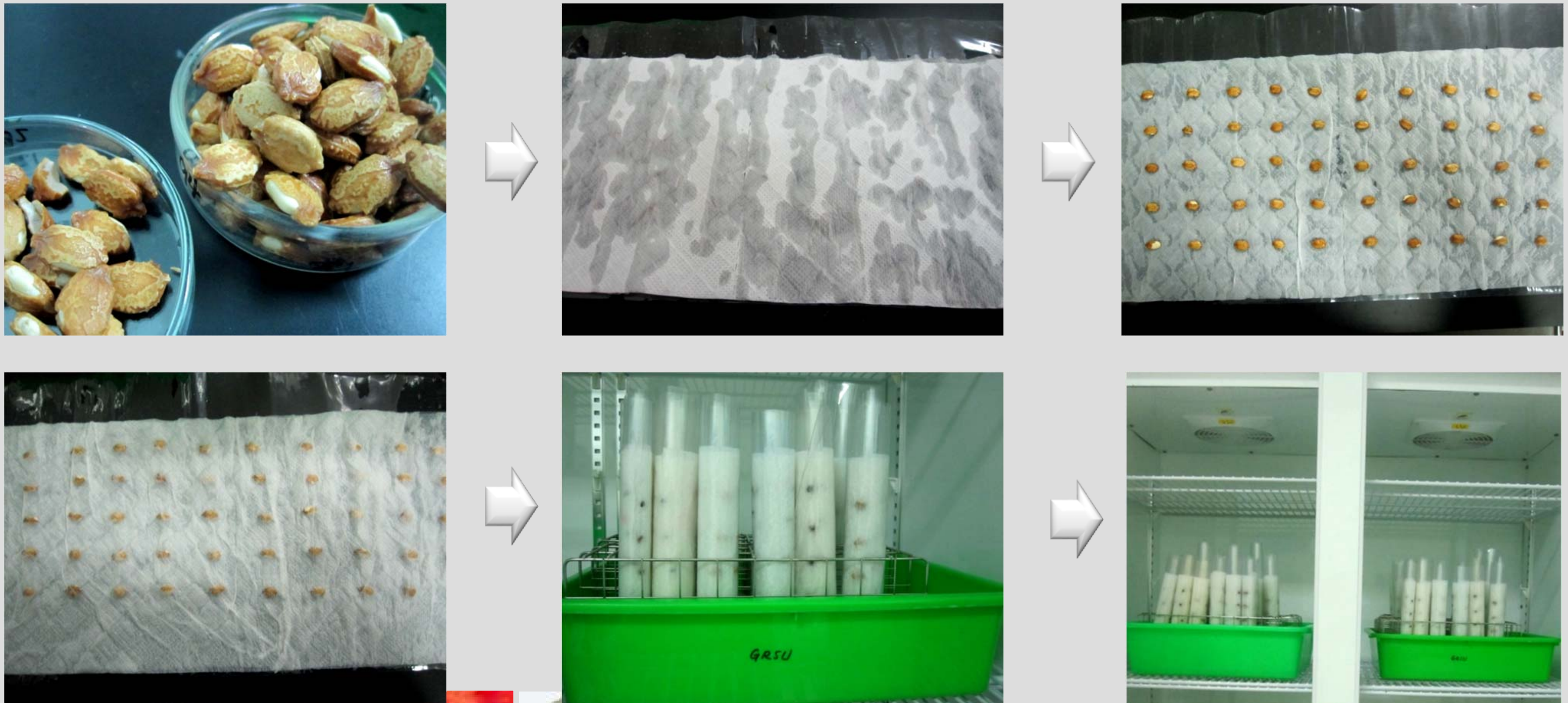


Top of Paper Method (TP) – *Brassica* seeds

Normal seedlings

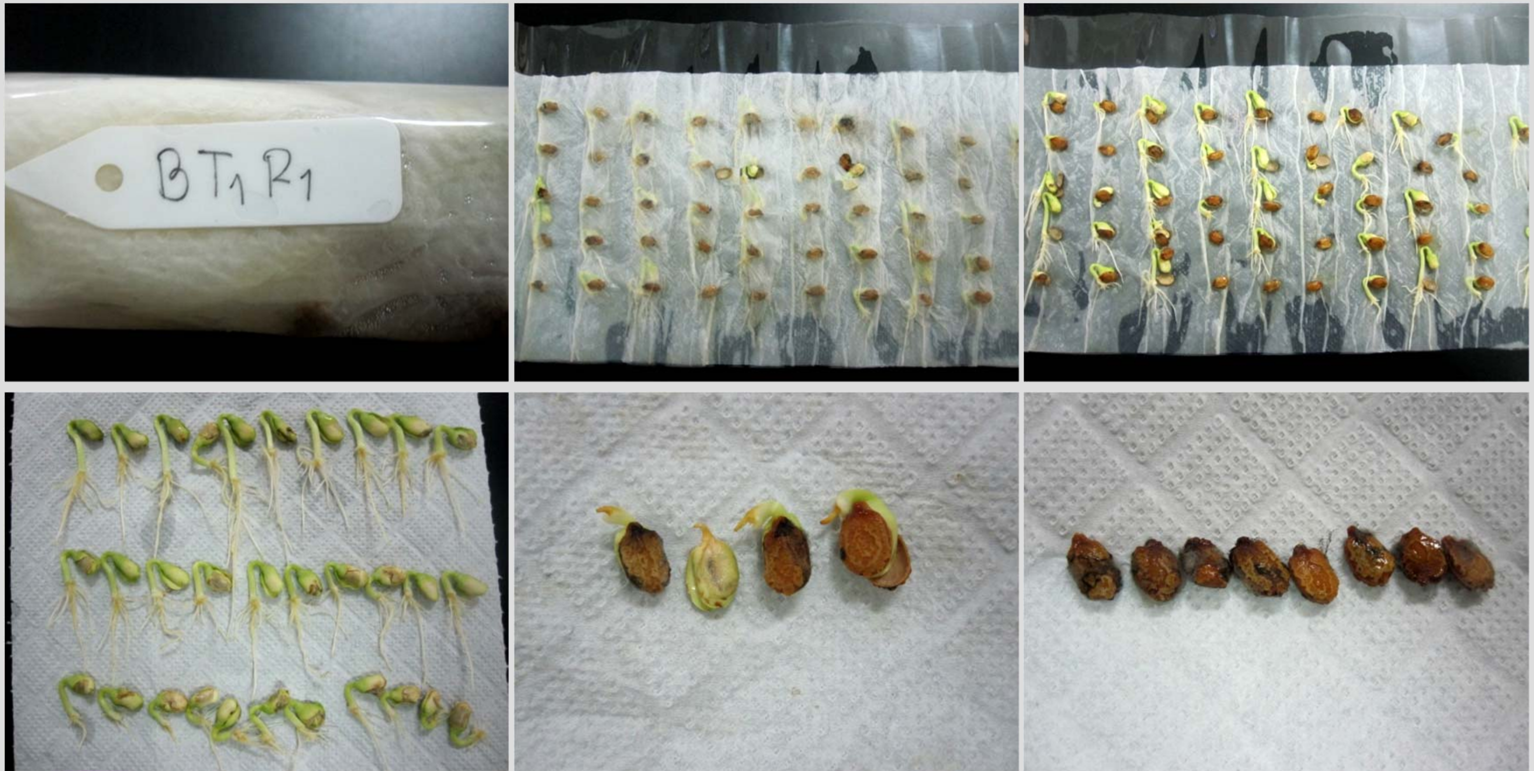
Abnormal seedlings

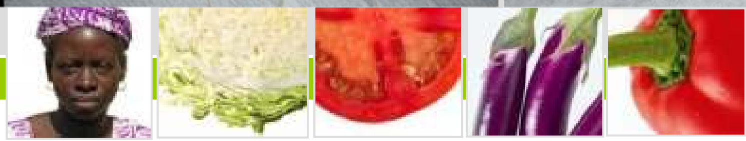
- **Between paper method (bitter gourd)**
- **Growth chamber with 20ºC/30ºC (8h/16h)**
- **Germination counting at 7+14 days**



Treatment 1; Control (no treatment)

Normal Seedling (%)	Ab-normal Seedling (%)	Dead Seed (%)	Hard Seed (%)
68.67	3.33	25.33	2.67



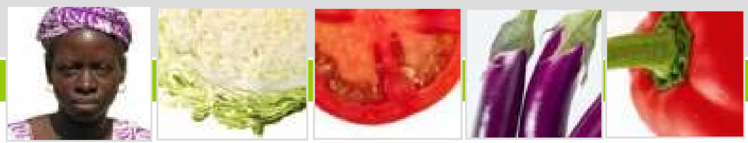


Predicted longevity of sel. species and monitoring frequency

Genus	Species	Predicted longevity (years)	Monitoring frequency (years)*
Lactuca	*sativa*	74.5	18.6
Allium	*cepa*	111	27.8
Glycine	*max*	112.5	28.1
Brassica	*napus*	131.5	32.9
Cucurbita	*pepo*	245	61.3
Cucumis	*melo*	266	66.5
Phaseolus	*vulgaris*	373	93.3
Pisum	*sativum*	446.5	111.6
Vigna	*unguiculata*	304	76.0
Vigna	*radiata*	1252	313

*Frequency: 4 intervals based on longevity

Source: 2nd Draft Genebank Standards



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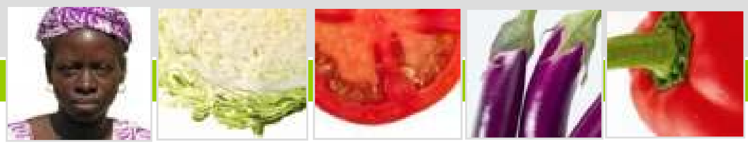
Thank you for your attention



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