



Guidelines for Eggplant Seed Regeneration and Storage

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Introduction to Eggplant

Solanum melongena, known as eggplant, aubergine, or brinjal belongs to Solanaceae family

- World production in 2007: 32 million t on > 1.8 million ha
- China (18.02 million t); India (8.45 million t); Egypt (1 million t); Turkey (863,737 t); Indonesia (390,846 t)
- South and Southeast Asia regarded as Center of origin and diversity
- Two to three crops per year; harvest starts at 60 days after planting; harvest period: 90-120 days
- Eggplant belongs to principal crops of AVRDC (3390 accessions)



Eggplant collection at WVC (cultivated, wild & weedy species)

Eggplant species	# Accessions	Species	# Accessions	Species	# Accessions
<i>S. aculeatissimum</i>	48	<i>S. lycopersicoides</i>	8	<i>S. scabrum</i>	60
<i>S. aethiopicum</i>	200	<i>S. macrocarpon</i>	54	<i>S. seaforthianum</i>	3
<i>S. americanum</i>	27	<i>S. mammosum</i>	6	<i>S. sepinum</i>	1
<i>S. anguivi</i>	22	<i>S. melongena</i>	1832	<i>S. sessiliflorum</i>	1
<i>S. atropurpureum</i>	1	<i>S. nigrescens</i>	2	<i>S. sisymbriifolium</i>	19
<i>S. aviculare</i>	2	<i>S. nigrum</i>	20	<i>S. sitiens</i>	5
<i>S. capense</i>	3	<i>S. nodiflorum</i>	4	<i>S. spp.</i>	716
<i>S. capsicoides</i>	3	<i>S. ocranthum</i>	1	<i>S. spinosissimum</i>	1
<i>S. chenopodioides</i>	3	<i>S. opacum</i>	3	<i>S. stramonifolium</i>	15
<i>S. cochabambense</i>	1	<i>S. parkinsonii</i>	18	<i>S. suaveolens</i>	4
<i>S. dasyphyllum</i>	3	<i>S. petinatum</i>	1	<i>S. surattense</i>	4
<i>S. eleagnifolium</i>	2	<i>S. pseudocapsicum</i>	3	<i>S. tarderemotum</i>	9
<i>S. ferox</i>	27	<i>S. quinquangulare</i>	1	<i>S. torvum</i>	110
<i>S. incanum</i>	11	<i>S. repandum</i>	1	<i>S. trilobatum</i>	10
<i>S. indicum</i>	36	<i>S. retroflexum</i>	2	<i>S. undatum</i>	2
<i>S. juglandifolium</i>	3	<i>S. rickii</i>	2	<i>S. viarum</i>	3
<i>S. laciniatum</i>	3	<i>S. rigescentoides</i>	1	<i>S. villosum</i>	32
<i>S. linnaeanum</i>	3	<i>S. rostratum</i>	1	<i>S. xanthocarpum</i>	25
<i>S. linociera</i>	11	<i>S. sarrachoides</i>	1		
Total					3390



Eggplant diversity at WVC





Contents of the regeneration protocol

- Which accessions to regenerate?
- Seed germination and treatment
- Seedling production
- Seedling characterization
- Crop rotation
- Land preparation
- Net cage construction
- Fertilizer application
- Transplanting to field
- Isolation



Contents of the regeneration protocol (2)

- Irrigation
- Weed control
- Staking and pruning
- Supplementary hand pollination
- Control of diseases and pests
- Characterization of vegetative plant growth
- Recording inflorescence data
- Fruit harvest and fruit characterization
- Seed extraction and pre-drying
- Seed drying
- Seed characterization
- Seed processing and storage

Decide which accessions to regenerate

Check the seed inventory and passport data to decide which accessions are of high priority for regeneration

- Accessions with less than 400 seeds or with low germination rates (lower than 80%) are of priority to regenerate.
- Pending internal and external seed requests for specific accessions



Locating the accessions for regeneration in the long-term cold storage

Seed germination and treatment (1)

Eggplant is a warm season crop and requires relatively high temperatures for optimum growth: 25-32 °C during the day and 21-27 °C at night.

The optimum temperature for eggplant seed germination ranges from 22 – 30 °C.



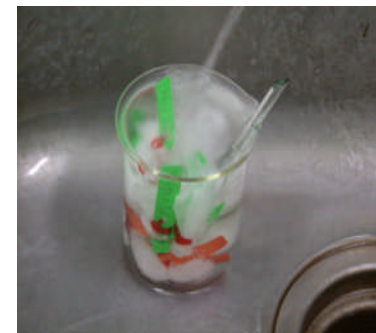
Seeds germinate in incubator.



Seed germination and treatment (2)

To prevent a build-up of massive virus infections in the field from seed-borne viruses, a treatment with tri-sodium phosphate is recommended. The steps are as follows:

1. Prepare a 10% $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ solution by dissolving 100 g of $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ in 1 liter H_2O (tap water).
2. Put dry seeds in a cloth or gauze bag and immerse it into the solution for two hours. A ratio of 100 g seed per 250 ml trisodium phosphate solution is recommended.
3. Seed is then rinsed in running tap water for 45 min under continuous stirring.
4. Seed is spread out on screens in thin layers with upward air movement for a few hours to remove excess moisture. Seed must be sown immediately thereafter



Seeds treated with 10% tri-sodium phosphate solution before sowing.



Seedling production

- Prepare sowing medium of peat moss and vermiculate in a ratio of 3: 1.
- Use plug trays with 50 cells and sow 2-3 seeds into the center of each cell.
- Sown seeds are covered with an additional thin layer of sowing medium.
- Seedlings are raised in a screenhouse and watered twice a day to keep sowing medium sufficiently moist.
- After 2 weeks, cells are fertilized with urea solution (2-3 g/L).
- Thinning to 1 vigorous seedling is done at the 1-2 true leaf stage.



Prepare sowing medium (peat moss : vermiculite = 3:1)



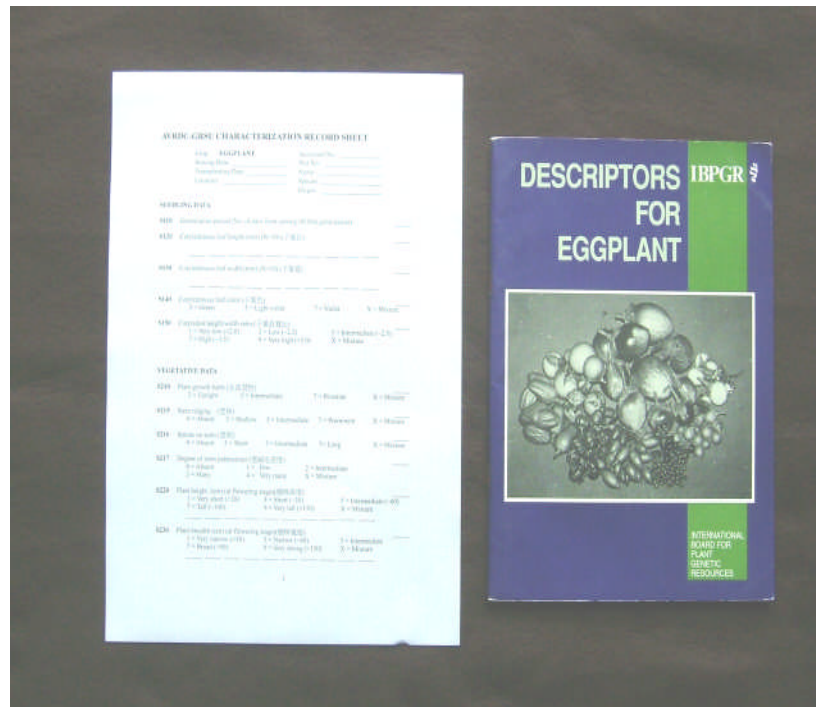
Sow seeds into plug trays w/ 50 cells



Watering after seeds are sown



Seedling characterization



Morphological characterization is based on a standard set of descriptors published by IBPGR (1990).
Descriptors for Eggplant

Recording data sheet and descriptors for eggplants

Seedling characterization (2)

Five traits are recorded at the seedling stage:

- germination period (no. of days from sowing to first germ.)
- cotyledon leaf length (mm)
- cotyledon leaf width (mm)
- cotyledon leaf color
- cotyledon length/width ratio



Seedling data collected at seedling stage



Seedlings nursed in screenhouse for optimum growth





Crop rotation

To avoid accumulation of diseases and insect pests, planting eggplants immediately after other crops of the Solanaceae family (tomato, pepper, potato) should be avoided.

After paddy rice, the incidence of bacterial wilt and nematodes is reduced and eggplant can be safely sown.





Land preparation

Selection of land: Eggplant grows best in rich, deep, well-drained, sandy loam or silt loam soils. The ideal pH value ranges from 5.5 - 6.8.

Field preparation: The field is plowed and harrowed, and 20-25 cm high beds are formed.

Bed mulching: The beds are covered with 1.5 m wide black polyethylene mulch sheets to reduce emergence of weeds, prevent soil compaction, and conserve soil moisture.



Select land and plow it



Plots prepared for planting



Bed mulched with black polyethylene sheets

Net cage construction

Net cages consisting of iron pipes and 32 mesh netting are built over the raised beds to prevent insect damage and the transmission of viruses by white flies.

Six accessions of eggplant can be grown in one net cage of 112.5 m² (22.5 m long x 5 m wide) in double-row plots with a spacing of 75 cm between rows and 40 cm within. The double-row plot size is 9 m² (6 m long x 1.5 m wide) consisting of 30 plants per accession.



Frames protected with special bitumen coating



Net cage door prepared



Placing the net over the cage structure



Net cage ready for transplanting of eggplants



Transplanting to double-row plots



Fertilizer application

Fertilizer is applied twice during regeneration in the field.

At the time at field plowing and harrowing, a basal fertilizer application is made with complex no. 1 (NPK = 20-5-10) at a rate of 500 kg/ha.

A supplementary application is made 6 weeks after transplanting with fertilizer complex no. 43 (NPK+Mg = 15-15-15-4) at 500 kg/ha.



Basal fertilizer application before transplanting



Transplanting to the field

About 5 weeks after sowing, seedlings are ready for transplanting to the field inside net cages.

About one week before transplanting, water is reduced to harden the seedlings and reduce transplanting shock. Seedlings are thoroughly watered 12-14 hours before transplanting. Out of the initial 50 seedlings, 30 seedlings of uniform growth will be selected for transplanting.



Seedlings are ready for transplanting to the field



Transplanting to the field (2)

Thirty eggplant seedlings per accession are transplanted into double-row plots with a spacing of 75 cm between rows and 40 cm within.

Holes are dug into the plastic mulch to bury the plants deep enough so that the first true leaf is just above the mulch surface. Soil is pressed firmly around the root.

Immediately after transplanting, the seedlings are watered.



Wooden labels are prepared for acc. identif. in the field



Double-row plots used to transplant eggplant seedlings into net cages in the field





Isolation

Eggplant's cone-like anthers contribute to high levels of self-pollination.

The stigma slightly protrudes beyond the anthers, giving rise to accidental outcrossing, mainly due to insect activity in open fields.

In open fields, outcrossing rates of 0 – 8.2% have been observed in the Center's fields. Outcrossing has not been observed in insect-free net cages used for regeneration by the Center.

In open fields, a minimum isolation distance of 200 m for foundation seed and 100 m for certified eggplant seed is recommended.



Eggplant flower



Irrigation

As weather is usually dry during regeneration months, furrow irrigation is applied about every 3 weeks.

Irrigation is critical during flowering and fruit set; lack of water during these periods may cause blossom-end rot and malformed fruit.



Furrow irrigation is used at WVC





Weed control

Eggplant establishes slowly and cannot compete with aggressive weeds. Plastic mulch effectively controls weeds.

Chemicals are used to control emerging weeds in furrows, approx. 3 weeks after transplanting.

Amex (butralin) and Roundup (glyphosphate) can be used as pre-emergence herbicides, while paraquat (bipyridyl) is used to control weeds already established.

Staking and pruning

Bamboo poles are set 3-4 weeks after transplanting to support the plant for the expected fruit load. To enhance fruit color and quality, three branches per plant are maintained and all other lateral branches are periodically removed.

Removal of older leaves from lower third of the plants increases air circulation and enhances light penetration within the canopy.



Pruning of plants inside net cage



Staking to support plants for expected fruit load



Defoliation of older leaves



Supplementary hand pollination

Anther collecting:

The anthers are collected from the flowering plants during early morning hours. Necessary instruments: forceps, small plastic cups, parafilm, cotton, alcohol, glassine bag, and silk stocking.

The anthers inside glassine bags are placed in a dryer at 15 °C and 15 % RH for 48 hours. The anthers are then placed in a small plastic cup with cover and the pollen is extracted by shaking.



Anthers collected from flowers, placed inside glassine bags, and dried for 48 hours.



Supplementary hand pollination (2)

Pollination: Done during morning hours by dipping a finger tip into the collected pollen and touching the stigma with the pollen-covered finger. Unused pollen can be kept in a freezer up to one month.



Plastic cups with collected pollen, storage in freezer and hand pollination



Control of diseases and pests

Grey mould rot (*Botrytis cinerea*) results in loss of leaf area and fruit quality. Control with weekly fungicide sprays (Milcrub-super 25 % 1000 x).

Thrips (*Thrips palmi*) occur frequently during the regeneration cycle and cause browning of the leaves, especially on the lower leaf surface. When thrips scratch fruit surfaces, irregular discoloration occurs. Weekly sprays with (1) Oxamyl 24 EC (dil. 1: 500) or (2) Carbosulfan 48 EC (dil. 1: 1000).

Cotton leafhoppers (*Amrasca biguttula biguttla*) feed mainly on the underside of eggplant leaves. Weekly sprays of (1) Carbosulfan 48 EC (dil. 1: 1000) or (2) Bifenthrin 2.8 EC (dil. 1: 1000).



Control of diseases and pests (2)

Two-spotted spider mite (*Tetranychus urticae*) feeds on large colonies on the underside of eggplant leaves, creating white specks on leaves and causing leaf withering. Weekly sprays with Bifenthrin 2.8 EC (dil. 1: 1000) or Cyhalothrin 2.8 EC.

Aphids (*Aphis gossypii*) colonize and feed on tender shoots and the underside of young leaves by sucking the plant juice, weakening infested plants. Weekly sprays with Carbosulfan 48 EC (dil. 1: 1000), Bifenthrin or Primicarb.

Eggplant fruit and shoot borer (*Leucinodes orbonalis*): The larvae attack the terminal shoots and young fruits resulting in withering of the shoots; attacked fruits become unmarketable. Weekly sprays with (1) Bifenthrin 2.8 EC (dil. 1: 1000) or Lannate 90 % WP (dil. 1: 2000).



Characterization of vegetative growth

Vegetative data (16 traits) on plant growth are collected at fifty percent of flowering:

Plant growth habit, stem ridging, spines on stem, degree of stem pubescence, plant height, plant breadth, plant branching, petiole color, petiole length, leaf blade length, leaf blade width, leaf blade lobing, leaf blade tip angle, leaf blade color, leaf prickles, leaf hairs.

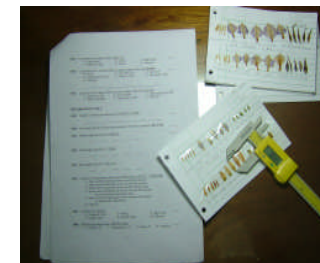


Characterization data of plant vegetative growth traits collected

Recording of inflorescence data

Ten inflorescence traits are recorded:

Number of flowers per inflorescence, flowering time (no. of days from sowing till first flower opening), stamen length (cm), petal length (cm), sepal length (cm), no. of hermaphrodite flowers per inflorescence, corolla color, relative style length (mm), pollen production, and style exertion.



Observation of inflorescence data in eggplants

Flower specimen collected for characterization and filing



Fruit harvest and characterization

Fruits are harvested at commercial maturity for fruit composition analysis (% dry matter, % sugar, % fiber) by the Nutrition Unit. To ensure that all plants of a given accession contribute equally to the seed harvest, an equal number of comparable, healthy fruits per plant must be harvested.

For fruit characterization, fruits are stored under shade for up to one week until the flesh softens. This allows the seeds to reach physiological maturity.



Fruits harvested in the field



Fruits kept for ripening after harvest



Diversity of fruits harvested



Fruit harvest and characterization (2)

Twenty-five fruit traits were characterized at immature stage or at physiological maturity:

Fruiting date (50% of the plants have mature fruits), fruit length, fruit breadth, fruit length/breadth ratio, fruit curvature, fruit pedicel length, fruit pedicel thickness, fruit pedicel prickles, fruit shape, fruit apex shape, fruit color at commercial ripeness, fruit color distribution at commercial ripeness, etc.



Characterization of both immature and mature fruits



Seed extraction and pre-drying

Seeds are extracted from the harvested fruits after one week in storage (physiological maturity of seeds) by hand or using a small grinder machine. After seed extraction, water is added and the content is mixed well. Water is added several times and poured off, together with the pulp. Pulp and empty seed float.



Step1



Step2



Step3



Step4



Step5



Step6



Step7



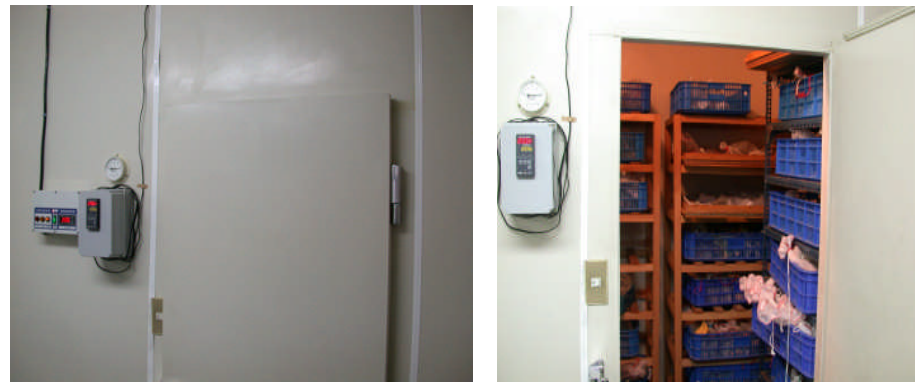
Step8

Eggplant seed extraction and drying procedures



Seed drying

Seed is dried as quickly as possible by spreading the wet seed in the shade on screens for pre-drying. The temperature should not exceed room temperature; sun drying should be avoided. Pre-drying is followed by continuous drying on seed screens in a drying room where cool and dry air (15 °C and 15 % RH) is forced through the seeds. After 7-10 days, seeds have reached the final moisture content of 5-6%.



Seed drying room (15 °C and 15 % RH)

Seed characterization

After drying of seeds, five seed traits are measured:
Seed color, no. of seeds per fruit, seed density, seed size (mm)
and 100 seeds weight.



Determination of eggplant
seed characteristics





Seed processing and storage

After seed drying, seeds are visually inspected and sorted by hand to remove foreign materials (debris), seeds with defects, injuries, and of substandard size.

A sample of the cleaned seed lot is used for seed health inspection and initial viability testing. Seed is then packed in moisture-proof aluminum foil pouches and sealed for preservation in the active (5°C), base (-15°C), and safety duplicate collections (-18°C).



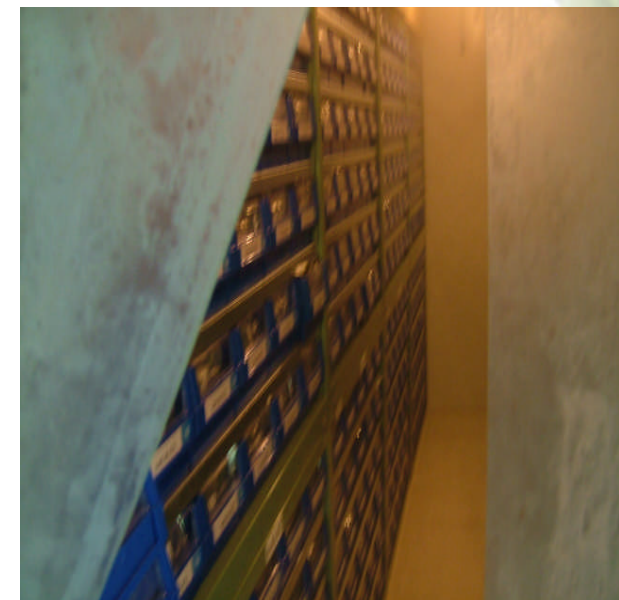
Seeds packed in water-proof aluminum foil pouches are heat-sealed for cold storage



Seed processing and storage (2)



Seeds stored as active collection for frequent access and use at 5 °C, 45% RH



Seeds stored as base collection at -15 °C