REF. NO. 10 MARCH 1997

CULTIVATION AND EVALUATION OF FORAGE CROP PGR

TECHNICAL ASSISTANCE ACTIVITIES FOR GENETIC RESOURCES PROJECTS

JAPAN INTERNATIONAL COOPERATION AGENCY

REF. NO. 10 CULTIVATION AND EVALUATION OF FORAGE CROP PGR GENETIC RESOURCES

Published

REF. NO. 1	Preservation of Plant Genetic Resources
REF. NO. 2	Exploration and Collection of Plant Genetic Resources Part I Seed-propagated Crops
REF. NO. 3	Exploration and Collection of Plant Genetic Resources Part II Vegetatively Propagated Crops
REF. NO. 4	Evaluation and Classification of Plant Genetic Resources
REF. NO. 5	Utilization of Plant Genetic Resources for Crop Improvement
REF. NO. 6	Cryopreservation of Plant Genetic Resources
REF. NO. 7	Cultivation Methods for the Evaluation of Characteristics of Genetic Resources and Evaluation of Genetic Resources (Cereal, Pulse and Root Crops)
REF. NO. 8	Cultivation and Evaluation of Vegetable PGR
REF. NO. 9	Cultivation and Evaluation of Fruit Tree PGR

Editorial Office: Agriculture, Forestry & Fisheries Technical Information Society (AFFTIS)
6F Seifun-kaikan, 15-6 Kabuto-cho, Nihonbashi,
Chuo-ku, Tokyo 103, JAPAN

CONTENTS

Introduction

In 1994, the editorial board of GRP REF sponsored by Japan International Cooperation (JICA) planned to publish a series of technical manuals for cultivation and evaluation of PGR. The board published PGR REF NO.7 for cereal, pulse and root crops in 1994, published PGR REF No.8 for vegetables in 1995, and published PGR REF No.9 for fruit trees. In 1997, the board planned to publish PGR REF No.10 for forage crops.

I was asked from the board to cooperate in the arrangement of the REF No.10. As the number of useful forage crops is large, and they are cultivated in a wide range of climate, from tropical zone to cold zone, I hesitated in deciding the kind of forage crops to be described in PGR REF No. 10. Consulting with Drs. Torao Goto and Shigeru Suzuki, AFFTIS (Agriculture, Forestry & Fisheries Technical Information Service) I selected five kinds of grasses, and four kinds of legumes.

Italian ryegrass was selected to represent annual grass: orchardgrass was selected to represent perennial grasses: Kentucky bluegrass was selected to represent prostrate grasses: panicum was selected to represent grasses adapted to temperate zone: sorghum was selected as one of the important grasses for concentrated feedstuff, as corn was already described in PGR REF No.7.

Chinese milk vetch was selected to represent annual type of legumes: alfalfa was selected to represent perennial legumes: white clover was selected to represent prostrate legumes: tropical legumes were also described.

In order to utilize this booklet for the characterization of a specific forage crop, readers are advised to choose one kind of crop from nine crops in this booklet as the most resembling, and apply the descriptions to the specific crop.

Koichi Nakashima
Director of Breeding Department,
National Grassland Research Institute,
MAFF

I. Italian Ryegrass

I-1. Cultivation of Italian Ryegrass Genetic Resources for Evaluation of Characteristics

I-2. Evaluation of Characteristics of Italian Ryegrass Resources

by

Yasufumi Ueyama

Ibaraki Prefectural Livestock Experiment Station, Tomobe, Ibaraki 307-19 Japan

I-1. Cultivation of Italian Ryegrass Genetic Resources for Evaluation of Characteristics

Italian ryegrass (*Lolium multiflorum*) is cultivated from fall to spring or summer as a winter annual forage grass. For the selection of the experimental site, winter survival of genetic resources is the most important factor. The mean air temperature in the coldest month should be above 0 °C and moderate rainfall is necessary in the growing season.

The data of primary and secondary characters are obtained from space planting plots and those of tertiary ones which deal with productivity are obtained from row planting or broadcasting plots. Space planting is convenient for the evaluation of characteristics and field management. For space planting seedlings are usually transplanted.

1. Land preparation

Field is plowed and harrowed so as to obtain a smooth soil surface. Italian ryegrass germinates easily and shows an adequate growth at the early stage compared to the other temperate grasses.

Before sowing or transplanting, basal fertilizer is applied. The rate of fertilizer application is usually N: 1.0, P_2O_5: 1.0, K_2O: 1.0 kg/a, although the rate of fertilizer depends on the fertility of soil. Soil with high acidity should be amended by the application of lime.

2. Sowing and transplanting

For space planting plots, seeds are sown around the middle of September, when the daily mean temperatures are less than 25 °C. Usually seeds are sown in seed beds. After germination, seedlings are thinned to separate individuals from each other. Seedlings which are grown for 30 to 40 days, are transplanted in open field. Plant individuals are separated by a distance of 0.3 m to 0.4 m, with a row distance of 0.6 m to 0.7 m.

For the productivity test, seeds are sown in October, when the daily mean temperature is around 15 °C. Seeds are planted in rows, with a spacing of 0.3 m to 0.4 m, or broadcasted. Seeding rate is adjusted based on germination tests to obtain the following stand rate (g/m^2): for diploid entries 4.0, for tetraploid ones 5.0. After seeding, soil is packed.

3. Experimental design for the evaluation of characteristics

Evaluation test is conducted according to a randomized complete block design with three replications. Each plot consists of 20 or more individuals for space planting test. Size of plot for productivity test should exceed 6.0 m^2.

4. Additional application of fertilizer

An additional amount of nitrogen and potassium is applied in early spring before the flushing stage and after harvest as side-dressing. The standard rate is N: 0.3, K_2O: 0.3 kg/a.

5. Weed control

Mechanical weed control should be applied carefully not to impede the growth of entries. Herbicide is applied from late fall to early spring. Growth of sprouts is rarely suppressed by herbicides.

6. Harvest

The first harvest for the productivity test usually corresponds to the heading stage in spring. When the plant height of the entries exceeds 60 cm before overwintering, harvest should be performed in late November or early December. The second harvest should take place 30 to 40 days after the first harvest. Frequency of harvests for very early or early maturing entries is two, and that for middle or late maturing ones is three or four. Entries for summer survival test are not harvested during July to early September.

I-2. Evaluation of Characteristics of Italian Ryegrass Genetic Resources

1. Primary characters

<Essential items>

Plant type

Plant type is observed at the heading stage. Plant type is evaluated based on the angle formed by outer stems with the horizontal line, and is classified into 1: extremely erect (right angle), 3: erect, 5: intermediate, 7: prostrate (acute angle), 9: extremely prostrate. Refer to Photo. 2.

Culm length

Culm length is measured from the ground to the neck node of spike of the main stem at the full heading stage .

Plant height at early stage

Plant height at early stage is measured from the ground to the tip of the longest leaf before overwintering.

Spike length

Distance from the neck node to the tip excluding awns of spike is measured 7 to 10 days after the full heading time.

Stem thickness

Diameter of internode just below the neck node of spike is measured.

Leaf length

Length of the first leaf below the flag leaf is measured.

Leaf width

Maximum width of the first leaf below the flag leaf is measured.

Heading date

The date when 50% of productive stems head is recorded.

Number of spikes

The number of spikes is counted at the full heading stage. Simple estimation by observation can replace the counting. This character is classified into 1: no spikes, 3: few spikes, 5: intermediate, 7: many, 9: very large number.

<Optional items>

Plant height in early spring

The distance from the ground to the tip of the longest leaf is measured in early spring or 30 days after snow melt.

Number of spikelets

The number of spikelets in a spike is counted for two spikes.

Anthocyanin pigmentation at early stage

Anthocyanin pigmentation of the stem base is observed before overwintering and classified into 1: no pigmentation, 3: light, 5: intermediate, 7: dark, 9: very dark.

Anthocyanin pigmentation at maturing stage

Anthocyanin pigmentation of node is observed from the head emergence stage to the full heading stage, and classified into 1: no pigmentation, 3: light, 5: intermediate, 7: dark, 9: very dark.

Weight of 1000 seeds

Weight of 100 seeds consisting of a mixture of seeds from 20 individuals is measured with 4 replications, and weight of 1,000 seeds is computed.

Presence of awn and its length

The presence of awns is observed. If awns are present, length of two floret awns on the top of the spike per plant is measured.

Fluorescence reaction of seedling roots

Percentage of seedling roots reacting to fluorescent light is determined 18 days after exposure of the seedlings to darkness for 16 hours at 20 °C and 8 hours at 30 °C, alternately. In one lot, 25 seeds are tested.

Number of stems

Number of stems is counted at the full heading stage. Instead of counting, simple estimation by observation can replace the counting. This character is classified into 1: no stems, 3: few stems, 5: intermediate, 7: many, 9: very large number.

2. Secondary characters

<Essential items>

Crown rust resistance

The resistance to *Puccinia coronata* is evaluated based on the appearance of lesions after artificial inoculation or in infected field and the accession is classified into 1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant, 9: highly resistant.

Lodging resistance

The degree of lodging resistance is evaluated when lodging occurred, and the accession is classified into 1: highly susceptible, 5: intermediate, 9: highly resistant.

Growth period

Growth period is estimated based on the percentage of stubs which survived in early summer and classified into 1: very short (0%), 3: short (25%), 5: intermediate (50%), 7: long (75%), 9: very long (100%).

Regrowth

Regrowth is evaluated a few weeks after the first cutting in spring and classified into 1: very poor, 3: poor, 5: intermediate, 7: good, 9: very good.

<Optional items>

Net blotch resistance

The resistance to *Drechslera dictyoides* is evaluated based on the appearance of lesions in the field and the accession is classified into 1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant, 9: highly resistant.

Halo blight resistance

The resistance to *Pseudomonas syringae* is evaluated based on the appearance of lesions in the field and the accession is classified into 1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant, 9: highly resistant.

Brown patch resistance

The resistance to *Rhizoctonia solani* is evaluated based on the appearance of lesions in the field and the accession is classified into 1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant, 9: highly resistant.

Blast resistance

The resistance to *Pyricularia grisea* is evaluated based on the appearance of lesions in the

field and the accession is classified into 1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant, 9: highly resistant.

Snow blight resistance

The resistance to snow mold disease is evaluated based on the appearance of lesions in the field and the accession is classified into 1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant, 9: highly resistant.

Ergot resistance

The resistance to *Claviceps purpurea* is evaluated based on the appearance of sclerotia (ergots) in spikes in the field and the accession is classified into 1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant, 9: highly resistant.

Leaf blight resistance

The resistance to *Drechslera siccans* is evaluated based on the appearance of lesions in the field and the accession is classified into 1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant, 9: highly resistant.

Spring habit

Spring habit is evaluated based on the percentage of heading plants seeded in spring and classified into 1: very low (0%), 3: low (25%), 5: intermediate (50%), 7: high (75%), 9: very high (100%).

Cold hardiness

Overwintering ability is evaluated based on the degree of winter damage without snow covering, and is classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Snow tolerance

The overwintering ability under snow cover is evaluated 10 to 15 days after snow melt and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Tolerance to high temperature

Tolerance to high temperature is evaluated based on the percentage of plants surviving after summer and the vigor in early fall, and the accession is classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

3. Tertiary characters

<Essential items>

Green yield

Green yield is determined based on the measurement of the fresh weight of grass harvested from over 2.0 m^2 plot at each cutting time.

Dry matter percentage

The percentage of dry matter is determined based on the measurement of dry weight of approximately 500 g of fresh sample after drying at 70 °C for 48 hours at each cutting.

Dry matter yield

Dry matter yield is determined as follows: (green yield) × (dry matter percent) / 100.

<Optional items>

Dry matter digestibility

Percentage of digestible dry matter is determined by *in vivo* method or *in vitro* enzyme method.

Crude protein content

Crude protein content is determined by the Kjeldahl method or a Near Infra-red Analyzer and expressed on a dry matter basis.

Acid detergent fiber (ADF) content

ADF content is determined by the acid detergent-acetone washing method and expressed on a dry matter basis.

Neutral detergent fiber (NDF) content

NDF content is determined by the neutral detergent-acetone washing method and expressed on a dry matter basis.

Acid detergent lignin (ADL) content

Percentage of ADL content is determined by the acid detergent method and expressed on a dry matter basis.

Mono- and oligosaccharides

Mono- and oligosaccharide content is determined by thin layer chromatography after ethanol extraction, and expressed on a dry matter basis.

Photo. 1 Field for evaluation of Italian ryegrass genetic resources.

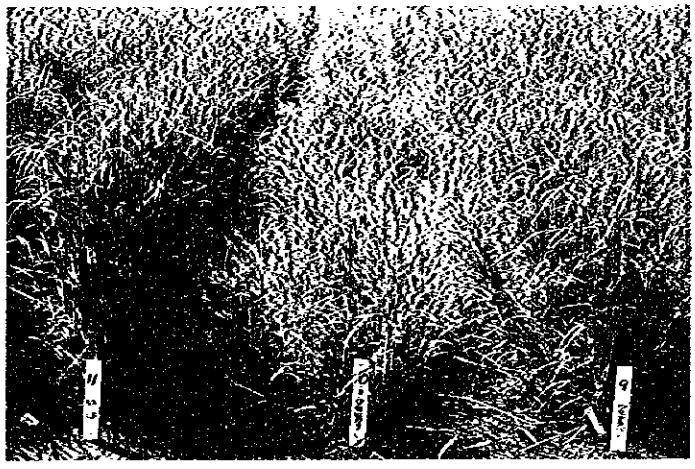

Photo. 2 Plant type of Italian ryegrass.
From left : erect, prostrate, intermediate.

II. Orchardgrass

II-1. Cultivation of Orchardgrass Genetic Resources for Evaluation of Characteristics

II-2. Evaluation of Characteristics of Orchardgrass Genetic Resources

by

Shin-ichi Sugita

National Grassland Research Institute, MAFF, Nishinasuno, Tochigi 329-27 Japan

II-1. Cultivation of Orchardgrass Genetic Resources for Evaluation of Characteristics

Cultivation methods described here for orchardgrass can be applied to similar temperate grasses such as tall fescue *(Festuca arundinacea* Schreb.*)*, meadow fescue *(Festuca pratensis* Huds.*)*, reed canarygrass *(Rhalaris arundinacea* L.*)* and timothy *(Phleum pratense* L.*)*. Temperate grasses except for timothy should be exposed to winter conditions (low temperature and/or short daylength) for differentiation of inflorescence.

Temperate grasses which are long-day plants will not start flowering until a critical day length is reached in spring. Critical daylength varies depending on the species and strains.

1. Space planted plot for evaluation of primary and secondary characters

1) Raising of seedlings

Seeds are sown in petri dishes in the same way as in the germination test and kept in a chamber at about 20 °C until germination. Germinated seeds are planted in paper pots or seedling boxes at a spacing of 2 × 3 cm.

2) Planting of seedlings

45 ~ 50 days old seedlings are planted in well-prepared fields. Seedlings should be transplanted before the daily mean temperature decreases to 16 °C in order to be able to withstand cold stress in winter. Distance between rows is usually 80 cm and distance between plants in a row is 50 to 80 cm to avoid competition with neighboring plants and to identify a single plant easily. Each plot consists of more than 12 plants and is laid out with two replications based on a randomized block design. Plants on the borders of rows are excluded from the evaluation of characteristics.

3) Application of fertilizers (kg/a/year)

(1) Year of establishment

Basal dressing N: 0.5, P_2O_5: 0.5, K_2O: 0.5, Lime: 10

(2) Years for observation

Spring dressing N: 0.5, P_2O_5: 1.0, K_2O: 0.5

Top dressing N: 0.3, K_2O: 0.3 (after each cutting)

4) Cutting

Plants are cut back to 10 cm height after evaluation of all entries at the heading stage and thereafter cutting is carried out at intervals of 40 ~ 50 days during the growing season in the second and third years.

5) Duration of characterization test

Three years (one year for establishment and two years for characterization).

2. Row-seeded plot for evaluation of tertiary characters

1) Seeding

Seeds are drilled at a depth of 0.5 ~ 1.5cm and at a rate of 100 ~ 150 g/a for characterization of forage yield and quality, and for seed productivity at a rate of 50 g/a. Soil should be carefully compressed to enable close contact of seeds with available moisture, since the seed size of temperate grasses is very small. In order to obtain strong seedlings that can withstand cold stress in winter, sowing should be carried out while the daily mean temperatures remain above 18 °C. Each plot consists of 6 rows 3 m long with a spacing of 30 cm. Plots are arranged in a randomized block design with three replications.

2) Application of fertilizers (kg/a/year)

(1) Year of establishment

Basal dressing N: 0.5, P_2O_5: 1.0, K_2O: 0.5, Lime: 10

(2) Years for observation

Spring dressing N: 0.5, P_2O_5: 1.0, K_2O: 0.5

Top dressing N: 0.5, K_2O: 0.5 (after each cutting)

3) Mowing

Plots are mowed at a height of 5 ~ 10 cm immediately after all entries have fully headed and thereafter at intervals of 40 ~ 50 days during the growing season.

4) Duration of characterization test

Three years (one year for establishment and two years for characterization).

II-2. Evaluation of Characteristics of Orchardgrass Genetic Resources

1. Primary characters

<Essential items>

Plant type

Plant type is evaluated based on the angle formed by outer main stems with the horizontal line at the heading stage. The angle is recorded at intervals of 10° and the accession is classified into 1: extremely erect, 9: extremely prostrate.

Plant length

Plant length is measured from the ground to the top of the plant at the heading stage or at the time of the first cutting (cm).

Head length

Length of head is measured from the lowest rachis-branch base (for *Phleum pratense*, neck node) to the top of the head (cm).

Leaf length

Leaf length is measured using the first leaf blade below the flag leaf (cm).

Leaf width

Leaf width is measured using the widest part of the first leaf blade below the flag leaf (mm).

Date of first heading

The average date when the first head of each plant emerges is recorded as date of first heading. Observation is carried out twice a week.

Stem thickness

Longer diameter of the longest stem just below the head is measured at the heading stage or at the time of the first cutting, and the stem is classified into 1: very slender, 9: very thick.

Number of stems

Number of stems is counted at the heading stage or at the time of the first cutting, and classified into 1: very few stems, 9: very many stems.

Number of heads

Number of heads is counted at the heading stage or at the time of the first cutting, and classified into 1: very few heads, 9: very many heads.

Width of plant

Width of plant is recorded as the average length of two horizontal orthogonal axes of the base of a plant at the heading stage, and classified into 1: very small, 9: very large. For *Festuca arundinaceae* and *Phalaris arundinacea*, rhizome formation is included in the measurement.

<Optional items>

Culm length

Culm length is measured for the main stem from the ground to the base of head at the heading stage (cm).

Leaf color

Leaf color is evaluated based on the greenness of the leaf blade at the heading stage, and classified into 1: very light, 9: very dark.

Percentage of headed stems

Percentage of headed stems to the total number of stems is counted at the regrowth stage after the first cutting, and classified into 1: very low, 9: very high.

Heading behavior in autumn

Heading behavior in autumn is evaluated based on the number of headed stems in autumn after regrowth, and classified into 1: very few headed stems, 9: very large number of headed stems.

Texture of leaves

Texture of the leaf blade is estimated by touching the leaf at the heading stage, and classified into 1: very smooth, 9: very rough.

Weight of 1000 seeds

Weight of 1000 seeds (g) is measured by sampling 100 seeds from a mixture of seeds from 20 plants with 4 replications.

Weight of 20 heads

Weight of 20 heads (g) is measured using 20 fully matured and dried heads.

2. Secondary Characters

<Essential items>

Plant vigor in spring

Amount of grass in early spring is observed and classified into 1: very small, 9 very large.

Tolerance to summer depression

Tolerance to summer depression is evaluated based on the number of dead plants, degree of regrowth and plant vigor in early autumn, and classified into 1: very low, 9: very high.

Winter hardiness

Degree of winter damage is evaluated based on the number of dead plants and injury of stems and leaves in early spring, and classified into 1: very low, 9: very high.

Regrowth

Regrowth is evaluated based on the apparent amount of grass one to three weeks after the first cutting and classified into 1: very poor, 9: very good.

Lodging resistance

Degree of lodging resistance is evaluated at the heading stage, and the accession is classified into 1: highly susceptible, 9: highly resistant.

<Optional items>

Resistance to rhynchosporium scald

Resistance to *Rhynchosporium orthosporum* is evaluated based on the degree of infection, and the accession is classified into 1: highly susceptible, 9: highly resistant.

Resistance to rust

Resistance to *Puccinia* spp. is evaluated based on the degree of infection, and the accession is classified into 1: highly susceptible, 9: highly resistant.

Resistance to snow blight

Resistance to snow mold fungi is evaluated based on the degree of infection, and the accession is classified into 1: highly susceptible, 9: highly resistant.

Resistance to leaf streak

Resistance to *Scolecotrichum graminis* is evaluated based on the degree of infection, and the accession is classified into 1: highly susceptible, 9: highly resistant.

Resistance to summer blight

Resistance to *Rhizoctonia solani* is evaluated based on the degree of infection, and the accession is classified into 1: highly susceptible, 9: highly resistant.

Resistance to anthracnose

Resistance to *Colletotrichum graminicola* is evaluated based on the degree of infection, and the accession is classified into 1: highly susceptible, 9: highly resistant.

Resistance to powdery mildew

Resistance to *Erysiphe graminis* is evaluated based on the degree of infection, and the accession is classified into 1: highly susceptible, 9: highly resistant.

Resistance to leaf blotch

Resistance to *Stagonospora arenaria* is evaluated based on the degree of infection, and the accession is classified into 1: highly susceptible, 9: highly resistant.

Main diseases of important temperate grasses other than orchardgrass are listed below.

For timothy (*Phleum pratense* L.)

Purple Spot : *Cladosporium phlei*
Brown Stripe : *Scolecotrichum graminis*
Stem Rust : *Puccinia graminis*
Choke : *Epichloe typhina*
Snow Blight : *Sclerotinia borealis, Typhula* spp., *Pythium* spp., *Fusarium nivale*

For tall and meadow fescue (*Festuca arundinaca* and *pratensis* L.)

Chocolate spot : *Pseudomonas coronafaciens*
Net Blotch : *Drechslera dictyoides*
Leaf Spot : *Bipolaris sorokiniana*
Crown Rust : *Puccinia coronata*
Summer Blight : *Rhizoctonia solani*
Snow Blight : *Sclerotinia borealis, Typhula* spp., *Pythium* spp.
Ergot : *Claviceps purpurea*

For reed canarygrass (*Phalaris arundinacea* L.)

Buff Spot: *Stagonospora foliicola*
Crown Rust: *Puccinia coronata*
Snow Blight: *Sclerotinia borealis*
Summer Blight: *Rhizoctonia solani*
Ergot: *Claviceps purpurea*

Insect resistance

Resistance to insects is evaluated based on the degree of occurrence (note the name of insect), and the accession is classified into 1: highly susceptible, 9: highly resistant.

Plant vigor in autumn

Plant vigor is evaluated based on the amount of grass in autumn and classified into 1: very low, 9: very high.

3. Tertiary characters

<Essential items>

Fresh yield of spring

Fresh yield in spring cutting is calculated based on the weight of fresh grass harvested from an area of more than 2 m^2 in the central zone of a plot at each cutting in spring.

Dry matter percentage of spring

Dry matter percentage in spring cutting is determined by sampling 300 to 500 g of fresh grass and drying it at 70 °C for 48 hours.

Dry matter yield of spring

Dry matter yield in spring cutting is calculated as follows: fresh weight × dry matter percentage / 100.

Fresh yield of summer

Fresh weight in summer is calculated in the same way as the fresh yield of spring.

Dry matter percentage of summer cutting

Percentage of dry matter in summer cutting is determined in the same way as the dry matter percentage in spring.

Dry matter yield of summer cutting

Dry matter yield in summer cutting is calculated in the same way as the dry matter yield of spring.

Fresh yield of autumn

Fresh yield in autumn cutting is calculated in the same way as the fresh yield in spring.

Dry matter percentage of autumn cutting

Dry matter percentage in autumn cutting is determined in the same way as the dry matter percentage in spring.

Dry matter yield of autumn cutting

Dry matter yield in autumn cutting is calculated in the same way the dry matter yield in spring.

<Optional items>

Dry matter digestibility

Dry matter digestibility is determined by calculating the percentage of digestible dry matter based on *in vivo* method or *in vitro* enzyme method.

Mono- and oligosaccharide

Mono- and oligosaccharide content (dry matter basis) is determined by thin layer chromatography after ethanol extraction.

Crude protein content (CP)

Crude protein content (dry matter basis) is determined by the Kjeldahl method or a Near Infra-red Analyzer.

Acid detergent fiber (ADF)

Acid detergent fiber content (dry matter basis) is determined by the acid detergent-acetone washing method.

Neutral detergent fiber (NDF)

Neutral detergent fiber content (dry matter basis) is determined by the neutral detergent-acetone washing method.

Acid detergent lignin (ADL)

Acid detergent lignin content (dry matter basis) is determined by the acid detergent-acetone washing method.

Seed productivity

Seed productivity is based on the weight of pure seed (g) harvested from an area of more than 1 m^2.

Palatability

Palatability is determined based on the degree (observation) or percentage (calculation) of cattle intake by grazing or cafeteria feeding and classified into 1: very low, 9: very high.

III. Kentucky Bluegrass

III-1. Cultivation of Kentucky Bluegrass Genetic Resources for Evaluation of Characteristics

III-2. Evaluation of Characteristics of Kentucky Bluegrass Genetic Resources

by

Yoshiro Tsurumi

National Grassland Research Institute, MAFF, Nishinasuno, Tochigi 329-27 Japan

III-1. Cultivation of Kentucky Bluegrass Genetic Resources for Evaluation of Characteristics

Kentucky bluegrass belongs to the genus *Poa*, which consists of about 200 species. It is considered to be native to Europe up to Central Asia. The name of this grass is related to the fact that the importance of the grass was first recognized in Kentucky, USA and seeds harvested from the bluish fields of the grass were sold to other states.

This grass which spreads by underground rhizomes and produces a dense sod, is widely used as one of the main pasture grasses for grazing and one of the lawn grasses in parks and golf courses. Erect types are suitable for grazing, while creeping types for lawn.

It grows well under the cool climatic conditions and in relatively wet regions. It is highly resistant to low temperature and shade, but is severely damaged by high temperatures and drought. It is adapted to a wide range of soils and shows a good persistency.

Reproduction is characterized by facultative apomixis at a high percentage, but the rate fluctuates with the growth conditions. Number of chromosomes was reported to range from 2n=28 to around 150.

Leaves are smooth, glabrous, green to dark green, slender, about 3 mm wide, and long (10 ~ 30 cm long), with a boat-shaped tip which is peculiar to *Poa* spp. The plant produces many erect culms, usually 30 ~ 75 cm long. The inflorescence consists of an open panicle, bearing small seeds with hairs on the base.

This manual can be used for other grasses with rhizomes.

1. Space planted test

1) Raising of seedlings

Seeds are sown in petri dishes and kept in a chamber at about 20 °C until germination. Germinated seeds are planted one by one in paper pots or seedling beds. Seedlings are raised for a few months in a green house.

2) Planting of seedlings

Each seedling is planted in a 100 cm × 100 cm space in the well prepared field.

3) Size of plot

One dozen plants per plot, including two plants for borders, are planted by a randomized block design with two replications.

4) Amount of fertilizers (kg/a/year)

(1) First (sowing) year

Basal dressing N: 0.5, P_2O_5: 1.0, K_2O: 0.5, Lime: 10

Top dressing N: 0.3, K_2O: 0.3

(2) Second to third years
Spring dressing N: 0.5, P_2O_5: 1.0, K_2O: 0.5
Top dressing N: 0.3, K_2O: 0.3 (after each cutting)

5) Cutting times

Plants are cut at 10 cm height once in the first year and a few times in the second and third years.

6) Duration of the test

Two to three years.

2. Sod plot test

1) Seeding

Seeds are scattered in each plot at the rate of 1.0 g/m^2 and covered slightly by soil and pressed well with a roller.

2) Size of plot and number of replications

Plot size is 6 m^2 and plots are arranged in a randomized block design with three replications.

3) Amount of fertilizers (kg/a/year)

(1) First (sowing) year
Basal dressing N: 1.0, P_2O_5: 1.0, K_2O: 1.0, Lime: 10
Top dressing N: 0.5, K_2O: 0.5

(2) Second to third years
Spring dressing N: 1.0, P_2O_5: 1.0, K_2O: 1.0
Top dressing N: 0.5, K_2O: 0.5 (twice a year after cutting)

4) Mowing frequency

Plots are mowed at 5 cm height a few times in the first (sowing) year and several times in the second and third years.

5) Duration of the test

Three years (persistency and tolerance to frequent mowing are observed in the third year).

III-2. Evaluation of Characteristics of Kentucky Bluegrass Genetic Resources

1. Primary characters

<Essential items>

Plant type

Angle between outer main heading stems and the horizontal line is measured during the internode elongation stage to the heading stage and the accession is classified into extremely erect, 3: semi-erect, 5: intermediate, 7: semi-prostrate and 9: extremely prostrate.

Plant length

Plant length is measured (cm) from the ground to the tip of main stems at the heading stage or at the time of first cutting.

Panicle length

Panicle length is measured (mm) from the base to the tip of the panicle at the full heading stage.

Leaf length

Leaf length is measured (cm) for the first leaf blade below the flag leaf.

Leaf width

Leaf width is measured (0.1 mm) for the widest part of the leaf blade below the flag leaf.

Date of first heading

The date when the first panicle of each plant emerges is recorded.

Stem thickness

Longer diameter of the internodes just below the panicle of the longest stem is measured at the heading stage or at the time of the first cutting, and the accession is classified into 1: very slender, 3: slender, 5: intermediate, 7: thick and 9: very thick.

Spreading of plant

Plant spreading is estimated by measuring two diameters of the plant crosswise and the accession is classified into 1: very limited, 3: limited, 5: intermediate, 7: wide and 9: very wide.

<Optional items>

Seedling vigor

Seedling vigor observed based on the growth four weeks after seeding is classified into 1: very low, 3: low, 5: intermediate, 7: high and 9: very high.

Leaf color

Greenness of leaf blade is recorded at the heading stage under standard fertilizer application and is classified into 1: very light, 3: light, 5: intermediate, 7: dark and 9: very dark.

Texture of leaf

Texture of leaf blade evaluated by touching at the heading stage is classified into 1: very smooth, 3: smooth, 5: intermediate, 7: rough and 9: very rough.

Number of panicles

Number of panicles at the full heading time or at the time of the first cutting is classified into 1: very few, 3: a few, 5: intermediate, 7: many and 9: very large number.

Anthocyanin pigmentation of leaf sheath

Anthocyanin pigmentation of the leaf sheath is classified into 1: very light, 3: light, 5: intermediate, 7: dark and 9: very dark.

Anthocyanin pigmentation of seed

Anthocyanin pigmentation of seed is classified into 1: very light, 3: light, 5: intermediate, 7: dark and 9: very dark.

Seed size

Seed size is measured crosswise for two diameters or observed, and is classified into 1: very small, 3: small, 5: intermediate, 7: large and 9: very large.

Weight of 1000 seeds

Weight of 1000 seeds is estimated (0.01 mg) by sampling 100 seeds with 4 replications using a mixture of seeds from 20 plants.

2. Secondary characters

<Essential items>

Stem rust resistance

Resistance to *Puccinia graminis* is evaluated based on the degree of infection by artificial inoculation or planting in infected field and the accession is classified into

1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant and 9: highly resistant.

Stripe rust resistance

Resistance to *Puccinia striiformis* is observed and the accession is classified in the same way as for stem rust resistance.

Yellow leaf rust resistance

Resistance to *Puccinia poae-nemoralis* is observed and the accession is classified in the same way as for stem rust resistance.

Greening in early spring

Earliness of greening is observed in early spring based on the sprouting date in the sod plot and the accession is classified into 1: very early, 3: early, 5: intermediate, 7: late and 9: very late.

Plant vigor in autumn

Plant vigor observed based on the growth in autumn is classified into 1: very low, 3: low, 5: intermediate, 7: high and 9: very high.

Coloring in early winter

Degree of yellow coloration is observed after frost fall, and is classified into 1: no coloration or very light, 3: light, 5: intermediate, 7: dark and 9: very dark.

Regrowth

Regrowth is observed after first cutting, and is classified into 1: very poor, 3: poor, 5: intermediate, 7: good and 9: very good.

<Optional items>

Melting-out resistance

Resistance to *Drechslera poae* is evaluated based on the degree of infection and the accession is classified into 1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant and 9: highly resistant.

Powdery mildew resistance

Resistance to *Erysiphe graminis* is evaluated based on the degree of infection and the accession is classified into 1: highly susceptible, 3: susceptible, 5: intermediate, 7: resistant and 9: highly resistant.

Snow mold disease resistance

Resistance to main snow mold fungus is evaluated based on the degree of infection (note the name of snow blight disease) and the accession is classified into 1: highly susceptible,

3: susceptible, 5: intermediate, 7: resistant and 9: highly resistant.

Shade tolerance

Shade tolerance estimated based on the degree of growth under shading conditions is classified into 1: very low, 3: low, 5: intermediate, 7: high and 9: very high.

Drought tolerance

Drought tolerance estimated based on the degree of growth under drought conditions is classified into 1: very low, 3: low, 5: intermediate, 7: high and 9: very high.

Termination of growth

The date when plants ceased growing is recorded during late autumn to early winter, and the time of growth termination is classified into 1: very early, 3: early, 5: intermediate, 7: late and 9: very late.

Tertiary characters

<Essential items>

Fresh yield of spring cutting

Fresh plant weight harvested from at least 2 m^2 area of 6 m^2 plot is measured (kg) at each cutting time during spring.

Dry matter percentage of spring cutting

Percentage of dry matter is determined (0.1%) by drying 300 to 500 g of fresh sample at 70 °C for 48 hours at each harvest during spring.

Dry matter yield of spring cutting

Dry matter yield during spring is calculated based on fresh yield and dry matter percentage.

Fresh yield of summer cutting

Fresh weight during summer is calculated in the same way as that for spring.

Dry matter percentage of summer cutting

Dry matter percentage during summer is calculated in the same way as that for spring.

Dry matter yield of summer cutting

Dry matter yield during summer is calculated in the same way as that for spring.

Fresh yield of autumn cutting

Fresh yield during autumn is determined in the same way as that for spring.

Dry matter percentage of autumn cutting

Dry matter percentage during autumn is calculated in the same way as that for spring.

Dry matter yield of autumn cutting

Dry matter yield during autumn is calculated in the same way as that for spring.

<Optional items>

Tolerance to mowing

Mowing tolerance is observed based on the sod density after the last mowing in the second or third year under frequent mowings at low-level cutting, and is classified into 1: very low, 3: low, 5: intermediate, 7: high and 9: very high.

Sod density

Sod density in the plots under frequent mowings at low-level cutting is classified into 1: very low, 3: low, 5: intermediate, 7: high and 9: very high.

Crude protein

Crude protein content on a dry matter basis is determined (0.1%) by the Kjeldahl method or a Near Infra-red Analyzer.

Acid detergent fiber (ADF)

ADF content on a dry matter basis is determined (0.1%) by the acid detergent-acetone washing method.

Neutral detergent fiber (NDF)

NDF content on a dry matter basis is determined (0.1%) by the neutral detergent-acetone washing method.

Acid detergent lignin (ADL)

ADL content on a dry matter basis is determined by hydrolysis of the sample with 72% sulphuric acid after acid detergent-acetone washing.

Mono- and oligosaccharides

Mono- and oligosaccharide content on a dry matter basis is determined (0.1%) by colorimetry with anthrone reagent after extraction in ammonium oxalate solution from non-structural carbohydrates.

Cyanogenetic glucosides

Content of cyanogenetic glucosides on a dry matter basis is determined (0.001ppm) by colorimetry in alkali picrate solution.

Saponin

Content of saponin on a dry matter basis is determined (0.01ppm) by thin layer chromatography after ethanol extraction.

Development of rhizomes

Development of rhizomes based on the number and weight of rhizomes and spreading of the plant is classified into 1: very poor, 3: poor, 5: intermediate, 7: good and 9: very good.

Degree of apomixis

Apomixis degree estimated based on the uniformity of 50 progenies is classified into 1: very low, 3: low, 5: intermediate, 7: high and 9: very high.

References

Fergus, E. N. and R. C. Buckner (1976) The bluegrasses and redtop, Forages, pp 243-253, Iowa State Univ. Press, USA (3rd edition).

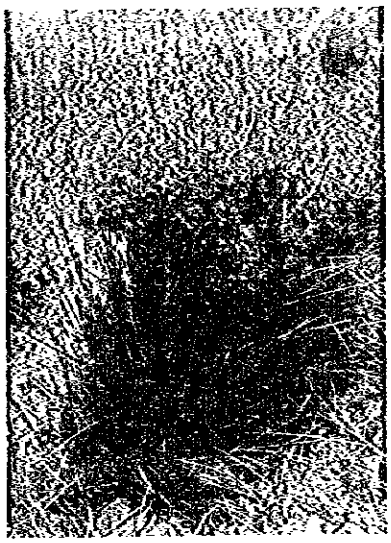

Photo. 1 Kentucky bluegrass at full heading time.

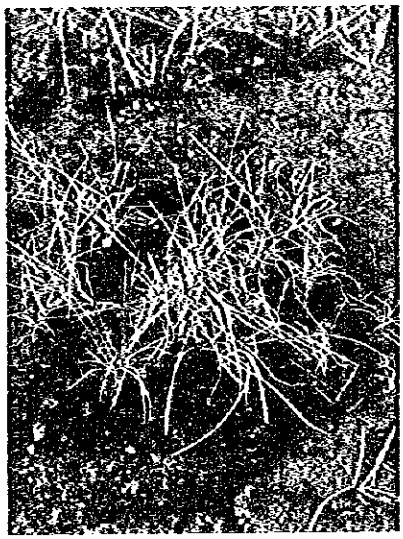

Photo. 2 Rhizomous plant infected with melting-out disease.

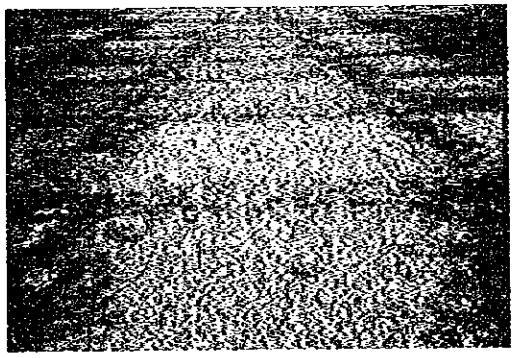

Photo. 3 Differences among strains in earliness of greening.

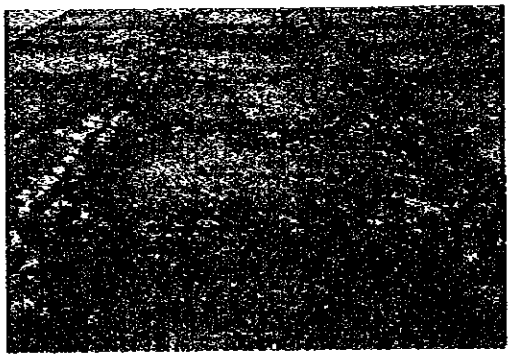

Photo. 4 Differences among strains in stripe rust infection.

IV. Panicum

IV-1. Cultivation of Panicum Genetic Resources for Evaluation of Characteristics

IV-2. Evaluation of Characteristics of Panicum Genetic Resources

by

Hidemichi Matsuoka

Kyushu National Agricultural Experiment Station, Nishigoshi 861-11 Japan

IV-1. Cultivation of Panicum Genetic Resources for Evaluation of Characteristics

In the case of tropical pasture plants, especially volunteer seedlings of native/wild panicum, special attention should be given to seed shattering. Volunteer seedlings may contaminate the experimental plot and it will become difficult to separate true genetic resources from volunteer seedlings in the experimental plots. PGR experimental field should be set up and maintained so as to be free from contamination with native/wild panicum volunteers.

1. Land preparation

Experimental field is carefully plowed and harrowed so that the soil surface is smooth, especially in the case of direct sowing, to obtain accurate data for morphological and/or productivity characteristics. Application of barnyard manure or compost is recommended at a rate of 200-400 kg/a two weeks before transplanting or seeding. Basal application of nitrogen fertilizer ranging from 0.5 to 1.0 kg/a depending on the soil fertility is recommended. Nitrogen fertilizer is applied at the rate of 0.5 kg/a after each cutting or grazing for the productivity test. Basal application of phosphorus fertilizer is often more effective than nitrogen fertilizer in phosphate-deficient soils.

2. Planting time

As panicum plants require a high temperature for germination and rapid growth, mean temperatures of over 20 °C are necessary for direct seeding to fields. It is recommended to use seed beds made of paper pots and to transplant seedlings at the 4 - 5 leaf stage for trial fields especially when experimental fields are contaminated with volunteer seedlings of panicum.

3. Planting density

Space planting is suitable for the evaluation of PGR. Row spacing is usually set at 1.5 m and intrarow spacing at 0.5 m. In case of stoloniferous species such as rhodesgrass and creeping signal grass, interrow spacing of 3.0 m is recommended. Five to ten seeds are sown in one hill depending on the seed quality and dormancy, and thinned to a single plantlet per hill at the 4 - 5 leaf stage. Split tuft or stem cutting is planted instead of seed for vegetatively propagated cultivars like Coloniao.

For the productivity test, clean seeds that can germinate are sown at the rate of 0.1 ~ 0.2 kg/a by broadcasting or drilling. The use of a land roller or packer after seeding leads to good germination and establishment.

4. Protection from insect injury

Seedlings that are transplanted and/or have germinated are often attacked by insects such as armyworms or cutworms. Abnormal heading often occurs when the main stems are killed due to the attack of insects. It is recommended to broadcast a systemic insecticide for the protection of the seedlings after transplanting or seeding.

5. Experimental design for evaluation of characteristics

For the evaluation of the morphological and physiological characteristics, it is preferable to use more than 20 plants with two replications. In the test for productivity, size of each trial should be above 6 m^2, consisting of 4 rows with an interrow spacing of 0.5 - 0.6 m. Two outer border rows should be excluded from the productivity test. Yield and characterization tests should be carried out based on a randomized complete block design with two replications.

6. Sampling inspection for evaluation of characteristics

Most of the morphological characteristics are usually analyzed and/or observed at around the heading stage. Observation of waxy and pubescence characteristics must be performed before the heading stage. For the productivity test, materials are cut when check cultivars reach the booting stage or the heading stage, or when plants become about 1.5 m tall.

IV-2. Evaluation of Characteristics of Panicum Genetic Resources

1. Primary characters

Twenty plants should be used for the determination of each character.

<Essential items>

Plant type

The angle between the outer shoots and the horizontal shoot is measured, and plant type is classified into 1: erect, 5: intermediate, 9: prostrate.

Culm length

Length of the longest culm from the ground level to the neck node of panicle is measured (cm).

Panicle length

Length of panicle from the neck node of panicle to the top is measured using the longest stem (cm).

Culm diameter

Diameter of internode is measured below the neck node of panicle of the longest stem (mm).

Leaf length

Length of the leaf blade next to the flag leaf is measured (cm) using the longest stem.

Leaf width

Width of the leaf blade next to the flag leaf is measured (cm) using the longest stem.

Heading date

The date when in more than 10% of the plants panicles emerge is recorded.

Seed shattering

Shattering degree of mature seeds from rachis-branch is recorded and is classified into 3: low, 5: intermediate, 7: high.

Panicle number

The number of panicles is recorded at the heading stage or at the time of the first cutting and classified into 0: no panicles, 1: very few, 5: intermediate, 9: many.

<Optional items>

Pubescence

Presence and amount of hairs on node, leaf sheath, leaf blade and ligule are observed at the heading stage, and classified into 1: absent or very few, 3: few, 5: intermediate, 7: many, 9: very large number.

Wax

Presence and amount of wax is observed on leaf and culm at the heading stage and classified into 1: absent, 3: little, 5: intermediate, 7: abundant, 9: extremely abundant.

Leaf color

Color of leaf blade is observed at the heading stage and classified into 3: light green, 5: intermediate, 7: dark green.

Leaf hardness

Hardness or softness of leaf blade is observed at head emergence and the leaf blade is classified into 1: extremely soft, 3: soft, 5: intermediate, 7: hard, 9: extremely hard.

Anther color

Color of anther is observed at the inflorescence and classified into 1: white, 3: yellow, 5: brown, 7: purple, 8: dark purple, 9: other colors.

Seed weight (Thousand seed weight)

Random samples of 100 cleaned seeds are weighed with 4 replications. The data obtained are expressed as thousand seed weight (g).

2. Secondary characters

Twenty plants should be examined with 2 replications for each character.

<Essential items>

Regrowth

Regrowth is evaluated 1 ~ 2 weeks after cutting and classified into 1: very poor, 3: poor, 5: intermediate, 7: good, 9: very good.

Overwintering survival

General aspects of overwintering ability are estimated based on the percentage of dead plants and the loss of stand and overwintering survival is classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Vigor in spring

Vigor is evaluated 30 days after sprouting in spring and the accession is classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Vigor in autumn

Vigor or regrowth after cutting in autumn is classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

<Optional items>

Disease resistance

Disease severity is evaluated based on artificial inoculation or field infection. The kinds of diseases such as virus diseases, brown stripe, ergot, anthracnose, brown patch and leaf blight are recorded. Resistance is classified into 1: extremely low, 3: low, 5: intermediate, 7: high, 9: extremely high.

Insect resistance

Insect damage is evaluated based on artificial inoculation or natural attack in the field. The kinds of insects are recorded, and the resistance is classified into 1: extremely low, 3: low, 5: intermediate, 7: high, 9: extremely high.

Persistency

Percentage of plants surviving in the second year after establishment is recorded and persistency is classified into 1: extremely low 3: low, 5: intermediate, 7: high, 9: extremely high.

Apomixis

Percentage of apomicts is determined based on the embryo sac analysis or the off-type analysis in progeny test, and the degree of apomixis is classified into 0: no apomixis, 1: extremely low, 3: low, 5: intermediate, 7: high, 9: extremely high.

Self-fertility

Self-fertility is estimated based on the amount of seeds harvested by forced pollination using bagging or an isolation field. Self-fertility is classified into 0: absent, 1: extremely low, 3: low, 5: intermediate, 7: high, 9: extremely high.

3. Tertiary characters

<Essential items>

Samples for determination should be collected from 2 plots for each character. The size of the experimental plot should exceed 6 m^2. Forage is cut at a height of 10 cm from the ground level during the booting or heading stage, or when plants reach a height of 1.5 m.

Fresh yield

The whole fresh forage is harvested from plots excluding borders and weighed. Fresh yield is expressed in kg/a.

Dry matter percentage

About 500 g of fresh forage is dried at the temperature of 70 °C for 48 hours. Dry matter percentage is calculated from the ratio of dry matter weight to fresh weight.

Dry matter yield

Dry matter yield is determined by multiplying the fresh yield by the dry matter percentage and expressed in kg/a.

<Optional items>

Samples for determination are collected from 2 plots with 2 replications for each character. Content of each chemical constituent is expressed on a dry matter basis.

Dry matter digestibility

Dry matter digestibility is determined by *in vivo* digestibility test using animals or *in vitro* digestibility test using enzymes.

Crude protein

Crude protein content is determined by the Kjeldahl procedure, measuring the amount of total N in a forage sample and multiplying by 6.25. Near infrared reflectance spectroscopy method can also be applied.

Acid detergent fiber (ADF)

Acid detergent fiber is determined as residual fiber using acetic detergent followed by acetone washing.

Acid detergent lignin (ADL)

Acid detergent lignin is determined in the same way as ADF lignin.

Neutral detergent fiber (NDF)

Neutral detergent fiber is determined as residual fiber using neutral detergent followed by acetone washing.

Mono- and oligosaccharides

Mono- and oligosaccharides are quantitatively analyzed by ethanol extract using thin layer chromatography.

Alkaloids

Alkaloids are separated and determined by thin layer chromatography and expressed in ppm on a dry matter basis.

Hydrocyanic acid

Hydrocyanic acid is determined by colorimetric analysis using alkali picrate solution and expressed in ppm on a dry matter basis.

Nitrate nitrogen (NO_3-N)

Nitrate nitrogen is determined by the phenol di-sulphuric acid method and expressed in ppm on a dry matter basis.

Herbage intake

Herbage intake is estimated by grazing or cafeteria feeding and classified into 1: extremely low, 3: low, 5: intermediate, 7: high, 9: extremely high.

Palatability

Palatability is estimated by grazing or cafeteria feeding and classified into 1: extremely low, 3: low, 5: intermediate, 7: high, 9: extremely high.

Photo. 1 Guinea grass at the heading stage. Most of the morphological/physiological characteristics are evaluated at the heading stage. Space planting is used with interrow spacing of 1.5 m and intrarow spacing of 0.5 m.

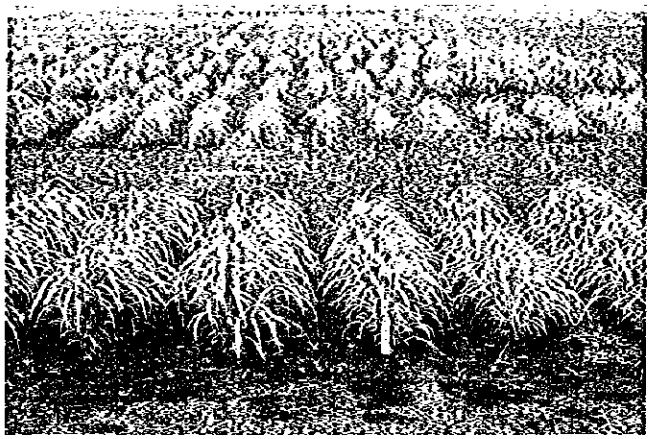

Photo. 2 Productivity test. Size of each plot consisting of 4 rows with an interrow spacing of 0.6 m is 6 m^2.

Photo. 3 Symptoms of virus disease. Space planted guinea grass is infected with sugar cane mosaic virus.

V. Sorghum

V-1. Cultivation of Sorghum Genetic Resources for Evaluation of Characteristics

V-2. Evaluation of Characteristics of Sorghum Genetic Resources

by

Masahiro Matsuura

Kyushu National Agricultural Experiment Station, Nishigoshi 861-11 Japan

V-1. Cultivation of Sorghum Genetic Resources for Evaluation of Characteristics

1. Land preparation

Sorghum exhibits a wide adaptability to various soil conditions, but sometimes growth is reduced when the crop is cultivated continuously for many years in the same field, particularly in sandy soil or volcanic ash soil. Therefore, continuous cultivation in the same soil should be avoided as much as possible. Sorghum can grow under a wide range of pH values but the optimum pH values range from 6.5 to 7.0. Therefore, lime application is necessary for acid soil.

Since sorghum is deep-rooted, deep plowing and careful harrowing are recommended to ensure good germination and growth.

2. Planting time

Sorghum is usually sown when the average daytime temperature exceeds 17 °C. The optimum temperature for germination of sorghum is higher than that for maize, and early season seeding at a low temperature may result in poor germination and rot of seeds in soil. The raising of seedlings in small pots (5 × 5 × 5 cm) and transplanting to experimental fields is recommended for PGR evaluation, to achieve uniform growth. Transplanting is carried out at the 3 to 4 leaf stages.

In addition, special care is necessary to avoid rainfall and strong wind during the growing season. When the soil moisture is lower than 20 percent, germination of sorghum is poor and delayed. Sorghum plants can recover from lodging when the plant height is lower than 1.5, but it can not recover when the plant becomes taller after the heading stage.

3. Seeding and transplanting

Seeds must be dressed with chemicals such as Benlate or Captan to protect germinating seeds from fungi infection and seedlings from bird injury. Two or three seeds are sown to a hill or a pot and thinned to a single plant at the 3 to 4 leaf stages. Spacing between rows is usually 60 cm and between plants 15 to 20 cm both in the case of direct seeding and transplanting. Seeding depth varies depending on the soil conditions, but it must not exceed 5 cm to secure good germination. Tamping of the covered soil is usually practiced. For clayey soil, special care is necessary for the determination of the sowing time. When it rains soon after sowing and the soil surface becomes dry due to strong sunshine, a hard soil layer is formed on the soil surface, which prevents the penetration of seedlings through the soil layer and germination becomes very poor.

4. Experimental design for the evaluation test

Each plot for the evaluation of characteristics must consist of more than 15 plants and the design of the experimental plot is a randomized block with two replications.

5. Fertilizer application

Although sorghum can grow under various soil conditions, it grows best on fertile soil at pHs ranging from 6.5 to 7.0. Therefore, lime application is necessary depending on the pH of the field. The recommended rate of basal fertilizer application for N, P and K ranges from 10 to 15, 15 to 20 and 10 to 12 kg/10a depending on the soil fertility. Additionally, 8 to 10 kg/10a of N and K are applied at the 6 to 8 leaf stages and after cutting when regrowth is used. The timing of fertilizer application is determined based on the leaf color. Additional fertilizer should be applied between rows. When fertilizer stays on a spot between the blade and leaf sheath, plant growth is delayed. Application of manure promotes the growth of sorghum.

6. Weed and pest control

Chemicals and mechanical cultivation are usually applied for weed control of sorghum fields. However, hand weeding is recommended for a small-sized test field. The application of herbicides such as Gesaprim and Pendimethalin immediately after sowing is effective to control the germination of weeds which may depress the early growth of sorghum. Seedlings may be injured when Pendimethalin is applied at high temperatures. Other herbicides can be used during the early growth stage of sorghum. Intertillage is also effective but must be practiced when the plants are still short.

Sorghum plants are often attacked by aphids under hot and dry conditions after heading. Dysyston granules can be applied to control them if necessary. Sumithion should not be applied for pest control of sorghum, because of serious leaf damage.

7. Harvest

Harvest stage for the evaluation of sorghum genetic resources varies depending on the type of utilization. As grain crop sorghum is harvested at the full-ripe stage and as silage crop at the dough-ripe stage, in the case of grain sorghum, harvest in the rainy season should be avoided because *Sorghum bicolor* shows a high degree of viviparity under wet and warm conditions. Bird injury becomes serious after the milky stage.

V-2. Evaluation of Characteristics of Sorghum Genetic Resources

Sorghum for practical use consists of two species, *Sorghum bicolor* and *S. sudanense*, in addition to their hybrids. The number of characteristics for evaluation amounts to 40 for essential items and 44 for optional items. These items are grouped into three categories: primary characters consisting mostly of morphological ones, secondary characters consisting mostly of disease and insect pest resistance and tertiary characters related to yield. The characters in each category are grouped into two, essential and optional. The characters to be evaluated vary depending on the species and type of utilization, e. g. grain sorghum or silage sorghum.

1. Primary characters

<Essential items>

The important morphological characters are included in this category.

Length of culm and panicle

Measurement takes place at maturity as shown in Fig. 1.

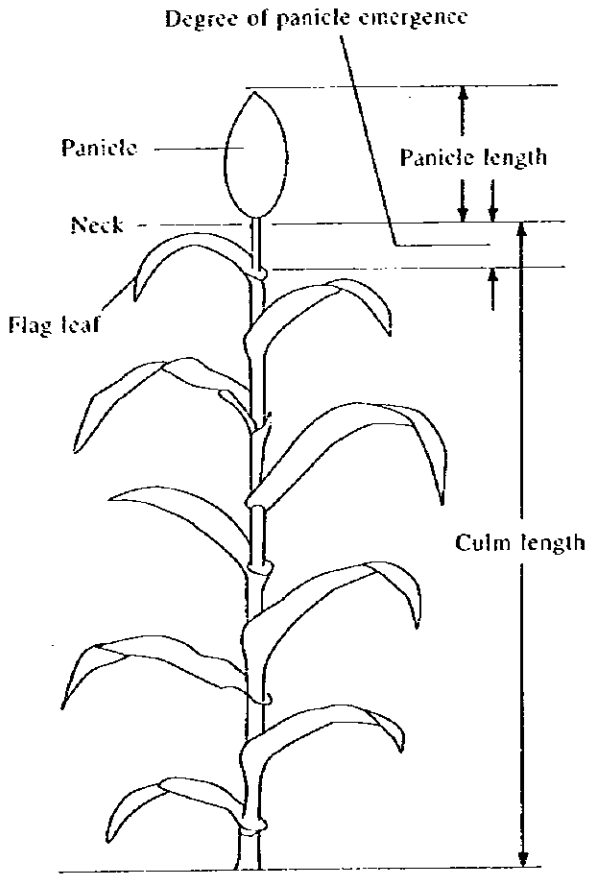

Fig. 1 Sorghum plant.

Panicle shape

Panicle shape is classified into six types, 1: broom, 2: lax cone, 3: cone, 5: spindle, 7: cylinder and 9: short cylinder as indicted in the pictures drawn in Fig.2.

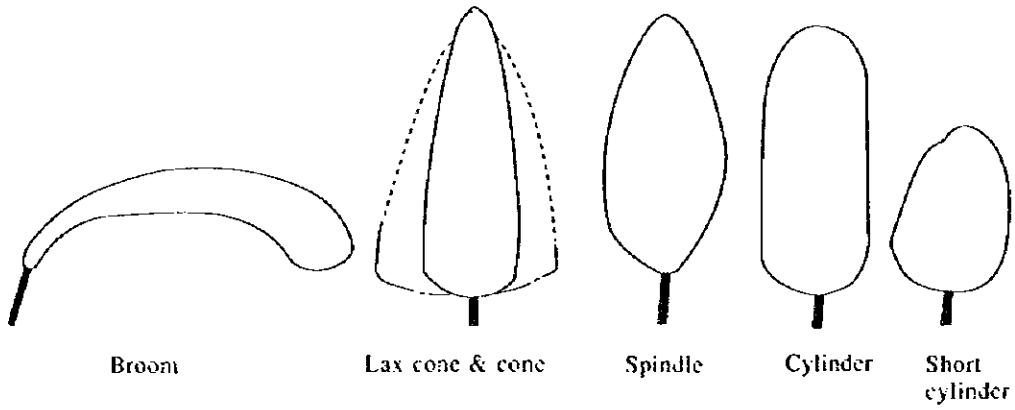

Fig. 2 Panicle shape.

Type of panicle

Type of panicle is classified into three types based on compactness, 1: open, 5: medium and 9: compact.

Diameter of culm

Diameter of culm is measured at a height of 10 to 15 cm from the base as shown in Fig.3.

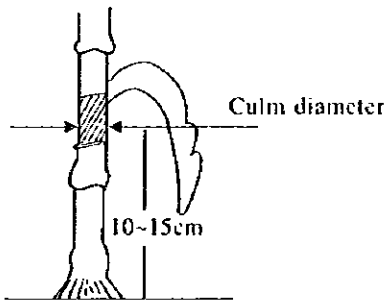

Fig. 3 Area for measurement of the culm diameter.

Length and width of leaf

Length and largest width of the longest leaf blade are measured at maturity.

Number of tillers per plant

Number of stems which are longer than 1/3 of the main stem is counted.

Grain weight

Weight of cleaned grains per panicle on the main stem is recorded only for grain sorghum.

Date of heading

Date of heading is recorded when 50 percent of the plants begin to head.

Date of maturity

Date of maturity is recorded when the grains on 1/3 portion from the base of a head of most panicles become as hard as wax.

<Optional items>

Quantity of lipid white powder on stems and sheaths

Quantity of lipid white powder on stems and sheaths is evaluated 50 days after sowing, and classified into 0: no powder, 3: small amount, 5: intermediate, 7: large amount. This character affects the digestibility of plants.

Color of midrib

Color of midrib of two or three leaves below the longest leaf of a plant is recorded at the heading stage, and classified into 1: white, 2: light green, 3: green, 5: orange, 7: brown and 9: variegated. Brown midrib is related to the digestibility of a plant and is genetically controlled.

Juiciness of stem

Juiciness of stem is estimated based on the observation of cross sections of stems at the heading stage, and classified as follows: 1: dry (when more than 70 percent of cross section is dry), 5: mixed (when mixed), 9: juicy (when more than 70 percent of cross is juicy). This character is related to the dry matter percentage of a plant.

Color of coleoptile

Color of coleoptile is observed at germination, and classified into 1: green, 5: variegated, 9: purple.

Number of leaves on the main stem

Angle formed by a leaf with stem

Angle formed by a leaf with stem is measured as shown in Fig.4.

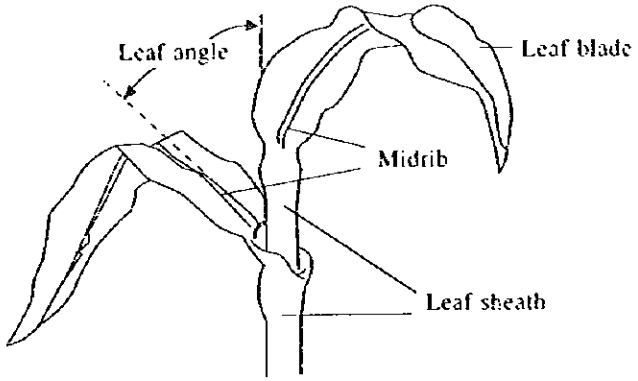

Fig. 4 Leaf blade and leaf sheath.

Number of panicles per plant

Panicle emergence

Distance from the junction of the flag leaf with the stem to the base of the neck is measured as shown in Fig.1.

Awn presence

Glume color

Glume color is classified into 1: grey, 2: wheat straw color, 3: yellow to brown, 4: orange, 5: red, 6: reddish brown, 7: brown, 8: purplish brown, 9: black. Refer to Fig. 5.

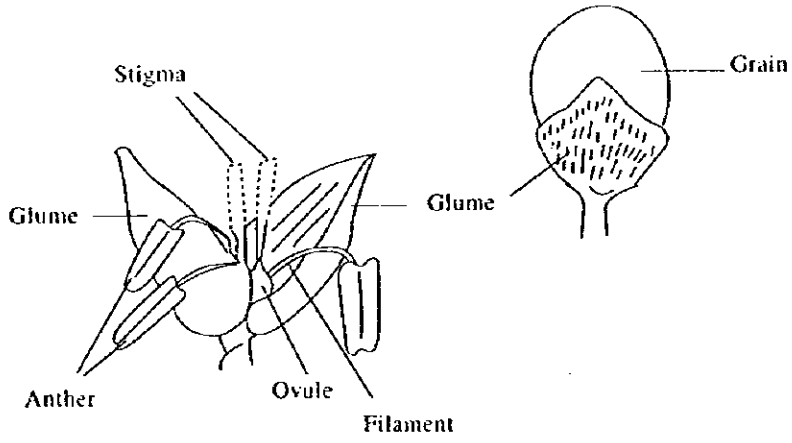

Fig. 5 Appearance of glume.

Polyembryony

Presence of two or three embryos in one grain is observed, and classified into 0: absent, 9: present.

Grain shape

Shape of grains is classified into 1: boat-shaped, 3: egg-shaped, 5: long round-shaped, 7: round-shaped and 9: fan-shaped as shown in Fig. 6.

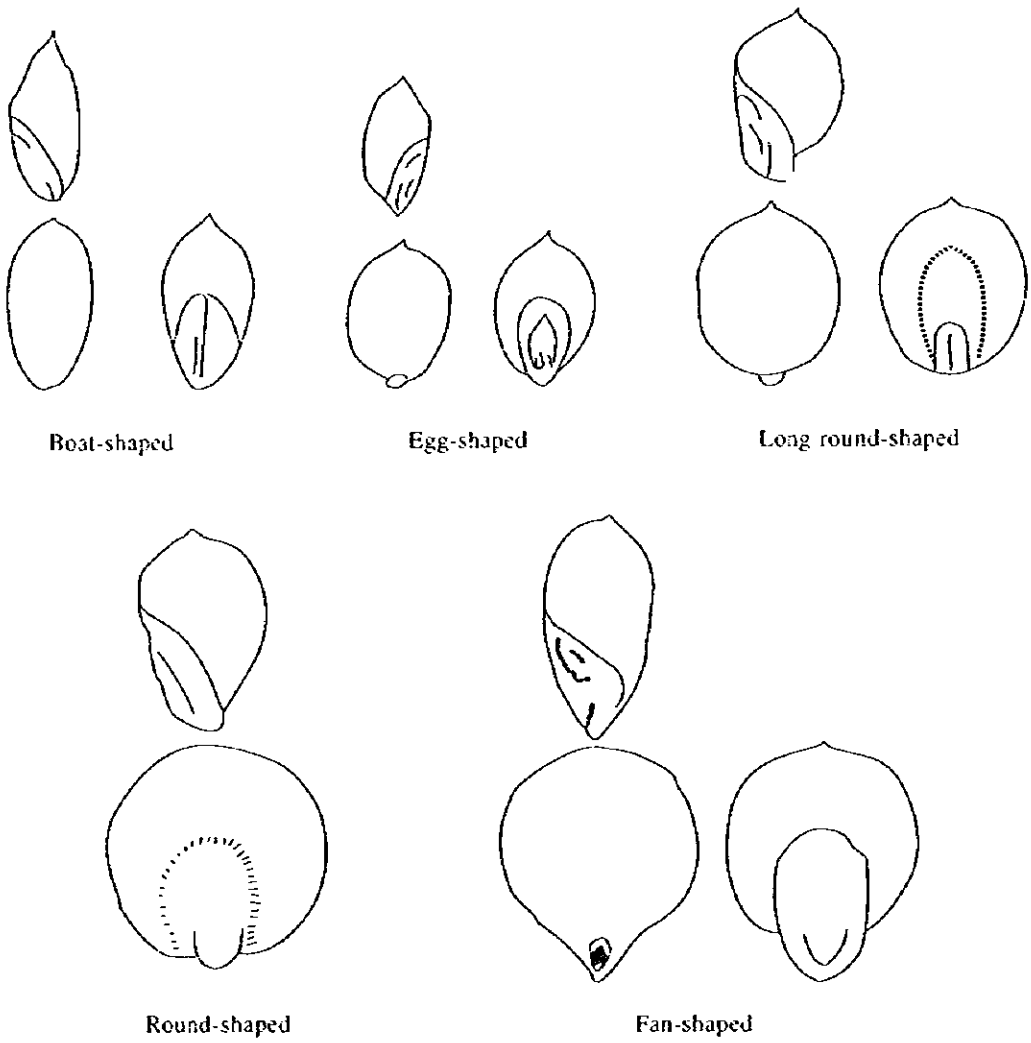

Fig. 6 Grain shape.

Weight of 1,000 grains

Presence of rhizome

Presence of rhizomes is observed soon after maturity, and classified into 0: absent and 9: present.

Vigor at early stage

Vigor at early stage is observed 30 ~ 40 days after sowing.

Flowering date

The date when about 50% of heads have flowered is recorded.

Shattering habit

Grain texture

The rate of the opaque portion is determined for the evaluation of the grain texture and is classified into 1: extremely low, 3: low, 5: intermediate, 7: high, 9: extremely high.

Degree of gluten

2. Secondary characters

<Essential items>

Resistance to leaf blight

Resistance to *Trichometaphaeria turcica* is recorded.

Leaf sheath blight

Resistance to *Rhizoctonia solani* is recorded.

Aphid

Resistance to aphid (*Rhopalosiphum maidis*) is recorded.

Lodging

Lodging is recorded when it occurs. Degree of lodging is classified into nine grades, 1: extremely high to 9: extremely low.

Vigor of plant regrowth

Vigor of plant regrowth is evaluated 20 days after cutting. Degree of vigor is classified into nine grades in the same way as for lodging.

<Optional items>

Resistance to diseases

Resistance to bacterial stripe (*Pseudomonas andropogonis*), zonate leaf spot (*Gloeocercospora sorghi*), target spot (*Helminthosporium sorghicola*), rust (*Puccinia purpurea*), anthracnose (*Colletotrichum graminicola*), ergot (*Claviceps purpurea*) is recorded.

Resistance to pests

Resistance to oriental corn borer (*Ostrinia furnacalis*) and other insect pests, and degree of bird injury are recorded when the damage is apparent. The resistance is classified into nine grades, 1: extremely low to 9: extremely high.

Resistance to viviparity

Resistance to viviparity is observed and nine grades are recorded as in the case of other injuries.

3. Tertiary characters

<Essential items>

All the characters in this category are related to the yield.

Fresh yield

Fresh yield of more than 15 plants is determined at each harvest. For silage, sorghum is harvested at the dough-ripe stage and for grain, at the full-ripe stage. Plants are divided into heads and other parts in necessity.

Dry matter percentage

At least five sorghum plants more than 1 kg in fresh weight are sampled and dried at 70 °C in the oven for 48 hours. Dry matter yield is calculated by multiplying the fresh yield by the dry matter percentage.

Brix value

Brix value of internode sap is measured at harvest in the middle of the main stems or in sap squeezed from whole stem.

<Optional items>

Main characters in this category are related to the nutritive value of the crop.

Grain weight

Weight of cleaned grains is measured for grain type sorghum.

Dry matter digestibility

Dry matter digestibility is determined by *in vivo* method or *in vitro* enzyme method.

Crude protein content

Crude protein content is determined by the Kjeldahl method or a Near Infra-red Analyzer.

Acid detergent fiber (ADF)

Content of acid detergent fiber (ADF) is determined by the acid detergent-acetone washing method.

Acid detergent lignin (ADL)

Content of acid detergent lignin (ADL) is determined by the acid detergent-acetone washing method.

Neutral detergent fiber (ADF)

Content of neutral detergent fiber (ADF) is determined by the neutral detergent-acetone washing method.

Mono- and oligosaccharide content

Mono- and oligosaccharide content is determined by the ethanol extraction and phenol sulphuric acid method.

Hydrocyanic acid content

Hydrocyanic acid content is determined by colorimetry with an alkali picrate solution.

Nitrate nitrogen content

Nitrate nitrogen content is determined by the phenol di-sulphuric acid method.

The above two characteristics are important when sorghum is harvested at the early growth stage, since the components are toxic to cattle.

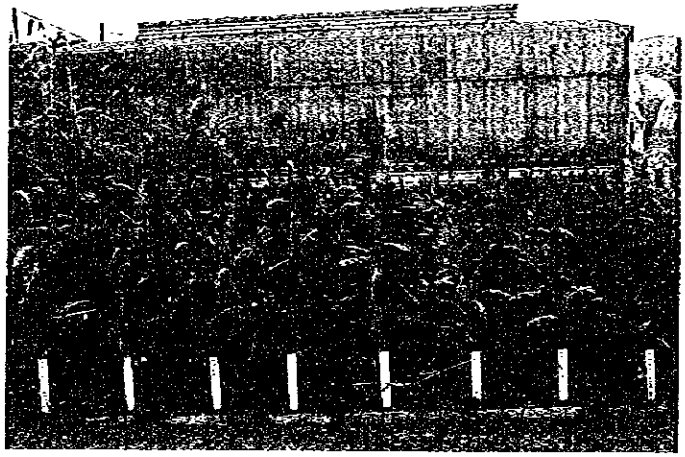

Photo. 1 **Plant height of sorghum lines with different number of dwarf genes. From left, five rows: lines with two genes, two rows: lines with three genes, and one row: lines with four genes.**

Photo. 2 **Spindle-shaped panicle of *Sorghum bicolor.***

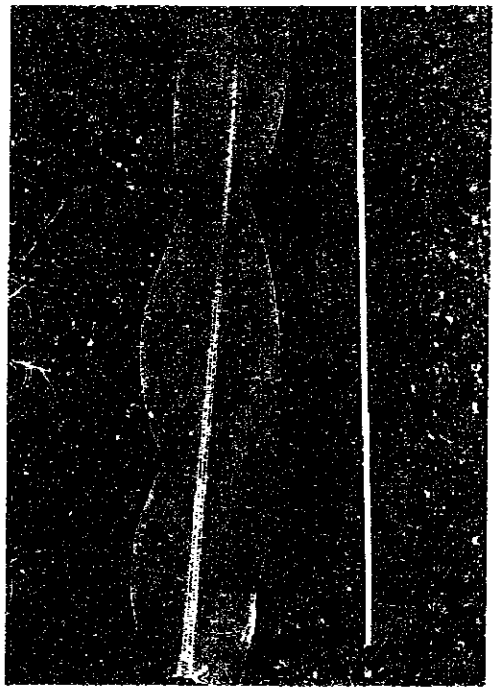

Photo. 3 **Brown midrib (left) of *Sorghum bicolor.***

VI. Chinese Milk Vetch

VI-1. Cultivation of Chinese Milk Vetch Genetic Resource for Evaluation of Characteristics

VI-2. Evaluation of Characteristics of Chinese Milk Vetch Genetic Resources

by

Tomoyuki Takai

Hokkaido National Agricultural Experiment Station, MAFF, Sapporo 062 Japan

VI-1. Cultivation of Chinese Milk Vetch Genetic Resources for Evaluation of Characteristics

Chinese milk vetch which is an annual legume, is generally sown in autumn (from September to October in the northern hemisphere), flowering occurs in the following spring (from April to May), and seeds ripe in early summer (from May to June). Exposure to low temperatures at the early developmental stage is necessary for the flowering of Chinese milk vetch.

Chinese milk vetch is cultivated for use as green manure, forage and for collecting honey.

1. Land preparation

Fertilizer application program should be based on the results of a local soil test. The usual standard rate in Japan is N: 0.5, P: 2.0, K: 2.0 kg/a. Before sowing, soil amendment materials such as lime and phosphatic fertilizer are applied depending on the soil conditions. Plowing is necessary to bury the residues of the preceding crops and weeds on the soil surface. In this case deep plowing of more than 25 cm is usually practiced.

2. Seed preparation

In areas where Chinese milk vetch had not been grown previously, seeds are usually inoculated with Chinese milk vetch root nodule bacteria (*Rhizobium astragali*).

3. Sowing time

Sowing time of Chinese milk vetch depends on the precipitation pattern and temperature. Chinese milk vetch is usually sown in fall one or half a month before the first frost. The optimum temperature for seed germination of alfalfa is about 20 ~ 25 °C.

4. Sowing of seeds

a) Space planted plot for the evaluation of primary and secondary characters

In case of direct seeding, 2 – 4 seeds are sown to one hill and thinned to one plant 2 – 3 weeks after germination. Spacing between plants is 40 cm and row spacing is 80 cm. Twenty plants per plot with two replications are necessary for the evaluation.

b) Broadcasted plot for the evaluation of tertiary characters

Standard seeding rate is 200 ~ 400 g per are. After broadcasting, seeds are covered with soil.

5. Field management

Weed control is necessary. For weed control, cultivation before sowing is most effective. Disease and insect outbreaks should be controlled by spraying of chemicals, except for the test for the evaluation of resistance to sclerotinia.

6. Harvest

Chinese milk vetch for silage and green manure is harvested at the full flowering time.

VI-2. Evaluation of Characteristics of Chinese Milk Vetch Genetic Resources

1. Primary characters

<Essential items>

Plant type

Plant type is classified into 3: erect, 5: intermediate, 7: prostrate (Fig. 1).

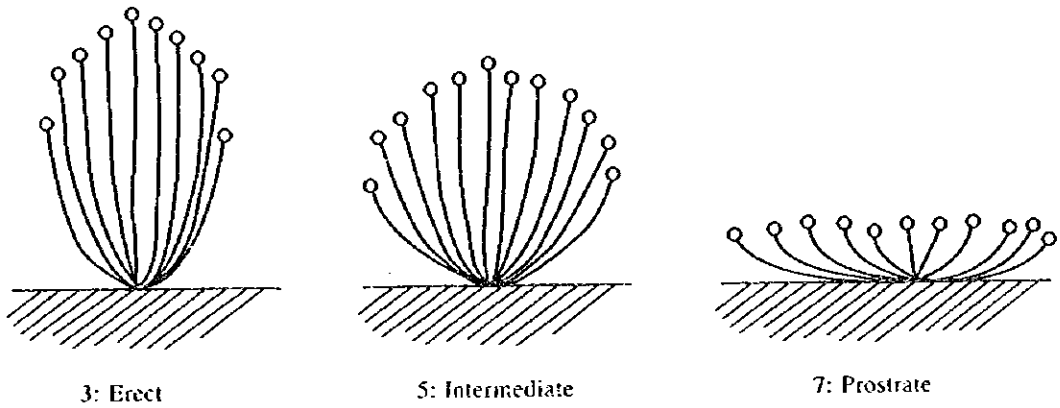

Fig. 1 Plant type.

Plant height

Plant height is measured at the full flowering time.

Stem thickness

Diameter of stem is measured or observed at the full flowering time and the stem is classified into 3: slender, 5: intermediate, 7: thick.

Leaf length

Length of the largest leaf is measured from the base of the petiole to the top leaflet at the flowering time (Fig. 2).

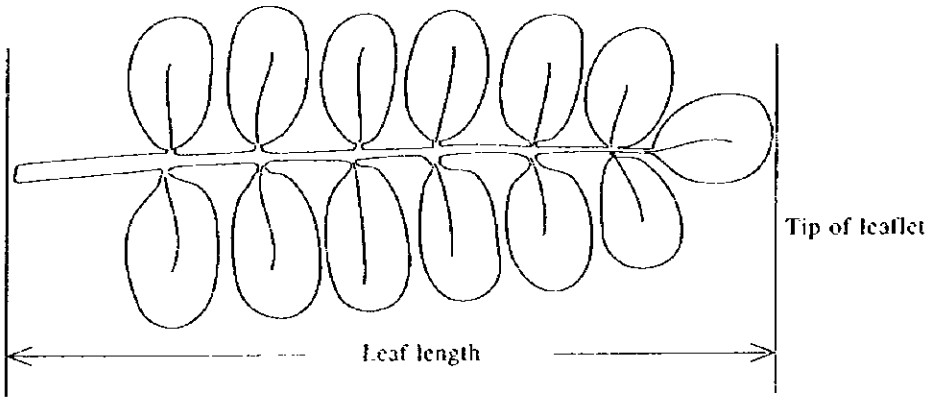

Fig. 2 Leaf length.

Leaflet size

Width and length of the top leaflet of the largest leaf are measured or observed at the flowering time and classified into 3: small, 5: intermediate, 7: large (Fig. 3).

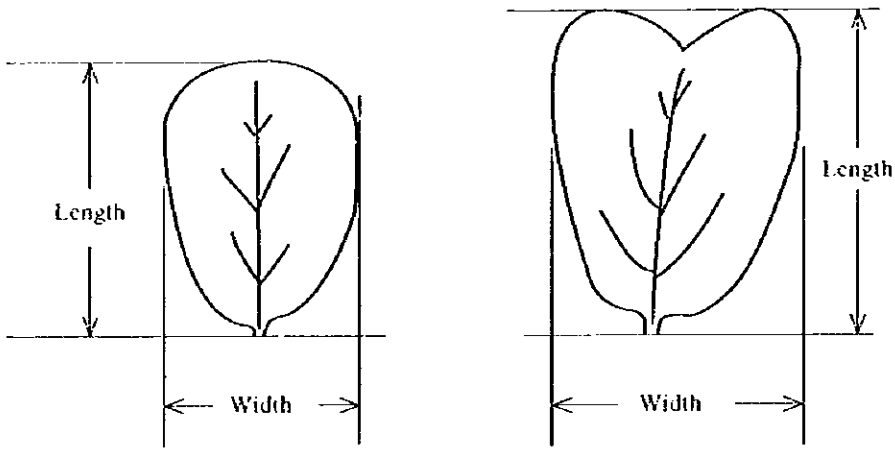

Fig. 3 Leflet size.

First flowering date

The date when plants begin to flower is recorded.

Flower color

Color of standard and keel petals is observed immediately after flowering and classified into 1: white, 2: yellowish white, 4: bright purple, 5: red purple, 7: deep red purple.

<Optional items>

Stem color

Degree of coloring of stem by anthocyanin pigment is observed under sunshine at the flowering time and classified into 1: green, 3: light red, 5: red brown, 7: dark red purple.

Number of stems

Number of primary stem branches is counted at the beginning of the flowering time and classified into 3: few branches, 5: intermediate, 7: many branches.

Length of flower stalk

Length of flower stalk on the longest stems is measured at the flowering time.

Number of florets per cluster

Number of florets per cluster is counted and classified into 3: few florets, 5: intermediate, 7: many florets.

Pod color

Pod color is observed after ripening and classified into 3: brown, 5: dark brown, 7: black.

Seed shape

Shape of seed samples from mature pods is observed and classified into 1: circle, 3: egg, 5: ellipse, 7: diamond (Fig. 4).

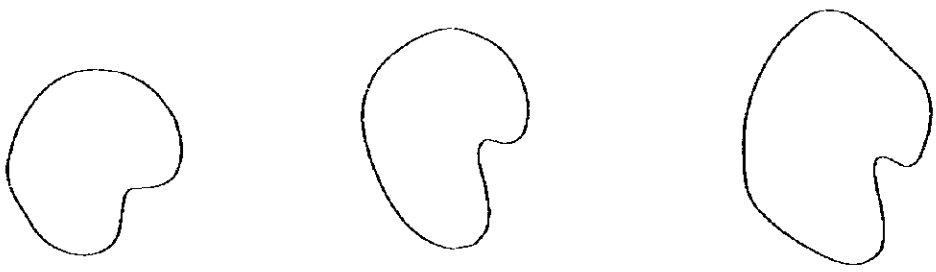

Fig. 4 Seed shape.

Weight of 1000 seeds

Weight of 1000 seeds is measured.

2. Secondary characters

<Essential items>

Resistance to *sclerotinia* root rot and crown rot

Resistance to *Sclerotinia trifolii* is evaluated based on the degree of damage and the frequency of dead plants when the infection became apparent, and the accession is classified into 3: susceptible, 5: intermediate, 7: resistant.

Overwintering ability

Tolerance to overwintering is evaluated based on the percentage of plants which survived immediately after overwintering and classified into 3: low, 5: intermediate, 7: high.

Plant vigor in spring

Plant vigor is observed one month after overwintering and classified into 3: low, 5: intermediate, 7: high.

<Optional items>

Disease resistance

Resistance to diseases is evaluated based on the degree of damage and the frequency of dead plants when the damage became apparent (record the name of disease) and the accession is classified into 3: susceptible, 5: intermediate, 7: resistant.

Insect resistance

Resistance to insects is evaluated based on the degree of damage and the frequency of dead plants when damage became apparent (record the name of insect) and the accession is classified into 3: susceptible, 5: intermediate, 7: resistant.

Spring habit

Percentage of flowering plants which are sown in spring is observed and classified into 3: low, 5: intermediate, 7: high.

Plant vigor in autumn

Plant vigor is observed before winter and classified into 3: low, 5: intermediate, 7: high.

3. Tertiary characters

<Optional items>

Dry matter yield

Dry matter yield is calculated as follows: fresh weight x dry matter percentage/100.

Dry matter percentage

Dry matter percentage is determined for 300 g fresh sample dried at 70 °C for 48 hours.

Green yield

Green yield is determined based on the fresh weight of the plant harvested from an area of more than 2 m^2.

Dry matter digestibility

Percentage of digestible dry matter is determined by *in vivo* method or *in vitro* enzyme method.

Crude protein content

Crude protein content is determined on a dry matter basis by the Kjeldahl method or using a Near Infra-red Analyzer.

Acid detergent fiber (ADF) content

ADF content is calculated on a dry matter basis by the neutral detergent-acetone washing method.

Neutral detergent fiber (NDF) content

NDF content is calculated on a dry matter basis by the acid detergent-acetone washing method.

Mono- and oligosaccharide content

Mono- and oligosaccharide content is determined on a dry matter basis by thin layer chromatography after ethanol extraction.

Seed productivity

Seed productivity is determined based on the yield of fertile seeds per m^2.

Seed weight per flower head

Seed weight per flower head is determined as weight of pure seeds per head for 20 mature heads.

Seed fertility

Percentage of seed fertility is determined for 20 mature flower heads.

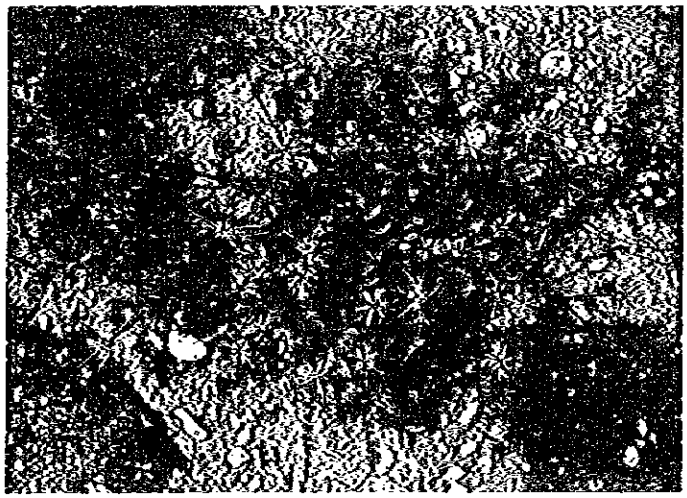

Photo. 1 Chinese milk vetch genetic resources in evaluation field.

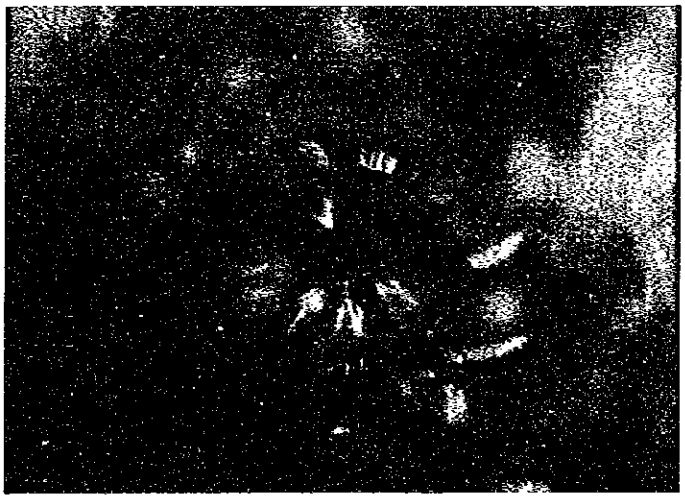

Photo. 2 Flowers of Chinese milk vetch.

VII. Alfalfa

VII-1. Cultivation of Alfalfa Genetic Resources for Evaluation of Characteristics

VII-2. Evaluation of Characteristics of Alfalfa Genetic Resources

by

Mitsuru Gau

Hokkaido National Agricultural Experiment Station, MAFF, Sapporo 062 Japan

VII-1. Cultivation of Alfalfa Genetic Resources for Evaluation of Characteristics

Alfalfa (*Medicago sativa* L.), a perennial forage legume, requires a fertile soil and adequate supply of water. Removal of weeds is necessary because of its low competitive ability for moisture and light at the stand establishment stage. Difference in characteristics among genetic resources is maximized with good management practices.

1. Land preparation

Alfalfa is so sensitive to soil acidity that pH values of 6.5 – 7.0 are necessary for adequate growth. If the pH is lower than 5.5, it is necessary to use lime. Seedbed should be moist and compact, especially when seeds are sown in summer. Plowing is necessary to bury existing vegetation and plant residues that interfere with sowing, and for amendment and fertilization. Since shallow seed placement is necessary to secure a good emergence, compaction of seedbeds before sowing or planting usually results in better stands particularly when the soil moisture is low.

2. Sowing or planting

The time of sowing depends upon the precipitation pattern and temperature. Alfalfa is sown mostly in early spring or in late summer and fall. In case of spring sowing, weed control is usually necessary. In case of late summer or fall sowing, weed competition usually can be avoided, but adequate growth is necessary prior to the onset of winter.

Alfalfa should be sown at a depth of 1.3 cm to a compact seedbed. The sowing depth decreases in the case of fine soil and increases in the case of coarse soil. The use of a roller after sowing is recommended for dry soil.

3. Seed preparation

Inoculation of alfalfa seeds with *Rhizobium meliloti* is necessary even for areas where alfalfa had been planted previously. In the case of a high percentage of hard seed, scarification of seedcoat by mechanical abrasion is necessary. Sandpaper is generally used for seedcoat scarification.

4. Planting density

A mature alfalfa plant usually reaches a height of 60 – 100 cm. Therefore, rows are spaced usually 50 – 100 cm with intrarow spacing of 50 – 100 cm for the evaluation of individual plant. For the evaluation of the prostrate type, wider spacing is necessary. For the evaluation of characters related to yield, 1 g/m^2 of seeds is necessary. In the case of space planting, 4 – 5 seeds are sown to one hill to get good emergence, and thinned to single plant after establishment.

5. Experimental design for evaluation of characteristics

Usually two border rows and two outer plants in a row are excluded from the evaluation of characteristics. Number of plants and areas for measurement depends upon the characteristics. Evaluation test should be laid out based on randomized blocks with

replications.

6. Application of fertilizer

Fertilizer should be applied based on data of local soil test. In addition to Ca application as lime, important nutrients for alfalfa are P and K. N is applied at seeding time or not applied because high N status reduces or prevents nodulation and N_2 fixation. Standard fertilization in Japan is N: 0.50, P_2O_5: 1.90, K_2O: 0.75 kg/a in the first year, N: 0.44, P_2O_5: 1.30, K_2O: 1.34 kg/a in the second year and subsequently.

7. Field management

Water requirement depends on the climatic conditions, cultivar and soil fertility. Irrigation is necessary to achieve adequate growth, and it is essential for semi-arid and arid regions. Weeds should be removed chemically or mechanically. Suitable herbicides should be applied to alfalfa before sowing. After sowing, intertillage by machine or hand weeding should be carried out.

VII-2. Evaluation of Characteristics of Alfalfa Genetic Resources

1. Primary characters

Seven essential and eight optional items are listed as primary characters for the evaluation of alfalfa genetic resources. The evaluation of primary characters should be based on 20 individuals except for the flower color which should be based on 50 individuals.

<Essential items>

Plant type

Plant type is classified at the flower budding stage into 1: erect, 9: prostrate.

Plant height

Plant height is measured (cm) from the ground surface to the top of the plant at the blooming stage.

Stem diameter

Stem diameter is measured (mm) in the middle part of the stem.

Length of leaflet

Length of leaflet of the largest leaf around the center of the plant is measured (mm) at the blooming stage.

Width of leaflet

Width of leaflet of the largest leaf around the center of the plant is measured (mm) at the blooming stage.

Flowering time

Flowering time corresponds to the day when 50% of the plants have bloomed.

Flower color

Color of flower petal is observed soon after flowering, and classified into 1: white, 2: white-yellow, 3: yellow, 4: variegated (yellow-green), 5: blue-purple, 6: red-purple, 7: purple, 8: dark purple, 9: other.

<Optional items>

Plant height at establishment

Plant height at the establishment is measured (cm) from the ground surface within two months after sowing only when the seeds are sown in late summer or fall.

Sprouting date

Date of sprouting is recorded after overwintering in the cold region.

Leaf color

Leaf color is observed at the blooming stage, and is classified into 1: pale green, 5: intermediate, 9: dark green.

Pubescence

Density of pubescence of stems is observed and classified into 1: low, 5: intermediate, 9: high.

Pod shape

The number of spirals of pod is counted and the pod shape is classified according to the number of spirals into 1: sickle shape (no spirals), 2: less than one spiral, 5: about three spirals, 9: more than five spirals. Refer to Fig. 1.

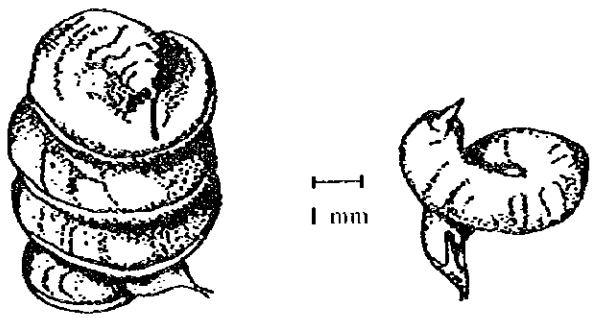

Fig. 1 Shape of alfalfa pods.

Seed number in a pod

Average number of seeds in a pod is counted at the seed maturing stage for ten pods per plant.

Thousand seed weight

Weight of one hundred seeds is measured for 20 plants with four replications to obtain the thousand seed weight.

Variation in flower color

Variation in flower color is classified into 1: when more than 98% of the plants exhibit yellow flowers, 5: when 25 – 75% of the plants exhibit purple flowers, 9: when more than 98% of the plants exhibit purple flowers.

2. Secondary characters

Eight essential and eight optional items are listed as secondary characters for alfalfa. In the evaluation of secondary characters 20 plants are used with two replications.

<Essential items>

Resistance to leptosphaerulina leaf spot (*Leptosphaerulina briosiana*)

Resistance to leptoshaerulina leaf spot is classified based on the number of lesions into 1: extremely low, 5: intermediate, 9: extremely high.

Resistance to spring black stem (*Ascochyta imperfecta*)

Resistance to spring black stem is classified based on the severity of the symptoms into 1: extremely low, 5: intermediate, 9: extremely high.

Resistance to blue alfalfa aphid (*Acyrthosiphon kondoi*)

Resistance to blue alfalfa aphid is visually observed based on the number of aphids and the degree of wilt, and classified into 1: extremely low, 5: intermediate, 9: extremely high.

Regrowth vigor

Regrowth vigor is evaluated based on the herbage mass within three weeks after the first cutting, and is classified into 1: extremely low, 5: intermediate, 9: extremely high.

Vigor in spring

Vigor in spring is evaluated based on the herbage mass two weeks after the budding stage and is classified into 1: extremely low, 9: extremely high.

Vigor in summer

Vigor in summer is evaluated based on the herbage mass in mid-summer (usually August), and is classified into 1: very low, 9: excellent.

Vigor in fall

Vigor in fall is evaluated based on the herbage mass after the cutting in fall (usually October or later), and classified into 1: extremely low, 9: extremely high.

Lodging resistance

Resistance to lodging is evaluated based on the degree of lodging (especially when lodging occurred), and is classified into 1: extremely low, 9: extremely high.

<Optional items>

Resistance to virus diseases

Resistance to virus diseases is evaluated based on the appearance of yellow or discolored mottles, or mosaic lesions on plants undergoing regrowth after the first cutting, and the accession is classified into 1: extremely low, 9: extremely high.

Resistance to southern anthracnose (*Colletotrichum trifolii*)

Resistance to southern anthracnose is evaluated based on the general symptoms of mottles on leaves and stems, and the frequency of dead plants, and the accession is classified into 1: extremely low, 9: extremely high.

Resistance to southern blight (*Corticium rolfsii*)

Resistance to southern blight is evaluated based on the general symptoms and the frequency of dead plants, and the accession is classified into 1: extremely low, 9: extremely high.

Resistance to sclerotinia crown and stem rot (*Sclerotinia trifoliorum*)

Resistance to sclerotinia crown and stem rot is evaluated based on the general symptoms on stems and the frequency of dead plants, and the accession is classified into 1: extremely low, 9: extremely high.

Resistance to root-knot nematode

Resistance to root-knot nematode is evaluated based on the growth inhibition in general and the frequency of club root, and the accession is classified into 1: extremely low, 9: extremely high.

Resistance to bug

Resistance to bug is evaluated based on the extent of injury on buds and fruits (especially in the seed production plot), and the accession is classified into 1: extremely low, 9: extremely high.

Wet soil tolerance

Tolerance to wet soil is evaluated based on the growth inhibition and discoloration of leaves in wet fields during or after the rainy season, and the accession is classified into 1: extremely low, extremely high.

Acid tolerance

Tolerance to acid soil is evaluated based on the growth vigor on soil with a pH below 5.0 and the accession is classified into 1: extremely low, 9: extremely high.

3. Tertiary characters

Nine essential and eleven optional items are listed as tertiary characters. The evaluation of essential items is carried out with two replications in plots with an area of more than 2 m^2. The evaluation of optional items is carried out in two plots with three replications.

<Essential items>

Green yield of spring cutting

Green yield in spring cutting is determined based on the fresh weight (kg/a) of the first cutting in spring.

Dry matter percentage of spring cutting

Percentage of dry matter of spring cutting is determined by drying 300 g of fresh herbage at 70 °C for 48 hours.

Dry matter yield of spring cutting

Dry matter yield (kg/a) in spring cutting is calculated based on the fresh weight of herbage × dry matter percentage.

Green yield of summer cutting

Green yield in summer cutting is determined based on the fresh weight (kg/a) of summer cutting (usually June - August).

Dry matter percentage of summer cutting

Dry matter percentage of summer cutting is determined by drying 300 g of fresh herbage at 70 °C for 48 hours.

Dry matter yield of summer cutting

Dry matter yield (kg/a) in summer cutting is calculated based on the fresh weight of herbage × dry matter percentage.

Green yield of fall cutting

Green yield in fall cutting is determined based on the fresh weight (kg/a) of herbage cut after September.

Dry matter percentage of fall cutting

Dry matter percentage of fall cutting is determined by drying 300 g of fresh herbage at 70 °C for 48 hours.

Dry matter yield of fall

Dry matter yield (kg/a) in fall cutting is calculated based on the fresh weight of herbage × dry matter percentage.

<Optional items>

Leaf ratio

Leaf ratio is expressed as the percentage of dry weight of leaf to dry weight of total matter weight determined in a 20 g sample of fresh herbage at each cutting.

Dry matter digestibility

Dry matter digestibility is expressed as the percentage of digestible dry matter determined by digestion trial or enzyme method.

Crude protein percentage

Crude protein percentage (dry matter weight basis) is determined by the Kjeldahl method or using a Near Infra-red Analyzer.

Acid detergent fiber (ADF)

Content of acid detergent fiber is expressed as the percentage of ADF (dry matter weight basis) determined by the acid detergent-acetone washing method.

Neutral detergent fiber (NDF)

Content of neutral detergent fiber is expressed as the percentage of NDF (dry matter weight basis) determined by the neutral detergent-acetone washing method.

Acid detergent lignin (ADL)

Content of acid detergent lignin is expressed as the percentage of ADL (dry matter weight basis) determined by the acid detergent-acetone washing method.

Mono- and oligosaccharides

Content of mono- and oligosaccharides is expressed as the percentage of mono- and oligosaccharides (dry matter weight basis) determined by the phenol-sulfuric acid method after alcohol-extraction.

Saponin

Saponin content is expressed as the percentage of saponin (dry matter weight basis) determined by the phenol-sulfuric acid method after ethanol-extraction.

Persistency

Persistency is classified based on the degree of decrease of annual yield after sowing or

percentage of remaining plants at the last cutting each year into 1: extremely low, 9: extremely high.

Number of racemes

Number of racemes is counted at the blooming stage or at the first cutting and classified into 1: extremely few racemes, 9: extremely large number of racemes.

Seed weight in a raceme

Seed weight in a raceme is expressed as the average weight of seeds (mg) per raceme for 20 racemes.

References

Barnes, D. K., E. T. Bingum, R. P. Murphy, O. J. Hunt, D. F. Beard, W. H. Skrdla and L. R. Teuber (1977) Alfalfa germplasm in the United States: Genetic vulnerability, use, improvement and maintenance. USDA Tech. Bull. 1571. U. S. Government Printing Office, Washington, D. C.

Hanson, A. A., D. K. Barnes and R. R. Hill, Jr. (1988) Alfalfa and alfalfa improvement. Number 29 in the series AGRONOMY. Am. Soc. Agron., Madison, Wisconsin, USA.

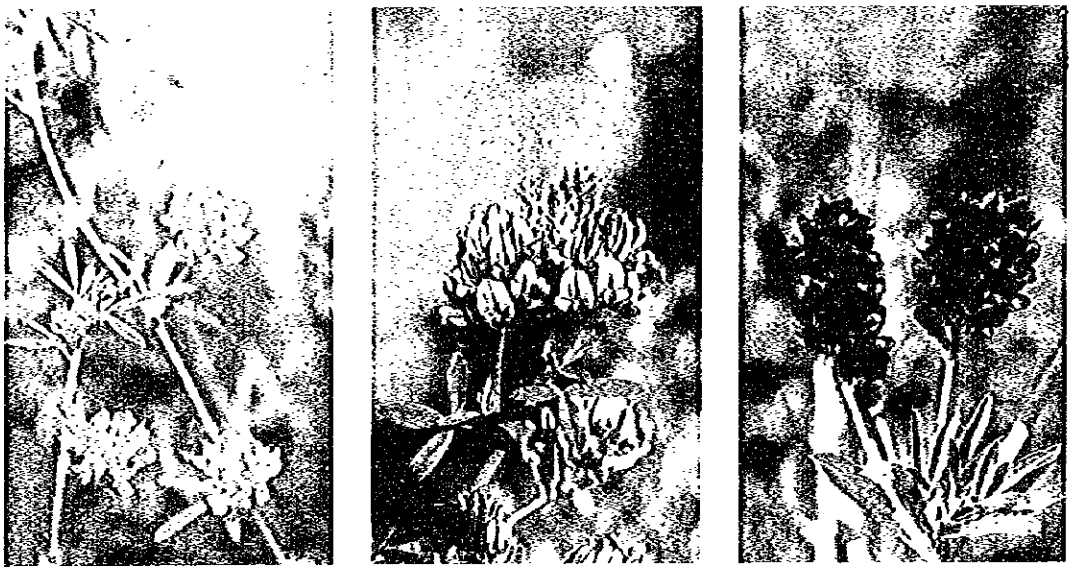

Photo. 1 Variation in alfalfa flower color.
Left: yellow, center: variegated, right: purple

Photo. 2 Field for evaluation of alfalfa genetic resources.

VIII. White Clover

VIII-1. Cultivation of White Clover Genetic Resources for Evaluation of Characteristics

VIII-2. Evaluation of Characteristics of White Clover Genetic Resources

by

Toshihiko Yamada

Yamanashi Prefectural Dairy Experiment Station, Nagasaka, Yamanashi 408, Japan

VIII-1. Cultivation of White Clover Genetic Resources for Evaluation of Characteristics

1. Design of experiment

Randomized complete block design with more than two replications is recommended. For the evaluation of the primary and secondary characters, space planting trial which is carried out when the seedlings are transplanted is often used. For tertiary characters such as forage yield, white clover is seeded directly in the plots with or without grass cultivars.

2. Space planting trial

1) White clover seeds

White clover seeds are very small, approximately 1,374,000 - 1,764,000 / kg. Seeds are round to ovate, about 1.1 - 1.2 mm long, and 0.9 - 1.0 mm wide. The seeds are smooth and dull and their color ranges from yellow to red-brown, depending on the age and environment. Most of the white clover seeds are hard or impermeable to moisture when ripened under dry conditions. Seed will generally germinate under relatively cool conditions but will remain dormant at high temperatures. Scarification or alternating temperature stimulates the germination of dormant hard seeds. It is recommended that seeds be inoculated with appropriate *Rhizobium* culture immediately prior to sowing.

2) Preparation of seedlings

Autumn or winter is the optimum sowing time. Seedling cases (15 × 5 cm) with drainage holes are used for growing the seedlings. White clover is adapted to well-drained clayey and loamy soil. Although white clover grows even in slightly acid soil, soil with pH 6.0 - 6.5 is suitable. For the alleviation of soil acidity, lime is applied. Approximately 50 seeds are sown per seedling case. Application of vermiculite on the soil surface is recommended to preserve the moisture since young seedlings are sensitive to desiccation.

White clover is grown in a green house at a minimum temperature of 5 °C. The first leaf developed from the terminal bud is simple, but thereafter leaves become normally trifoliate, when the number of seedlings is reduced by thinning to 20 per seedling case.

3) Land preparation

Continuous cultivation of white clover in a field should be avoided because of contamination of volunteer plants and lack of soil uniformity due to the symbiotic N fixation of the preceding white clover plants.

Soil pH needs to be adjusted between 6.0 - 6.5 by the application of lime. Basal fertilizer is applied by broadcasting. Recommended rate of fertilizer depends on the soil fertility. Performance of white clover often depends on ample supply of

phosphorus and potassium. Except for basal application, white clover does not require N fertilizer since symbiotic N fixation provides plants with all necessary nitrogen.

Recommended plowing depth ranges from 20 to 30 cm. Rotary harrowing is applied repeatedly to obtain fine soil particles and to flatten the soil surface. Experimental field should be smooth and free of weeds.

4) Transplanting

Suitable time for transplanting varies with the location. The optimum time in Japan is from around mid-April to mid-May when mean temperatures become higher than 10 °C. The 4 - 5 leaf stage is optimum for transplanting. One plot consists of single rows of 10 plants with 100 cm intrarow spacing, arranged in a randomized complete design with more than two replications. Interrow spacing of 1.8 - 2.0 m is suitable because of the ease of weed control by rotary harrowing at the early growth stage.

5) Weed control

At the early growth stage, intertillage is applied using rotary harrowing. As white clover spreads horizontally with stolons, weed control becomes difficult. As a result, preemergence herbicides should be applied after the last rotary plowing. Thereafter, hand weeding is applied.

6) Defoliation practices

Regrowth of white clover is rapid after defoliation. Considering environmental factors, plants are cut with a harvester or grazed by animals several times a year.

2. Broadcasting trial

1) Land preparation

Land preparation for broadcasting trial is the same as that for the space planting trial. Seedbeds should be compact, smooth and free of weeds.

2) Sowing

Late-summer to early-autumn is the optimum sowing time since rapid root development occurs during the cool season and weed control in this season is easier than in the case of spring sowing. However, in cold climatic areas such as Hokkaido, spring sowing is more effective. Suitable seeding rate is 2 - 5 kg/ ha. Seeds should be inoculated with the appropriate *Rhizobium* culture immediately prior to sowing.

Plot size of more than 6 m^2 is recommended. Plots are arranged in randomized complete blocks with more than two replications. Since the seed is small, white clover should be seeded in the soil surface or at a depth of approximately 5 - 10 mm. Seeds are sometimes pressed into the soil with a roller.

3) Cutting or grazing

The clover forage is cut with a plot harvester or grazed by animals several times in a year. Harvested clover is dried and weighed. In the case of sowing with grasses, measurement on samples are performed after separation into clover and grasses.

VIII-2. Evaluation of Characteristics of White Clover Genetic Resources

1. Primary characters

This category includes mainly basic morphological characters. At least 20 plants per entry are necessary for evaluation with two replications.

<Essential items>

Stolon thickness diameter [mm]

Stolon thickness diameter is measured with a slide caliper in the middle of the 3rd or 4th internode counted from the distal end of a primary stolon.

Internode length [mm]

Internode length is measured for the 3rd or 4th internode counted from the distal end of a primary stolon.

Petiole length [cm]

Petiole length is measured for the 3rd or 4th leaf counted from the distal end of a primary stolon.

Leaflet length [mm]

Leaflet length is measured for the long diameter of the central leaflet of the 3rd or 4th leaf counted from the distal end of a primary stolon.

Leaflet width [mm]

Leaflet width is measured for the short diameter of the central leaflet of the 3rd or 4th leaf counted from the distal end of a primary stolon.

Number of stolons

Number of stolons arising from primary stems is counted at the first flower initiation stage, and classified into 1: few stolons, 5: intermediate, 9: many stolons.

Definition of leaf mark

Degree of definition of leaf mark is observed for the fully expanded 3rd or 4th leaf counted from the distal end of a primary stolon, and classified into 0: no mark, 1: indistinct, 5: intermediate, 9: distinct.

Date of first inflorescence

The average date when 5 flowering heads per plant developed is recorded as date of the first inflorescence.

Petal color of florets

Petal color of florets is observed at the time of flower initiation, and classified into 1: white, 5: whitish-pink, 9: red. Observation in summer should be avoided since red marking tends to fade or disappear at high temperatures.

Number of heads

The number of heads is counted one month after the first inflorescence, and recorded as number of heads per m^2.

Peduncle length [cm]

Length of peduncle is measured.

<Optional items>

Plant habit

Plant habit is evaluated based on the angle that stems form with the horizontal line, and classified into 1: erect 5. intermediate, 9: prostrate. Refer to Fig. 1.

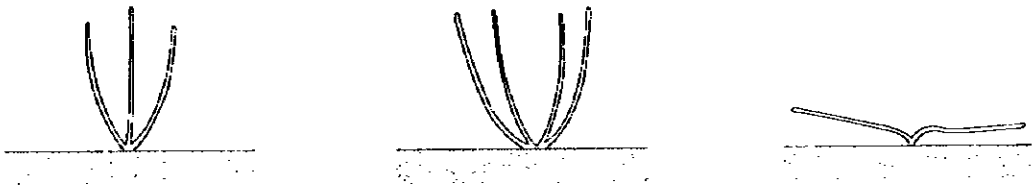

Fig. 1 Plant habit.

Leaflet shape

Shape of leaflet is evaluated for the central leaflet, and classified into 1: round, 2: oval, 3: obovate, 4: obcordate. Refer to Fig. 2.

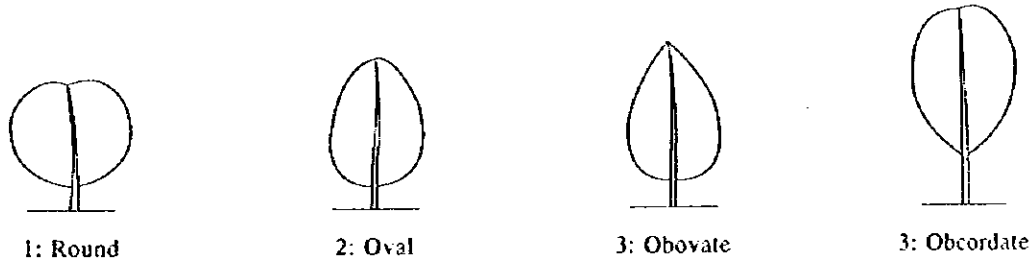

Fig. 2 Leaflet shape.

Stolon length [cm]

Stolon length is defined as the distance from the primary stem to the distal end of primary stolons.

Plant height [cm]

Plant height is defined as the distance from the soil surface to the top of the leaf canopy measured at the early flowering stage.

Number of nodes

Number of nodes is counted over a 10 cm section from the 1st developed node at the distal end of the primary stolon.

Number of rooted nodes

Number of rooted nodes is counted over a 10 cm section from the 1st developed node at the distal end of the primary stolon.

Number of leaves

Number of leaves is observed at flowering time, and classified into 1: few leaves, 5: intermediate, 9: many leaves.

Frequency of abnormal leaflets

Frequency of abnormal leaflets (other than trifoliate) is observed, and classified into 0: no abnormal leaflets, 1: low , 5: intermediate, 9: high.

Marking type

Type of markings on leaf is observed at the flowering time, and classified into 1: V-shaped, 2: both (V-shaped and another type), 3: other (type is specified in note).

Anthocyanin marks (Red flecking)

Frequency of red markings on leaf is observed at the flowering time, and classified into 0: no marking, 1: low frequency, 5: intermediate frequency, 9: high frequency. Since red markings tend to fade or disappear at high temperatures, evaluation of this character should be avoided in summer.

Leaf color

Leaf color is evaluated based on the greenness of leaf at the flowering time, and classified into 1: light green, 5: intermediate, 9: dark green.

Petiole thickness [mm]

Petiole thickness is measured at the midpoint of petiole produced from the 3rd or 4th section counted from the distal end of a primary stolon.

Average number of florets per head

Average number of florets is counted for 10 heads per plant.

Peduncle thickness [mm]

Peduncle thickness is measured at the midpoint of the peduncle.

Sprouting date

The date when white clover begins to sprout after overwintering is recorded as sprouting date.

1000-seed weight [g]

Weight of one hundred seeds is measured for a mixture of seeds from 20 plants with 4 replications.

Seedling vigor

Vigor of seedlings is observed one month after sowing, and classified into 1: low, 5: intermediate, 9: high.

2. Secondary characters

This category includes of traits mainly related to adaptability such as tolerance to environmental stress, pests and diseases.

<Essential items>

Resistance to virus diseases

Resistance to virus diseases is evaluated based on the presence of symptoms in the nursery or field test, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Sclerotinia trifolii* (Crown rot)

Resistance to *Sclerotinia trifolii* is evaluated based on the presence of symptoms in the nursery or field test, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Leptoshaerulina trifolii* (Pepper spot)

Resistance to *Leptoshaerulina trifolii* is evaluated based on the presence of symptoms in the nursery or field test and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Plant spread [cm^2]

Plant spread is calculated based on the product between the longest diameter and the

shortest diameter of spread of stolons of one plant.

Stolon density

Stolon density is calculated based on the number and length of stolons per unit area of sward (100 cm^2), and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Degree of stolon branching

Degree of stolon branching is calculated by counting the number of branches in a 15 cm core, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Overwintering ability

Overwintering ability is evaluated based on the number of dead plants and injury of stolons, regrowth in early spring, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Tolerance to summer depression

Tolerance to summer depression is evaluated based on the number of dead plants and injury of stolons, regrowth in early autumn, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

<Optional items>

Resistance to *Curvularia trifolii* (Curvularia leaf blight)

Resistance to *Curvularia trifolii* is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Rhizoctonia solani* (Summer blight)

Resistance to *Rhizoctonia solani* is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Corticium rolfsii* (Southern blight)

Resistance to *Corticium rolfsii* is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Uromyces trifolii* (Leaf rust)

Resistance to *Uromyces trifolii* is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Uromyces nerviphilus* (Stem rust)

Resistance to *Uromyces nerviphilus* is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Acrythociphon* spp. (Aphid)

Resistance to *Acrythociphon* spp. is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Agrotis* spp. (Cutworm)

Resistance to *Agrotis* spp. is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Plathypena scabra* (Green cloverworm)

Resistance to *Plathypena scabra* is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Spodoptera litura* (Armyworm)

Resistance to *Spodoptera litura* is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Coleophora frischella* (Clover seed moth)

Resistance to *Coleophora frischella* is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Sitona hispidulus* (Clover rot curculio)

Resistance to *Sitona hispidulus* is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Resistance to *Meloidogyne* spp. (Root knot nematode)

Resistance to *Meloidogyne* spp. is evaluated based on the natural infection, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Plant vigor in autumn

Plant vigor in autumn is evaluated based on the degree of regrowth in late autumn, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Tolerance to shading

Tolerance to shading is evaluated based on the growth under shading (light intensity is reduced by more than 50% using mesh cloths, etc.), and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

3. Tertiary characters

This category includes of traits related to productivity.

<Essential items>

Green yield of spring cutting [kg/a]

Green yield in spring cutting is determined based on the fresh weight of herbage harvested from 1 m^2 area per plot in spring.

Dry matter percentage of spring cutting [%]

Dry matter percentage in spring cutting is calculated based on the dry matter weight of about 300 g of fresh sample dried at 70 °C for 48 hours.

Dry matter yield of spring cutting [kg/a]

Dry matter yield in spring cutting is calculated based on the fresh weight × dry matter percentage.

Green yield of summer cutting [kg/a]

Green yield in summer cutting is determined based on the fresh weight of herbage harvested from 1 m^2 area per plot.

Dry matter percentage of summer cutting [%]

Dry matter percentage in summer cutting is calculated based on the dry matter weight of about 300 g of fresh sample dried at 70 °C for 48 hours.

Dry matter yield of summer cutting [kg/a]

Dry matter yield in summer cutting is calculated based on the fresh weight × dry matter percentage.

Green yield of autumn cutting [kg/a]

Green yield in autumn cutting is determined based on the fresh weight of herbage harvested from 1 m^2 area per plot.

Dry matter percentage of autumn cutting [%]

Dry matter percentage in autumn cutting is calculated based on the dry matter weight of about 300 g of fresh sample dried at 70 °C for 48 hours.

Dry matter yield of autumn cutting [kg/a]

Dry matter yield in autumn cutting is calculated based on the fresh weight × dry matter percentage.

<Optional items>

Dry matter digestibility [%]

Dry matter digestibility is evaluated based on the percentage of digestible dry matter determined by *in vivo* method or *in vitro* enzyme method.

Crude protein content [%]

Crude protein content (on dry matter basis) is determined by the Kjeldahl method or using a Near Infra-red Analyzer.

Acid detergent fiber (ADF) [%]

Acid detergent fiber content (on dry matter basis) is determined by the acid detergent-acetone washing method.

Cyanogenesis rating [%]

Cyanogenesis rating is evaluated based on the degree of color change of picrate paper, and scored into 0: no change = yellow to 5: dark orange along whole length of the paper. Cyanogenesis rating is recorded as the percentage of plants scoring 1 or more within an entry.

Saponin content [ppm]

Saponin content is determined by extraction with ethanol and separation and measurement by thin layer chromatography.

Adaptability to grazing

Adaptability to grazing is evaluated based on coverage, intake, clover ratio, etc. after grazing experiment, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Adaptability to mixed sowing

Adaptability to mixed sowing is evaluated based on the ratio of white clover in mixed sowing with grasses, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Persistency

Persistency is evaluated by determining the ratio of white clover plants 3 to 4 years after sowing, and classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high.

Head weight [g]

Head weight is determined based on the average weight of 20 ripe heads randomly sampled not later than one month after flowering.

Average number of seeds per pod

Average number of seeds per pod is determined by counting the number of pods and seeds in 20 ripe heads.

Seed yield per plant [g]

Seed yield per plant is obtained by determining the average seed yield of 10 randomly selected plants.

References

IBPGR. 1992. Descriptors for white clover (*Trifolium repens* L.) International Board for Plant Genetic Resources, p. 52, Rome, Italy.

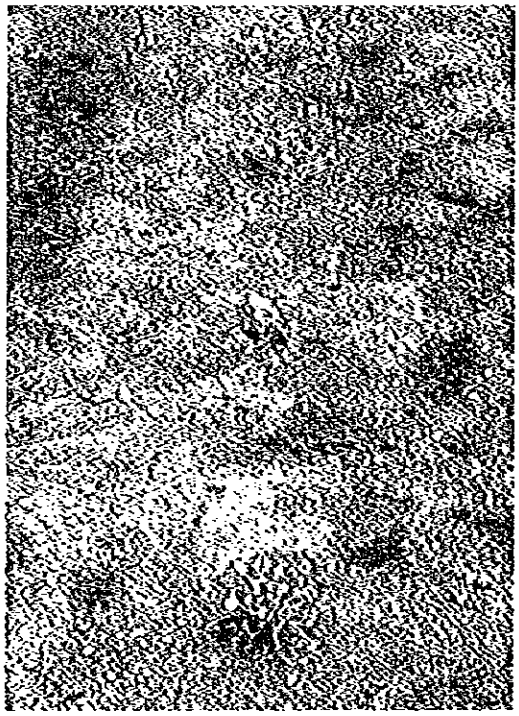

Photo. 1 White clover plants for evaluation one - two months after transplanting to field in space planting trials.

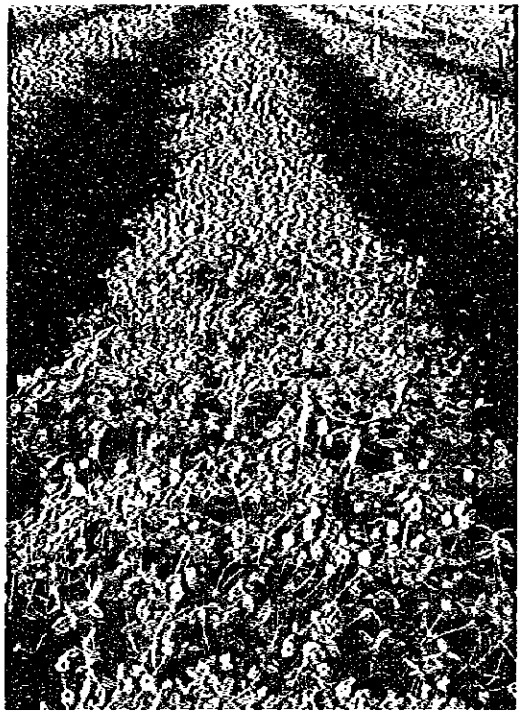

Photo. 2 White clover plants one year after transplanting.

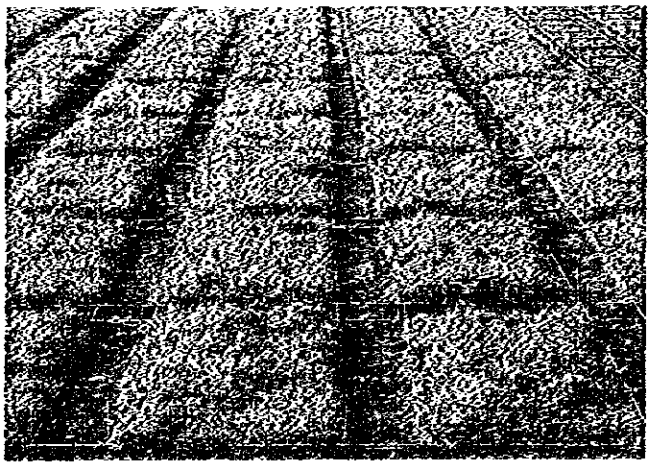

Photo. 3 Broadcasting trials in a mixture with grasses.

IX. Tropical Legumes

IX-1. Cultivation of Tropical Legume Genetic Resources for Evaluation of Characteristics

IX-2. Evaluation of Characteristics of Tropical Legume Genetic Resources

by

Hitoshi Nakagawa

Hiroshima Prefectural Agriculture Research Center, Higashihiroshima, Hiroshima 739-01, Japan

IX-1. Cultivation of Tropical Legume Genetic Resources for Evaluation of Characteristics

Tropical legumes

Legumes are important plants to humans as they provide food, fiber, timber and medicinal products. They are sown in grasslands and these legume-based grasslands play a major role in the meat and milk industries especially in tropical and subtropical regions. The legumes are rich in protein and increase substantially the nutritive value of fodder. They fix atmospheric nitrogen in symbiosis with *Rhizobium* bacteria occurring in the legume root nodules and enrich the soil with nitrogen, hence enabling to save nitrogen fertilizer.

Tropical legumes consist of 11 tropical tribes including about 6,200 species in 280 genera. Among them, Cassiae (shrub, herb), Mimosaceae (small tree, shrub, herb), Indigoferae (shrub, herb), Aeschynomeneae (shrub, herb), Desmodieae (shrub, herb), Phaseoleae (shrub, climber), Crotalariae (shrub, herb) are important forage species. The most important legume species in subtropical Japan are classified into three groups by the plant type as indicated below. The cultivation methods and the evaluation of characteristics are inclusively mentioned and some species are specifically described if necessary.

Group 1: Tree or small tree; Leucaena (*Leucaena leucocephala* (Lam.) de Wit).

Group 2: Shrub and herb; Joint vetch (*Aeschynomene falcata* (Poir.) D.C. Podr.), Alyce clover (*Alysicarpus vaginalis* (L.) DC), *Arachis glabrata* Benth., Stylo (*Stylosanthes guianensis* (Aubl.) Sw.), Townsville stylo (*S. humilis* H.B. and K.), Carribean stylo (*S. hamata* (L.) Taub.), *Cajanus cajan* (L.) Millsp.

Group 3: Creeping herb or climber; Calopo (*Calopogonium mucunoides* Desv.), Centro (*Centrosema pubescens* Benth.), Butterfly pea (*Clitoria ternatea* L.), Greenleaf desmodium (*Desmodium intortum* (Mill.) Urb.), Silverleaf desmodium (*D. uncinatum* (Jacq.) DC), Glycine (*Glycine wightii* (R. Grah. ex Wight & Arn.) Verdc., Lablab bean (*Lablab purpureus* (L.) Sweet), Siratro (*Macroptilium atropurpureum* (DC) Urb.), Phasey bean (*M. lathyroides* (L.) Urb.), Axillaris (*Macrotyloma axillare* (e. Mey.) Verdc.), Biflorus (*Macrot. uniflorum* (Lam.) Verdc.), Velvet bean (*Mucuna pruriens* (L.) DC var. *utilis* (Wight) Burck.), Puero (*Pueraria phaseoloides* (Roxb.) Benth.), Kudzu (*P. thunbergiana* (Sieb. and Zucc.) Benth.), Mung bean (*Vigna radiata* (L.) Wilczek), Cowpea (*V. unguiculata* (L.) Walp.)

For the cultivation and evaluation of tropical legumes, inoculation of certain strains of *Rhizobium* may be necessary in some soils of the tropical and subtropical regions. Many researchers reported that *Cajanus cajan*, Siratro, Phasey bean, Stylo and *Vigna* species can develop a symbiosis with almost any strain of Cowpea type found in the soil. The moderately specific species for which inoculation with certain strains of Cowpea *Rhizobium* is required are *Clitoria ternatea*, species of *Desmodium*, Lablab bean, Glycine and Puero. The highly specific species for which the right strain is necessary are Centro and Leucaena.

1. Land preparation

Seedbed is prepared in the usual way. The smaller the seeds, the finer the soil of the seed bed should be.

2. Seed treatment to break dormancy

Most legume seeds contain a high percentage of hard seeds (usually between 60 and 90%) which will not germinate even under favorable conditions. Some treatments can be applied to break the dormancy. Effectiveness of a particular treatment depends on the species.

a) Scarification

For small seed lots, scarification can be achieved by rubbing the seed with sand paper.

Siratro: mechanical scarification

b) Cutting or piercing of seed coat

Cutting or piercing the seed coat is also effective to break the dormancy.

(a) Pierce the seed coat: Alyce clover

(b) Cut the seed coat: Centro, Axillaris, Leucaena, Velvet bean, Cowpea

(c) Cut the seed pod: Townsville stylo

c) Chemical treatment

Chemical treatment with alcohol or acetone dissolves some substances that inhibit germination. Treatment with concentrated sulfuric acid is more common.

(a) Acid treatment for 20 minutes: Calopo, Phasey bean, Puero, Kudzu

(b) Acid treatment for 25 minutes: Glycine, Siratro

(c) Warm glycerin at 50 °C for 1 hour: Puero, Centro

d) Hot water treatment

Soaking seeds in hot (80 ~ 100 °C) water breaks the dormancy of some seeds.

(a) 80 °C for 4 minutes or 100 °C for half a minute: Leucaena

3. Inoculation

Most efficient inoculants will be obtained by using the seeds of tropical legumes preserved in the Seedbank of CSIRO and other research stations. The inoculants are usually sent in the form of peat culture and are mixed with seeds before sowing.

4. Planting time

As tropical legumes grow well under warm weather, seeds are sown in spring both in the temperate and in subtropical areas.

5. Planting of seeds

Seeds are sown in the seedbed or 5 cm pots and single seedlings are planted in the field in rows so that interrow cultivation can be applied to the plants. The spacing between plants depends on the plant type and size of plant. In general, one plot measures 2 m × 5 m, and width of interrow is 1.5 m. Each plot consists of 10 to 25 plants.

6. Field management

Application of fertilizer (N : P_2O_5 : K_2O = 5:13:5) at the rate of 370 kg/ha at planting is usual. It is most important that fertilizer should be spread evenly so that every plant grows to the same even height and flowers at the same time. Plots should be freed of weeds. However, use of herbicides sometimes exerts harmful effects on tropical legumes. Pest control is necessary.

Group 1: Leucaena grows up to a height of 20 m high. For forage use, trees will be cut at about 2 m height from the ground once or twice a year and evaluated.

Group 2: Stylo should not be cut below 10 cm from the ground.

Group 3: Vines will be cut when they elongate outside of the plot.

IX-2. Evaluation of Characteristics of Tropical Legume Genetic Resources

Evaluation tests will be conducted with two replications.

<Essential items>

Plant height

Plant height (cm) is measured from the ground for 20 plants at the flowering stage and the average height is recorded.

Stem diameter

The maximum stem diameter (0.1 mm) is measured for 20 plants at the flowering stage and the average values are recorded.

Occurrence of vines

Occurrence of vines is examined for 20 plants and classified into 0: absent, 9: present.

Twining ability

Twining ability is evaluated for 20 plants and classified into 3: no twining, 4: weak, 5: intermediate, 6: strong, 7: very strong.

Paler shiny area at mid-rib

Presence and brightness of paler shiny area at the mid-rib are observed and classified into 3: absent, 4: vague, 5: slightly clear, 6: clear, 7: very clear.

Hairiness

Number of hairs on the surface of stems and leaves are observed and classified into 3: no hairs or very few, 4: few, 5: intermediate, 6: many, 7: very large number.

Leaf shape

Shape of leaves is classified into 3: simple, 4: trifoliolate, 5: imparipinnate (a) or paripinnate (b), 6: pinnate, 7: others. Refer to Fig. 1.

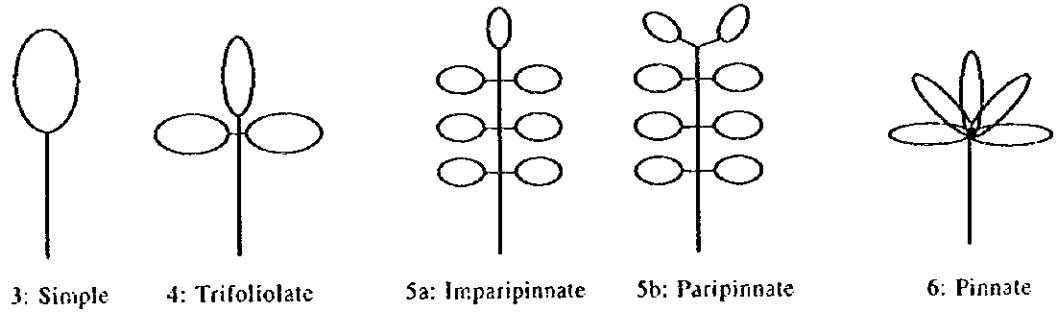

Fig. 1 Classification of leaf shape.

Length of leaflets

Length of leaflets (mm) in simple leaf or other compound leaves is measured for 20 plants and the average values are recorded.

Width of leaflets

Width (mm) of leaflets in simple leaf or other compound leaves is measured for 20 plants and the average values are recorded.

Flowering date

The date when 50% of the plants begin to flower is recorded.

Flower color

Standard petal color of flower is observed for 20 plants and classified into 1: white, 2: purple, 3: blue, 4: green, 5: yellow, 6: orange, 7: pink, 8: red, 9: others.

Plant type

Plant type is observed for 20 plants at the growing stage and classified into 3: erect, 4: comparatively erect, 5: intermediate, 6: comparatively prostrate, 7: prostrate.

Pod length

Length (mm) of 5 pods is measured for 20 plants and the average values are recorded.

Pod width

Width (mm) of 5 pods is measured for 20 plants and the average values are recorded.

Pod weight

Weight of 5 mature pods (mg) per plant is measured for 20 plants and the average values are recorded.

Number of seeds per pod

Number of seeds in 5 mature pods per plant is counted for 20 plants and the average values are recorded.

1,000 seed weight

Weight of 100 seeds (10 mg) is measured with more than 2 replications and the 1,000 seed weight is calculated.

<Optional items>

Regrowth

Regrowth after harvest is evaluated and classified into 3: no or very poor, 4: poor, 5: intermediate, 6: good, 7: very good.

Winter hardiness

Winter hardiness is evaluated for 20 plants based on the regrowth and number of dead plants in early spring and classified into 3: very low, 4: low, 5: intermediate, 6: high, 7: very high.

Resistance to diseases

Resistance to certain diseases (note the name of the disease) is evaluated for 20 plants based on the symptoms and frequency of infected plants within 20 plants, and the accession is classified into 3: susceptible, 4: comparatively susceptible, 5: intermediate, 6: comparatively resistant, 7: resistant.

Resistance to insect pests

Resistance to certain insect pests (note the name of the insect pest) is evaluated for 20 plants based on the symptoms and the damage caused to the plants, and the accession is classified into 3: susceptible, 4: comparatively susceptible, 5: intermediate, 6: comparatively resistant, 7: resistant.

Spring vigor

Vigor of overwintered plants is evaluated for 20 plants based on the amount of foliage in spring and classified into 3: very low, 4: low, 5: intermediate, 6: high, 7: very high.

Autumn vigor

Vigor of regrowth is evaluated for 20 plants based on the amount of foliage in autumn and classified into 3: very low, 4: low, 5: intermediate, 6: high, 7: very high.

Self-fertility rate

Rate of self-fertility is estimated for 20 plants based on the number of seeds per pod covered with a paraffin bag or artificially self-pollinated and classified into 3: no self-fertile plants, 4: low, 5: intermediate, 6: high, 7: very high.

Seed dormancy

Seed dormancy is estimated based on the germination test of 200 mature seeds under specified temperature conditions in a petri dish for a specified number of days and classified into 3: no, 4: light, 5: intermediate, 6: deep, 7: very deep.

Tertiary characters

<Essential items>

Fresh weight at the 1st harvest

Fresh weight including pods (0.1 kg /a) at the 1st harvest is determined by harvesting more than 2 m^2 of plot.

Dry matter percentage at the 1st harvest

Fresh foliage including pods at the 1st harvest (300 g) is dried at 70 °C for 48 hours and the dry matter content is determined. Dry matter percentage (0.01%) is calculated as follows: (dry matter weight / fresh weight) × 100.

Dry matter yield at the 1st harvest

Dry matter yield at the 1st harvest is calculated as follows: fresh yield at the 1st harvest × Dry matter percentage × 0.01 (kg/a).

Fresh weight of regrowth

Fresh weight of regrowth is calculated using the following data. Fresh weight determined by harvesting more than 2 m^2 at the first cutting, the fresh foliage weight with pods at the 2nd cutting, and that at the 3rd cutting, etc.

Dry matter percentage of regrowth

Dry matter percentage of regrowth is determined in the same way as for the 1st harvest.

Dry matter yield of regrowth

Dry matter yield of regrowth is determined in the same way as for the 1st harvest.

<Optional items>

Digestible dry matter

Percentage of digestible dry matter is determined by *in vivo* method or *in vitro* enzyme method.

Crude protein

Crude protein content is determined by the Kjeldahl method or using a Near Infra-red Analyzer and expressed on a dry matter basis.

Acid detergent fiber (ADF)

ADF content is determined by the acid detergent-acetone washing method and expressed on a dry matter basis.

Neutral detergent fiber (NDF)

NDF content is determined by the neutral detergent-acetone washing method and expressed on a dry matter basis.

Acid detergent lignin (ADL)

Percentage of ADL is determined by the acid detergent-acetone washing method and expressed on a dry matter basis.

Mono- and oligosaccharides

Mono- and oligosaccharide content is determined by thin layer chromatography after ethanol extraction and expressed on a dry matter basis.

Animal intake

Intake by animals by grazing or feeding per hour is evaluated and classified into 3: very low, 4: low, 5: intermediate, 6: high, 7: very high.

Palatability

Palatability by grazing or free cafeteria feeding is estimated and classified into 3: very low, 4: low, 5: intermediate, 6: high, 7: very high.

Appendix

Diseases

Cajanus cajan

wilt (*Fusarium udum*), root rot (*Phaeolus manihotis*), canker of stems (*Physalospora cajanae*)

Centrosema pubescens

leaf-spot (*Cercospora* sp.)

Clitoria ternatea

Rhizoctonia microsclerotia, Corticum solani

Desmodium uncinatum

anthracnose (*Colletotrichum dumatium*)

Lablab purpureus

stem rot (*Sclerotina sclerotiorum*)

Macroptilium atropurpureum

Rhizoctonia solani, violet root (*Rhizoctonia crocorum*), powdery mildew, orange-colored rust (*Uromyces phaseoli*)

Stylosanthes guianensis

anthracnose, *Corticum, Rhizoctonia solani, Diplodia*

Insect Pests

Cajanus cajan

root-knot nematodes (*Melidogyne* and other genera)

Centrosema pubescens

mite (*Tetranychum* sp.)

Clitoria ternatea

nematodes

Desmodium intortum

Amnemus weevil (*Amnemus quadrituberculatus*)

Desmodium uncinatum

Amnemus weevil (*Amnemus quadrituberculatus*), leaf-eating caterpillars, grass hoppers, pod-borers, nematodes (*Meloidogyne javanica, Radopholus similis*)

Glycine wightii

Amnemus weevil, *Bruchus* weevil

Lablab purpureus

nematodes (*Helicotylenchus dihystera, Meloidogyne hapla, M. incognita*), leaf eating insects

Leucaena leucocephala

leucaena psyllid (*Heteropsylla cubana*)

Macroptilium atropurpureum

nematodes (*Helicotylenchus dihystera*), bean fly (*Melanagromyza phaseoli*), meloid beetles, bean leaf roller (*Urbanus proteus*)

References

Bogdan, A. V. (1977) Tropical pasture and fodder plants, Longman Inc. New York: 475pp.

Hacker, J. B. (1990) A guide to herbaceous and shrub legumes of Queensland, University of Queensland Press, Box 42, St Lucia, Queensland, Australia: 351pp.

McIvor J. G. and R. A. Bray (ed.) (1983) Genetic resources of forage plants, CSIRO, 314 Albert Street, East Melbourne, Australia: 337pp.

Skerman, P.J. (1977) Tropical legumes, FAO, Rome, Italy: 609pp.

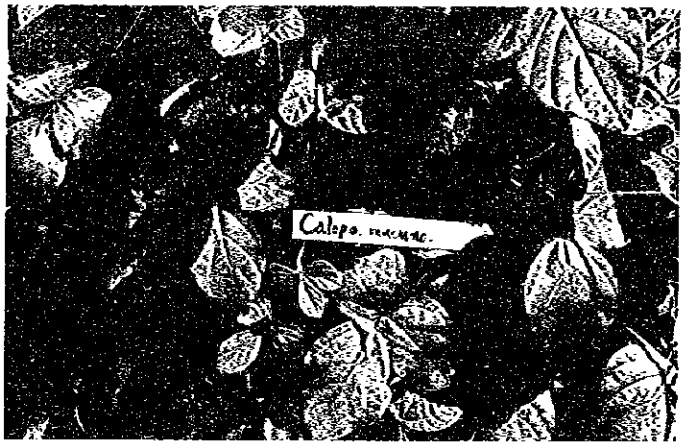

Photo. 1 Calopo (*Calopogonium mucunoides* Desv.).

Photo. 2 Stylo (*Stylosanthes guianensis* (Aubl.) Sw.).

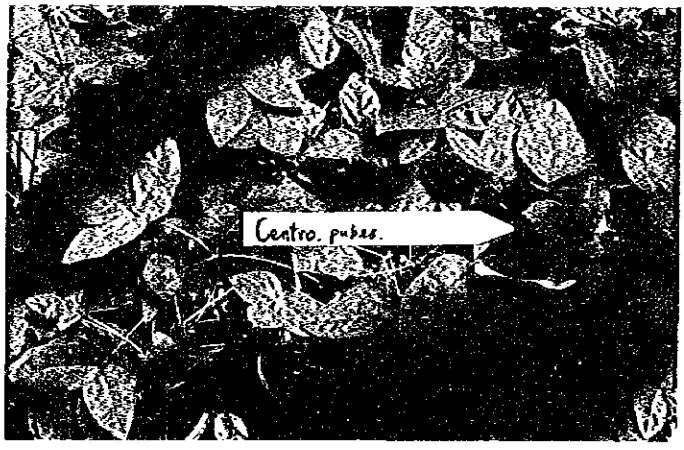

Photo. 3 Centro (*Centrosema pubescens* Benth.).

Photo. 4 Siratro (*Macroptilium atropurpureum* (DC) Urb.).

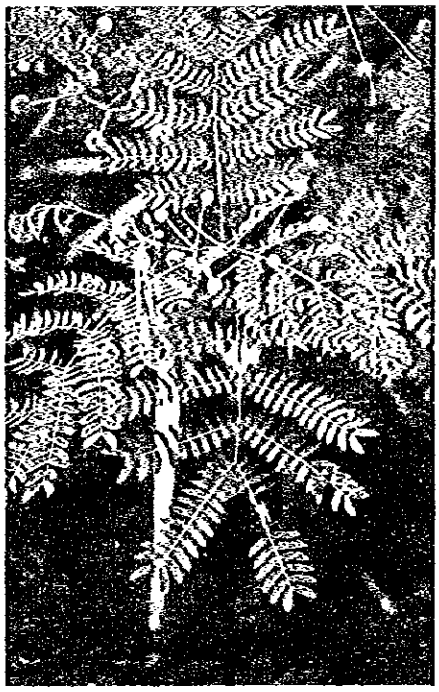

Photo. 5 Leucaena (*Leucaena leucocephala* (Lam.) De Wit) attacked by leucaena psyllids.