Regeneration Guidelines

Forage legumes

Jean Hanson[1], Ahmed Amri[2], Kenneth Street[2], Ali Shehadeh[2], Natalya Rukhkyan[2] and Richard Snowball[3]

[1] International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia

[2] International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria

[3] Genetic Resource Centre for Temperate Pasture Legumes (GRCTPL), Perth, Australia

Introduction

Forage legumes are members of the Fabaceae family. There are about 650 genera and 18,000 species widely distributed throughout the world, with over 1500 species of legumes commonly grazed or utilized by livestock. Forage legumes may be herbaceous plants, shrubs or trees and annuals and perennials, with species ranging in height from a few centimeters to over 5 m in height. Leaves are often pinnate or trifoliate and flowers show a wide variety of colours, usually with 5 petals. Flowers of the subfamily Papilionoideae comprise a standard, 2 wings and 2 petals fused into the keel and flowers of the subfamily Mimosoideae are usually arranged in racemes or heads and petals are basally united into a tube. Pods usually dehisce into 2 valves.

With such a wide range of diversity, specific conditions and methods are required for each species. These general guidelines are only indicative and specific information for each species should be sourced from literature.

GLOBAL CROP DIVERSITY TRUST
A Foundation for Food Security

Preparation for regeneration

When to regenerate

- When seed stocks are less than 1000 seeds
- When percent germination is reduced to 65% (FAO/IPGRI, 1994)

Other precautions

A population size of at least 100 plants should ideally be used for regeneration of forage legumes in order to maintain genetic variation. Transgenes have been reported for alfalfa (Medicago sativa).

Choice of environment and planting

Field selection and preparation

- Select the environment and soil type best suited for the species.
- Select the field based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different pests.
- Plough to invert soil, followed by 2-3 passes with the disc-harrow to obtain a well prepared and level seed bed prior to planting. Remove any weeds or other grass.
- Most forage and pasture legumes species are small seeded in size. Planting depth should not exceed more than 3 cm.

Planting season

- Plant in the rainy season to avoid water stress and ensure good establishment.

Method of Regeneration

Pre-treatments

Many forage legumes have hard seed and require scarification before planting to allow imbibition. Use one of the following methods:

- Gently rub seeds between two pieces of fine sand paper until the seed coat is scratched
- Use tweezers with well defined square edges and apply gentle but firm pressure to the seed coat with the arms of the tweezers open to about 2 mm so that a small section of the seed coat will chip off
- Use a scalpel to chip the seed coat
- Pour boiling water over the seeds and leave to cool and soak overnight

Planting layout, density and distance

- Aim for a final plant number of 100 in plots of approximately 25 m^2.
- Plant herbaceous materials in 11 rows of 5 m long, with rows 50 cm apart and 30-50 cm between plants giving a density of 100-150 plants per plot.
- Plant smaller herbaceous materials closer together with rows 30 cm apart and 25-20 cm between plants in smaller plots to avoid large empty areas of soil between plants where weeds will grow.

- Plant fodder trees in larger plots, with rows 1-2 m apart and 1-2 m between plants or in single rows 5 m apart and 1-2 m between plants. Increase the between plant spacing for large trees.
- Forages vary in their breeding systems and species are treated differently.
- Use an isolation distance of at least 100 m between accessions. Plant accessions of other species that do not hybridize with the legume or of other genera between the plots of one species to increase the isolation effect.
- Bee proof pollination cages are the best solution to isolate accessions and prevent insect pollination to avoid outcrossing but are expensive.

Planting method

Direct sowing for accessions with large size or many seeds

1. Count or weigh the number of seeds to be planted per row and place in separate envelopes/bags. Allow two seeds per hole if enough seeds available because not all of them will germinate. If there are only a few seeds, plant one seed per hole.
2. Label the plot with the accession number, planting date and plot number.
3. Lay out the plots at the chosen row spacing.
4. Mark rows or holes for sowing about 2-5 cm deep, 30-50 cm along the row.
5. Check that the accession number is correct and place the corresponding envelope/ bag on the end of the row.
6. Open the envelope and place 2 seeds per hole at 30-50 cm along the row by hand sowing. Small seeds can be scattered along the row rather than planted in holes.
7. Cover with soil and lightly compact the row.

Seedling transfer for accessions with small numbers of seeds

8. Germinate seeds in Petri dishes in an incubator using the right conditions for the species.
9. As soon as the radicles start to emerge, plant the young seedlings individually in seedling trays or pots filled with sterilized compost or forest soil.
10. Label the tray or pot with the accession number and planting date.
11. Keep the pots in a warm place away from direct sun but with good light intensity or in a greenhouse.
12. Water carefully so the soil remains moist but not wet.
13. Once seedlings are strong and growing well, place the pots outside so the seedlings can harden off; keep the soil moist.
14. Label the field plot with the accession number, planting date and plot number.
15. Peg out the plots at the chosen row spacing and make holes at 50 cm along the row.
16. Transplant the seedlings to the field, one seedling per hole, taking care not to damage the roots. Water after transplanting.
17. Maintain accessions with few plants or weak seedlings in 8" pots for regeneration in the greenhouse.

Thinning

- If direct sown, thin to one plant per hole at 4-6 weeks after establishment when plants are growing well to give a plant density of about 100-150 plants per plot and avoid competition that will result in weak plants and low seed yields. When thinning, do not remove only smaller or weaker plants as this will reduce genetic variation. Thinning can be done at the same time as the first weeding.

Fertilization

- Fertilizer application will depend on soil type and fertility. Follow local recommendations.
- Apply appropriate Rhizobia innoculant when needed in the holes at planting.
- It is possible to grow the crop without fertilizer, but apply phosphorous in the holes before planting using a fertilizer such as diammonium phosphate or other phosphorus rich fertilizer at 100 kg P/ ha. Apply 50-60 kg N/ ha as a top dressing at early flowering stage to ensure good seed quality.

Crop management

Weed management

- Early growth can be slow so weed by hand 4 weeks after establishment or transplanting to the field. Cultivate between rows twice during early stages of plant growth. Ensure field technicians know what young plants look like so they do not mistake them for weeds.
- Eliminate off-types and plants growing off-row.

Irrigation

- Irrigate the field after sowing or transplanting and then when needed. Do not allow leaves to wilt at any stage and ensure soil is moist at time of flowering.

Common pest and diseases:

Forage legumes are susceptible to a wide range of virus and fungal diseases similar to many pulse crops.

Pest and disease control

Coordinate periodic field inspections with pathologists and virologists during the growing season.

Spray with appropriate chemicals to control diseases. Spray with fungicide to control mildew during the rainy season or when using irrigation and with insecticide at the first sign of insect damage. Rogue material infected with virus before flowering to eliminate the disease and incinerate.

Harvesting

- Inspect the field daily once seeds start to mature to determine the right date for harvest. Seeds often ripen unevenly and may shatter once mature so harvesting at the right stage is critical.
- When equal number of seeds are required from each plant within an accession, hand harvest seed pods from each plant into individual cotton/cloth bags. Many forage legumes are indeterminate in growth habit and it is difficult to separate individual plants within the plot after some months of growth. In these cases hand harvest pods from the entire plot.
- Collect the seeds from each plant in labelled cloth or paper bags with an additional label inside each bag. Use paper bags in dry climates only.

- Thresh the pods on a tarpaulin by gently beating or thresh small quantities from individual plants by hand using rubber threshing board; return the seeds to their labelled bag.
- Thresh larger quantities from full plots in a threshing machine.
- Ensure that seed mixing does not occur during threshing by thoroughly cleaning all equipment and implements between each sample. Wash and dry cloth bags between each use and avoid reuse of paper bags. Where resources are short and bags have to be reused, check carefully in folds and remove trapped seeds and use bags for different species so any mixed seeds can easily and quickly be identified and removed.

Post-harvest management

1. Clean the seeds of debris by hand picking, hand winnowing or using a seed blower.
2. Hand pick over the seeds in trays to remove any shriveled, discoloured, infected or damaged seeds from each plant. Incinerate the waste to avoid spread of seed borne diseases.
3. Compare the harvested seed with original seed of that accession for seed characters to check for mistakes/correspondence.
4. Take equal quantities of seeds from each plant and mix in one paper bag labelled inside and outside. Once you have all the seeds needed, discard any extra.
5. Retain the bags of each accession in temporary storage until seed drying.
6. Take a sample of the seeds and carry out seed health tests for common diseases. If the material is annual and the seeds are infected with seed-borne diseases and more original seeds are available for a second regeneration, destroy the seeds by incineration. If no original seeds are available, resow from the harvested seeds and use the correct fungicide to control the disease and obtain clean seeds. If the material is perennial and the fresh seeds are infected with seed borne diseases, treat the plants with the correct fungicide for the disease until no symptoms are seen on the plants and harvest fresh seeds. Destroy the earlier harvested seeds by incineration once clean seeds have been harvested. Thermotherapy and tissue culture can be used to remove virus but are time consuming and expensive.
7. If the seeds are free from pests and diseases, dry the seeds in low relative humidity at 15°C until they reach between 3-7% moisture content.
8. Remove the seeds from the drying room, weigh and pack directly into storage containers. Options for medium term storage include using plastic containers or cans with sealed lids for storage in environments with humidity control or laminated aluminum foil packets for storage in environments without relative humidity control. Use of laminated aluminium foil packets is more suitable for long-term storage. Seal the containers or packets immediately.
9. Sample and test the viability of the seeds and record the results following standard germination methods (ISTA, 2008). If viability is high, proceed to storage, If viability is low, reschedule the accession for a further regeneration from the original seeds.
10. Store seeds in the genebank at 5-10°C in medium-term storage or at -18°C in long-term storage.

Monitoring accession identity

Comparisons with previous passport or morphological data
Most forage legumes are distinguished on the basis of flower colour, flower parts, number of sepals, number of leaf pinnae in compound leaves, pubescence and stem traits.

Documentation during regeneration

The following information should be collected during regeneration:

- Regeneration site name and map/GPS reference
- Name of data collector
- Field/plot/nursery/greenhouse reference
- Accession number; population identification
- Source of seed
- Generation or previous multiplication or regeneration (if generation is not known)
- Preparation of planting materials (pre-treatments)
- Sowing date
- Field layout and density used
- Field management details (watering, fertilizer, weeding, pest and disease control, stresses recorded, others)
- Environmental conditions (altitude, precipitation, soil type, others)
- Emergence in the field or green house (number of plants germinated)
- Number of plants established and harvested
- Isolation method used
- Harvest date and method
- Days from sowing to flowering
- Number of plants established and harvested
- Quantity of seeds harvested/accession
- Comparisons with reference materials (record any identification numbers or references of any samples or herbarium specimens taken from this regeneration plot)
- Agronomic evaluation; agro-morphological traits recorded
- Taxonomic identification
- Post harvest procedures

References and further reading

Bray RA. 1983. Strategies for gene maintenance. In: McIvor JG, Bray RA. (eds.) Genetic Resources of Forage Plants. CSIRO, Melbourne, Australia. p157-168.

Fairey DT, Hampton JG. (eds). 1997. Forage Seed Production volume I:Temperate Species. CABI International, Cambridge, UK.

FAO/IPGRI. 1994. Genebank Standards. Food and Agriculture Organization of the United Nations, Rome.

Humphreys LR, Riveros F. 1986. Tropical Pasture Seed Production. FAO Plant Production and Protection Paper 8, FAO, Rome, Italy.

ISTA. 2008. International Rules for Seed Testing. International Seed Testing Association. ISTA secretariat, Bassersdorf/Zurich, Switzerland.

Loch DS, Ferguson J. (eds). 1999. Forage Seed Production volume II:Tropical and Subtropical Species. CABI International, Cambridge, UK.

1 Flowers of Vigna vexillata (subfamily Papilionoideae).
Jean Hanson/ILRI

2 Dehiscent legume pods with seeds.
Jean Hanson/ILRI

3 Weeding forage legume regeneration plots.
Alexandra Jorge/ILRI

4 Harvesting Leucaena seeds from individual plants into separate bags.
Jean Hanson/ILRI

5 Hand cleaning seeds during regeneration.
Alexandra Jorge/ILRI

6 Transferring germinated seeds of Vigna unguiculata into seedling trays to raise seedlings for the field.
Alexandra Jorge/ILRI

Notes