Regeneration Guidelines

Forage grasses

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Introduction

Grasses are members of the Poaceae family. There are about 650 genera and 10,000 species widely distributed throughout the world, with over 600 species commonly used for grazing and livestock feeds. Grasses are herbaceous annuals and perennials, often with rhizomes or stolons, with species ranging in height from a few centimeters to over two metres in height. Flowers are composed of spikelets in a panicle or in racemes or spikes. Two sterile glumes support one to many florets. Each floret is composed of two bracts, the lemma and palea which enclose a small flower. While many grasses are out-crossing and wind-pollinated, a combination of apomixis and sexual reproduction is also common in the Paniceae tribe. Most forage grasses produce seeds except for few shy seeders that rarely or never produce caryopses and need to be maintained in the vegetative form.

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.

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Preparation for regeneration

When to regenerate

- When seed stocks are less than 1000 seeds.
- When percent germination is reduced to 65% or 85% of initial germination for wild species with low initial viability (FAO/IPGRI, 1994).

Other precautions

A population size of at least 100 plants should ideally be used for regeneration of out-breeding grasses in order to maintain genetic variation. For those reproduced vegetatively, one plant is sufficient to maintain the genetic variation but more plants are needed to mitigate the risk of loss of material. There are no reported transgenes in forage grasses.

Choice of environment and planting season

Field selection and preparation

- Select the environment and soil type best suited for the species.
- Plough and disc-harrow to obtain a well prepared and level seed bed prior to planting. Remove any weeds or other grass.

Planting season

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

Method of Regeneration

Planting layout, density and distance

- Aim for a final plant number of 100 in plots of about 25 m^2 for out-breeders propagated by seeds and 25 plants in plots of about 10 m^2 for vegetatively propagated grasses.
- Plant in rows, each row 50 cm apart with within row spacing of 50 cm giving a density of 25-100 plants per plot.
- Forages vary in their breeding systems and species are treated differently.
- For out-breeders use an isolation distance of at least 100 m between accessions. Plant accessions of other species that do not hybridize with the grass or of other genera between the plots of one species to increase the isolation effect.
- Use tall plants or artificial barriers as wind breaks to control pollen flow and prevent wind pollination.

Sowing method

1. Germinate seeds in Petri dishes in an incubator using the right conditions for the species.
2. As soon as the shoots start to emerge, plant the young seedlings individually in seedling trays or small pots filled with sterilized compost or forest soil.
3. Label the tray or pot with the accession number and planting date.
4. Keep the pots in a warm place away from direct sun but with good light intensity or in a greenhouse and cover at night to retain moisture.

5. Water carefully using a spray bottle so the soil remains moist but not wet.
6. Once seedlings are strong and growing well, place the pots outside so the seedlings can harden off; keep the soil moist.
7. Peg out the plots at the chosen row spacing and make holes at 50 cm along the row.
8. Transplant seedlings to the field, one seedling per hole, taking care not to damage the roots. Water after transplanting.

Fertilization

- Fertilizer application will depend on soil type and fertility. Follow local recommendations.
- 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 and after every cut for perennial species.

Crop management

Weed management

- Early growth can be slow so weed by hand 4 weeks after establishment and then monthly.
- Eliminate off-types and plants growing off-row.

Irrigation

- Irrigate the field after sowing 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 grasses are susceptible to many fungal diseases and few virus and phytoplasma depending on the species.

Pest and disease control

Spray with fungicide to control mildew during the rainy season or when using irrigation and with insecticide at the first sign of insect damage. Pay particular attention to army worm and spray at the first infestation.

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 some seed loss in grasses is normal. Harvest when at least 50% of the seeds on the head are mature and before shattering.
- When equal number of seeds are required from each plant within an accession, hand harvest seed heads from each plant into individual cotton/cloth bags. Many grasses are stoloniferous and it is difficult to separate individual plants within the plot after some months of growth. In these cases hand harvest heads from the entire plot.
- Harvest by cutting the stems or heads when seeds are mature but before fully ripe seeds start to dehisce and shatter.

- 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 seed heads on a tarpaulin by gently beating or in a threshing machine; return the seeds to their labelled bag.
- 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 seed from each plant and mix in one paper bag labelled inside and out. 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 them 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

- Distinctive traits are specific for each grass species. Grass accessions are usually distinguished on the basis of tiller, flower, spikelet, pubescence and stem traits.
- Digital images are useful for identification and comparison.

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 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
- 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)
- Post harvest procedures

References and further reading

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1 Brachiaria ruziziensis florets with anthers ready for pollination in isolated plots.
Jean Hanson/ILRI

2 Chloris gayana producing seed ready for harvest.
Jean Hanson/ILRI

3 Seeds shatter as they start to mature in Brachiaria dictyoneura.
Jean Hanson/ILRI

4 Stoloniferous perennial grass plots should be harvested from all heads.
Jean Hanson/ILRI

5 Weeding of grass regeneration plots.
Jean Hanson/ILRI

Notes