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CULTIVATION METHODS FOR THE EVALUATION OF CHARACTERISTICS OF GENETIC RESOURCES AND EVALUATION OF GENETIC RESOURCES (CEREAL, PULSE AND ROOT CROPS)

TECHNICAL ASSISTANCE ACTIVITIES
FOR GENETIC RESOURCES PROJECTS

JAPAN INTERNATIONAL COOPERATION AGENCY

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REF. NO. 1	Preservation of Plant Genetic Resources
REF. NO. 2	Exploration and Collection of Plant Genetic Resources Part I Seed-propagated Crops
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REF. NO. 4	Evaluation and Classification of Plant Genetic Resources
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I. Introduction

Genetic resources are invaluable materials for human beings. Human beings have been enjoying their lives totally depending on genetic resources which have undergone evolution and have attained the present status of diversity.

Systematic utilization of plant genetic resources (PGR) was initiated under the leadership of Dr. I. Vavilov in Russia, who, following the differential phytogeographical method, organized explorations for the collection of PGR throughout the world and suggested the existence of 8 centers of diversity of plants in the world.

PGR projects involve three processes. The first process is the exploration and collection of PGR, the second is the preservation of PGR and the third is the evaluation and multiplication of PGR.

Editorial board of GRP REF sponsored by Japan International Cooperation Agency (JICA), has planned the publication of a series of technical manuals for evaluation of PGR. The ultimate purpose of the Genetic Resources Project is the supply of genetic resources that can be used for the breeding of new cultivars.

Although the increase and preservation of PGR are important, PGR can not be utilized unless adequate information for their evaluation is supplied.

At present, JICA is sponsoring three Genetic Resources Projects; the Project of the Center for Plant Genetic Resources in Sri Lanka which was initiated in 1988; Genetic Resources Conservation Project in Chile which was initiated in 1989; Plant Genetic Resources Preservation and Research Laboratory Project in Pakistan which was initiated in 1993. The projects in Sri Lanka and Chile are now in the following-up phase. JICA has been also promoting several projects related to PGR for example at the Beijing Vegetable Research Center in China.

In addition to the overseas activities, JICA is organizing a Group Training Course on PGR, and is inviting foreign PGR researchers for training in Japan.

To support the above-mentioned activities, the Editorial Board of GRP REF plans to publish manuals for the evaluation of PGR. The editorial board asked plant breeders and retired plant breeders in Japan to provide guidance for the evaluation of genetic resources of food crops.

In REF No. 7, PGR evaluation techniques for rice, wheat, barley, soybean, sweet potato, potato and corn are described.

Description for each crop consists of two parts. In Part I, cultivation methods for analyzing the characteristics of PGR are described and in Part II, evaluation of genetic resources is described.

In Japan, descriptors for evaluating characters are grouped into three classes, primary, secondary and tertiary. Each descriptor is categorized into essential or optional items. Therefore, descriptors appear in a systematic order.

Editorial board is planning to publish similar manuals on vegetables, fruits and other crops in the further series of GRP REF.

Torao Goto
FARDA

II-1. Cultivation Methods for the Evaluation of Characteristics of Genetic Resources

Rice

by

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II-1. Cultivation methods for the evaluation of characteristics of genetic resources

Rice

A large number of entries are grown in a paddy field for the cultivation of rice germplasm. Each entry is planted in one or two rows with one plant per hill. Therefore, the method is different from usual rice cultivation in farmers' fields. Care should be taken in handling many entries to avoid contamination, and also because there is a wide range of morphological and physiological differences among the entries. Some entries might not head until the end of the rice season due to the high photo-sensitivity. In such a case, for getting seeds, it is necessary to plant the entries in a pot and take them into a glasshouse.

1. Experimental design

At first, the number of entries, size of field, time of planting and growing period should be determined. According to the experimental design, the schedule of cultivation is determined, and field notes and field maps are prepared.

2. Preparation of seedlings

Preparation of bed soil

Presently in Japan, rice seedlings are raised mostly in seedling boxes under upland conditions in a greenhouse.

Soil in seedling box

① Kind of soil; Loam or clay loam

② Volume of soil; 5 ℓ/box, 90 ~ 100 ℓ/10 a, 20 boxes/10 a

③ pH of soil; 4.5 ~ 5.0

Disinfection of soil

To protect seedlings from foot rot or seedling blight, bed soil should be disinfected by mixing 3 ~ 6 g of Tachigaren/box or 15 ~ 20 g of Daconil/box.

Fertilizer application to bed soil

Fertilizer should be mixed with bed soil one week before seeding. 1~ 2 g of N, P_2O_5 and K_2O for elements, 8 g of ammonium sulfate, 10 g of superphosphate of lime, 3 g of potassium chloride are mixed into soil of one seedling box.

Pretreatment of seeds

Selection of seeds

Fully ripe seeds can be selected based on a specific gravity of 1.13. However, selection by specific gravity is laborious for handling a small amount of seeds of a large number of entries. Winnowing is an easier way for the selection of fully ripe seeds.

Disinfection of seeds

Seeds are disinfected to prevent the occurrence of seed-borne diseases, such as (a) blast, (b) Bakanae disease, (c) bacterial grain rot, (d) rice leaf tip nematode. The type of disinfectant and disease are as follows:

Common name	Chemicals	Effective against
Thiram benomyl	Benlate-T	a b
Trifumizole	Trifumine	b
Copper hydroxide	Kocide	c
TPN	Daconil	c
Hydroxy-isoxazole	Tachigaren	c
Hydroxy-isoxazole-metalaxyl	Tachigare-ace	c
Thiocyclam	Evisect	d
MPP	Baycid	d
MEP	Sumithion	d

Sowing

The process of sowing of seeds in a box is as follows:

① Seeds are sown on a bed in the seed box uniformly.
Sowing rate; for soaked seeds 0.34 ℓ / box
for dry seeds 150 g / box

② After sowing, seeds are covered with soil uniformly.

③ The boxes are watered fully.

④ Nursing period required to get good seedlings for transplanting by hand is about 30 days.

Nursing management

Temperature

① To accelerate the emergence of seedlings, seedling boxes are put in an incubator and kept at 30°C 2 days after sowing and watering.

② To green the seedlings, after emergence, the boxes are put in a vinylhouse and the temperature is kept at 25°C (daytime) and 15°C (nighttime) for 3 ~ 4 days.

③ For hardening of seedlings, temperature in the house is kept at 20°C (daytime) and 10°C (nighttime) until transplanting.

④ To get healthy seedlings, room temperature should be controlled by opening and closing the windows and doors of the house, in taking care of ventilation and sunshine. A temperature range of 5 ~ 25°C is favorable for nursing.

Watering

Excess watering should be avoided because of spindly growth of seedlings.

3. Preparation of paddy fields

Application of manure

Approximately 70% of the total nitrogen absorbed by rice plants originates from soil, whose fertility increases by continuous application of barnyard manure. Manure not only supplies nitrogen to the soil but also various kinds of minor elements. For maintaining the soil fertility, 1t/10a of barnyard manure should be applied annually.

Plowing

Plowing is usually carried out with a plow drawn by a tractor in autumn or early spring. By plowing at a depth of 15 ~ 20 cm, manure and debris of the preceding crops are incorporated into the soil.

Puddling and levelling

After soil clods are saturated and softened by irrigation water, soil surface of the fields is puddled with a rotary harrow 2 ~ 3 times to crush clods.

Effects of puddling

Advantages of puddling are as follows;

① Levelling of soil surface

② Prevention of water leakage

③ Improved weed control

④ Ease of transplanting

⑤ Mixture of fertilizer with soil

Fertilizer application

The amount of fertilizer varies depending on the soil fertility and lodging response of entries. For cultivation of germplasm, care should be taken to avoid lodging. Usually, 4 ~ 5 kg of nitrogen per 10a is applied as basal dressing, in addition to 10 kg of phosphate and 8 kg/10a of potassium.

4. Transplanting

Layout of test field

To transplant seedlings with a constant spacing, strings or rulers are used for marking at definite intervals between and within rows. A marker or a special kind of frame is used in some parts of the Hokuriku and Tohoku districts instead of strings or rulers. In this case, after preparation of the paddy fields, water is drained and lines are drawn squarely with a hexagonal barrow or frame.

Distribution of seedlings

After each row is marked with a label, seedlings of each entry are placed at the base of the labels following the layout of the field.

Transplanting

Single seedling is transplanted in a hill by hand along guide strings or marks on the soil surface. For the observation of characters in the field, double row planting for each entry is preferable to single row. Spacing of 18 cm within entry and of 36 cm between entries is convenient for the evaluation of entries.

5. Management of paddy fields

Weed control

For effective and safe weeding, it is necessary to make the soil surface perfectly level, to transplant healthy seedlings and to avoid water leakage from levees.

Safe method of herbicide application

① After application of herbicides, water depth is kept at 3 ~ 4 cm without continuous irrigation and surface drainage for at least 4 ~ 5 days.

② As seedlings are easily damaged at high temperatures by herbicides such as Simetryne, application of herbicides at high temperatures should be avoided.

③ Herbicides such as MCPB and MCP should not be applied at temperatures lower than 18°C.

④ Herbicides such as Molinate should not be applied to fields near fish ponds.

Water management

Water depth

After transplanting, water depth is kept so as to raise the water temperature in warm regions, but in cool regions water depth is kept at 10 ~ 15 cm to protect young panicles from low temperatures.

Midseason drainage

Thirty ~ 40 days before heading, water is drained for 5 ~ 10 days in order to suppress excess tillering, to increase the utilization of soil fertility and to supply oxygen into the rooting zone. Although midseason drainage is effective for increasing rice yield, it is not always applicable for the cultivation of rice germplasm. As heading date varies a great deal with the entries, it is difficult to determine the appropriate time for midseason drainage. For instance, at the normal midseason drainage time, some early entries may have already reached the meiosis stage of pollen mother cells, when they need water supply.

Control of diseases and insect pests

Control of diseases

① Blast (*Pyricularia oryzae*): For controlling blast, reduction of nitrogen application, avoidance of dense planting and seed disinfection are effective methods. Kasgamycin (Kasumin), Phthalide (Rabcide), Blastciden-s (Bla-s), Probenzole (Oryzemate) and Isoprothiolane (Fiji-one) are effective chemicals to control blast.

② Sheath blight (*Rhizoctonia solani*): In the regions with frequent occurrence of sheath blight, cultivation of early entries should be avoided. Validamycine (Validacin), Mepronil and Fultoanil (Moncut) are effective chemicals to control sheath blight.

③ Bacterial blight (*Xanthomonas oryzae*): In Japan, bacterial blight became a minor disease since the cultivation of nursery changed from flooded nursery to seedling-cultivation in a box. Techlofthalam (Shirahagen S) is applied twice during one or two weeks before heading.

④ Stripe and dwarf can be avoided by controlling vectors (see the next section).

Control of insect pests

Major insect pests in Japan are, (a) rice water weevil (*Lissorhoptrus oryzophilus*), (b) rice stem borer (*Chilo suppressalis*), (c) brown planthopper (*Nilaparvata lugens*), (d) small brown planthopper (*Laodelphax striatellus*), (e) whitebacked planthopper (*Sogatella furcifera*), (f) green rice leafhopper (*Nephotettix cincticeps*) and vectors of dwarf. These insect pests are controlled by insecticides as follows:

Common name	Chemicals	Effective against
Diazinon, Ethylthiometon	Ethimeton	a b c d e f
Isoxathion	Karphos	a b
Ethylthiometon	Ekamart	a c d e f
Propaphos	Kayaposnac	a d e f
Carbosulfan	Advantage	a d f
MPP	Baycid	b d e f
Diazinon	Diazionon	b d e f
MEP	Sumithion	b d
Cartap	Padan	b
Malathion	Malathion	c d e f
BMPC	Bassa	c d e f

Top dressing

In conventional cultivation, 3 ~ 4 kg / 10 a of nitrogen is applied as topdressing 18 ~ 25 days before heading. However, in case of germplasm cultivation, topdressing is not applied because of the wide range of duration of growth among entries. The fertilization of genetic resources is carried out only by basal dressing.

Harvest

Harvest time

The optimum harvest time is determined based on the following criteria:

① By the summation of average temperatures after heading date: This value varies with temperatures at the ripening stage, for early entries about 900°C, for late entries 1,000 ~ 1,100°C.

② By the number of days after heading: For early entries 35 ~ 40 days, for intermediate entries 40 ~ 45 days, for late entries 45 ~ 50 days. However, the number of days after heading also varies with the weather conditions.

③ By the observation of panicles: Harvest time is determined by the observation of the color becoming yellow in 80 ~ 90% of the capsules on the panicles.

Harvest

After removal of offtypes, the plants in each plot are harvested except for 2 ~ 3 border plants in both rims of rows. Plants are cut off with a sickle and bundled with strings, to which a paper tag is attached to indicate the name of the entry. Care should be taken in handling entries which easily shatter. Such panicles are put carefully in paper bags with a name tag .

Postharvest operations

Drying

As rice grains contain 20 ~ 25% of moisture when harvested, it is necessary to reduce the moisture content of grains to 13 ~ 15%. For drying, reaped rice plants are hanged on racks under a roof, for protecting them from birds.

Threshing

The reaped rice plants are threshed by a miniature threshing machine. Care should be taken to avoid contamination of seeds.

Storage

Seeds of each entry are put in a paper bag with an attached tag and kept in a seed storage room which is maintained at a temperature of 10°C and a relative humidity of 40%. For the preservation of genetic resources, a note or card should be prepared for each entry to record the related information.

II-2. Cultivation Methods for the Evaluation of Characteristics of Genetic Resources Wheat and Barley

by

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II-2. Cultivation methods for the evaluation of characteristics of genetic resources

Wheat and Barley

Cultivation manual for wheat and barley is combined, because the cultivation methods for wheat and barley are almost the same.

1. Preparation of seeds

It is important to keep genetic resources pure. 'Off-type' seeds recognizable by the grain color, seed size and other traits should be discarded before sowing. Seeds affected by seed-borne diseases such as smut (*Ustilago* and *Tilletia*), Stripe (*Cephalosprium*) and scald (*Rhynchosporium*) need to be treated with fungicides before sowing. Since the viability of seeds stored for a long time or introduced from abroad may decrease, it is necessary to test the germination ability before sowing.

2. Land preparation

Usually wheat and barley are cultivated in rotation with other crops. However, fields used for wheat or barley in the preceding crop season should be avoided, because the characteristics of genetic resources are often distorted by continuous cultivation of wheat and barley. Moreover, fields where wheat and barley were grown as preceding crops should not be used because shattered grains of the preceding crops may germinate and contaminate the tested accessions. In areas where wheat or barley is grown in the rainy season, soil with high moisture level causes wet injury, the growth being suppressed due to insufficient root development. For such areas, underdrainage or open ditch drainage should be implemented. Drainage is particularly effective for barley because barley is more sensitive to soil wetness than wheat.

Test field is usually plowed and after plowing the field is harrowed for sowing. Way of plowing depends upon the soil conditions of the field.

Seeds can be sown on flat rows or low ridges in areas with relatively scarce rainfall or in fields with good drainage conditions. However, in areas where rainfall occurs frequently, seeds are sown on high ridges.

3. Sowing seeds

To get uniform emergence of seedlings, seeds are immersed in water for forced sprouting and only germinated seeds are sown. Sowing time varies with the locality and the degree of winter habit of cultivar. Winter wheat and barley should be sown early in the fall so as to achieve sufficient growth before wintering. However, too early sowing induces shoot elongation before winter, resulting in serious damage by cold temperatures. Spring wheat and barley are sown as early as possible after the winter season is over. When wheat and barley with a high degree of winter habit are sown in spring, seeds should be vernalized before sowing by exposing them to low temperature (lower than 8°C) for an appropriate period of time depending upon the winter habit.

Generally, genetic resources should be sown by hand. Hill seeding is suitable for identifying off-type plants. Drill seeding is practised for the evaluation of the yielding ability, although genetic purity should be ascertained by other sowing methods. Interrow space in hill seeding is usually 60 ~ 80 cm. In paired row seeding, interrow space is wider and space between rows is about 10 ~ 15 cm. Hill space within row is usually 8 ~ 12 cm. For the evaluation of characteristics, the number of plants per hill should be restricted to one. To cope with the occurrence of vacant hills, 2 ~ 3 seeds are sown per hill and after germination extra seedlings are thinned out to allow a single plant per hill to grow. Depth of soil cover depends upon the soil type and soil conditions. Usually, the sowing depth is 5 ~ 6 cm.

4. Fertilizer application

Recommended rate of fertilizer application depends on soil fertility. Main elements of fertilizer are nitrogen, phosphate and potassium. Particularly, the supply of nitrogenous fertilizer is most important. Wheat and barley can not grow normally if the nitrogen supply is insufficient, and wheat and barley display lodging if the nitrogen supply is excessive. Lime is also important for acid soils. Magnesium and manganese need to be supplied if the soil is deficient in them.

As the purpose of the cultivation of genetic resources is not to achieve high yield, fertilizer is usually applied only as basal dressing. If necessary, nitrogen is applied as top-dressing one or two times during growth.

5. Field management

Before sowing, weeds need to be controlled. The most effective method of control of weeds after sowing is pre-emergence application of herbicides, preferably immediately after sowing. If necessary, intertillage is practised.

Treading wheat and barley plants is effective to stimulate root growth and reduce frost heaving injury.

Fungus diseases such as leaf rust (*Puccinia*) and powdery mildew (*Erysiphe*) should be controlled by spraying of fungicides, except when the evaluation of resistance to these diseases is the object of the experiment.

6. Harvest

Removal of off-plants before harvest is essential to keep genetic resources pure. Off-type plants found at various growth stages should be removed at each stage. Plants with off-type spikes are cut down by hand or pulled out. Combine-harvester is not applicable, because contamination of seeds easily occurs inside machines. The optimum harvest time is when the water content of grains is lower than 25%. Spikes are threshed with a laboratory spike thresher. After being dried to 11 ~ 13% moisture content, seeds are stored in a storage house under low temperature and low humidity conditions.

Although wheat and barley are usually self-pollinated, out-crossing also occurs at a low frequency. The rate of out-crossing varies with accessions and environment. Therefore, for harvesting seeds of genetic resources it is recommended to cover spikes with a paper bag before flowering.

Photo. 1 Hill seeding of wheat genetic resources.

Photo. 2 Wheat genetic resources in the evaluation field.

Photo. 3 Small plot thresher for wheat genetic resources.

II-3. Cultivation Methods for the Evaluation of Characteristics of Genetic Resources

Soybean

by

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Soybean

II-3. Cultivation methods for the evaluation of characteristics of genetic resources

Soybean

Generally, the cultivation of soybean for obtaining normal grain yield is rather easy compared to other upland crops. However, the characteristics of soybean are easily affected by climatic and soil conditions. Therefore, experiments should be carried out during several years to obtain standard values for the evaluation of the characteristics.

1. Pre-sowing

Seed preparation

It is necessary to select accessions adapted to a particular area for normal growth of soybean plant. Selection of healthy seeds is also important.

Soil drainage

Drainage is necessary to keep the test field in good condition, in particular upland fields converted from paddy fields. For drainage of the subsoil, underdrain or subsoil crushing is used, and for surface drainage, circumference drain or small block drain is used. For the drainage of lowland, ridges are necessary.

Soil preparation

Before seeding, the following three procedures are usually applied. Plowing-in of debris of the preceding crops, correction of soil acidity, and fertilisation of soil. For the first step, debris of the preceding crops and compost are incorporated into the soil. Lime is applied to the soil to adjust the pH to 6. Before sowing, basal dressing of fertilizer (N; 2 ~ 3 kg, P; 10 kg, K; 10 kg) is applied for soil fertilization.

Table 1 Amount of fertilizer applied kg per 10a in Japan

District	N	P_2O_5	K_2O	Soil improvement*
Hokkaido	2.0	18.0	9.0	100
Tohoku	2.0	10.0	8.0	100
Kanto, Tokai	3.6	14.4	12.0	100
Hokuriku	3.2	8.0	12.0	100
Chugoku, Kinki	3.0	10.0	10.0	150
Shikoku	2.0	6.0	6.0	100
Kyushu	3.0	10.0	10.0	100

* Calcium carbonate, quick lime or magnesium lime.

Land preparation such as tillering and harrowing is necessary to secure good emergence of seedlings and for normal growth. Land preparation is carefully made to obtain fine soil particles on the surface and coarse soil particles in the lower layers.

2. Sowing

Sowing time

In northern Japan, the optimum sowing time is 15 ~ 20 May. The time of soybean sowing is very important. The later the sowing, the lower the yield and the more luxuriant the vegetative growth.

Recommended planting density is 70 cm × 20 cm × 2 plants, and it is adjusted due to the sowing time, and maturity of the cultivar. In case of early sowing and late maturing cultivars, planting density is lower. In case of late sowing and early maturing cultivars, planting density should be higher.

The number of stands is adjusted by intrarow spacing.

Table 2 Planting density and growth and development parameters

District	Cultivar	Planting density*	Parameters of growth and development					
			Stem length (cm)	Nodes/ plant	Branches/ hill	Pods/ hill	100 seed weight (g)	Yield (kg/10a)
Hokkaido	Kitamusume	A=16,600 B= 60×20 C= 2	55	14	5	90	31	340
Tohoku	Suzuyutaka	A=13,000 B= 75×20 C= 2	70	17	5	68	25	350
Kanto Tokai	Enrei	A=16,600 B= 80×15 C= 2	80	15	5	70	33	350
Hokuriku	Enrei	A=10,000 B= 70×15 C= 2	70	16	5	68	25	350
Chugoku Kinki	Tamahomare	A=10,000 B= 65×15 C= 2	70	14	4	80	33	450
Shikoku	Tamahomare	A=13,300 B= 75×20 C= 2	65	15	5	44	32	370
Kyushu	Fukuyutaka	A= 8,900 B= 75×30 C= 2	75	19	6	58	32	330

* A; Number of plants / 10a.
B; Row width(cm) × Interrow spacing(cm).
C; Plants/hill.

Sowing

Seeds with a moisture level that is too high or too low are not suitable. Soil cover depth over seeds should be three times as large as the seed size. In case of soil with a high moisture soil content, soil cover should be shallow, while, in case of soil with a low moisture content, soil cover should be deeper.

3. Management from emergence to flowering

Transplanting and re-sowing

To achieve a high yield, it is necessary to transplant or re-sow to vacant hills as early as possible after emergence.

Weed control

Herbicides are sprayed during the interval between sowing and emergence. To select a herbicide, determine if the predominant weeds are gramineous or broad-leaved. Special care should be taken to avoid chemical injury to soybeans, especially in the case of sandy soil. Spraying of herbicides immediately after heavy rain should be avoided.

Protection from dove injury

Care is necessary in an area where doves occur. Protection measures are taken during the period from germination to full emergence.

Intertillage and ridging

To prevent lodging, control weeds and promote the activity of roots, intertillage and ridging are performed two or three times in the early growth stage before flowering.

4. Management from flowering to ripening

Top dressing

To achieve a high yield, top dressing is applied at the flowering time. Top dressing is especially effective in fields after wheat cultivation. For top dressing at the last time of ridging, the use of slow-acting fertilizers is recommended.

Irrigation

If leaves begin to show wilting symptoms such as rolling inside, irrigation is necessary. For soybeans grown over a wide area, slow irrigation should be applied during two or three days.

Control of insect pests and diseases

The timing of control is important. Continuous cropping promotes the occurrence of soilborne diseases. The earlier the control, the more effective. The control measures should be taken also in the nearby fields.

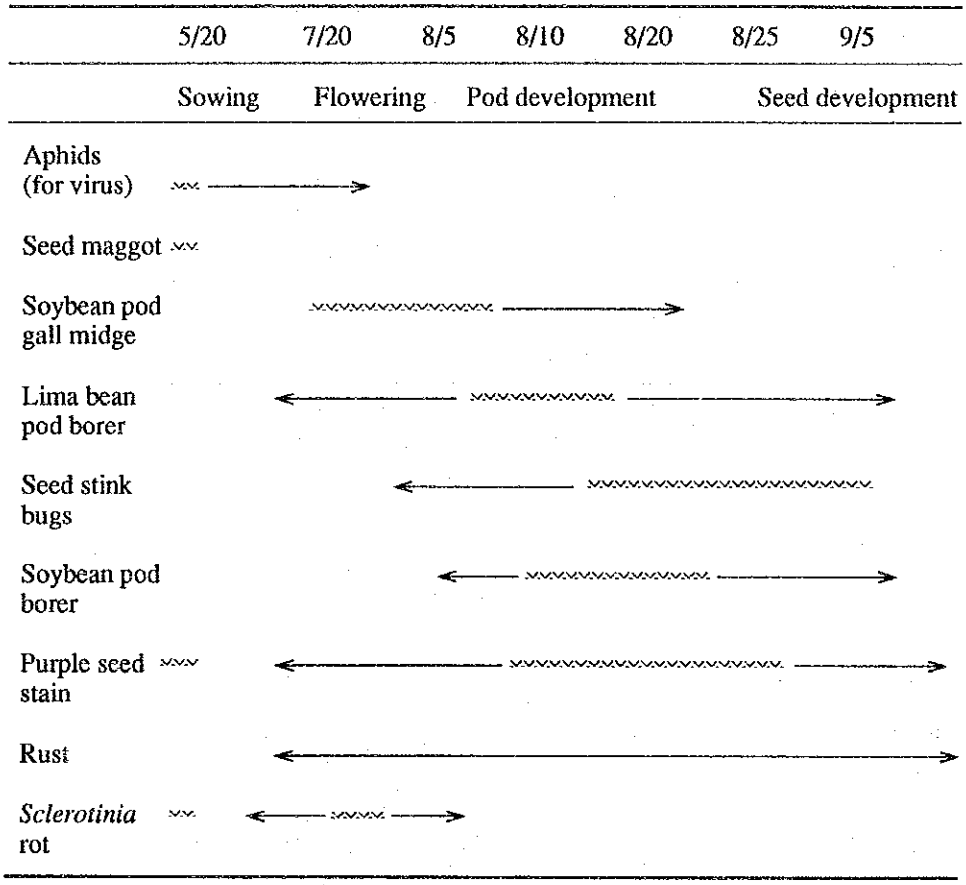
Table 3 Sowing time, growth stage and management

District	Cultivar	Growth stage and management
Hokkaido	Kitamusume	○ 5/20 — ◎ 7/28 — ◇ 8/10 — □ 8/20 — × 10/8 ▲△ 6/15 ▲ 7/8 ▲ 7/23 △ 8/5 △ 8/20 △ 9/25
Tohoku	Suzuyutaka	○ 5/20 — ◎ 8/5 — ◇ 8/15 — □ 8/25 — × 10/10 ▲ 6/25 ▲ 7/20 △ 8/5 △ 8/20 △ 9/5
Kanto Tokai	Enrei	○ 5/25 — ◎ 7/25 — ◇ 8/15 — □ 8/25 — × 10/8 ▲ 6/25 ▲ 7/20 △ 8/20 △ 9/5
Hokuriku	Enrei	○ 5/25 — ◎ 7/25 — ◇ 8/15 — □ 8/25 — × 10/8 ▲ 6/15 ▲ 7/15 △ 8/25 △ 9/5
Chugoku Kinki	Tamahomare	○ 6/10 — ◎ 8/1 — ◇ 8/15 — □ 8/30 — × 10/30 ▲ 7/20 △ 8/10 △ 8/25 △ 9/10
Shikoku	Tamahomare	○ 6/20 — ◎ 8/3 — ◇ 8/22 — □ 8/31 — × 10/27 ▲ 7/5 ▲ 7/20 △ 8/8 △ 8/23 △ 9/12
Kyushu	Fukuyutaka	○ 6/20 — ◎ 8/9 — ◇ 8/27 — □ 9/7 — × 10/26 ▲ 7/5 ▲ 7/20 △ 8/5 △ 8/29 △ 9/15

Note) Growth stage
○ ; Sowing
◎ ; Flowering
◇ ; Pod development
□ ; Seed development
× ; Maturity

Management
▲ ; Intertillage and ridging
△ ; Control of insect petsts and diseases

Table 4 Plant growth and time for control of main diseases and insect pests



Note) ——— ; Suitable time for control.
〰〰 ; Time for more effective control.

Main diseases of soybeans are as follows;

Virus diseases caused by soybean mosaic virus, soybean stunt virus and soybean dwarf virus.

Damping-off diseases caused by *Phytophthora* rot, root necrosis (*Calonectria crotalariae*) and *Corticium rolfsii* Curzi.

Other diseases include purple seed stain (*Cercospora kikuchii*), *Sclerotinia* rot, downy mildew (*Peronospora manshurica*), rust (*Phakospsora pachyrhizi*) and *Sphaceloma* scab.

Main insects of soybeans are as follows;

Insects in the early growth stage: seedcorn maggot (*Hylemya platura*), cut worm (*Agrotis segetum*) and Aphid (*Aphiis glycines*).

Insects attacking leaves: soybean beetle (*Anomala rufocuprea*) and common cutworm (*Spodoptera litura*).

Insects attacking pods and seeds: seed sting bug (*Riptortus clavatus* and others), soybean pod borer (*Leguminivora glycinivorella*) and limabean pod borer (*Etiella zinckenella*).

Insects attacking roots: soybean cyst nematode (*Heterodera elachista*) and soybean root miner (*Ophiomya shibatsujii*).

5. Harvest and drying

Harvest

Determination of harvest time is very important. Yield loss can be avoided largely by selecting the optimum harvest time and good quality seeds are obtained. Sun-drying before threshing should be performed rapidly to avoid damage by rainfall. During harvest with combines, soybeans should be harvested after the water content of stems decreases by exposure to dry weather, to avoid dusting of seeds by soil.

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II-4. Cultivation Methods for the Evaluation of Characteristics of Genetic Resources

Sweet Potato

by

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Sweet Potato

II-4. Cultivation method for the evaluation of characteristics of genetic resources

Sweet Potato

1. Preparation of plant materials

Sprouts up to 30 cm long are used as planting materials in the temperate zone. Emphasis is placed on the selection of healthy storage roots. In some instances it may be necessary to apply hot water treatment (47 ~ 48°C) for 40 minutes. Storage roots with medium-size are suitable seed roots. Nursery beds are set up in greenhouses or covered with a plastic film. It is generally recommended that storage roots should be presprouted by incubation at 30°C and 95% relative humidity for a week prior to bedding to accelerate sprouting. Electric wire is set up underground to maintain soil temperatures at 25 ~ 30°C. Seeding beds are fertilized with N-P-K and organic manure. Four to 5 storage roots are bedded for each cultivar at a 15 × 30 cm density and covered with 2 ~ 5 cm of soil. The sprouting potential of individual root depends on the genotype, cultural conditions, as well as latent diseases. Therefore, fungicides, pesticides, top dressing and irrigation should be applied carefully. In the tropical zone, where crops are grown continuously throughout the year, cuttings are obtained from the preceding crop. Cuttings are 20 ~ 30 cm long with 5 ~ 7 modes.

2. Land preparation

Experimental fields are plowed at a 20 ~ 30 cm depth, rotary-tilled and ridges 20 ~ 30 cm in height are formed. Ridges are covered with a plastic film in a rather cool zone. N-P-K and organic manure are applied as shown in Table 1. Nematode resistance is evaluated in a field inoculated with nematodes.

3. Planting

Ten ~ 20 cuttings per cultivar are planted vertically or obliquely in rows 70 ~ 100 cm apart, at an intrarow spacing of 25 ~ 35 cm. Tests for yielding ability and resistance to pests and diseases are carried out with two replications but tests for other characteristics are carried out without replication. For planting, 2 / 3 of a cutting in length is put inside soil. A small amount of soil is placed at the base of transplants to prevent them from wilting due to exposure to sunshine, when ridges are covered with a plastic film.

Table 1 Cutivation method for the evaluation of characteristics of sweet potato

Growing conditions	Temperate zone	Subtropical zone
Nursery bed		
Electric hot bed	Necessary	Desirable
Fertilizer rate (kg/a)	N :P :K :organic manure	N :P :K :organic manure
	2.3 :2.3 :2.3 :360	1.0 :1.7 :1.7 :50
Date of bedding	Beginning of April	End of March
Bedding density	20 × 30 cm	20 × 30 cm
Field		
Fertilizer rate (kg/a)	No application	N :P :K :organic manure
		1.3 :0.5 :0.5 :100
Date of transplanting	End of May to middle of June	End of May
Planting density	100 × 25 cm	71 × 35 cm
Planting method	Vertically on mulched ridge	Obliquely on ridges without mulch
Growth duration	120 to 150 days	120 to 150 days

4. Management

After rooted cuttings begin to grow, growth habit, leaf color and other specific characteristics are observed to identify the germplasm. If severe drought or pest damage occur, irrigation or pesticide is applied.

5. Harvest

Storage roots are usually harvested 120 ~ 150 days after transplanting. At harvest, foliage is removed by hand or machines, and after plowing storage roots are pulled up by hand carefully. Roots of infected plants can be removed and only roots of healthy plants are harvested. Roots are cut off from vines and kept in a container for each cultivar.

6. Storage

Storage roots are rapidly transferred to a storage room under the curing conditions of 30°C and 90% relative humidity for 5 ~ 7 days. These curing conditions stimulate the growth of the periderm layer on the surface of roots rapidly. Roots should not be removed from the storage room to prevent enlargement of wounds in the periderm. After curing, the roots are kept in a storage room at 12 ~ 14°C. At temperatures above 15°C roots begin sprouting and below 11°C roots are putrefied.

II-5. Cultivation Methods for the Evaluation of Characteristics of Genetic Resources

Potato

by

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II-5. Cultivation method for the evaluation of characteristics of genetic resources

Potato

§ 1 Ordinary cultivation

1. Field preparation

Time of ploughing

Autumn ploughing is recommended for mixing green manure or remains of preceding crop plants into soil and for regions where spring is corresponds to the peek for field works. However, in fields where autumn ploughing may cause soil erosion in spring or accelerate soil freezing, spring ploughing is preferable.

Ploughing and land levelling

Recommended ploughing depth ranges from 25 to 30 cm. For land levelling, rotary harrowing is carried out repeatedly to obtain fine soil particles and flatten the soil surface.

2. Potato tubers for seeding

Tuber size and method for cutting

Optimum tuber weight size for seeding ranges from 40 to 60 g. In the case of tubers lighter than 40 g, whole tubers are planted. In the case of tubers with a weight of 60 ~ 120 g, tubers are cut into half. In the case of tubers heavier than 120 g, tubers are cut into 3 ~ 4 pieces. To make the number of buds even, tubers are cut longitudinally (Fig. 1).

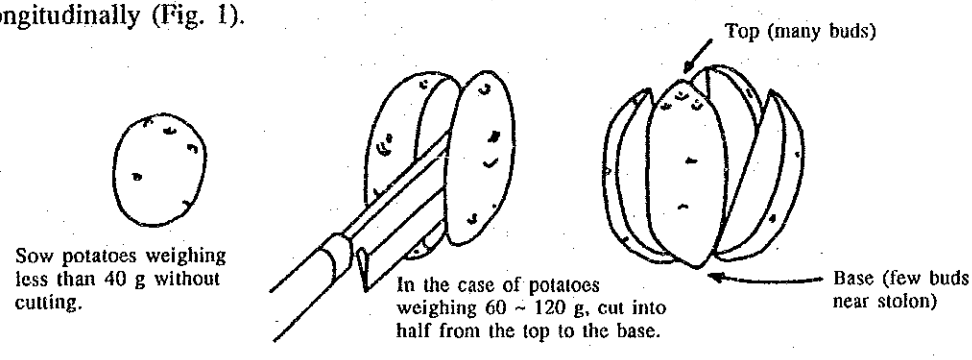


Fig. 1 Preparation of seed potatoes.

After cutting, potatoes are kept in a cool place for curing so as to obtain a corky cut surface.

Disinfection of knives

Knives should be disinfected before use to prevent the transmission of diseases such as ring rot (*Corynebacterium michiganense* pv. *sepedonicum*), soft rot (*Erwinia carotovora* ssp. *carotovora*) and black leg (*Erwinia carotovora* ssp. *atroseptica*). Knives are dipped for 5 seconds into 1/500 solutions of mercuric bichloride, or 10 times diluted Chemicharon (70% solution of calcium hypochloride). To prevent the transmission of X mosaic virus, knives are dipped into a saturated solution of calcium hydroxide or 25 ~ 30 seconds.

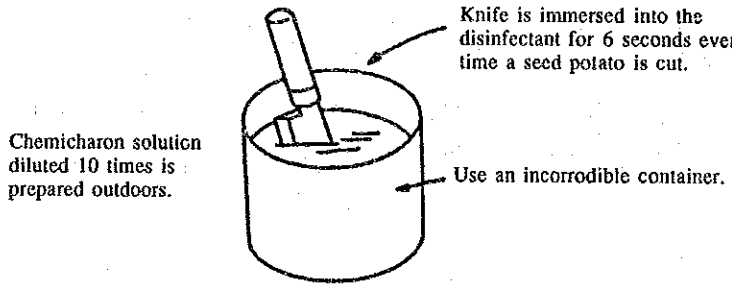
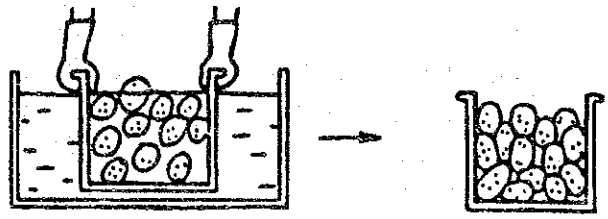


Fig. 2 Disinfection of knife.

Disinfection of seed potatoes

To prevent the transmission of *Streptomyces scabies*, *Erwinia carotovora* ssp. *atroseptica*, and *Spongospora subterranea*, seed potatoes are disinfected with chemicals such as streptomycin-thiophanate-methyl wettable powder.

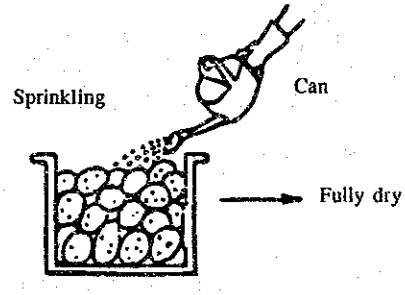
In case of immersion (disinfectants should be used on the recommendend concentration)



Immersion (10 ~ 20 sec.)

Fully dry

In the case of spraying (5 ~ 6 ℓ for 200 g of potatoes)



In the case of dusting (fully mix with disinfectants in a ratio of 0.3% to the weight of tuber)

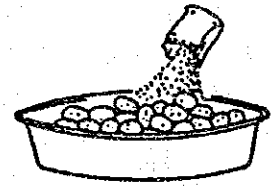


Fig. 3 Disinfection of seed potatoes.

Promotion of germination by exposure to light

To obtain a vigorous and uniform germination, seed potatoes are placed in a bright and dry place and exposed to warm scattered light. Exposure to light is carried out from 20 days to 30 days before planting. Recommended temperature during the exposure is in the range of 15 ~ 20°C. Too high (25 ~ 30°C) a temperature and too low (freezing) a temperature should be avoided. Exposure to light accelerates bud formation, flowering, vegetative growth, tuber formation and withering of plants.

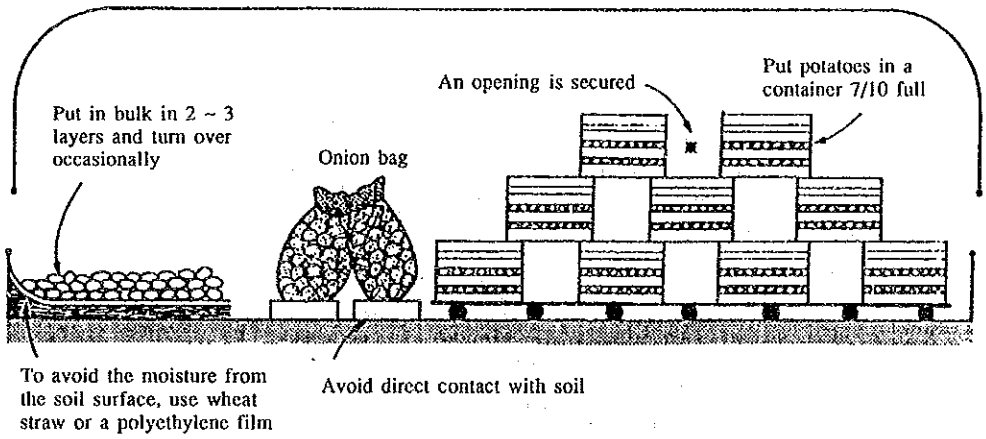


Fig. 4 Method for hastening of sprouting.

3. Fertilizer application

Amount of fertilizer

Amount of fertilizer applied depends on the soil fertility and yield expectation. The absorption rate of elements from applied fertilizer is 50 ~ 60% for nitrogen, 10 ~ 20% for phosphorus and 60 ~ 70% for potassium. Nitrogen application increases the number and weight of tubers. The higher the application of nitrogen, the heavier the tuber weight and the lower the content of starch. Phosphorus influences growth in the early stage as well as the number of tubers. Phosphorus is also necessary for the formation of cells and synthesis of starch. Potassium affects the number and weight of tubers. Potassium is necessary for the tranlocation and accumulation of assimilated products. The more potassium is applied, the less the starch content.

Application method

The total amount of fertilizer is applied to planting rows as basal fertilizer before planting. The applied fertilizer should be well mixed with soil on both sides of planted potatoes, in avoiding application upon or under potatoes.

Application of barnyard manure, green manure, soybean cake and fish cake

The above-mentioned fertilizers improve the chemico-physical properties of soil, hence the increase of soil fertility. Application of barnyard manure the rate of 1 ~ 2t per are is recommended.

4. Planting

Planting time

Tuber yield depends largely upon the planting time. The later the planting, the lower the tuber yield. Generally, budding begins at 5 ~ 6°C, and temperatures above 10°C are necessary for vegetative growth.

Planting method

Since direct contact of seed potato with fertilizer causes injury such as budding inhibition, seed potatoes are planted in the soil layer above fertilizer. Usually, cut planes of seed potatoes are placed upward, although the direction of the cut plane does not affect the tuber yield.

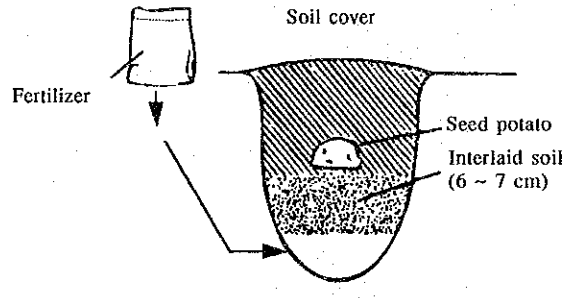


Fig. 5 Sowing method.

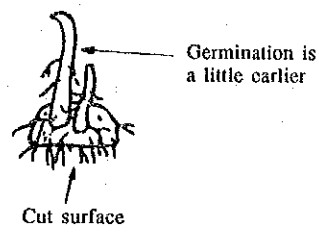


Fig. 5 Sowing method.

Depth of soil coverage above seed potato

A soil layer of 3 ~ 6 cm of soil for covering is recommended. Generally, in the case of dried and light soil, soil covering should be thick, and in the case of wet and heavy soil, shallow covering is preferable.

Planting density

Row space and intrarow spacing vary with the soil volume for ridging, and depend upon the traits of cultivars. Generally, row space ranges from 65 to 75 cm and intrarow spacing from 25 to 35 cm. Planting density varies in the range of 3,500 to 5,300 per are depending on the purpose. Dense planting reduces the tuber size, resulting in a high starch content. By scarce planting the tuber size enlarges, resulting in a low starch content.

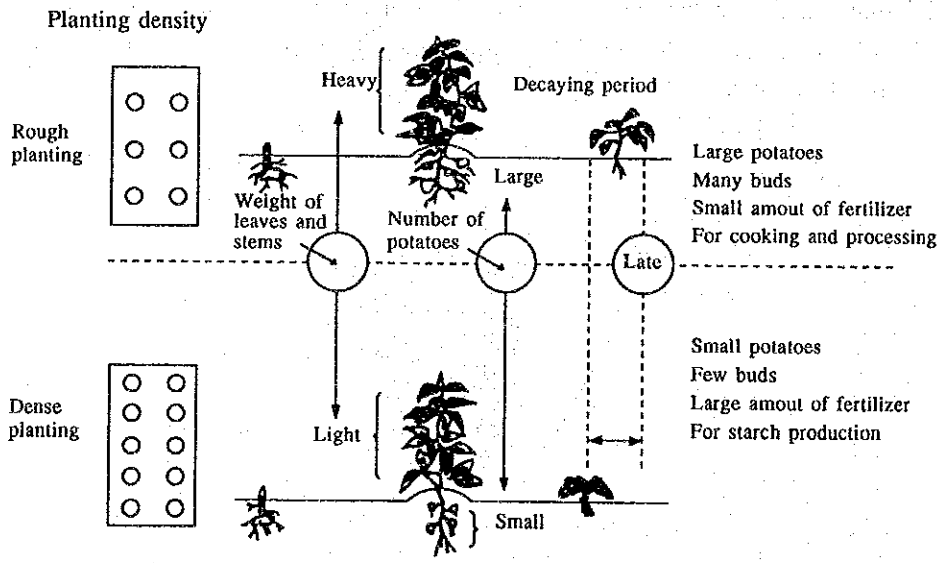


Fig. 7 Planting densities for different purposes.

5. Weeding

The first weeding is performed as early as possible after budding. The second weeding is performed one week before ridging. Herbicides should be sprayed effectively, following the application standard, after weed identification, and evaluation of the effect of herbicide.

6. Intertillage

Intertillage between rows is performed 1 ~ 2 times, which is also effective for weeding. The first intertillage, far from plants, should be performed as soon as possible after germination. The last intertillage should be applied so as to move soil toward plants around a week ahead of ridging.

7. Ridging

Ridging is performed 20 ~ 25 days after germination at the flower bud bearing stage. The height of the ridges is 20 ~ 25 cm from the base to the top, in a semicylindrical form like a mountain.

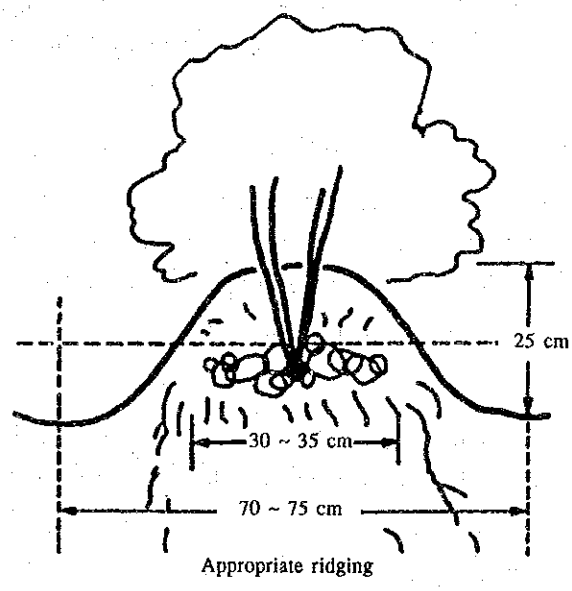


Fig. 8 Method of ridging.

8. Prevention of diseases and insect outbreaks

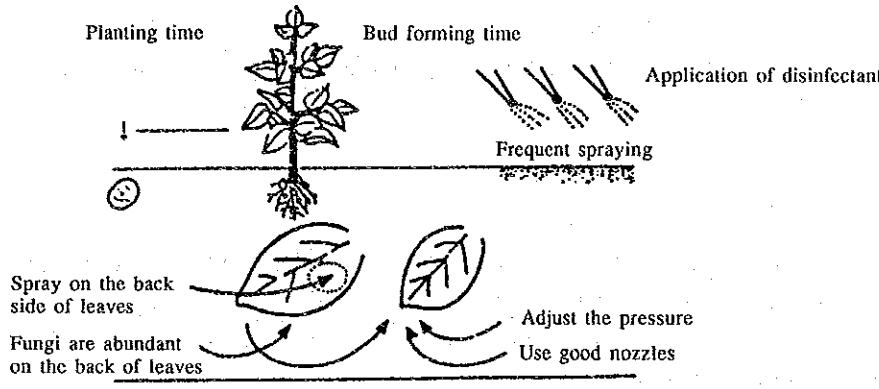


Fig. 9 Method for the control of diseases.

Aphids

Insecticide application at the bottom of planting rows is effective for aphids at the early growth stage. The effectiveness of the insecticide lasts about 2 months. For aphids appearing during the growth period, insecticide is mixed with fungicide.

Leaf blight disease (*Phytophthora infestanse*)

Fungicide is sprayed several times at an interval of 1 week to 10 days starting from the beginning of the flower bud stage so as to prevent disease development. Disease development is not frequent in a hot and dry climate, unlike in a cool and wet climate.

9. Harvest

Harvest takes place after stems and leaves become yellow and wilted, tuber coat becomes hard, and cannot be easily peeled, and stolon can be easily detached.

10. Storage

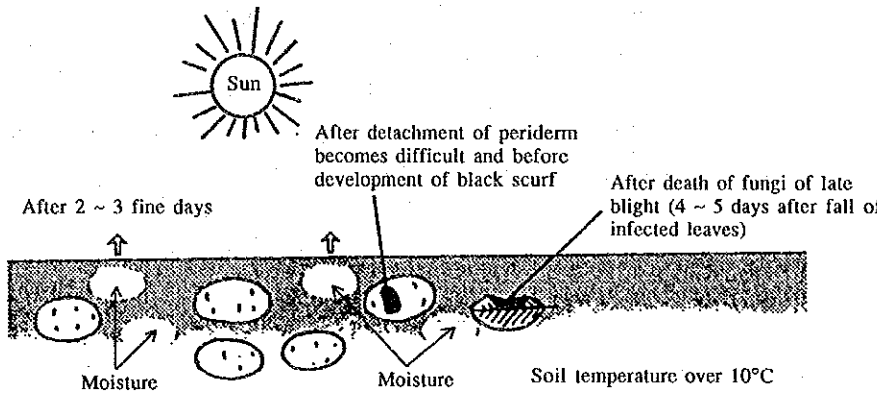


Fig. 10 Time of harvest.

Pretreatment for storage

Tubers are dug out carefully and stored when the surface of the tubers becomes dry by half-day exposure to sunshine after digging. After harvest, tubers continue to respire actively, resulting in the production of heat. Tubers become easily rotten by heat and humidity if heaped up in a large volume. After being dug, tubers should be kept in a windy and cool place for two weeks until the respiration rate decreases.

Storage method

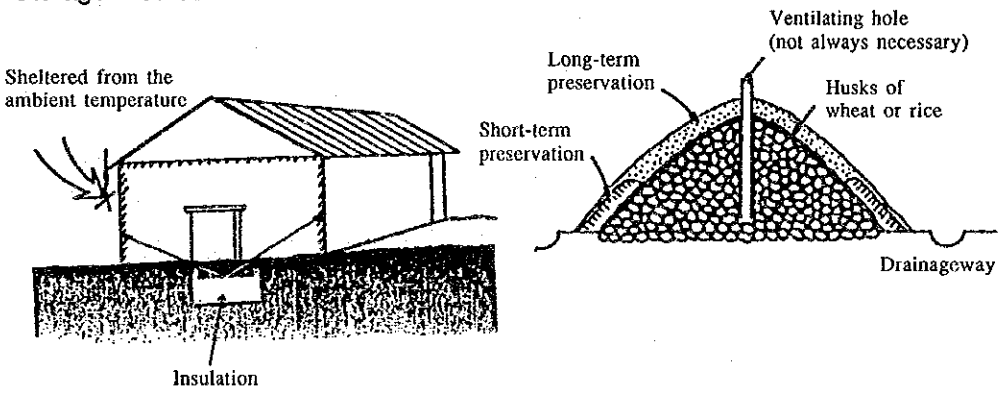


Fig. 11 Method of preservation of potatoes.

There are two types of storage methods; indoor and outdoor. Indoor storage can be applied on earth and half underground. In either cases, the plants should be fully insulated from heat in avoiding the penetration of cool air. In the case of outdoor storage, freezing should be avoided. Since overwintering is important, temperatures during storage should be maintained as close as possible to outdoor temperatures.

§ 2 Forcing cultivation

1. Suitable cultivars

Short duration cultivars, with a vigorous early growth and which produce plump and uniform tubers, are suited to forcing cultivation.

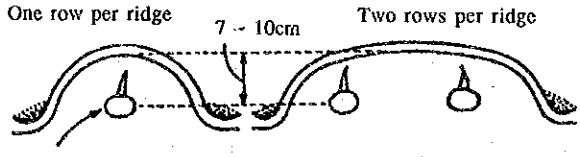
2. Preparation of seed tubers

Seed tubers, which are fully aged and have completed the dormancy period, should be prepared. Expose tubers to light for 2~4 weeks to accelerate germination.

3. Cultivation in plastic greenhouse or plastic tunnel

Seed tubers should be planted deeper than in standard cultivation so as to avoid freezing during the low temperature period.

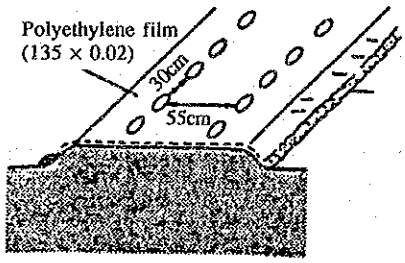
Normal planting Plant seed potatoes 15 ~ 20 days before the estimated sprouting time, and soon after ridging is applied and polyethylene film is spread



Seed potatoes are subjected to sprouting-promoting treatment by exposure to light before planting.
Try to make sprouts appear above soil surface after the risk of late frost is no longer present.

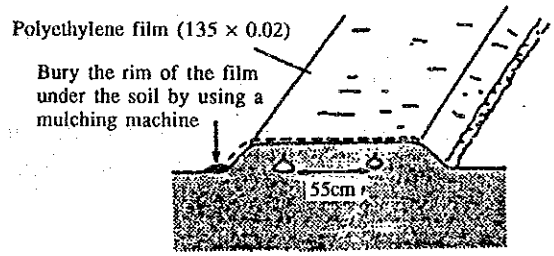
Spreading of holey sheet

Spreading before planting



Spreading of normal mulching film

Spreading after planting



Mulching culture

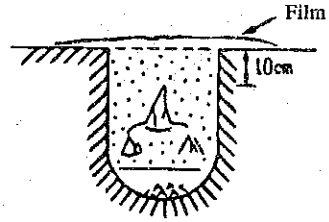


Fig. 12 Method of mulching using a film.

4. Cultivation by mulching

Immediately after planting, soil is earthed up upon seed tubers at a height of about 10 cm, and covered with a plastic film. Plastic film without holes is used and after germination holes are made. Sometimes a holey film is used. After soil is covered with a holey film, seed tubers are planted in the holes.

5. Application of fertilizer

All the fertilizer is applied as basal fertilizer. In forcing cultivation, quick-acting fertilizer is used to advance harvest time. Although the amount of fertilizer varies with fields, the average amount of N, P, K is 8 ~ 12 kg/a for each element.

6. Control of growth

For early season marketing, the number of stems per plant is reduced to one, by removing excess branches.

7. Harvest

After being dug, the tubers are kept on the field for several days. After the surface of the tubers becomes dry, tubers with a good appearance are selected, put in cartons and kept in a warehouse.

II-6. Cultivation Methods for the Evaluation of Characteristics of Genetic Resources

Corn

by

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II-6. Cultivation method for the evaluation of characteristics of genetic resources

Corn

Location of experimental field must be selected carefully in order to avoid xenia which appears on kernels as an immediate effect of outcrossing. Xenia effect on endosperm can be detected in sweet corn such as starchy vs. sugary endosperm and nonshrunken vs. shrunken endosperm.

1. Land preparation

Seed beds are prepared to obtain a fine and firm contact-zone between seeds and soil, to facilitate the water supply from soil to seeds.

Corn is a deep-rooted crop. Corn roots usually reach a depth of 50 cm and spread over a wide area. Soil is fully plowed to incorporate the residues of the preceding crops and weeds on the soil surface into soil. Usually, deep tillage of more than 25 cm is used. However, if deep tillage resulting in the spread of poor subsoil on the soil surface, deep tillage is not performed and compost or soil amendment matter is applied. Machines for tillage are selected for the purposes and soil types. The first rough tillage is performed with a disk harrow, and a rotary harrow is used next for flattening the soil for the seedbeds.

For heavy soil, soil with a high ground water level and upland fields converted from paddy fields, the following measures are adopted.

(1) Drainage by open ditch or underdrain.

(2) Subsoil breaking at ground water level with subsoiler to improve the efficiency of drainage.

(3) Deep-tillage with bottom plow or rotary tilling.

(4) Improvement of soil structure by the application of compost and organic matter.

2. Planting time

Planting time of grain corn varies with the locality and the kind of corn. Silage corn should be planted as early as possible after the mean temperature increases to and remains at 10°C for a few days. Sweet corn should be planted after the mean temperature exceeds 12 ~ 15°C. Suitable seeding temperature for sweet corn of

shrunken type is higher than that for sugary corn. However, slightly delayed seeding time is preferable for the evaluation of corn characteristics and acceleration of germination to facilitate the comparison between silage corn and grain corn. In mainland Japan, the seeding time is around mid-April to late May, when the mean temperature reaches and remains at 12 ~ 15°C for a few days .

3. Planting depth, replanting and thinning

Planting depth varies from 1.5 to 5 cm depending on the soil conditions. In case harrowing of soil is insufficient, soil cover should be rather deep. Two to four seeds of corn are planted to one hill. For sweet corn, a larger number of seeds is planted compared with silage corn. Replanting of missed hills is performed at the 3 to 4 leaf stage. Thereafter, thinning to single plant per hill is performed by hands or hoe, in removing excess plants including growing points from the soil surface. Replanted plants are marked to be excluded from the evaluation of characteristics, because they usually grow poorly. If replanting is not performed, two plants adjacent to a missing hill should be omitted in the evaluation of the characteristics.

4. Planting density

Row space is usually 70 to 90 cm and intrarow spacing 20 to 35 cm, plant population being 4,000 to 7,000 plants/10 a, depending on the duration of growth, corn type, soil fertility and climate. The earlier the maturity, the more fertile the soil. The warmer the climate, the denser the population. A low population is preferable for sweet corn. Before seeding, seeds should be disinfected.

5. Experimental design for evaluation of characteristics

Size of test plot should exceed 10 m^2 and row number should exceed four. Two border rows are excluded from the measurement of the characteristics and measurement of more than 10 plants is necessary for the evaluation of the characteristics. Evaluation test is based on randomized complete-block design with three replications.

6. Application of fertilizer

Corn plants grow in a relatively wider range of pH compared with other crops. However, the optimum pH for corn is 6.0 to 6.5. Therefore, limestone should be

applied to fields with a pH less than 5.0. Fertilizer containing N, P and K is applied before planting at 3 to 5 cm below seeds and 5 cm apart from seeds on both sides. This basal application of fertilizer is important to stimulate growth in the early stage. Recommended application rate for N, P and K ranges from 14 to 16kg/10a, from 15 to 20kg/10a, from 10 to 12kg/10a and barnyard manure application ranges from 3 to 5t/10a. As for N, split application is recommended to avoid salt injury. The rate of N in basal application ranges from 6 to 10kg/10a and additional N is applied in the center of the rows during the period from germination to the 6 ~ 8 leaf stage. If barnyard is not applied, the amount of N, P, K is increased by 5, 3.5, 10kg/10a.

7. Weed control

Two methods of weed control are used, chemical and mechanical. Herbicide is applied to corn at three different stages, preplanting, preemergence and postemergence. In the field where herbicide is applied, intertillage is performed only one time during the growth period. For a field with a small size, hand weeding is used.

8. Harvest

Harvest date varies with the locality, planting time and maturity time. Corn for silage is harvested at the full dent stage of kernels, from 40 ~ 50 days after silking, when the dry matter percentage of the whole crop is less than 30%. Sweet corn is harvested when kernels reach the maximum size, being plump and full of milk. Harvest takes place 14 ~ 20 days after silking, when the color of silks outside husks turns dark brown and they become dry. Grain corn is harvested as soon as grains mature, to avoid freezing risk and damage by putrefaction and insect attacks. Grain corn matures 45 ~ 60 days after silking.

9. Storage

Harvested ears are shelled after drying at 38 ~ 43°C. Grains with a moisture content of 17 ~ 22% can be safely stored if the temperature is kept below 10°C. For safe storage in warm areas, the moisture content of grain should be kept under 13 ~ 14% and some measures of protection against insect attacks should be taken.

III-1. Evaluation of Genetic Resources

Rice

by

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Rice

III-1. Evaluation of genetic resources

Rice

The database management system for rice genetic resources has been developed in MAFF, Japan. For the evaluation of each accession, descriptors have been developed for the database on a decimal system.

Descriptors are classified into three groups; primary, secondary and tertiary. The primary descriptors are related to morphological and physiological characters. The secondary descriptors are related to reaction to biotic stresses. The tertiary descriptors are related to yield and quality. Each descriptor is categorized into essential or optional item.

Prior to the evaluation of the characters, accession number, name, former designation, seed source and country of origin should be listed in the field notes.

1. Primary characters

Thirteen essential and 18 optional items are listed as the primary ones for rice genetic resources.

<Essential items>

Culm length

Distance from the ground level to the base of panicle is measured in centimeter (n=5)*. The tallest culm of a hill is measured at the ripening stage.

Panicle length

Distance from the base to the tip of panicles is measured in centimeter (n=5).

Number of panicles

Number of panicles per plant is counted at the ripening stage (n=5).

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* Minimum number of measurements.

Apiculus color

Apiculus color is observed three weeks after heading and classified into 1: straw, 2: tawny, 3: brown, 4: red brown, 5: light red, 6: red, 7: light purple, 8: purple, 9: black purple.

Length of caryopsis

Length of caryopsis (n=5) is measured using a projector or dial gauge (in millimeter) as the distance from the base of the lowermost sterile lemma to the tip (apiculus) of the fertile lemma or palea, whichever is longer.

Width of caryopsis

Width of caryopsis (n=5) is measured using a projector or dial gauge (in millimeter) as the distance across fertile lemma and palea at the widest level. A modified photo-enlarger is used for measuring the grain dimension.

Length of brown rice

Length of brown rice (n=5) is measured using a projector or dial gauge (in millimeter) as the longitudinal distance.

Width of brown rice

Length of brown rice (n=5) is measured using a projector or dial gauge (in millimeter) as the distance across at the widest level.

Endosperm type

Type of endosperm is classified by the reaction to Iodin potassium solution or visually into 2: non-glutinous, 8: glutinous.

Heading date

Heading date corresponds to the day when 50% of the plants in an accession headed.

Lemma and palea color

When the terminal spikelet becomes ripe, the color of the lemma and palea is observed and classified into :1 straw, 2: yellow, 3: gold, 4: reddish yellow to orange, 5: brown, 6: reddish brown, 7: purple, 8: black, 9: others.

Appearance of awn

After full heading, appearance of awn is observed and classified into 0: absent, 1: extremely scarce, 3: scarce (10 %), 5: intermediate (25 %), 7: abundant (40 %).

Awn length

Length of awn is classified into 2: extremely short, 3: short, 4: rather short, 5: intermediate, 6: rather long, 7: long, 8: extremely long.

<Optional items>

Plant type

Plant type is visually classified into 2: extreme panicle weight type, 3: panicle weight type, 4: partial panicle weight type, 5: intermediate type, 6: partial panicle number type, 7: panicle number type, 8: extreme panicle number type.

Culm thickness

Thickness of culm is visually classified into 2: extremely fine, 3: fine, 4: rather fine, 5: intermediate, 6: rather thick, 8: extremely thick.

Culm hardness

Hardness of culm is classified into 2: extremely hard, 3: hard, 4: rather hard, 5: intermediate, 6: rather soft, 7: soft, 8: extremely soft.

Blade pubescence

Condition of blade surface at tillering stage is classified into 0: glabrous (smooth), 1: extremely scarce, 2: scarce, 3: little, 4: rather little, 5: intermediate, 6: rather abundant, 7: abundant, 8: considerably abundant, 9: extremely abundant.

Flag leaf angle

Flag leaf angle is observed at the dough-ripe stage near the auricle as the angle between the flag leaf blade and the main panicle axis and is classified into 2: extremely erect, 3: erect, 4: rather erect, 5: intermediate, 6: rather descending, 7: descending, 8: extremely descending.

Blade color

Leaf blade color at the tillering stage is observed and classified into 1: yellow, 2: yellowish blotched 3: pale green, 4: green, 5: dark green, 6: purple blotched, 7: purple margins, 8: purple, 9: others.

Basal leaf sheath color

Color of outer surface of leaf sheath at the tillering stage is classified into 1: yellow, 2: yellowish blotched, 3: pale green, 4: green, 5: dark green, 6: purple blotched, 7: purple margins, 8: purple, 9: others.

Spikelet density

Number of spikelets per 10 cm of panicle axis (n=5) is measured using the longest panicle of individual plants.

Degree of panicle exsertion

The degree of exsertion of panicles, the distance from the top of the flag leaf sheath to the panicle base, is measured at the ripening stage and classified into 2: extremely short, 3: short, 4: rather short, 5: intermediate, 6: rather long, 7: long, 8: extremely long.

Panicle type

Based on the type of branching, angle of primary branches and spikelet density, panicle type is classified into 1: lancet, 3: spindle type, 5: clavated, 7: broom type, 9: open.

Pubescence of lemma and palea

Pubescence of hulls is classified into 0: none (glabrous), 1: rare, 2: scarce, 3: little, 4: rather little, 5: intermediate, 6: rather abundant, 7: abundant, 8: considerably abundant, 9: extremely abundant.

Sterile lemma

Color of sterile lemma at the ripening stage is classified into 0: white, 1: light yellow, 3: orange, 5: yellowish brown, 7: red, 0: purple.

Phenol reaction

Dipping 5 grains into 1 % phenol solution for 48 hours, the reaction to phenol is classified into 0: negative, 9: positive.

Awn color

Color of awn at the maturity date is classified into 1: straw, 2: yellowish brown, 3: brown, 4: reddish brown, 5: light red, 6: red, 7: light purple, 8: purple, 9: blackish purple.

Brown rice color

Color of brown rice is classified into 0: white, 1: light brown, 2: brown, 3: reddish brown, 4: red, 5: brownish purple, 6: purple, 7: dark purple, 8: blackish purple, 9: others.

Degree of luster of brown rice

The degree of luster of brown rice is evaluated by the shade of amber color of brown rice and classified into 2: extremely light, 3: light, 4: rather light, 5: intermediate, 6: rather shaded, 7: shaded, 8: dark shaded.

Maturity date

The time when more than 90 % of grains on panicles become ripe is recorded as maturity date. Conventionally, maturity date is set by adding 40 days to the date of full heading.

Days from first heading to full heading

Number of days is calculated by subtracting the date of head emergence from the date of full heading.

2. Secondary characters

As for the secondary characters, the following 10 essential and 3 optional items are listed.

<Essential items>

Estimated genotypes for blast (*Pyricularia oryzae*) resistance

Blast is one of the most severe diseases of rice in Japan. As shown in Table 1, the relationships between resistance genes of rice and Japanese races of blast have been identified. Judging from the reaction pattern of rice seedlings to races, the genotypes of blast resistance can be estimated. This resistance is referred to as "true resistance". The procedure of the test for true resistance to blast is as follows;

Preparation of test materials

① Seeds of each entry are sown using a spoon in a hole on the soil of seedling boxes.

② After covering seeds with soil and watering, seedling boxes are put into an incubator to accelerate germination.

③ As differential checks, Shin 2 (+), Aichi-asahi (*Pi-a*), Ishikari-shiroke (*Pi-z*), Kanto 51 (*Pi-k*), Tsuyuake ($Pi\text{-}k^m$), Fukunishiki (*Pi-z*), Yashiromochi (*Pi-t*), Pi No. 4 ($Pi\text{-}ta^2$), Toride 1 ($P1\text{-}z^t$), BL 1 (*Pi-b*), K 59 (*Pi-t*) are also sown.

④ After emergence of the seedlings, the seedling boxes are transferred into a glasshouse.

Table 1 System for differentiating blast races in Japan, and reaction of some races to the differentials

Differential	Shin 2	Aichi-asahi	Ishikari-shiroke	Kanto 51	Tsuyu-ake	Fuku-nishiki	Yashiro-mochi	Pi No. 4	Toride 1
Gene	*Pi-k*^{s}	*Pi-a*	*Pi-i*	*Pi-k*	*Pi-k*^{m}	*Pi-z*	*Pi-ta*	*Pi-ta*^{2}	*Pi-z*^{t}
Code	1	2	4	10	20	40	100	200	400
Race									
001	S	R	R	R	R	R	R	R	R
003	S	S	R	R	R	R	R	R	R
007	S	S	S	R	R	R	R	R	R
017	S	S	S	S	R	R	R	R	R
031	S	R	R	S	S	R	R	R	R
033	S	S	R	S	S	R	R	R	R
037	S	S	S	S	S	R	R	R	R
101	S	R	R	R	R	R	S	R	R
102	R	S	R	R	R	R	S	R	R
103	S	S	R	R	R	R	S	R	R
107	S	S	S	R	R	R	S	R	R
137	S	S	S	S	S	R	S	R	R
303	S	S	R	R	R	R	S	S	R
333	S	S	R	S	S	R	S	S	R

S: Susceptible reaction, R: Resistant reaction.

Inoculation

① Preparation for inoculation; The concentration of inoculum is adjusted so that 30 ~ 50 spores are observed in one microscope field (150 magnification).

② Although the 3-leaf stage is most suitable, seedlings older than the 2.5 leaf-stage can be used.

③ Inoculation is carried out by spraying.

④ After inoculation, seedling boxes are put in an incubator at 24 ~ 26°C for 15 hours.

Evaluation

Seven ~ 10 days after inoculation, lesions are formed on leaf blades, when lesions on each entry are observed. Entries with any susceptible lesion are classified as susceptible, while others are classified as resistant to the used isolate.

Field resistance to leaf blast

Since the breakdown of true resistance to blast often occurred a few years after the release of resistant cultivars, it has become necessary to incorporate field resistance, which does not include specific resistance to races, to suppress blast outbreaks.

The test procedure for the field resistance is as follows:

① One entry is sown in a row in the upland nursery together with checks enclosed with a spreader variety (Fig.1). Differential checks should be planted in some rows of the test nursery.

② Sowing time in each region is as follows: Hokkaido, late June: Tohoku, Hokuriku and Kanto, early or middle June: Chugoku and Kyushu late May.

③ The inoculation of a specific race should be carried out before the occurrence of infection with natural races.

④ Fourty ~ 50 days after inoculation (5 or 6 leaf stage), the nurseries are attacked by blast and the epidemic becomes apparent.

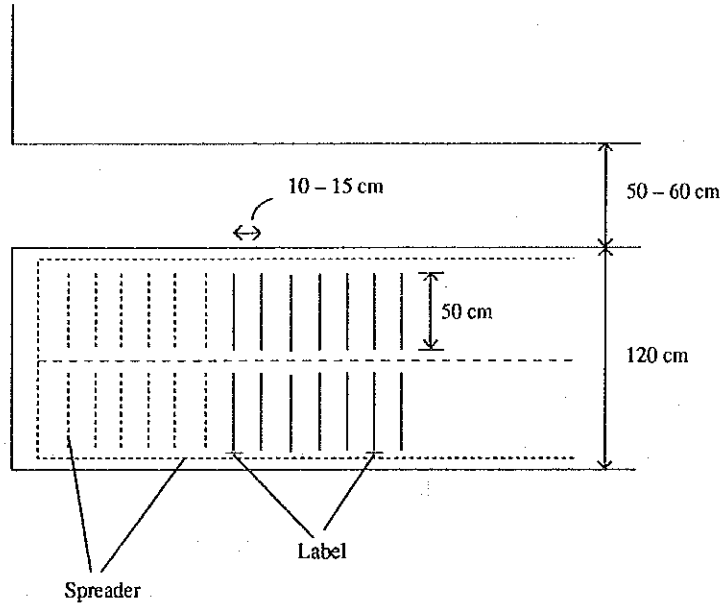


Fig. 1 Upland nursery for test of field resistance to leaf blast.

⑤ The number and size of susceptible lesions on the leaves in each row are determined compared with check varieties (Table 2). In this case, varieties with the same genotype for true resistance must be compared with each other.

Table 2 Scores of resistance in leaf blast nursery

Score	General description of symptoms	% of infected leaf area
0	No susceptible lesions of leaf blast	0
1	Few susceptible lesions	1
2	A few susceptible lesions	2
3	Several susceptible lesions	5
4	Many susceptible lesions	10
5	A large number of susceptible lesions with few dead leaves	20
6	A few dead leaves	40
7	Several dead leaves	60
8	Many dead leaves	80
9	Almost all leaves dead	90
10	All leaves and stems dead	100

⑥ If varieties with different genotypes are compared with each other for field resistance, races which make susceptible lesions on both varieties should be inoculated beforehand.

Since resistance to neck blast usually accompanies the resistance to leaf blast, resistance to neck blast can be usually estimated from the test on leaf blast.

Varietal group of bacterial blight (*Xanthomonas oryzae*) resistance

Inoculation and evaluation

The inoculation method is as follows;

① About 5 cm below the tip of the uppermost leaf, a fully developed leaf is clipped with scissors smeared with the inoculum.

② The concentration of the inoculum is 10^8 cells/ ml.

③ The inoculation at the booting stage is most suitable for the evaluation of resistance.

④ Size of lesion is measured 18 ~ 21 days after inoculation.

Classification of varietal groups

Based on the reaction to five races of bacterial blight predominant in Japan, the resistance pattern is classified into five groups; (1) Kinmaze, (2) Kogyoku, (3) Rantai Emas, (4) Wase-Aikoku, (5) Java. If other reaction patterns are found, they are recorded in the note. The relationship between varietal groups and bacterial blight races in Japan is shown in Table 3.

Table 3 Relationship between varietal groups and bacterial blight races in Japan

Varietal group	Resistance gene	Reaction to Japanese races of BB				
		I	II	III	IV	V
Kinmaze	None	S	S	S	S	S
Kogyoku	*Xa-1, Xa-12*	R	S	S	S	R
Rantai Emas 2	*Xa-1, Xa-2, Xa-12*	R	R	S	S	R
Wase Aikoku 3	*Xa-3*	R	R	R	S	S
Java 14	*Xa-1, Xa-3, Xa-12*	R	R	R	S	R

Field resistance to bacterial blight

To test the field resistance to bacterial blight, the foremost two plants in each row are inoculated by the clipping method at booting stage. Based on the symptoms, the field resistance to bacterial blight is classified into 1: no symptoms or limited necrosis at the leaf tip, 3: necrosis or chlorosis on 1/4 of the leaf tips, 5: necrosis or chlorosis on half of the leaves, 7: necrosis or chlorosis on 3/4 of the leaves, 9: all leaves are dead.

Resistance to stripe

Stripe, the most serious virus disease in Japan, is transmitted by the small brown planthopper (*Laodelphax striatellus*). Resistance to stripe is tested by natural infection in an area with epidemics or by artificial inoculation using vectors.

Resistance to green rice leafhoppers (*Nephotettix cincticeps*)

Green rice leafhoppers (GRLH) can suck the juice of susceptible plants from both sieve tubes and vessels, while they can suck juice only from the vessels of resistant plants. To test the resistance to GRLH, an antibiosis test is selected. Five GRLH nymphs (2nd instar) are caged with one seedling of rice (2nd leaf stage) in a test tube with water 1 cm in depth. Three ~ 4 days after caging, the number of surviving individuals is counted. Usually, no or one nymph survives on resistant seedlings for 3 days, while most nymphs survive on susceptible seedlings.

Resistance to brown planthoppers (*Nilaparvata lugens*)

To test the resistance to brown planthoppers (BPH), the bulk seedling method has been developed at IRRI since 1969. In Japan, the bulk seedling method was modified to meet the conditions of Japan. Until now, 9 genes resistant to BPH have been detected (Table 4).

Table 4 Relationship between resistance genes to brown planthopper and BPH biotypes

Resistance gene	Variety	Reaction to BPH biotype			
		1	2	3	4
Bhp-1	Mudgo	R	S	R	S
bhp-2	ADS 7	R	R	S	S
Bhp-3	Rathu Heenati	R	R	R	–
bhp-4	Babawee	R	R	R	–
bhp-5	ARC 10550	S	S	S	R
Bhp-6	Swarnalata	S	S	S	R
bhp-7	T 12	S	S	S	R
bhp-8	Chin saba	R	R	R	–
Bhp-9	Balamawee	R	R	R	–
None	TN1	S	S	S	S

Bulk seedling method

① To accelerate germination, seeds of test entries are put in a petri dish with water.

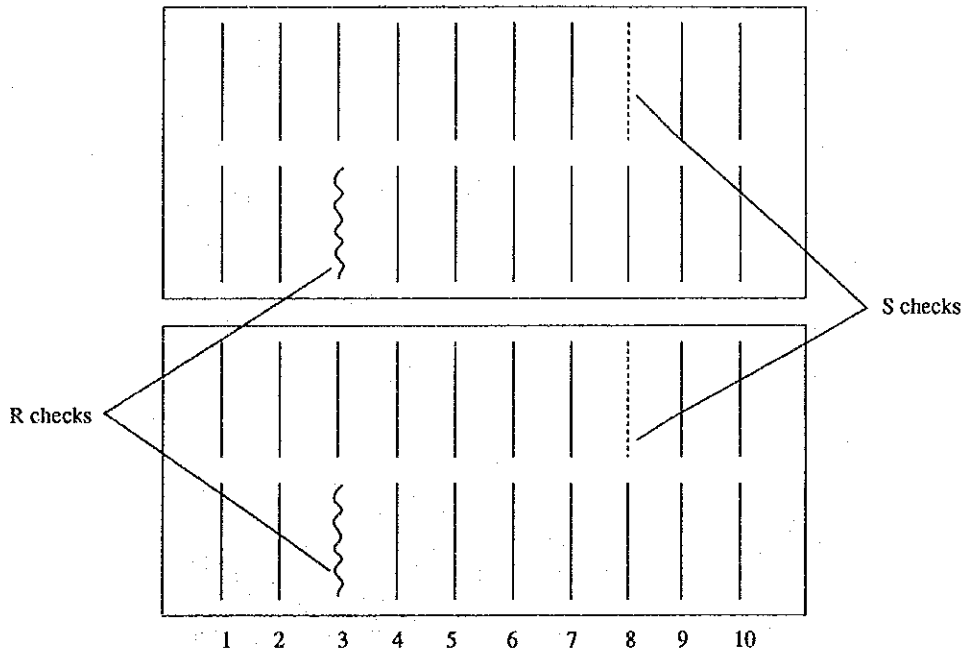
② Germinated seeds are sown in a row in a seedling case.

③ As shown in Fig. 2, 20 rows including resistant and susceptible checks are sown in each seedling case.

④ After covering with soil and watering, cases are kept in a daylight incubator for 3 days.

⑤ Two cases are put in a cage. When seedlings reach the first leaf stage, 5 ~ 7 BPH nymphs (2nd or 3rd instar) per seedlings are caged.

⑥ Five ~ 7 days after caging, susceptible checks are sucked by BPH nymphs and killed. Usually, since resistant plants survive the bulk seedling test, while the susceptible ones are killed, the resistance of the test entries can be evaluated.



Each row consists of 15 or 17 seedlings. Two cases with 20 rows including resistant and susceptible checks are accommodated in a cage.

Fig. 2 Bulk seedling test for brown planthopper resistance in a cage.

Test for factors of BPH resistance

To test the mechanism of BPH resistance of rice, tests for anti-xenosis (non-preference), antibiosis and tolerance of test materials are carried out.

Cold tolerance

There are two types of damages caused by low temperatures. In the first type, panicle sterility is caused by low temperatures (lower than 17°C) during reproductive growth and at heading time. In the second type, heading time is delayed due to growth retardation during vegetative growth caused by low temperature, resulting in yield decrease associated with incomplete grain filling.

Cold tolerance at the booting stage is tested in a paddy field irrigated with cool water by the following procedures;

① Temperature of water under continuous irrigation is kept at 19 °C by circulating underground water (Fig. 3).

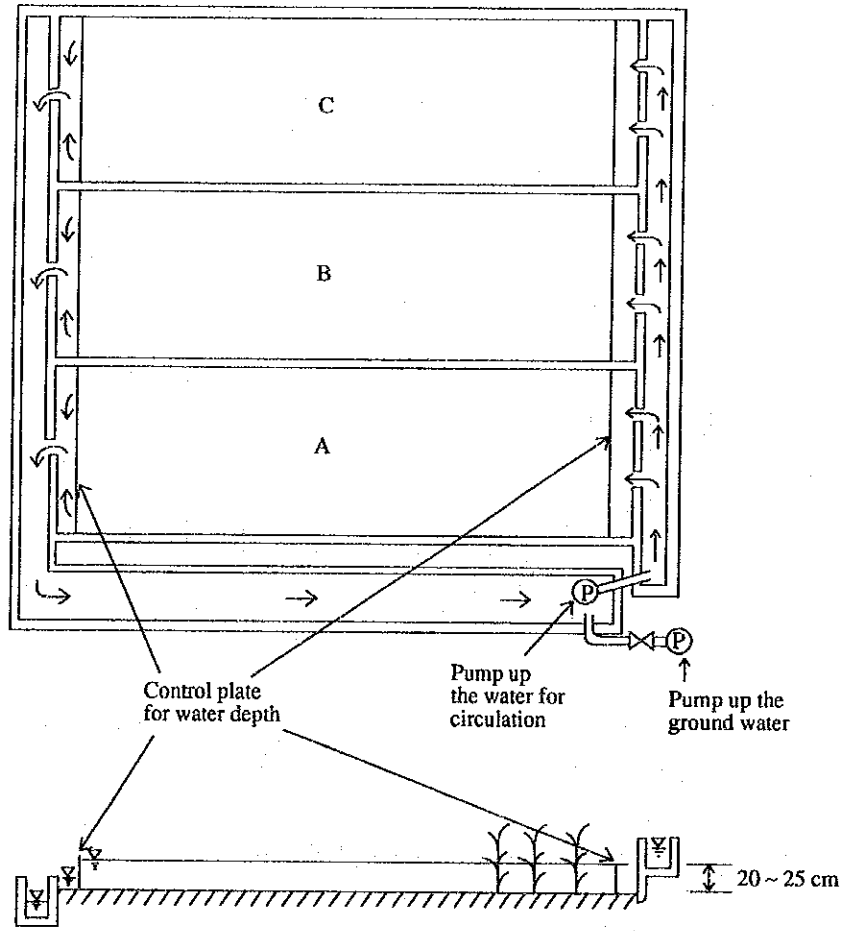


Fig. 3 Scheme of paddy field irrigated with cool water.

② Water depth is kept at 20 ~ 25 cm.

③ Cold treatment is applied starting from the panicle-initiation stage of the earliest entry to the heading time of the latest entry, for 50 days ~ two months.

④ Suitable number of plants per entry is 5 ~ 7.

⑤ Tolerance to cool-weather damage is estimated from the fertility of 10 ~ 15 panicles in each entry in each plot.

Lodging resistance

Culm strength is rated by recording the standing features of plants at maturity and classified into 1: extremely high (no lodging), 3: high (most plants leaning), 5: intermediate (most plants show moderate lodging at angles of about 45°), 7: low (most plants nearly flat), 9: extremely low (all plants flat).

Viviparity:

① Three panicles per entry are harvested 30 ~ 35 days after heading.

② Tagged panicles are put in a glasshouse or in an incubator with saturated humidity.

③ Germination percentage is determined for on each entry compared with check varieties.

<Optional items>

Resistance to Helminthosporium leaf spot (*Cochliobolus miyabeanus*)

Resistance to Helminthosporium leaf spot is classified into: 1 extremely high, 3: high, 5: intermediate, 7: low, 9: extremely low.

Resistance to dwarf

Reaction to dwarf is classified into 1: high, 9: low.

Resistance to rice waika virus

Resistance to rice waika virus is classified into 1: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 9: extremely low.

Resistance to sheath blight (*Rhizoctonia solani*)

Resistance to sheath blight is classified into 1: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 9: extremely low.

Resistance to rice stem maggots (*Chlorops oryzae*)

Resistance to rice stem maggot is classified into 1: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 9: extremely low.

Resistance to rice water weevils (*Lissorhoptrus oryzophilus*)

Resistance to rice water weevils is classified into 1: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 9: extremely low.

Salinity tolerance

Salinity tolerance is classified into 3: high, 5: intermediate, 7: low.

Low temperature germinability

Based on the germination percentage of seeds tested at 12°C for 10 days, low temperature germinability is classified into 1: extremely high (90%), 3: high (70%), 5: intermediate (50%), 7: low (30%), 9: extremely low (10%).

3. Tertiary characters

<Essential items>

Yield

Evaluation of yielding ability of an entry is carried out usually in an observation plot. Observation plot consists of 4 rows, 5 ~ 7 m long without replication. To facilitate roguing of off-types, hills are planted with a single plant spaced at 30 × 15 cm or 30 × 20 cm. Check varieties are planted every several rows as references to be used for adjustment of yield depending on soil heterogeneity and other environmental factors.

1,000 kernel weight

A random sample of 1,000 fully ripened whole grains with 15 % moisture content is weighed using a precision balance.

Panicle threshability

Panicles harvested at maturity are rubbed by hand. Based on the amount of detached grains, threshability is classified into 2: extremely low (few or no grains detached), 3: low (less than 5% of grains detached), 4: rather low (less than 10% of grains detached), 5: intermediate (25% of grains detached), 6: rather high (30 ~ 40% of grains detached), 7: high (more than 50% of grains detached), 8: extremely high (most of grains detached).

Grain quality

The most important factor for the evaluation of grain quality is the appearance of brown rice grains, which is often called simply "grain quality". However, grain quality is a general character, with the following parameters: percentage of white belly and white core, thickness of seed coat, depth of grooves, percentage of perfect grains, color and gloss of grains, and size and shape of grains. As the milling rate is affected by the grade of grain quality, grain quality is an important factor determining the commercial value of rice.

Since the grain quality of rice is affected by the climatic conditions during the growth period, large differences in grain quality sometimes can be observed among early, medium and late maturing entries. Early maturing entries may be of poor quality in certain years, while late maturing entries may be of poor quality in other years. Therefore, it is necessary to compare entries with check varieties belonging to the same maturity class.

Based on the translucency, uniformity of size and color of grains, apparent grain quality is classified into 9 classes from 1: high-high; no or few brown rice grains with white belly and white core, in addition to the high translucency, uniformity in size and shape, clear amber color with high glossiness to 9: low-low; 100 % white belly or white core brown rice with low glossiness.

Amount of white belly grains

Frequency and size of white belly in grains are classified into 2: extremely scarce and small, 3: scarce, 4: rather scarce, 5: intermediate, 6: rather abundant, 7: abundant, 8: extremely abundant and large.

Cracked rice

Based on the percentage of cracked rice, entries are classified into 2: extremely scarce, to 8: extremely high.

Eating quality

In Japan, the most important factor determining the eating quality is the stickiness. Based on a panelist test, eating quality is classified into 1: high-high, 2: high-intermediate, 3: high-low, 4: intermediate-high, 5: intermediate, 6: intermediate-low, 7: low-high, 8: low-intermediate, 9: low-low. Usually, Koshihikari is classified into 2, Hatsuboshi into 3, Nipponbare into 4, Akihikari into 5, Toyonishiki into 6.

<Optional items>

Top weight

Total dry weight of shoot at maturity is measured and expressed as kg/a.

Amylose content

Amylose content is the trait most closely linked to the eating quality. It is usually expressed as a percentage to milled dry weight of rice rather than on a starch basis. Amylose content is classified into waxy (0 ~ 3%), dull (4 ~ 15%), low (16 ~ 20%), intermediate (21 ~ 25%) and high (≥ 26%).

Content of protein

The percentage of protein content in brown rice is measured.

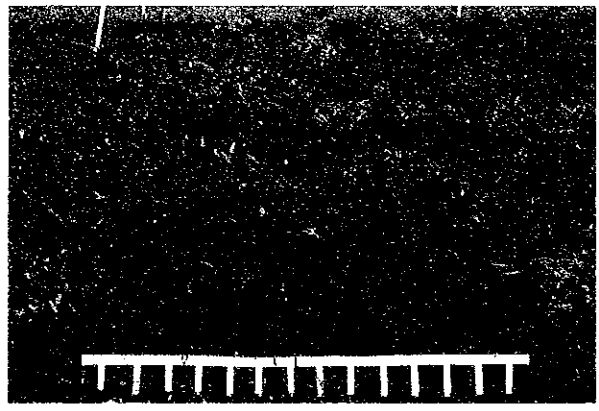


Photo. 1 Field test for resistance to leaf blast.

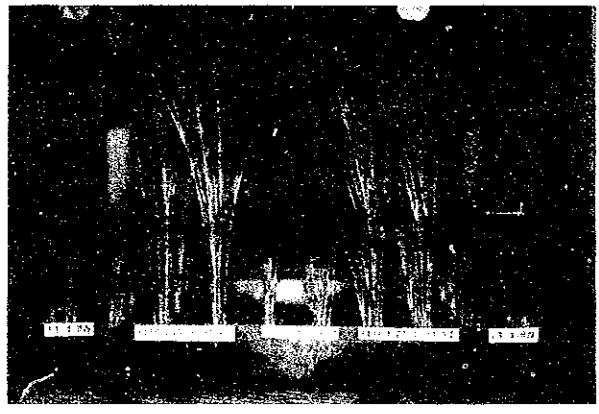


Photo. 2 Seedling test for resistance to brown planthoppers.

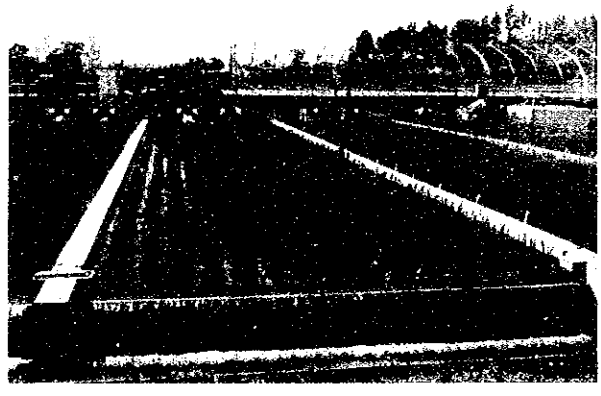


Photo. 3 Test field for cold tolerance at Fujisaka branch of Aomori AES.

III-2. Evaluation of Genetic Resources

Wheat

by

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CONTENTS

Wheat

III- 2. Evaluation of genetic resources

Wheat

1. Early stage observation

Germinability

Based on the percentage of germination, classify into 3: low (lower than 60%), 5: intermediate (between 3 and 7), 7: high (higher than 80%).

Germination rate

Percentage of grains germinated or rooted at 20°C within 72 hours after sowing. Usually, culture dishes 9 cm in diameter and Toyo filter papers No. 22 are used. 4 - 5 drops of pure water are added to 100 grains. Tests are carried out with more than 3 replications.

Germination percentage

Percentage of grains germinated or rooted at 20°C within 7 days after sowing. 100 grains are tested with more than 3 replications.

Degree of seedling emergence

If the percentage exceeds 80%, seedling emergence is rated as high, if less than 60% as low, and between 80 or 60% as intermediate.

Anthocyanin pigment in coleoptile

Color of coleoptile changes by the presence or absence of anthocyanin pigment on seedlings at the germination stage. Accessions are classified into 0: absent, 1: present. Horoshirikomugi and Norin 61 are classified into 0, Norin 64 into 1.

Stem number

Branches with the tip appearing above a leaf sheath or from the slit of the leaf sheath are counted as tillers. The number includes the main stem plus tillers. In the case of

row seeding, measure the stem number in 4 areas within a distance of 50 cm. In the case of broadcasting, count the stem number in 5 areas more than 50 cm^2.

Stem number per m^2

Count stem numbers per m^2.

Active leaf number

Count numbers of active leaves (less than half of leaves dead). Generally, leaf activity is estimated by dividing the leaf blade into 5 equal parts. Developing leaves are not counted. Parts where the colour turned yellow and assimilating ability was lost are considered as dead parts.

Life span of individual leaf

Duration from emergence to death when the dead part accounts for more than half of the leaf. Express by 1 day unit.

Plant age based on leaf number

The growth stage of a crop is expressed by the leaf numbers in the main culm. Measure length (m) of leaf blade appearing above the leaf sheath of the preceding leaf (n-1) of the main culm (or tiller culm), and measure the leaf blade length (M) when fully unfolded. Calculate plant age by the following formula: Plant age (leaf number) = (n-1) + m/M.

Leaf blade length

Measure the length of a leaf blade (0.1 cm) which appeared from the tip of leaf to auricle and is fully developed. Be careful to take data while the leaf tip does not yet wither.

Leaf area

Measure leaf weight and leaf area in 4 areas wider than 0.25 m^2, and get the ratio of leaf area to leaf weight (R). Calculate the total leaf area over more than 1000 cm^2 by

the following formula: Leaf area (A) = R × leaf weight (W). For the measurement of the leaf area, leaf area meter can be used. Expressed by 0.1 cm^2 unit.

Leaf area index (LAI)

Leaf area index which is the ratio of leaf area to the unit ground area is calculated by the following formula. LAI = plant numbers per unit ground area × leaf area per plant.

Description of damage during experiment

Describe the kind of damage and severity indicating the date when the damage occurred. Damage can be caused by many factors, such as, lodging, diseases, insects, cold, drought, water-logging, snow pile, frost, frost heave, wind and soil acidity. Degree of damage is described as follows; very heavy (100% damaged), heavy (over 90%), intermediate (over 70%), light (over 50%), rare (over 30%), none (0%).

2. Primary characters

<Essential items>

Growth habit

Growth habit is observed based on the plant type before the beginning of internode elongation. Growth habit is classified into 2: extremely erect, 3: erect, 4: rather erect, 5: intermediate, 6: rather prostrate, 7: prostrate, 8: extremely prostrate. Norin 61 is classified into 4, Nanbukomugi into 7.

Plant height

The distance between the ground surface and the leaf tip in the field. After the sampling measurement, the distance between the root crown to the tip of the highest leaf. The plant height at maturity is represented by the culm length plus spike length. In case of row seeding, measure the highest height at intervals of 10 cm per 50 cm in 4 areas with normal growth. In case of broadcasting, measure the highest height of 5 plants per $50m^2$ in 4 areas with normal growth. In total, measurement is carried out 20 times.

Culm length

The distance from the ground level up to the neck of spike is measured while standing, and after pulling up plants the distance from the rooting node to the neck of spike is measured. In case of row seeding, 20 culms are measured within a distance 10 cm in selected 4 areas with normal growth. In case of hill seeding, measurement is carried out in 20 hills. Culm length is classified into 2: extremely short, 3: short, 4: rather short, 5: intermediate, 6: rather long, 7: long, 8: extremely long. Norin 10 and Asakazekomugi are classified into 3, Horoshirikomugi into 5, Norin 61 into 6 or 7.

Internode length

Internodes which are longer than 0.5 cm are numbered and their length is measured. The first number is given to the node from the neck of spike to the uppermost node. Expressed by 0.1 cm.

Spike length

The distance from the panicle base to the ear tip is measured on the same stem where the plant height is measured.

Spike number

The number of spikes including late emerging heads and damaged heads is counted. In case of row seeding, counting is carried out in 4 areas at a distance of 50 cm. In case of broadcasting, count in 5 areas of more than 50 cm^2.

Spike number per m^2

Calculated from the above value.

Effective spike number

Number of spikes excluding late emerged heads and damaged ones.

Awnedness

Awnedness is classified into 0: none, 2: extremely scarce, 3: scarce, 4: rather scarce,

5: intermediate, 6: rather abundant, 7: abundant, 8. extremely abundant. Horoshirikomugi is classified into 0, Aobakomugi into 2, Norin 61 into 5, Asakazekomugi into 6.

Color of glume

Color of glume is classified into 1:light yellow, 2: yellow, 3: yellow brown, 4: brown, 5: red brown, 6: red, 7: red purple, 8: purple, 9: dark purple or black. Asakazekomugi and Horoshirikomugi are classified into 2, Norin 61 into 4.

Grain size

Grain size is closely correlated to 1,000 grain weight. By comparing with standard samples or measuring weight, classify into 2: extremely small (1,000 grain weight is less than 29g), 3: small (30~32g), 4: rather small (33~35g), 5: intermediate (36~38g), 6: rather large (38~41g), 7: large (41~42g), 8: extremely large. Norin 61 and Asakazekomugi are classified into 5~6, Horoshirikomugi into 7.

Grain colour

Classified into 0: white, 1: light yellow, 2: yellow, 3: yellow brown, 4: brown, 5: red brown, 6: red, 7: red purple, 8: purple, 9: dark purple. Asakazekomugi is classified into 2, Horoshirikomugi into 3 and Norin 61 into 4~5.

Jointing stage

Jointing stage is the time when stems start elongating. It coincides with the beginning of the internode elongation, when the length of the main stem reaches 2 cm. The date is recorded.

Earliness of internode elongation

Earliness of internode elongation is classified into 2: extremely early, 3: early, 4: rather early, 5: intermediate, 6: rather late, 7: late, 8: extremely late. Norin 61 is classified into 3 or 4, Asakazekomugi into 3.

Date of first heading

The date of first heading corresponds to the appearance of an ear tip from a leaf sheath not related to awn. Enough care should be taken not to record variants. The date is recorded.

Heading stage

Heading stage occurs when 40-50% of ears headed. The date is recorded and heading stage is classified into 2: extremely early, 3: early, 4: rather early, 5: intermediate, 6: rather late, 7: late, 8: extremely late. Asakazekomugi is classified into 3, Norin 61 into 5.

Number of days to heading

The number of days is counted from the sowing date to the heading date.

Full heading date

The date when 80-90% of ears headed.

Flowering date

The date when several flowers of a spike which started flowering is recorded. For assessing a population, the date when 40~50% of total ears flowered is recorded.

Maturity stage (Maturity date)

The date when the color of more than 80% of ears, leaves, stems and necks of spikes turned yellow, and when the color of rachises and grains became faded and grains became so hard that they could be notched with a nail is recorded as maturity date. It occurs usually about 40 days after flowering. Maturity is classified into 2: extremely early, 3: early, 4: rather early, 5: intermediate, 6: rather late, 7: late, 8: extremely late. Asakazekomugi is classified into 2 and Norin 61 into 5. The optimum time for mechanical harvest falls 3~4 days after the maturity date.

<Optional items>

Culm diameter

Measure the widest culm diameter at the maturity stage and classify into 2: extremely slender, 3: slender, 4: rather slender, 5: intermediate, 6: rather thick, 7: thick, 8: extremely thick. Norin 61 is classified into 5, Asakazekomugi into 6, Hiyokukomugi into 7.

Culm angle

The degree of culm angle to the perpendicular direction is measured at intervals from full heading to maturity and classified into 2: extremely acute, 3: acute, 4: rather acute, 5: intermediate, 6: rather obtuse. 7: obtuse, 8. extremely obtuse. Horoshirikomugi is classified into 3, Norin 61 into 5, Asakazekomugi into 7.

Degree of waxiness

The degree of waxiness on culm and spike is observed at the heading date (indicate the specific organs where data are taken) and classified into 0: none, 2: extremely scarce, 3: scarce, 4: rather scarce, 5: intermediate, 6: rather abundant, 7: abundant, 8: extremely abundant. Horoshirikomugi is classified into 0, Norin 61 into 3, Aobakomugi 7.

Leaf colour

Leaf colour is observed at the time when growth habit data are taken, at the vegetative stage (tillering stage) and at the reproductive stage (booting stage). The date should be recorded. Classify into 2: very light, 3: light, 4: rather light, 5: intermediate, 6: rather dark, 7: dark, 8: very dark. Asakazekomugi is classified into 4, Norin 61 into 5.

Degree of leaf sheath waxiness

The degree of waxiness is observed on the upper most leaf sheath at the heading stage, and classified into 0: none, 2: extremely scarce, 3: scarce, 4: rather scarce, 5: intermediate, 6: rather abundant, 7: abundant, 8: extremely abundant. Horoshirikomugi is classified into 0, Norin 61 is into 3~4, Aobakomugi into 7.

Degree of leaf sheath pubescence

The degree of pubescence is observed on the leaf sheath and classified into 0: none, 2: extremely scarce, 3: scarce, 4: rather scarce, 5: intermediate, 6: rather abundant, 7: abundant, 8: extremely abundant. Horoshirikomugi and Nanbukomugi are classified into 0, Norin 61 into 5, *T. timopheevi* into 7.

Leaf blade angle

The angle of the leaf blade is measured at the full heading stage, and classified into 0: none, 2: extremely acute, 3: acute, 4: rather acute, 5: intermediate, 6: rather obtuse, 7: obtuse, 8: extremely obtuse. Norin 61 is classified into 5, Horoshirikomugi into 7.

Leaf flecking

The amount of yellow spots on leaf blades is observed at the full heading stage, and classified into 0: 1 none, 2: extremely rare, 3: rare, 4: rather rare, 5: intermediate, 6: rather abundant, 7: abundant, 8: extremely abundant. Horoshirikomugi is classified into 0, Norin 61 into 2~3, Omasekomugi into 5~6.

Spike shape

Spike shapes are classified into 1: gimlet type, 3: spindle type, 5: stick type, 7: club type, 9: fan type. Nanbukomugi is classified into 1, Norin 61 into 3, Horoshirikomugi into 5.

Spikelet density

By counting the number of internodes on a rachis and dividing by the length (cm) of a rachis on 10 spikes, or by visual estimation, classify into 2: extremely low, 3: low, 4: rather low, 5: intermediate, 6: rather high, 7: high, 8: extremely high. Hiyokukomugi is classified into 3, Norin 61 into 4~5, Asakazekomugi into 6, Horoshirikomugi into 7.

Spike exsertion

The distance from the tip of the flag leaf sheath to the neck of the spike is measured at the maturity stage, and classified into 2: extremely short, 3: short, 4: rather short, 5: intermediate, 6: rather long, 7: long, 8: extremely long. Norin 61 is classified into 5.

Awn length

Measure the longest awn out of 20 spikes or compare with awns of standard cultivars, and classify into 2: extremely short, 3: short, 4: rather short, 5: intermediate, 6: rather long, 7: long, 8: extremely long. Nanbukomugi and Aobakomugi are classified into 2, Norin 61 into 5.

Glume pubescence

Pubescence of glume is classified into 0: absent, 1: present. Horoshirikomugi and Norin 61 are classified into 0, Norin 44 and Mikunikomugi into 1.

Grain shape

Grain shape is classified by the ratio of length to width into 2: extremely round, 3: round, 4: rather round, 5: intermediate, 6: rather long, 7: long, 8: extremely long. Asakazekomugi is classified into 4, Norin 61 into 5, Haruhikari into 7.

Winter survival percentage

Winter survival percentage is the ratio of plant number after overwintering to the plant number before winter. In case of row seeding, take data from 4 areas at a distance of 50 cm. If the total area is less than 1 m^2, add sampling area up to more than 1 m^2. In case of broadcasting, measure in 5 areas over 50 cm^2.

3. Secondary characters

<Essential items>

Degree of winter habit

Degree of winter habit is estimated by the heading response to successive sowings at 10 day intervals from early spring, compared with checks. Classified into 1: I (extremely low), 2: II, 3: III, 4: IV, 5: V, 6: VI, 7: VII (extremely high). Norin 61 is classified into 2, Aobakomugi into 4, Nanbukomugi into 5.

Winter or spring wheat

Classified into 2: spring wheat, 8: winter wheat. Norin 61 is classified into 2, Nanbukomugi into 8.

Susceptibility to pre-harvest sprouting

About 10 spikes taken several days before maturity are kept under low temperatures (about 20°C) and high moisture conditions. One week after, the degree of sprouting is evaluated and classified into 2: extremely low, 3: low, 4: rather low, 5: intermediate, 6: rather high, 7: high, 8: extremely high. Norin 61 is classified into 3, Aobakomugi into 6.

Threshability

Data are taken at the maturity stage, and classified into 2: extremely low, 3: low, 4: rather low, 5: intermediate, 6: rather high, 7: high, 8: extremely high. Norin 61 is classified into 3, Horoshirikomugi into 5, Aobakomugi into 6.

Lodging resistance

Lodging resistance can be evaluated when lodging occurred in the field. The growth stage corresponding to the lodging occurrence and the degree of lodging are recorded and lodging resistance is classified into 2: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 8: extremely low. Asakazekomugi is classified into 2, Norin 61 into 5~6, Nanbukomugi into 7.

Resistance to yellow mosaic virus

Resistance is evaluated based on the presence of disease symptoms in infested fields and the uniformity of heading at full heading stage. Classified into 2: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 8. extremely low. Asakazekomugi is classified into 3, Norin 61 is into 5~6, Nanbukomugi into 7.

Resistance to scab (*Giberella zeae*) disease

Resistance is evaluated based on the presence of symptoms in ears from the dough-ripe stage to the maturity stage. Classified into 2: extremely high, 3: high, 4: rather high,

5: intermediate, 6: rather low, 7: low, 8: extremely low. Nanbukomugi is classified into 4, Norin 61 into 5.

Resistance to powdery mildew (*Erysiphe graminis*) disease

Resistance is evaluated based on the lesion density and classified into 2: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 8: extremely low. Ushiokomugi is classified into 3, Norin 61 into 5.

Resistance to leaf rust (*Puccinia recondita*) disease

Resistance is evaluated based on the presence of symptoms during the ripening period. Classified into 2: extremely high, 3: high, 4:. rather high, 5: intermediate, 6: rather low, 7: low, 8: extremely low. Ushiokomugi is classified into 2, Norin 61 and Horoshirikomugi into 5, Asakazekomugi into 7.

<Optional items>

Presence and percentage of black point grains

Percentage of black point grains is observed and classified into 0: none, 2: extremely rare, 3: rare, 4: rather rare, 5: intermediate, 6: rather abundant, 7: abundant, 8: extremely abundant. Nanbukomugi is classified into grade 0, Norin 61 into 2 and Fujimikomugi into 3.

Cold tolerance

Cold tolerance is evaluated based on the degree of winter survival and classified into 2: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 8: extremely low. Nanbukomugi is classified into 3, Horoshirikomugi into 5, Norin 61 into 7.

Wet endurance

Wet endurance is estimated based on the wet injury observed in the field. Growth stage of injury occurrence should also be recorded. Classified into 2: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 8: extremely low. Norin 50 is classified into 3, Norin 61 into 5.

Snow tolerance

Snow tolerance is evaluated based on the degree of damage by snow mold after snowbreak, and classified into 2: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 8: extremely low. Nanbukomugi is classified into 3, Horoshirikomugi into 5, Norin 61 into 7.

Tolerance to soil upheave by frost

Classified into 2: extremely high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 8: extremely low. Mikunikomugi is classified into 3, Norin 61 into 7.

4. Tertiary characters

<Essential items>

Yielding ability

Grain yield is measured in gram and converted into kg per are. Yielding ability is classified into 2: extremely low, 3: low, 4: rather low, 5: intermediate, 6: rather high, 7: high, 8: extremely high. Norin 61 is classified into 5, Asakazekomugi into 6.

Weight per spike

Measure the weight of spikes fully air-dried and cut at the neck. In case of row seeding, data are taken in 4 areas at a distance of 50 cm. In case of hill seeding, measurement are made in 20 plants in 4 areas, and in case of drill seeding, measurements are made in an area more than 1.2 m^2.

Grain weight per spike

Measurements are made in the same spikes as those in which weight per spike was measured.

Total weight

Total dry matter weight of plants above ground is measured after sun curing. Total fresh weight and sampling area should also be recorded.

Grain weight

After removing screenings, fully sun-cured grains are weighed. The sampling area which varies with the experimental purposes should also be recorded.

Spike weight per plant

The mean weight of spikes excluding late appearing heads is measured in the same materials for which the total weight was measured.

Grain weight per plant

The grain weight per plant is measured after threshing. Use the same materials for which the total weight was measured.

Percentage of dry matter

Put fresh plant materials in weighing bottles, and record the weight. Fresh weight is obtained by subtracting the weight of the containers. Put the weighing bottles containing the materials in a drier at 100~102°C, and dry them until the weight becomes almost constant. Measure the weight after cooling in a desiccator. Dry matter weight is calculated by subtracting the container weights.

Dry weight ratio

The ratio of dry matter to fresh weight is calculated.

Culm base weight

The weight of the culm base 10 cm in length from the lowest internode longer than 0.5 cm is measured after the materials are fully sun-dried. Sampling numbers vary with the purpose of the experiment. The measuring unit is 0.1g.

Spikelet number per spike

Count for 10 or more spikes.

Mean fertile spikelet number per spike

Count for more than 10 spikes.

Fertile spikelet percentage

The percentage of fertile spikelets to the total number of spikelets is calculated using more than 10 spikes.

Sterile spikelet number

Sterile spikelets per spike are counted for more than 10 spikes excluding damaged spikelets.

Grain number per spike

Grain numbers are counted for more than 10 spikes. Grain numbers are also calculated by the following formula: grain number per spike = total grain weight/spike number/1,000 grain weight × 1,000.

Grain number per spikelet

For the calculation the following formula is used: grain number per spikelet = grain number per spike/spikelet number per spike.

Plump grain percentage

Grains with a width exceeding 2.0 mm are considered as plump grains. The weight ratio of plump grains, which remain on the sieve after sieving with a vertical screen wider than 2.0 mesh for 5 minutes, to the weight of original grains is measured using grains weighing more than 200 g.

Grain yield per are

After the water content of grains is adjusted to 12.5%, grain yield is obtained (0.1kg/a).

1,000 grain weight

Counting of grain numbers for 20 g grains with 12.5% water content is repeated 3~5 times to obtain the 1,000 grain weight. Classify into 2: extremely light, 3: light, 4: rather light, 5: intermediate, 6: rather heavy, 7: heavy, 8: extremely heavy. Aobakomugi is classified into 3, Norin 61 into 5~6, Asakazekomugi into 6.

Test weight

One liter weight of grains is obtained as average values of three times measurements using a special vessel with a capacity of exactly 1 liter. Water content of grains should be adjusted to the standard water content of 12.5%. Classified into 2: extremely light, 3: light, 4: rather light, 5: intermediate, 6: rather heavy, 7: heavy, 8: extremely heavy. Aobakomugi is classified into 3, Asakazekomugi into 4, Norin 61 into 5, Haruhikari into 7.

Grain quality

Based on visual inspection, grain quality is classified into 1: extremely high, 2: considerably high, 3: high, 4: rather high, 5: intermediate, 6: rather low, 7: low, 8: considerably low, 9: extremely low. Norin 61 and Horoshirikomugi are classified into 4.

Grain hardness

Grain hardness depends upon the amount of hard starch particles. Based on microscopic observation or on the value of BM ratio, grains are classified into 2: extremely soft, 3: soft, 4: rather soft, 5: intermediate, 6: rather hard, 7: hard, 8. extremely hard. Horoshirikomugi is classified into 4, Norin 61 into 5, Asakazekomugi into 6, Aobakomugi into 7.

Glassiness of grain

Grains are classified into 3: floury (in case glassy kernels account for less than 30%), 5: medium (intermediate), 7: glassy (in case glassy kernels account for more than 70%). Norin 61 and Asakazekomugi are classified into 3, Horoshirikomugi is into 5, Haruhikari into 7.

Crude protein content of grain

Total nitrogen content of grains is usually obtained by the Kjeldahl method and converted into protein content using the coefficient 5.70.

Crude protein content of 60% flour

Calculation is made based on the following formula: crude protein content of 60% flour = total nitrogen content of 60% flour × 5.70. Crude protein content of 60% flour is classified into 2: extremely low, 3: low, 4: rather low, 5: intermediate, 6: rather high, 7: high, 8: extremely high. Asakazekomugi is classified into 4, Norin 61 into 5, Haruhikari into 7.

<Optional items>

Flour yield

Flour yield is calculated by the following formula: flour yield = flour weight / total (flour+bran+shorts) weight, and classified into 2: extremely low, 3: low, 4: rather low, 5: intermediate, 6: rather high, 7: high, 8: extremely high. Norin 61 is classified into 5, Asakazekomugi into 7.

Milling score

Flour yield is adjusted by the content of ash by the following formula: milling score = 100 - {(80 - flour yield) + 50 × (ash content of straight flour - 0.30)}. Expressed by the figures up to the first decimal points and classified into 2: extremely low, 3: low, 4: rather low, 5: intermediate, 6: rather high, 7: high, 8: extremely high. Norin 61 is classified into 5, Asakazekomugi is into grade 7.

Ash content of 60% flour

Ash content of 60% flour is obtained by measuring the weight of remained ash after burning 3g of 60% wheat flour in a muffle at 600 ±50°C for 3 ~ 4 hours adding alcoholic solution of magnesium acetate.

III-3. Evaluation of Genetic Resources

Barley

by

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CONTENTS

Barley

III-3. Evaluation of genetic resources

Barley

1. Primary characters

<Essential items>

Uzu or normal type

Uzu is one of the dwarf types. Uzu type: 8 can be distinguished from normal type: 2 by the appearance of a coleoptile (Fig. 1).

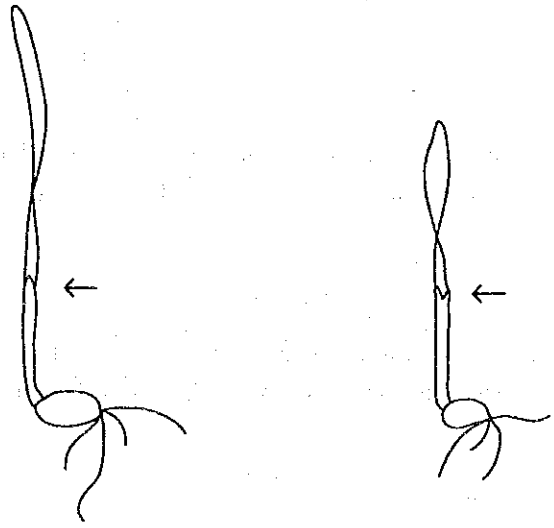
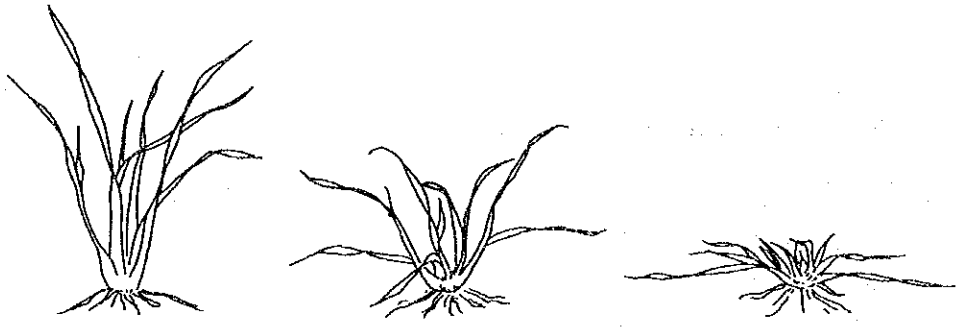


Fig. 1 Uzu or normal type.

Growth habit

Plant type is observed before the internode elongation stage. In areas with continuous snow cover, the plant type is observed before snow cover. This character is classified into 3: erect, 5: intermediate, 7: prostrate (Fig. 2).



Erect Intermediate Prostrate

Fig. 2 Growth habit.

Culm length

Distance from the ground level to the panicle base is measured in centimeter. More than 5 average culms are measured more than 10 days after heading.

Spike length

Distance from the panicle base to the top of the panicle (except awn) is measured. More than 5 spikes are measured at the same time as the culm length.

Number of rows of spikes

Spikes in which lateral caryopses are sterile and only the central ones ripen are referred to as two-row type (2). Spikes in which all caryopses become ripe are referred to as six-row type (8). Six-row type with a low spikelet density is classified as sparse six-row type (5).

Awn length

Length of awn is classified into 2: short, 5: intermediate, 8: long.

Glume color

Glume color of mature caryopsis is classified into 0: white, 2: yellow, 4: brown, 6: red, 8: purple, 9: black.

Naked or covered barley

Types in which glumes adhere to a grain are classified into 2: covered barley, while types in which glumes can be easily separated from grains are classified into 8: naked barley.

Heading date

The date, when 40 ~ 50% of productive tillers have headed, is recorded as heading date. The exsertion of the base of the panicle from the leaf sheath is used as the criterion of heading.

Maturity date

The date, when the base color of more than 80% of the panicles turned yellow, is recorded as maturity date. At this stage, grains become as hard as wax.

<Optional items>

Thickness of culm

Thickness of the central part of the top internode is observed at the maturity stage and classified into 3: low, 5: intermediate, 7: high.

Waxiness of culm

Waxiness of internode on the top internode is observed at heading time and classified into 0: absent, 9: present.

Auricle

Presence of auricle, which is a ramus on the basal part of a lamina, is observed and classified into 0: absent, 9: present.

Leaf color

Leaf color is recorded at the same time as the growth habit, and classified into 3: light, 5: medium, 7: dark.

Waxiness of leaf

Waxiness of flag leaf is observed at heading time and classified into 0: absent, 9: present.

Waxiness of panicle

Waxiness of panicle is observed at heading time and classified into 0: absent, 9: present.

Pubescence of leaf sheath

Pubescence on leaf sheath is observed and classified into 0: absent, 9: present.

Spike shape

Spike shape of two-rowed barley is classified into 2: stick type, 5: intermediate type, 8: arrow feather type (Fig. 3).

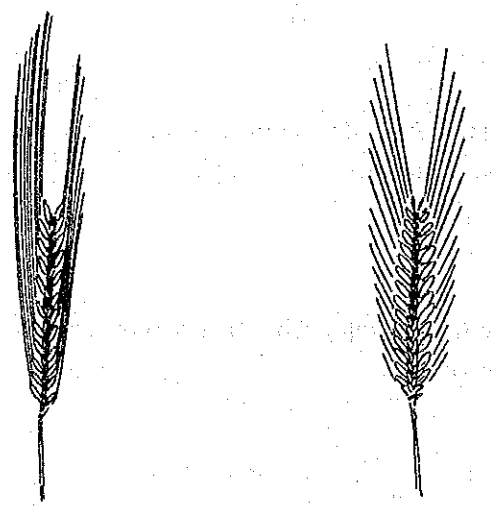


Fig. 3 Spike shape.

Spike density

Number of nodes per unit length of rachis is counted and spike density is classified into 3: sparse, 5: intermediate, 7: dense.

Nutation of spike

Degree of nutation is observed at the maturity stage and classified into 3: erect, 5: intermediate, 7: nutant.

Awn-spike length ratio

Based on the ratio of the distance between the panicle base and top of awn to the length of panicle, this character is classified into 2: less than 2.0, 8: over 2.0.

Flowering habit

Flowering habit is classified into 2: cleistogamic, 8: chasmogamic.

Grain shape

Ratio of length to width of grains is observed and classified into 3: spherical, 5: intermediate, 7: slender.

Grain size

Grain size is checked and classified into 3 : small, 5: intermediate, 7: large.

Rachilla hair length

Length of hair of rachilla, which is attached to the base of the rachilla, is observed and classified into 2: short, 5: intermediate, 8: long.

2. Secondary characters

«Essential items»

Degree of winter habit

Winter barley requires a certain period of exposure to chilling temperature to head. In the late spring sowing, heading time of barley entries with a high degree of winter habit is delayed and in extreme cases they remain in the rosette state. This character is examined by observing the heading behaviour of entries sown several times, compared with check cultivars. They are graded into 1: I extreme spring type to 7: VII extreme winter type.

Lodging resistance

Based on the time of occurrence of lodging and the degree of lodging, lodging resistance is classified into 3: high, 5: intermediate, 7: low.

Resistance to yellow mosaic

Based on the severity of mosaic lesions, yellowing and reduction of plant height, resistance to yellow mosaic is classified into 3: high, 5: intermediate, 7: low. If possible, reaction to a particular race should be tested.

Resistance to scab (*Gibberella zeae*)

Severity of scab lesions occurring from the dough-ripe stage to the maturity stage, resistance is classified into 3: high, 5: intermediate, 7: low.

Resistance to powdery mildew (*Eryshiphe graminis*)

Based on the severity of the lesions occurring from heading time to flowering time, resistance is classified into 3: high, 5: intermediate, 7: low.

«Optional items»

Resistance to leaf blotch (*Rhynchosporium secalis*)

Based on the severity of lesions at the full heading stage, the resistance is classified into 3: high, 5: intermediate, 7: low.

Earliness of internode elongation

Beginning of internode elongation is observed and classified into 3: early, 5: intermediate, 7: late.

Pre-harvest sprouting

Based on the rate of sprouted grains at maturity, the tolerance to pre-harvest sprouting is classified into 3: high, 5: intermediate, 7: low.

3. Tertiary characters

Parameters related to general quality, such as 1,000 grain weight, test weight,etc. are evaluated. For specific quality, pearling test and malting test are conducted.

<Essential items>

Grain quality

This item is evaluated based on the general appearance of grains, by observing the plumpness, homogeneity, shape and luster and classified into 3: high, 5: intermediate, 7: low.

Wrinkle of crust

The amount of wrinkles on hulls of grains is evaluated and classified into 3: large, 5: intermediate, 7: small. Cultivars with a thin hull of grains show a large amount of wrinkles, while cultivars with a thick hull show a small amount of wrinkles.

1,000 grain weight

Count the number of grains with 12.5% of moisture content in a 20 g sample, and deduce the 1,000 grain weight.

Liter weight

Measure the weight of grains in a liter sample with 12.5% of moisture content.

Content of crude protein

Analyze the whole nitrogen content of barley grains by the Kjeldhal or a similar method, and multiply the value obtained by the conversion coefficient of 6.25.

Pearling quality

Pearling time

Measure the time required to pearl grains up to 55% of the original weight with the Satake Pearler TM-05 type. For one pearling test, 180 g grains of hulled barley and 200 g grains of naked barley are used.

Whiteness of pearled grain

Measure the whiteness of pearled grains with the Whiteness Meter.

Malting quality

It is necessary to make malt for the evaluation of the malting quality. Malt is made using the Micro Malting Equipment. In the first step, barley grains are soaked in water at 13 ~ 15°C, until the water content of the grains reaches a value of 41 ~ 42%. In the second step, grains are put in a germination chamber to accelerate germination for 4 ~ 6 hours, so as to produce green malt. Germination is interrupted when bud elongating reaches 70% of the length of grains. Germinated grains become malt, after being kilned and roots are removed. Temperature for kilning is raised from 40 to 85°C. Time for kilning is 20 hours.

Steeping time

Soaking time required for the water content of grains to reach a value of 42% corresponds to the steeping time. For estimating the steeping time, water content of grains is measured at intervals of about 10 hours after the start of steeping (For example, 40 hours, 50 hours and 60 hours). Steeping time is estimated from the regression curve obtained.

Total nitrogen content of malt

Total nitrogen content of malt is measured by the Kjeldhal or a similar method.

Content of soluble nitrogen of malt

After grinding, malt is mixed with water for 30 min. at 45°C. Then, the temperature is raised stepwise by 1°C/1 min up to 70°C. Then, the temperature decreases to the room temperature, when the malt mixture is filtered to obtain wort. Nitrogen content of wort is expressed as soluble nitrogen content of malt.

Malt extract

By measuring the specific gravity of malt, the percentage of soluble extract is estimated.

<Optional Items>

Diastatic power

Starch saccharification enzyme extracted from malt reacts to starch and based on the reaction, the enzyme activity is estimated.

III-4. Evaluation of Genetic Resources

Soybean

by

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CONTENTS

Soybean

III-4. Evaluation of genetic resources

Soybean

1. Primary characters

<Essential items>

Stem length

Length of the main stem from the cotyledonous node to the terminal node (excluding terminal raceme) of a plant with normal growth is measured. Average values of 20 plants are described in cm.

Number of nodes on main stem

Total number of nodes on the main stem are counted from the cotyledonous node to the terminal node. Average values of 20 plants are described.

Leaflet shape

Shape of fully opened terminal leaflet is observed in the middle of the main stem and classified into 3: round (length/width 1.8 $\geqq$), 5: intermediate, 7: long (length/width 2.2 $\leqq$), 9: narrow.

Flower color

Color of flower on the day of flowering is classified into 1: white, 9: purple.

Hypocotyl color

Color of hypocotyl is observed before defoliation of the primary leaf and classified into 1: green, 9: purple.

Seed coat color

Color of seed coat is observed at the later stage of ripening (in case of mottled seed, lighter color is defined as the basic color irrespective of occupied area, and the basic

color and mottling color are recorded), and classified into 1: yellowish white, 2: yellow, 3: yellowish green, 4: green, 5: light brown, 6: brown, 7: black, 8: mottled.

Hilum color

Color of hilum part is classified into 2: yellow, 3: light brown, 4: brown, 5: dark brown, 6: green, 7: gray, 8: black, 9: others.

Color of cotyledon in seed

After removal of the seed coat or cutting seeds in half, color of cotyledon is observed and classified into 2: yellow, 9: green.

Flowering time

The date when 40 ~ 50% of plants have begun to flower is recorded.

Date of maturity

The date when most pods change color, seed color turns into the original one and pods make a sound by shaking in 80 ~ 90% of plants is recorded as the date of maturity.

100 seed weight

Air-dried 100 fully grown seeds are weighed with 2 replications.

Growth type

Growth type is determined based on the presence or absence of terminal raceme on the main stem and classified into 3: determinate (a large terminal raceme on the main stem), 5: intermediate, 7: indeterminate (no terminal raceme).

<Optional items>

Number of branches

Number of branches which have more than 2 nodes is counted on 20 plants with normal growth and expressed as No./ plant (up to 0.1).

Pubescence color

Color of pubescence is observed at the middle stage of growth and classified into 1: white, 8: brown.

2. Secondary characters

<Essential items>

Resistance to virus diseases

Resistance is evaluated based on the symptoms on plants at the growing stage in infested fields and the appearance of harvested seeds.

1) Preparation of test field

It is important to conduct the test in fields where severe viral diseases frequently occur. The races of the virus in the test field must be identified. No orchards or vegetable fields should be located nearby because spraying of chemicals is sometimes necessary to control pests which may adversely affect the insect vector of virus.

2) Layout of test plot

For each test cultivar, 20 ~ 30 plants are planted in a plot about 4.5 m^2 with 2 replications. It is necessary to set up several bordering rows surrounding the test field.

3) Cultivation method

Test cultivars are grown as one plant per hill following standard cultivation methods. Infected seeds of susceptible cultivar (eg. Darumamasari) are planted in about every 10th row. Control of pest other than virus vectors is conducted if necessary.

4) Observation method and evaluation method

(1) Evaluation of symptoms during the growth stages

Observe the presence of symptoms of viral disease in 20 plants of test cultivar at flowering time when the symptoms are distinct.

The severity is classified based on the following criteria.

Criterion	Symptoms		Score
A	No symptoms	No symptoms	0
B	Doubtful	Symptoms are not distinct	0
C	Mild	Slight infection	1
D	Intermediate	Leaves shrink moderately	2
E	Severe	Leaves shrink markedly	2
F	Very severe	Leaves shrink severely and plant growth stops	4

Severity = (1C + 2D + 3E + 4F) × 100 / 4N

Note: C, D, E, F: No. of plants in each score; N: Total number of observed plants

(2) Evaluation based on mottled grains

Take 300 normal grains randomly after removing damaged grains and immature grains, and count the number of mottled grains. Mottling color varies depending on the color of hilum. In black hilum cultivars, mottling color is black. In brown hilum cultivars, mottling color is brown. In yellow hilum cultivars, mottling color is pale brown. However, mottling of black seed coat can not be detected. Shape of mottling is radiate or faint in soybean mosaic virus (SMV) disease, and ring-spotted in stunt virus disease. Resistance to dwarf virus, Chinese milk vetch stunt virus and alfalfa mosaic virus can not be detected by the observation of grains, because these virus diseases do not cause mottling.

The severity is classified based on the following criteria.

Criterion	Symptoms		Score
A	No symptoms	No mottling	0
B	Mild	Mottling area 1 ~ 15% of seed surface	1
C	Intermediate	15 ~ 30%	2
D	Severe	30 ~ 50%	3
E	Very severe	50%≦	4

Severity = (1B + 2C + 3D + 4E) × 100 / 4N

Note: B, C, D, E: No. of plants in each score, N: Total number of observed plants

Resistance of test cultivars to virus disease is classified based on the severity of the symptoms on plant and mottling of seeds as follows.

Classification of entries	Severity
Highly susceptible	80 ≦
Susceptible	50.1 ~ 80.0
Intermediate	20.1 ~ 50.0
Resistant	0.1 ~ 20.0
Highly resistant	0

2) Inoculation test

Reaction to the pathogenic virus is tested by inoculation to soybean seedlings.

(1) Multiplication of virus race

It is necessary to multiply and preserve each virus race. Soybean viruses harbour many pathogenic races. At present, 5 races (A, B, C, D, E) have been identified for SMV virus in Japan.

For the multiplication of the virus, soybean cultivars susceptible to the viruses are sown in pots and virus races are inoculated to the potted seedlings. Leaves which show typical symptoms are collected and kept under low temperature (-80°C) or freeze-dry conditions.

(2) Inoculation of virus

<1> Sow entries in a pot and inoculate the virus to the primary leaves of seedlings which are half to fully unfolded.

<2> Grind preserved leaves with virus infection in a mortar by adding 10 times volume (fresh weight basis) of 0.1 M phosphate buffer solution (pH 7).

<3> Add appropriate amount of 600 mesh carborundum to the ground leaf solution.

<4> Inoculate virus to primary leaf of entry by rubbing 2 ~ 3 times with absorbent cotton immersed in the ground pathogen virus solution.

<5> After inoculation, carborundum on leaves of test cultivar is washed out with fresh water.

<6> Reaction to virus is evaluated by the appearance of symptom on upper leaves 1~2 weeks after inoculation.

For the virus test, attention should be paid to the following aspects:

① Seeds of entry should be free of virus.

② The experiment should be carried out in a greenhouse or net-house to prevent invasion of virus vectors such as aphids and it is also necessary to prevent the escape of inoculated pathogen outside.

③ It is necessary to disinfect the mortar by boiling before use and incinerate absorbent cotton after inoculation.

④ After the test, plant material is dried and incinerated.

Reaction of check cultivars to viruses and races

Cultivars	SMW					CMV				
	A	B	C	D	E	A	B	C	D	AE
Peking	R	R	R	(R)	(R)	R	R	R	R	R
Harosoy	R	S	R	R	S	(R)	(R)	R	S*	R
Oou 3	(R)	(R)	S	S*	S**	R	R	S	R	R
Tokachinagaha	S	S	S	S	S	R	R	R	R	R
Nemashirazu	R	R	S	S	S	R	R	S	R	S
Fukusennari	R	R	(S)	R	R	S	R	S	R	S
Norin 4	S	S	S	S	S	S	S	S	S	S
Tsurunotamago	S	S	S	S	S	S	S	S	S	S
Shiromame	(R)	S	R	(S)	S	S	S	S	S	S
Dewamusume	R	R	R	R	S	R	R	R	R	R

SMV: Soybean Mosaic Virus. CMV: Cucumber Mosaic Virus.

* Accompanied with stunted leaf. ** Stunting at the plant top.

3) Check cultivars

About 10 cultivars with a positive reaction to the virus based on inoculation test are used as checks. It is recommended to use checks which are adapted to a specific location and grow normally. In Japan, cultivars listed below are used as checks.

For virus test in Yamagata prefecture:

Peking, Harosoy, Oou 3, Tokachinagaha, Nemashirazu, Fukusennari, Norin 4, Tsurunotamago 1, Shiromame, Dewamusume.

For virus test in Nagano prefecture:

Oguradaizu, Hill, Harosoy, Enrei, Shin 4, Ayahikari, Fukusennari, Tachinagaha.

Resistance to cyst nematode (*Heterodera glycines*)

1) Preparation of test field

Test fields are prepared by scattering soil markedly infested with cyst nematodes into furrows, and continuous planting of susceptible cultivars (eg. Kitamusume, Enrei). Test fields are maintained by rotation cultivation of <oat or wheat → soybean cultivar susceptible to cyst nematode → entries of soybean>. It is recommended to use test fields where soybeans show severe symptoms of cyst nematode. The uniformity of infection is important for accurate testing. Planting of susceptible non-pubescent varieties along with pubescent entries is recommended in order to evaluate the infection based on the nematode density. The degree of nematode parasitism can be evaluated based on the amount of cysts on susceptible indicator plants which were planted along with the entries.

2) Size of test plot

A plot of 1.3 ~ 2.4 m^2 is necessary for one entry and the test is replicated 2 times.

3) Check cultivars

The cultivars for which the reaction to the nematode has already been determined are used as checks. As the reaction of the cultivars to the nematode differs depending on the races of nematodes, it is necessary to select checks from the following list.

Strongly resistant checks to Gedenshirazu related race:

Toyomusume, Toyokomachi, Toyosuzu, Nemashirazu, Raiden, Nasushirome, Suzuyutaka.

Strongly resistant checks to Peking related race:

Suzuhime.

Susceptible checks to both races:

Kitamusume, Tachisuzunari, Enrei, Tachinagaha, Fujimijiro.

4) Cultivation method

Entries are grown as a single plant per hill according to standard methods of cultivation. Check cultivars are planted in about every 10th row to observe the density of nematode infection and used for evaluation. In case the reaction to the nematode is estimated only based on the leaf color and grain yield, entries are planted in normal fields for comparison, in addition to infected fields.

5) Observation method and evaluation method

(1) Evaluation based on parasitic rate of cyst nematode

In the middle of the growing stage (60 ~ 90 days after planting) when a large number of nematode cysts are formed on the roots of the susceptible check, pull out carefully 10 plants from every entry. Count the number of cysts on plants and rank them according to the degree of parasitism into 0: number of cysts/plant = 0, 0.5: 5 $\leqq$, 1: 10, 2: 50, 3: 100, 4: 500. Following the next formula, the parasitic index is calculated.

Parasitic index (%) = {Σ (rank $\times$ No. of plants) $\times$ 100/4 (4 $\times$ total No. of plants)}

Based on the parasitic index, the degree of resistance to the cyst nematode is classified into 1: extremely low (index= 66% $\leqq$), 3: low (40 ~ 65 %), 5: intermediate (21 ~ 40 %), 7: high (1 ~ 20%), 9: extremely high (0%).

(2) Evaluation based on leaf color and grain yield

The degree of resistance is determined based on the degree of yellowing of leaves during growth and grain yield at harvest time (mainly from the grain yield in infested field). Color of leaves is observed 3 times around the flowering stage and classified into 5 grades, from 1: deep green to 5: yellow. Grain yield is graded according to the following two indices.

① (grain yield of entry in infested field) / (that of resistant check in infested field) $\times$ 100 (%).

② ①/(ratio of yield of checks in infested field to that in normal field) $\times$ 100 (%)

For the general evaluation, the effects on growth parameters such as flowering time, date of maturity, stem length, number of branches, number of normal pods per plant and 100 seed weight are observed and recorded.

General evaluation

Classification grade	1	3	5	7	9
Degree of yellowing of leaves	3.1≦	2.0 ~ 3.0	1.1 ~ 3.0	1.1 ~ 30	0.4 ≧
Grain yield ratio:					
①*	30≧	31 ~ 50	51 ~ 70	71 ~ 90	91 ≦
②*	30≧	31 ~ 50	51 ~ 80	81 ~ 100	101 ≦

* Explanation is given above.

<Optional items>

Resistance to root necrosis (*Calonectria crotalaria*) like disease

1) Preparation of test field

The experimental plot is set in a field where severe root necrosis like disease occurs every year. To maintain the experimental field, plant residues infected with root necrosis in the previous year are left and allowed to decompose in the field so as to become easily buried in soil, and subjected to shallow cultivation without ploughing just before planting. After planting, drainage ditches are made around the field to keep the soil moisture uniform. Since the occurrence of other diseases and pests may make it difficult to keep the experimental field, it is necessary to prepare a spare field for experimentation by planting susceptible cultivars such as Harosoy and ploughing in the plant debris.

2) Setting of test plot and method of cultivation

Test cultivars are grown following standard methods. Plot for each entry consists of about 10 plants (1.5 m^2) with 5 replications. Two seeds of entry and one seed of Harosoy (susceptible to root necrosis) are sown in one hill.

3) Observation and evaluation

The degree of damage by root necrosis is observed only on soybean plants from the hill where Harosoy showed symptoms of root necrosis five times every 7 ~ 10 days from the pod elongation stage. Every time, 10 plants per cultivar are examined, particularly around the base of plant stems.

The degree of damage is graded based on the following criteria:

Score	Symptoms
0	No symptoms
1	Mild symptoms only at the base of stems
2	Brown symptoms around the base of stems
3	Long brown symptoms around the base of stems
4	The main root becomes rotton and lateral roots become scarce
5	Withered

After calculating the ratio of infected hills (%) (A), average degree of damage (0~5) (B), ratio of degree of damage to Harosoy in the same hill (%) (C), resistance to root necrosis is classified into high, intermediate, low, based on the value of $B \times C$, or $B \times C \times A$. Due to yearly fluctuations of disease occurrence, no definite relationship can be obtained between the specific value and the degree of resistance.

4) Check cultivars

Harosoy is used as a susceptible check. In addition, several common cultivars are used as comparison. In Japan, Shirosennari, Tachiyutaka, Miyagishirome, Nanbushirome are used for this purpose.

Resistance to purple speck (*Cercospora kikuchii*)

1) Preparation of test field

The experimental plot is recommended to be set in a field where the occurrence of purple speck is frequent and soybean is planted continuously.

2) Setting of test plot

The following three plots are set up for the experiment.

Plot A	Standard sowing	Natural infection
Plot B	Late sowing	Natural infection
Plot C	Late sowing	Scattering infected seeds & sprinkling water

3) Method of cultivation

Test cultivars are grown following standard methods. Plot for each entry is about 3.2 m^2. In Plot B and Plot C, test materials are sown about 1 month after standard sowing time. In Plot C, seeds infected with purple speck which were harvested previously are scattered at the pod elongation stage at a rate of 60 g per row and water is sprinkled several times in a month after seed scattering in order to accelerate inoculation.

4) Observation and evaluation

100 g seeds are taken randomly after harvest at maturity and processing, and the severity of purple specks is observed (2 replications). The severity of the symptoms is evaluated regardless of the area of infection based on the criteria described below on the plot where the symptoms are the most severe.

The degree of severity is classified by the following criteria:

Criterion	% of infected seeds	Score
No infection	0	0
Doubtful	0.1 ~ 5%	1
Mild	5.1 ~ 15%	2
Intermediate	15.1 ~ 30%	3
Severe	30.1 ~ 50%	4
Very severe	50.1%≦	5

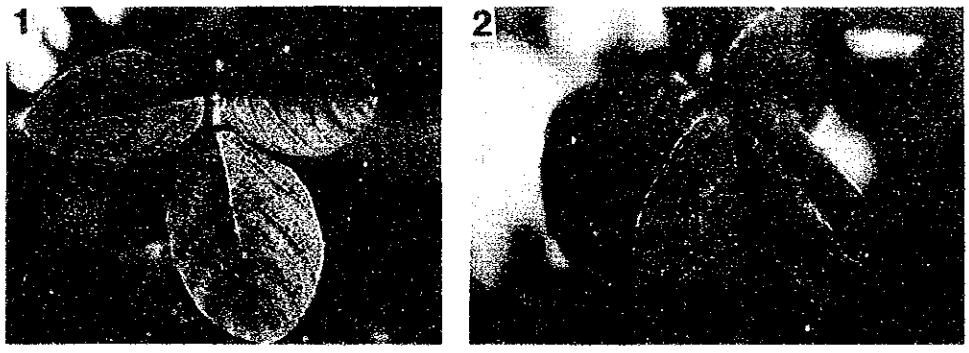
These scores can be easily determined by comparing with standard pictures which were taken on materials already inspected and graded.

5) Check cultivars

As the annual fluctuations of purple speck occurrence are large, standard checks cannot be used. However, some appropriate cultivars are used as for comparison, i. e. Norin 3, Shinmejiro, Fukumejiro, Hatsukari, Norin 2, Ibarakihanayome 1 and Ani in Japan.

6) Pathogenic seeds for the following year

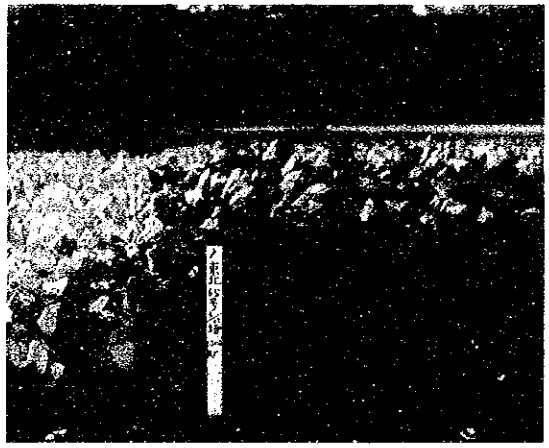
Severely infected seeds are selected and kept as pathogenic seeds for the following year.



1: Inoculated with C race

2: Inoculated with D race

Photo. 1 Mosaic symptoms on upper leaves of cultivar 'Enrei' inoculated with different races of SMV.



Left: Susceptible cultivar 'Tachisuzunari'

Right: Highly resistant cultivar 'Suzuyutaka' (Tohoku 65)

Photo. 2 Varietal differences in resistance to cyst nematode.

III-5. Evaluation of Genetic Resources

Sweet Potato

by

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CONTENTS

III-5. Evaluation of genetic resources

Sweet potato

1. Primary characters

<Essential items>

Plant type

Measure vine length 50 ~ 60 days after planting and classify the plant type into 1: erect, 2: bush, 3: semi-erect, 5: semi-spreading, 7: spreading and 9: markedly spreading.

Twining

Ability of vines to climb adjacent stakes showing twining characteristics. Classify by observation 80 ~ 90 days after planting into 1: non-twining, 3: slightly twining, 5: moderately twining, 7: twining, 9: markedly twining.

Flowering habit

Classify flowering habit under natural conditions into 1: none, 3: scarce, 5: moderate, 7: profuse. Most cultivars do not flower under natural conditions in Japan.

Node pigmentation

Classify the degree of anthocyanin (purple) pigmentation at around the 10th node from the tip 50 ~ 60 days after planting into 1: no pigmentation, 3: few purple spots, 5: many dark purple spots, 7: mostly dark purple, 9: totally dark purple.

Vine pigmentation

Anthocyanin (purple) pigmentation present on vines besides green colour. By observation 50 ~ 60 days after planting classify into 1: no pigmentation, 3: few purple spots, 5: many dark purple spots, 7: mostly dark purple, 9: totally dark purple.

Vein pigmentation

Classify vein pigmentation by the presence of anthocyanin (purple pigment) on the back side of leaves at around the 10th node from the tip of the vine 50 ~ 60 days after planting into 1: no pigmentation, 3: few purple spots, 5: partially purple, 7: mostly purple, 9: totally purple. Kokei 14 is classified into grade 1, Norin 1 into grade 3, Minamiyutaka and Koganesengan into grade 5.

Leaf shape

Classify the predominant shape of leaves at around the 10th node from the tip 50 ~ 60 days after planting into 1: heart-shaped (cordate), 2: teeth lobed heart-shaped, 3: triangular, 4: teeth lobed triangular, 5: single moderate lobed, 6: teeth single lobed, 7: single deep lobed, 8: double moderate lobed 9: extremely deep lobed (Fig. 1).

Colour of vine tip

Classify predominant colour of apex of vine 50 ~ 60 days after planting into 1: light green, 2: green, 3: dark green 4: yellow green, 5: light brown, 6: brown, 7: light purple, 8: purple, 9: purplish brown.

Nectary pigmentation

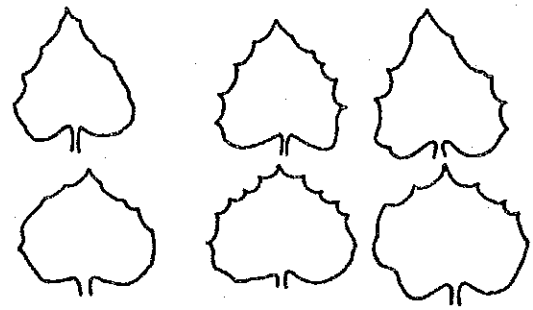
Classify nectary pigmentation of leaves at around the 10th node from the tip of the vine 50 ~ 60 days after planting into 1: no pigmentation 3: slightly pigmented, 5: moderately pigmented, 7: mostly purple, 9: totally purple. Shirosengan is classified into grade 1, Minamiyutaka into grade 5 and Koganesengan into grade 7.

<Optional items>

Vine internode length

Measure vine length and number of nodes 50 ~ 60 days after planting and divide vine length by number of nodes.

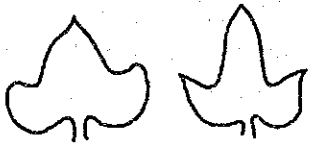
1. Heart-shaped



2. Teeth-lobed heart-shaped

3. Triangular

4. Teeth-lobed triangular



5. Single moderate lobed

6. Teeth single-lobed

7. Single deep-lobed

8. Double moderate-lobed

9. Very deep lobed

Fig. 1 Leaf shape.

Vine tip pubescence

Classify the degree of hairiness at the apex of a vine at 50 ~ 60 days after planting into 1: absent, 3: scarce, 5: moderate, 7: abundant, 9: extremely abundant.

Leaf colour

Classify predominant colour of leaves at around the 10th node the tip 50 ~ 60 days after planting into 1: yellow, 2: yellow green, 3: light green, 4: green, 5: deep green, 6: dark green, 7: light brown, 8: brown, 9: purple.

Leaf size

Classify size of leaves at around the 10th node tip 50 ~ 60 days after planting into 3: small, 5: medium, 7: large. Norin 2 and Beniaka are classified into grade 3, Kokei 14 into grade 5 and Benikomachi into grade 7.

Petiole length

Classify the average petiole length from the base to the insertion with the blade of 10 leaves from the tip of the vine 50 ~ 60 days after planting into 1: very short, 3: short, 5: intermediate, 7: long, 9: very long. Beniaka and Norin 2 are classified into grade 3, Koganesengan into grade 5 (Fig. 2).

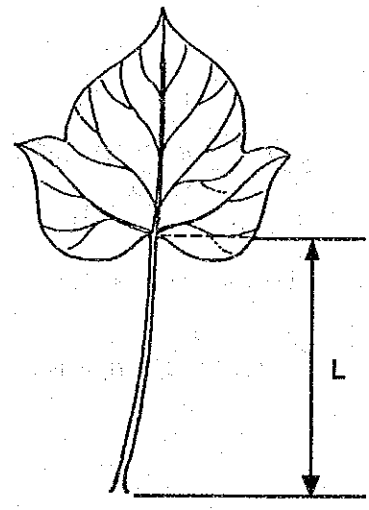


Fig. 2 Petiole length.

Petiole thickness

Classify petiole thickness on the same petioles as above-mentioned into 1: very thin, 3: thin, 5: intermediate, 7: thick, 9: very thick. Beniaka and Norin 2 are classified into grade 3, Kokei 14 into grade 5.

Length of joint part of storage roots

Average length of joint part of storage roots measured for 10 plants is classified into 1: very short, 3: short, 5: intermediate, 7: long, 9: very long. Tamayutaka is classified into grade 3, Norin 2 into grade 5.

Shape of storage roots

Medium to large-sized storage roots of several plants are used for classification. Shape of storage roots is classified into 1: round, 2: round elliptic, 3: short elliptic, 4: elliptic, 5: long elliptic, 6: long oblong, 7: lump. Tamayutaka and Norin 2 are classified into grade 3, Koganesengan into grade 4, Kokei 14 into grade 5 (Fig. 3).

Storage root size

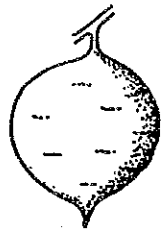
Classify the average storage root weight of 10 plants into 1: very light, 3: light, 5: intermediate, 7: heavy, 9: very heavy. Norin 2 is classified into grade 3, Koganesengan into grade 5 and Tamayutaka into grade 7.

Colour of skin of storage root

Classify predominant skin colour of storage roots which were washed and dried before evaluation into 1: white, 2: yellow, 3: brown, 4: orange, 5: pink, 6: red, 7: purple-red, 8: purple, 9: other colour.

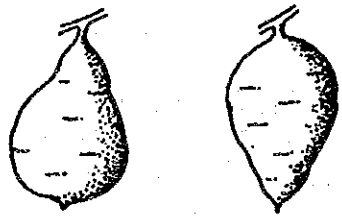
Colour of flesh of storage root

Classify predominant flesh colour of a cross section performed in about the middle of freshly harvested storage roots into 1: white, 2: light yellow white, 3: yellow white, 4: light yellow, 5: yellow, 6: light orange, 7: orange, 8: light orange, 7: orange, 8: light purple, 9: purple.



1. Round

2. Round elliptic



3. Short elliptic

4. Elliptic

5. Long elliptic

6. Long oblong

7. Lump

Fig. 3 Shape of storage root.

Anthocyanin (purple) pigmentation of cross section of storage root

Classify the degree of anthocyanin (purple) pigmentation of a cross section of storage roots into 1: no pigmentation, 3: slight pigmentation, 5: medium pigmentation, 7: mostly pigmented, 9: totally pigmented.

Carotene pigmentation of cross section of storage root

Classify the degree of carotene pigmentation of a cross section of storage roots into 1: no pigmentation, 3: slight pigmentation, 5: medium pigmentation, 7: mostly pigmentated 9: totally pigmented.

Longitudinal grooves

Classify longitudinal grooves of storage root surface into 1: no grooves, 3: slight grooves, 5: medium grooves, 7: deep grooves, 9: very deep grooves. Okimasari and Norin 2 are classified into grade 3, Okinawa 100 and Koganesengan into grade 7.

Vein-like surface of storage root

Classify the degree of vein-like surface of storage root into 1: none, 3: slight, 5: intermediate, 7: heavy, 9: very heavy.

2. Secondary characters

<Essential items>

Grafting compatibility

Grafting test of scions from 3 plants per cultivar to morning-glory stocks. Grafting compatibility is classified into 1: none, 3: low, 5: intermediate, 7: high.

Cross-incompatibility

Based on the observation of the pollen tube elongation of the test cultivar in the stigma of the check cultivar belonging to all the incompatibility groups 4 ~ 5 hours after pollination, cross-incompatibility is classified into 1: A group, 2: B group, 3: C group, 4: D group, 5: E group, 9: others.

Self-compatibility

Based on the observation of pollen tube elongation in the stigmas 4 ~ 5 hours after self-pollination, self-incompatibility is classified into 1: none, 3: low, 5: intermediate, 7: high, 9: very high.

Sprouting ability

Based on the observation of the number of sprouts per storage root, the rate of sprouting, sprouting variability and rate of sprouting growth, sprouting ability is classified into 3: low, 5: intermediate, 7: high. Nakamurasaki is classified into grade 3, Koganesengan, Tamayutaka and Norin 2 into grade 5 and Kanto 90 and Kyushu 77 into grade 7.

Storability

Based on the observation of storage roots kept in a storage room, storability is classified into 3: low, 5: intermediate, 7: high. Koganesengan is classified into grade 3, Okinawa 100 and Norin 2 into grade 5, Tamayutaka and Gifu 1 into grade 7.

Resistance to sweet potato weevil

Based on weevil (*Cylas formalis* Faust) damage in a field, the resistance is classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high. It is difficult to evaluate the resistance to the weevil in Japan, due to the absence of weevils, except in Okinawa and Amami islands.

Resistance to stem rot

Based on the symptoms in a test field infected with stem rot (*Fusarium oxysporum* f. *batatas* (Wr.) Synd. & Hans), the resistance is classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high. Benikomachi is classified into grade 3, Norin 10 and Koganesengan into grade 5, Norin 2 into grade 7.

Resistance to black rot

Based on the symptoms of the inoculated plants in the field, the resistance to black rot (*Ceratocystis fimbriate* Elliot) is classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high. Koganesengan and Kokei 14 are classified into grade 3, Norin 2 into grade 5, Kanto 84 into grade 7. The inoculation is carried out by soaking storage roots into the fungus solution.

Resistance to root-knot nematode

Based on the symptoms in a field infected with root-knot nematodes (*Meloidogyne incognita* var. *acrita* Chitwood), the resistance to the root-knot nematodes is classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high. Koganesengan and Norin 1 are classified into grade 3, Okinawa 100 and Nakamurasaki into grade 5, Norin 2 and Minamiyutaka into grade 7. The test field for root-knot nematode is based on the multiplication of the nematodes after planting of a susceptible cultivar (Norin 1) in the test field.

<Optional items>

Flower colour

Classify flower colour into 1: white, 2: pale purple limb with purple throat, 3: purple, 4: others.

Flower shape

Classify flower shape into 1: rounded, 2: intermediate, 3: pentagonal.

Flower length

Classify flower length based on the measurement of ten flowers into 1: very short, 3: short, 5: intermediate, 7: long, 9: very long (Fig. 4).

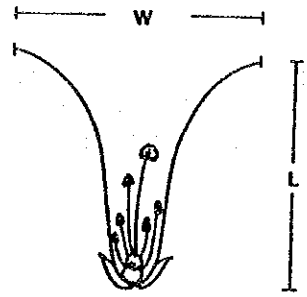


Fig. 4 Flower length and width.

Flower width

Classify flower width based on the measurement of ten flowers into 1: very narrow, 3: narrow, 5: intermediate, 7: wide, 9: very wide (Fig. 4).

Height of anther and stigma

Classify the relative height of anthers compared to the stigma into 1: all anthers are lower than the stigma, 2: outer two anthers are lower than the stigma, 3: equal height.

Stigma colour

Classify stigma colour into 3: white, 5: pale purple, 7: purple.

Homogeneity of sepal length

Classify the homogeneity of sepal length into 1: different, 2: similar.

Sepal shape

Classify sepal shape into 1: ovate, 3: elliptic, 5: obovate, 7: oblong, 9: lanceolate (Fig. 5).

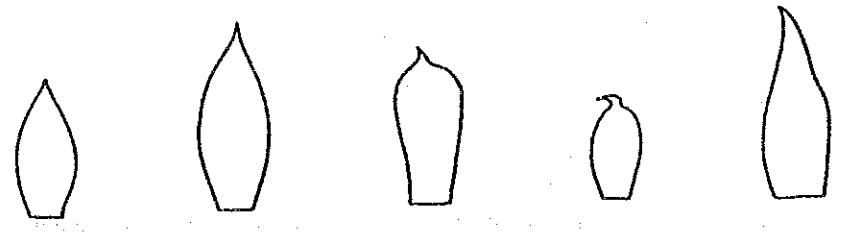


Fig. 5 Sepal shape.

Sepal apex

Classify the sepal apex based on observation into 1: acute, 3: obtuse, 5: acuminate, 7: caudate (Fig. 6).

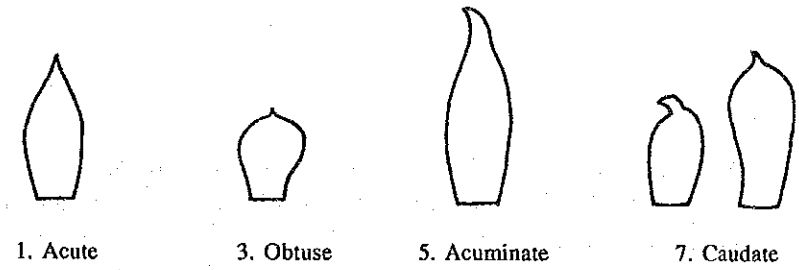


Fig. 6 Sepal apex.

Resistance to root-lesion nematode

Based on the symptoms in a field infected with root-lesion nematodes (*Pratylenchus coffeae* (Zimmermann) Shev. et. Allen), the resistance is classified into 1: very low, 3: low, 5: intermediate, 7: high, 9: very high. Norin 2 is classified into grade 3, Nakamurasaki into grade 5, Minamiyutaka into grade 7. The test field for root-lesion nematode is based on the multiplication of the nematodes after planting of a susceptible cultivar (Norin 2) in the test field.

3. Tertiary characters

<Essential items>

Number of storage roots per plant

Based on the determination of the the average number of storage roots per plant for 10 plants, the number of storage roots per plant is classified into 3: small, 5: intermediate, 7: large. Tamayutaka is classified into grade 3, Okinawa 100 and Norin 2 into grade 5, Koganesengan into grade 7.

Weight of storage roots

Based on the determination of the average weight of storage roots per unit area for 10 plants, the weight of storage roots is classified into 3: light, 5: intermediate, 7: heavy. Beniaka is classified into grade 3, Kokei 14 and Norin 2 into grade 5, Koganesengan into grade 7.

Dry matter content

Fresh roots weighing 2 kg are washed, air-dried and chopped into threads. The chopped potato samples (100 g) are dried with an electric drier. Dry matter content is obtained by measuring the weight of dried potato for classification into 3: low, 5: intermediate, 7: high. Okinawa 100 and Shirosengan are classified into grade 3, Tamayutaka and Norin 2 into grade 5, Kyushu 76 and 79 into grade 7.

Starch content

Samples of chopped potatoes (100 g) are smashed with an electric smasher at about 10,000 r/m for 90 seconds. After overnight sedimentation, take out sedimented matter and dry it with an electric drier. Starch content is calculated by measuring the weight of the dried sample, and classified into 3: low, 5: intermediate, 7: high. Okinawa 100 and Shirosengan are classified into grade 3, Tamayutaka and Norin 2 into grade 5, Koganesengan into grade 7.

Eating quality

Based on the evaluation of boiled storage roots by a panel, the eating quality is classified into 3: poor, 5: intermediate, 7: good. Okinawa 100 is classified into grade 3, Kokei 14 and Minamiyutaka into grade 5, Benikomachi, Beniaka and Beniazuma into grade 7.

<Optional items>

Vine weight

Based on the measurement of the vine weight in a unit area, the vine weight is classified into 3: low, 5: intermediate, 7: high. Okinawa 100 is classified into grade 3, Koganesengan into grade 5, Tamayutaka into grade 7.

Adaptability to early harvest

Based on the measurement of the vine and storage roots weight at early harvest time, the adaptability to early harvest is classified into 3: low, 5: intermediate, 7: high. Beniaka and Minamiyutaka are classified into grade 3, Okinawa 100 and Norin 2 into grade 5, Kokei 14 and Koganesengan into grade 7.

Adaptability to late planting

Based on the measurement of the vine and storage roots weight of cultivars planted late and harvested at the ordinary harvest time, the adaptability to late planting is classified into 3: low, 5: intermediate, 7: high. Beniaka is classified into grade 3, Tamayutaka into grade 5, Okinawa 100 into grade 7.

Adaptability to heavy manuring

Based on the measurement of the vine and storage root weight in a plot with heavy manuring, the adaptability to heavy manuring is classified into 3: low, 5: intermediate, 7: high. The amount of manuring is twice the ordinary amount. Beniaka and Norin 1 are classified into grade 3, Tamayutaka and Norin 2 into grade 5, Koganesengan and Okinawa 100 into grade 7.

III-6. Evaluation of Genetic Resources

Potato

by

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III-6. Evaluation of genetic resources

Potato

1. Primary characters

<Essential items>

Color of young buds

Color of young buds germinated over 2 ~ 3 cm is examined in 5 tubers and classified into 1: white, 2: slightly red, 3: pale red, 4: red, 5: purple red, 6: purple, 8: blue, 9: others. Color of Nishiyutaka is classified into 1, Dejima into 2, Norin 1 into 3.

Color of stem

The dominant color of the main stem at 10 cm above the ground level at the flowering stage (40 ~ 50% of plants have flowered) is examined in 3 ~ 5 plants and classified into 2: green, 3: red, 4: dark red, 5: purplish red, 6: reddish purple, 7: purple, 9: others. Color of Norin 1, Dejima and Toyoshiro is classified into 2.

Number of flowers

Number of flowers in the first flower cluster at the main stem at the full flowering stage (80 ~ 90% of plants have flowered) is examined in 5 plants.

Shape of flower petals

Largest flower in the first flower cluster of the main stem at the full flowering stage is examined and classified into 1: acute, 3: slightly acute, 5: intermediate, 7: slightly obtuse (Fig. 1). Toyoshiro and Danshakuimo are classified into 5, Norin 1 and Waseshiro into 7.

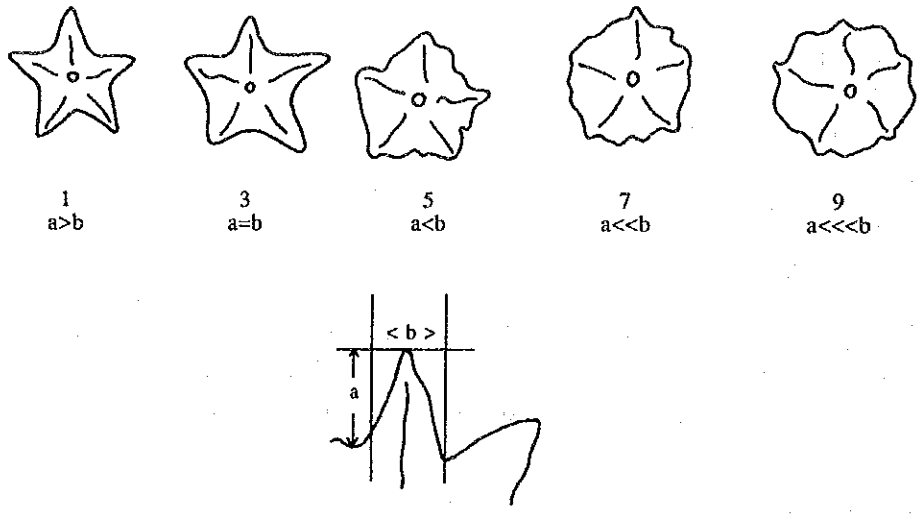


Fig. 1 Shape of flower petals.

Flower color

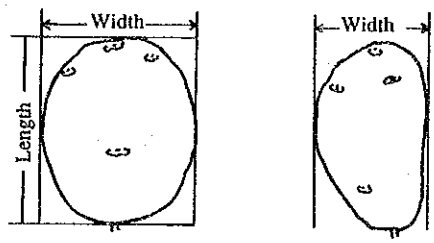
Predominant color of flower petals at the flowering stage is examined for 3 ~ 5 plants and classified into 2: white, 3: red, 4: reddish reddish purple, 5: purple, 6: bluish purple, 7: blue, 8: orange, 9: yellow. Norin 1 and Dejima are classified into 2, Danshakuimo into 4, May queen into 5.

Number of tubers

Count the number of tubers, which are heavier than 20 g at the yellowing stage (when 80% of leaves and stems have turned yellow). Express as number of tubers per plant and classify into 1: extremely small (less than 5), 2: slightly small, 3: small, 4: rather small, 5: intermediate, 6: rather large, 7: large, 8: larger, 9: extremely large.

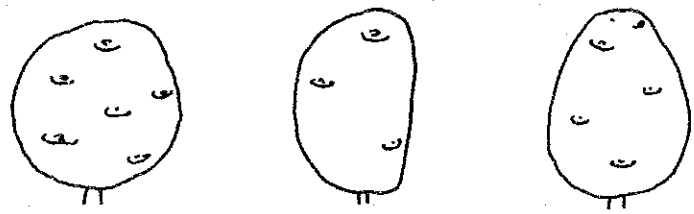
Shape of tuber

15 ~ 20 medium-sized tubers are examined and classified into 1: globular, 2: flat round, 3: ovate, 4: obovate, 5: ellipsoidal, 6: long ellipsoidal, 7: cylindrical, 9: others (Fig. 2). Danshakuimo is classified into 1, Dejima, Norin 1 and Toyoshiro into 2, Dejima into 3, May queen into 6.



Shape index of tuber:
L = length/width
T = thickness/width

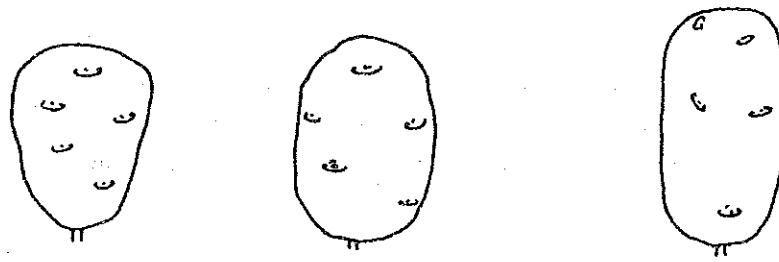
Base (point where stolen is attached)



1: Globular (L ≒ 1.0, T> 0.8)

2: Flat round (L ≒ 1.0, T< 0.8)

3: Ovate



4: Obovate

5: Ellipsoidal (L = 1.5)

6: Long ellipsoidal (L>1.5)

7: Cylindrical (L> 2.0)

Fig. 2 Shape of tubers.

Number of eyes

Count the number of eyes in 10 tubers and express as number of eyes per tuber. Score 1: extremely small, 2: slightly small, 3: small (less than 5), 4: rather small, 5: intermediate, 6: rather large, 7: large (more than 20), 8: larger, 9: extremely large. Waseshiro is classified into 3, Danshakuimo into 5.

Depth of eyes

Examine the depth of eyes in 10 tubers, express by the mean value and classify into 1: extremely shallow, 2: slightly shallow, 3: shallow, 4: rather shallow, 5: intermediate, 6: rather deep, 7: deep. Dejima is classified into 2, May queen into 3, Norin 1 into 5, Danshakuimo and Waseshiro into 7.

Color of tuber coat

Examine the predominant color of the tuber coat and classify into 2: white, 3: whitish yellow, 4: yellow, 5: yellowish brown, 6: brown, 7: pale red, 8: red, 9: purple. Danshakuimo is classified into 3, Norin 1 and Toyoshiro into 5, Benimaru into 7.

Color of flesh of tubers

Cut longitudinally 10 tubers, and examine the color of the flesh of tubers and classify into 2: white, 3: yellowish white, 4: pale yellow, 5: yellow, 6: orange, 7: red, 8: purple, 9: others. Danshakuimo, Norin 1 and Toyoshiro are classified into 2, Dejima and May queen into 3, Nishiyutaka into 4.

<Optional items>

Stem length

Length of main stem from the ground level to the growing point is measured for 5~10 plants at the flower falling stage (when almost all the flowers have dropped) and classified into 1: extremely short, 2: considerably short, 3: short, 4: rather short, 5: intermediate, 6: rather long, 8: considerably long, 9: extremely long. Danshakuimo and Nishiyutaka are classified into 3, Norin 1 into 5, Dejima into 7.

Number of branches

Count the number of branches of 5 ~ 10 plants at the flower falling stage, express as number per plant and classify into 1: none, 2: extremely small, 3: small, 4: rather small, 5: intermediate, 6: rather large, 7: large, 8: considerably large, 9: extremely large. Dejima and Danshakuimo are classified into 3, Norin 1 into 5.

Number of fruits

Examine the number of fruits for 10 plants at the yellow-ripe stage and classify into 1: none, 2: extremely small, 3: small, 4: rather small, 5: intermediate, 6: rather large, 7: large, 8: considerably large, 9: extremely large. Nishiyutaka, Benimaru and Danshakuimo are classified into 1, Norin 1 into 3.

Length of runners

Observe the longest runner for 5 plants at harvest time, and classify into 1: extremely short, 2: considerably short, 3: short, 4: rather short, 5: intermediate, 6: rather long, 7: long, 8: considerably long, 9: extremely long. Danshakuimo and Norin 1 are classified into 3, Benimaru into 5, Dejima into 7.

Secondary color of tuber coat

Examine the secondary predominant color of the tuber coat for 3 ~ 5 plants and classify into 1: none, 2: white, 3: whitish yellow, 4: yellow, 5: yellowish brown, 6: brown, 7: pale red, 8: red, 9: purple.

Appearance of secondary color of tuber coat

Examine the appearance of the secondary color of tuber coat, and classify into 1: none, 2: eye-shaped, 3: eyebrow-shaped, 5: spotted, 6: mottled.

Roughness of tuber coat

Examine the roughness of tuber coat for 3 ~ 5 plants and classify into 3: smooth, 4: rather smooth, 5: intermediate, 6: rather rough, 7: rough. Waseshiro is classified into 4, Norin 1, Danshakuimo and Nishiyutaka into 5, Toyoshiro into 6.

Secondary color of tuber flesh

Cut tubers longitudinally, examine the secondary predominant color of tuber flesh in 3 ~ 5 plants, and classify into 1: none, 2: white, 3: yellowish white, 4: pale yellow, 5: yellow, 6: orange, 7: red, 8: purple. Danshakuimo and Dejima are classified into 1, Benimaru into 7.

Appearance of secondary color of flesh tuber

Cut tubers longitudinally, examine the appearance of the secondary color of flesh of tubers for 3 ~ 5 plants, and classify into 1: none, 2: spotted, 3: linear, 4: mottled, 5: central, 6: ring. Danshakuimo is classified into 1, Benimaru into 6.

Size of tuber

Record the number and weight of tubers heavier than 20 g for 3 ~ 5 plants, and classify into 1: extremely small, 2: considerably small, 3: small, 4: rather small, 5: intermediate, 6: rather large, 7: large, 8: considerably large, 9: extremely large. Danshakuimo is classified into 3, Toyoshiro into 5, Nishiyutaka into 6, Dejima, Norin 1 and Waseshiro into 7.

Internal brown spots

Cut longitudinally 10 ~ 15 tubers which were harvested after the yellow-ripe stage, examine the degree of development of internal brown-spots (Fig. 3) and classify into 1: none, 2: very low, 3: low, 4: rather low, 5: intermediate, 6: rather high, 7: high. Toyoshiro and Waseshiro are classified into 1, Danshakuimo into 2, Norin 1 into 3, Benimaru into 7.

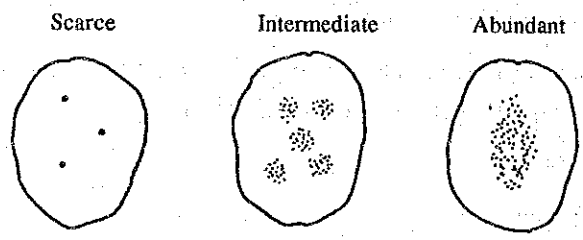


Fig. 3 Diagram of development of internal brown spot.

Hollow heart

Cut longitudinally 10 ~ 15 rather large tubers, examine the development of hollow hearts and classify them compared with Fig. 4 into 1: none, 2: scarce, 3: few, 4: rather few, 5, intermediate, 6: rather abundant, 7: abundant. Benimaru and Nishiyutaka are classified into 1, Norin 1 and Toyoshiro into 2, Danshakuimo into 3.

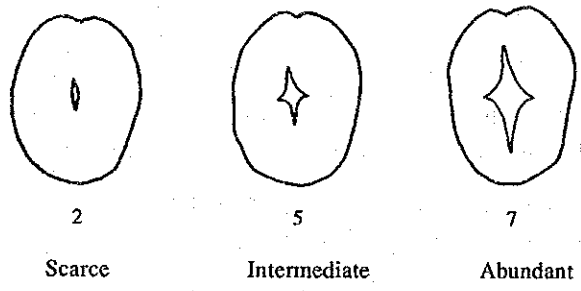


Fig. 4 Diagram of development of hollow heart.

2. Secondary characters

<Essential items>

Storability

Check the decrease of weight of tubers during ordinary storage (weight difference before and after storage), elongation of eyes and the degree of putrefaction (dry rot, late blight etc.) for 10 ~ 20 tubers and classify into 1: extremely poor, 2: considerably poor, 3: poor, 4: rather poor, 5: intermediate, 6: rather good, 7: good, 8: considerably good, 9: extremely good. Dejima is classified into 3, Nishiyutaka, Danshakuimo and Norin 1 into 6.

Resistance to bacterial wilt (*Pseudomonas solanacearum*)

Plant accessions in a field injected with the bacterial wilt pathogen together with check cultivars (Danshakuimo as susceptible check and Norin 1 as resistant check), and evaluate the resistance based on the germinability, percentage of infected plants, amount of dead stems and leaves, degree of browning of vascular bundles in tuber and percentage of putrid tubers. The test is carried out in 10 plants in a plot with 2

duplications. Classify accessions into 1: extremely susceptible, 2: considerably susceptible, 3: susceptible, 4: rather susceptible, 5: intermediate, 6: rather resistant, 7: resistant. Danshakuimo is classified into 1, Benimaru and Dejima into 3, Nishiyutaka into 5, Norin 1 into 7.

Genotype in relation to reaction to late blight (*Phytophthora infestans*):

Test in laboratory

① Preserve a series of *P. infestans* hyphae from all races (genotype r plant is susceptible to all hyphae belonging to race 0 and genotype R_1 plant is resistant to hyphae belonging to race 0 and susceptible to all hyphae belonging to race 1).

② Put filter papers wetted with water at the bottom of a tray. Place 10 ~ 20 pieces of detached vigorous leaves upon the filter paper.

③ 2 ~ 3 drops of a culture solution of zoospores separated at 12 ~ 14°C from hyphae are added to the leaf and are covered with a polyethylene film. To diagnose symptoms, leaves of checks (for example, Danshakuimo which has a r genotype and Waseshiro which has a R_1 genotype) are put together.

④ Keep the samples in a wet room or in a box at about 20°C

⑤ After several days, evaluate the symptoms on detached leaves of the cultivars to determine the genotype related to the resistance (Fig. 5).

Test in field

① Plant test cultivars in a field infested with late blight together with check cultivars. Compared with the first appearance of symptoms on checks, determine the genotype resistant to late blight of the accessions. For example, if the initial time of infection of the accession coincides with that of Danshakuimo, the genotype of the accessions is estimated as r and if it coincides with that of Waseshiro, the genotype is estimated as R_1.

The genotype is classified into 1: r, 2: R_1, 3: R_2, 4: R_3, 5: R_4, 6: R_1R_2, 7: R_1R_3, 8: R_2R_3, 9: others. Norin 1 and Danshakuimo are classified into 1, Toyoshiro, Waseshiro and Dejima into 2.

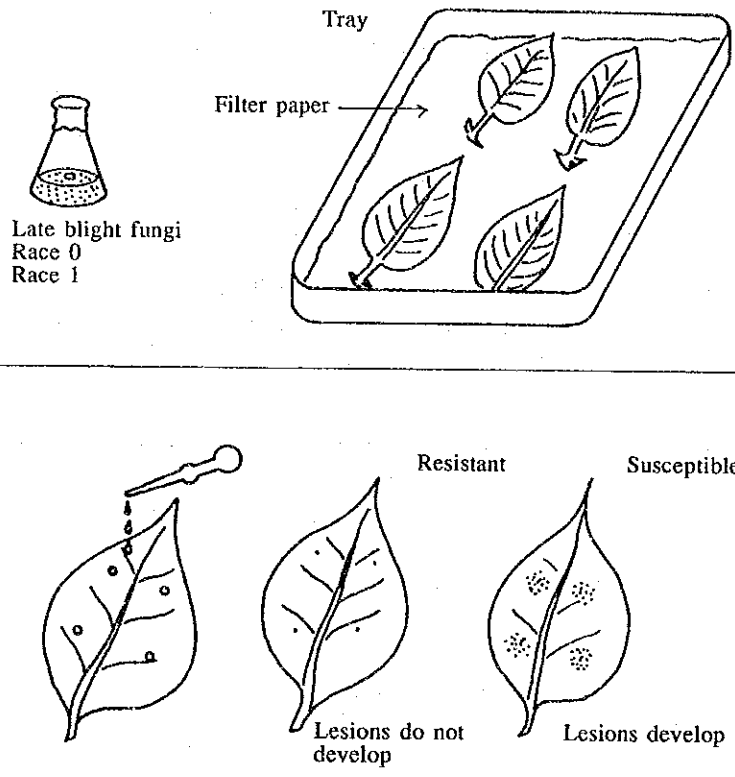


Fig. 5 Diagram of late blight inoculation test.

Resistance to bacterial soft rot (*Erwinia carotovora* spp. *carotovora*):

Test in laboratory

Inoculate bacterial soft rot on the cut surface of tubers and keep them in a wet room at 20 ~ 30°C. Examine the degree of putrefaction of the tubers and evaluate the resistance. Classify accessions into 1: extremely susceptible, 2: very susceptible, 3: susceptible, 4: rather susceptible, 5: intermediate, 6: rather resistant, 7: resistant. Norin 1 and Danshakuimo are classified into 5, Nishiyutaka into 7.

Resistance to tuber putrefaction

Plant accessions in an infested field (when late blight does not appear clearly, potato fields are submerged to accelerate the development of the disease). Examine the degree

of tuber putrefaction at harvest time and at the beginning of storage. Ten plants are tested in a plot with 2 duplications. Classify accessions into 1: extremely susceptible, 2: very susceptible, 3: susceptible, 4: rather susceptible, 5: intermediate, 6: rather resistant, 7: resistant. May queen and Danshakuimo are classified into 3, Norin 1 into 5.

Resistance to powdery scab (*Spongospora subterranea*)

Plant test cultivars in a field infested with powdery scab together with checks (Danshakuimo as susceptible check and Norin 1 as medium check). Evaluate the degree of resistance, by examining the symptoms on tubers at harvest time. Classify accessions into 1: extremely susceptible, 2: very susceptible, 3: susceptible, 4: rather susceptible, 5: intermediate, 6: rather resistant, 7: resistant. Danshakuimo and Benimaru are classified into 3, Norin 1 and Dejima into 5.

Genotypic test for cyst nematode resistance

Test in greenhouse

① Rear eggs and larvae of cyst nematodes.

② Inject eggs and larvae into soil of pot when stems of potatoes grow to a length of 10 ~ 20 cm.

③ Harvest potato plants at the end of the flowering stage and evaluate the resistance based on whether cyst nematodes are attached to potato roots.

④ One plant in one plot, 3 replications (Fig. 6).

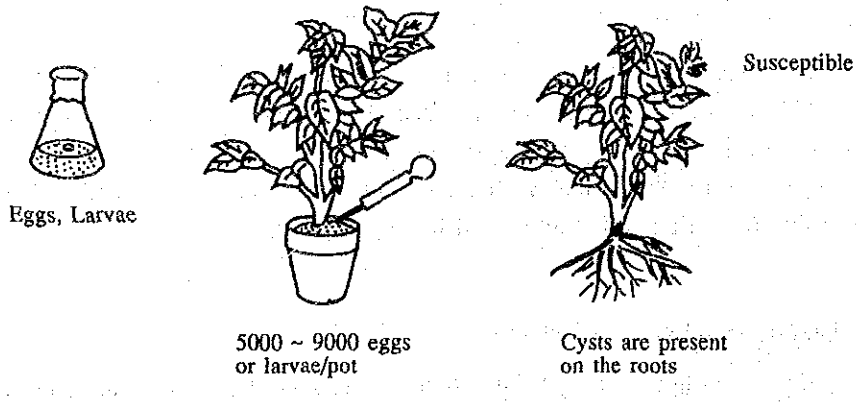


Fig. 6 Diagram of cyst nematode inoculation test.

Test in field

① Plant test cultivars in a field infested with cyst nematodes.

② Harvest potato plants at the end of the flowering stage, and evaluate the resistance based on whether cyst nematodes are attached to potato roots.

③ Five plants in one plot, 2 replications.

Classify accessions into 1: h, 2: H_1, 3: H_2, 4: H_3, 5: H_4. Norin 1 and Danshakuimo are classified into 1, Kitaakari and Musamaru into 2.

<Optional items>

Resistance to leaf roll virus

Test in greenhouse

① Rear aphids infected with leaf roll virus.

② Make the infected aphids fly to the potato seedlings when stems grow to more than 10 cm.

③ Evaluate the resistance by examining the symptoms on the top leaf at the end of the flowering stage.

Test in field

① Plant test potato accessions in a field together with virus-infected potatoes.

② Make aphids visit the potato field without protection.

③ Evaluate the resistance based on the symptoms on the top leaves at end of the flowering stage.

④ Ten plants in a plot, 2 replications.

In case the evaluation of resistance is difficult, plant the harvested potatoes in the next season, to facilitate the evaluation.

Classify accessions into 3: susceptible, 4: rather susceptible, 5: intermediate, 6: rather resistant, 7: resistant, 9: immune. Norin 1 and Danshakuimo are classified into 3, Dejima into 6.

Resistance to Y mosaic virus and X mosaic virus

Test for Y mosaic virus

① Extract the exudate from leaves infected with Y mosaic virus crushed with a mortar.

② Scratch vigorous leaves with carborundum and put the exudate from Y mosaic virus on to the scratched zone.

③ Evaluate the resistance based on the symptoms several days after the inoculation.

Classify accessions into 3: susceptible, 4: rather susceptible, 5: intermediate, 6: rather resistant, 7: resistant, 9: immune. Danshakuimo is classified into 3, Konfubuki into 7.

Test for X mosaic virus

① When cutting seed tubers, alternately cut tubers of test cultivar and virus-infected tubers (sap infection).

② Plant tubers of the accessions together with tubers infected with X mosaic virus and make them come into contact with each other (contact infection).

③ Evaluate the resistance based on the appearance of X mosaic virus symptoms on leaves at the end of the flowering stage.

Classify accessions into 3: susceptible, 4: rather susceptible, 5: intermediate, 6: rather resistant, 7: resistant, 9: immune. Danshakuimo is classified into 3, May queen into 7.

Resistance to black scurf (*Rhizoctonia solani*)

Test by inoculation

① Plant tubers of accessions in pots filled with soil mixed with black scurf pathogen.

② Evaluate the resistance based on the symptoms on the epidermis of tubers after harvest.

③ Ten plants in a plot, 2 duplications.