# Breeding of New Cultivars of Perennial Ryegrass in Japan

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#### Abstract

Persistency of perennial ryegrass is lower as compared with that of other cool temperate grasses because it is more susceptible to summer and winter stresses. To raise its persistency, it is required to increase not only resistance to summer damages caused by drought, high temperature and diseases, but also to winter losses caused by cold temperature and snow mold disease. Some cultivars are practically free from crown rust and have a high-yielding capabilities in the warm regions. Their present level of resistance to other stresses, however, are still to be improved yet to raise their persistency. Resistance to snow mold is independent from the resistance to summer stresses, while it is correlated with cold hardiness. The method employed for testing cold hardiness in the breeding program of the Yamanashi Prefectural Dairy Experiment Station, Japan, proved to be also effective for improving snow mold resistance. However, none of the selections for resistance to summer stresses showed higher levels of resistance to drought and high temperature than the existing cultivars. However, genetic variations within the strains or cultivars were much larger than those among them. Taking those variations into account, a maternal line selection method was adopted to improve stress resistance. Since there was a significant correlation between the parents and their offsprings, the maternal line selection would be an effective procedure. For the purpose of improving the adaptability of perennial ryegrass to warm regions in Japan, a great number of introductions from diverse origins are presently under test, including an attempt to introduce new genes from tall fescue. Some materials would be promising in achieving greater adaptability.

Discipline: Grassland

Additional key words: persistency, summer stress, winter stress

# Introduction

Perennial ryegrass (Lolium perenne L.) was first introduced to Japan from the United States during the 1870's together with some other grasses and legumes. For many years since then, however, its cultivation by farmers had been extremely limited. In the 1960's and beyond, a marked increase in grasslands under the promotion program of the Japanese Government has encouraged them to cultivate this species in large areas. In recent years, about 150 t of its seeds have been sown a year. It has been well recognized that perennial ryegrass has good palatability and high capability in spring and fall growth, both of which adequately meet the grazing purpose. However, some disadvantages have been pointed out in a wide range of yield trials, including low adaptability to the climatic conditions of Japan in general, and low resistance to environmental and biological stresses in particular. In 1964, which was relatively early time of increase in grassland acreage, a breeding work was initiated at the Yamanashi Prefectural Dairy Experiment Station as part of the national forage crops breeding program, under the recognition that the cultivar improvement was of the primary importance for stable and extensive cultivation of perennial ryegrass.

# Breeding new cultivars

# 1) Climatic conditions and breeding objectives

Under the climatic conditions of Japan, the grass cultivars that are introduced from the cool temperate

| Country     | C1           | Mea  | n temperature | e (°C) | Pre        | cipitation (mm) |       |  |  |
|-------------|--------------|------|---------------|--------|------------|-----------------|-------|--|--|
| Country     | Site         | Jan. | July          | Annual | Max.       | Min.            | Total |  |  |
| Japan       | Sapporo      | -6.4 | 20.5          | 7.0    | 156 (Aug.) | 32 (Feb.)       | 887   |  |  |
|             | Morioka      | -3.6 | 22.3          | 9.0    | 185 (July) | 64 (Feb.)       | 1,399 |  |  |
|             | Nagasaka     | 0.5  | 23.8          | 11.8   | 196 (June) | 39 (Dec.)       | 1,253 |  |  |
|             | Kuju         | 0.0  | 24.5          | 12.4   | 449 (July) | 17 (Feb.)       | 1,972 |  |  |
| Netherlands | De Bilt      | 2.0  | 16.5          | 9.2    | 89 (Aug.)  | 50 (Feb.)       | 802   |  |  |
| U.K.        | Manchester   | 3.7  | 15.6          | 9.5    | 85 (Aug.)  | 50 (Apr.)       | 805   |  |  |
| U.S.A.      | Portland     | 4.2  | 20.1          | 12.0   | 173 (Dec.) | 12 (July)       | 996   |  |  |
| New Zealand | Christchurch | 16.6 | 5.7           | 11.5   | 67 (July)  | 37 (Feb.)       | 628   |  |  |
| Australia   | Canberra     | 20.1 | 5.4           | 12.8   | 75 (Oct.)  | 38 (June)       | 639   |  |  |

Table 1. Climatic data of the selected sites in Japan and overseas

regions are generally not well suited for direct use. This is especially true with perennial ryegrass. The temperature and precipitation in Japan are shown in Table 1. Those sites in Japan listed in Table 1 are located in the relatively suitable areas for the cultivation of this species. One of the differences in climatic conditions of those areas is characterized by a greater variation of temperature between the summer and the winter seasons, as compared with the other countries where perennial ryegrass is well grown as the most important forage grass. The winter temperature in the northern part of Japan and the summer temperature in the southern part are not well suited for the ryegrass cultivars from overseas, causing great losses in persistency. In addition, those cultivars are exposed to severe attacks of summer diseases, such as crown rust (Puccinia colonata Cda. sp. lollii Erlcs) and rhizoctonia rot (Rhizoctonia solani Kühn), under a long rainy condition in the central and southern regions of the country. In the northern areas, the snow fall is usually rather deep and ryegrass is seriously attacked by snow mold. The breeding program of the Yamanashi Pref. Dairy Exp. Sta. has fully taken into account such stresses in improving resistance to summer damages caused by drought, high temperature and diseases, and also resistance to winter losses caused by cold temperature.

# 2) Cultivars early developed

When the breeding work was initiated, its major emphasis was placed on resistance to crown rust. The perennial ryegrass cultivars available in those days were quite often damaged by this disease, resulting in poor yield and low persistency. In 1972, however, two cultivars having crown rust resistance, named Kiyosato and Yatsugane, were officially released by the Japanese Government.

Kiyosato is a diploid synthetic cultivar of early heading composed of 10 clones, which were selected under the polycross progeny tests following both individual and clonal selections. The parental clones were selected from the five cultivars introduced from the Netherlands, the United Kingdom, New Zealand and Australia. Kiyosato has an elect plant type and is suited mainly for hay and silage making. Since it is very susceptible to snow mold, it is recommended for cultivation in the central and southern regions.

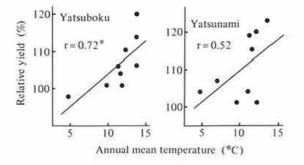
Yatsugane is a synthetic cultivar of very late heading composed of seven clones, which were selected from the two Dutch tetraploid cultivars through the same method as Kiyosato. Because of its very late heading, Yatsugane has an adequate seasonal distribution pattern of production required in grazing use. It has very high resistance to crown rust and moderate resistance to snow mold. It is recommended for cultivation in wide areas, except in Hokkaido, the northernmost island of Japan.

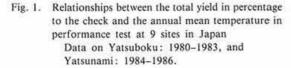
#### 3) Cultivars recently developed

Two cultivars, named Yatsuboku and Yatsunami, were released in 1984 and 1988, respectively.

Yatsuboku is a synthetic cultivar of medium-early heading composed of eight clones, which were derived also from the Dutch tetraploid cultivars. Its parental clones were selected only on the basis of clonal performance without progeny test. Its advantages over the check material, i.e. a Dutch cultivar Reveille, are higher in yielding ability in the warm region as well as in resistance to crown rust (Fig. 1). Yatsuboku shows higher resistance to snow mold and rhizoctonia rot, but slightly lower cold hardiness, as compared with Grimalda, another medium-early cultivar introduced (Table 2). It is widely adapted in Japan except Hokkaido and suited for hay-cut in spring, followed by aftermath grazing.

Yatsunami is a tetraploid synthetic cultivar of late heading, which is about 10 days earlier than Yatsugane at the Yamanashi Pref. Dairy Exp. Sta. Its 10 parental clones were selected, with the same way as the case in Yatsuboku, for resistance to summer stresses, vigorous growth in autumn, and cold hardiness. Nine clones were derived from Dutch cultivars and the remaining one was selected from hybrid ryegrass strain, i.e. Michigan 4N. Yatsunami includes about 20% of fluorescent plants. Its major advantages as compared with the check Yatsugane are higher forage yield in the warm region, resistance to rhizoctonia rot, cold hardiness and seed yield (Fig. 1 & Table 2). However, its snow mold resistance is more susceptible than Yatsugane. In com-





parison with other two late heading cultivars, Yatsunami is superior in cold hardiness and resistance to rhizoctonia rot, but shows slightly lower resistance to snow mold (Table 2). It is adapted to wide regions, except Hokkaido.

It is generally concluded that Yatsuboku and Yatsunami are superior to the other prevailing cultivars in terms of persistency, especially in the warm region of Japan. However, the level of resistance, except crown rust resistance, is not high enough to be qualified as a leading cultivar. Further improvement in this respect is required.

# Efforts for developing new cultivars

#### 1) Crown rust and snow mold resistance

The performance tests of many introductions undertaken at the beginning of the breeding program revealed that a crown rust disease influenced most seriously on forage yield, especially in the autumnal season (Table 3). The evaluation trials of the introduced cultivars from diverse origins carried out in Sapporo indicated that winter damages caused by snow mold were the most important factor of reducing yield and persistency. Tetraploid cultivars, which were introduced from Denmark and the Netherlands, showed high resistance to both crown rust and snow mold (Table 4). For this reason, these tetraploid cultivars were fully used in the breeding program to develop widely adapted cultivars.

# 2) Relationships among the causal factors of persistency

The achievements in yield and its stability of Japanese bred cultivars in the northern region of

| Trait :                    | Yatsuboku | Grimalda | Yatsunami | Yatsugane | Friend | Petra   |
|----------------------------|-----------|----------|-----------|-----------|--------|---------|
| Date of heading            | May 18    | May 13   | June 7    | June 17   | June 5 | June 17 |
| Snow mold resistance       | 2.4       | 3.5      | 3.1       | 2.6       | 3.0    | 2.5     |
| Cold hardiness             | 3.2       | 2.8      | 2.8       | 3.4       | 3.2    | 3.7     |
| Summer injury resistance   | 2.5       | 2.6      | 2.3       | 2.4       | 2.7    | 2.7     |
| Crown rust resistance      | 1.4       | 1.7      | 1.2       | 1.0       | 1.8    | 1.8     |
| Rhizoctonia rot resistance | 3.2       | 3.8      | 2.1       | 3.1       | 2.7    | 2.9     |

Table 2. Heading date and resistance<sup>a)</sup> to diseases and stresses of Japanese and foreign-originated cultivars of perennial rycgrass<sup>b)</sup>

a): Resistance; 1 (high) to 5 (very low).

b): The data from the results in the period 1984 to 1986.

Japan were not satisfactory. It has been one of the concerns in the breeding program that selection for summer stress resistance might have resulted in the loss of snow mold resistance. Snow mold resistance of the clones selected however proved not to be correlated significantly with resistance to crown rust, rhizoctonia rot and other summer stresses in the tests undertaken at the Yamanashi Pref. Dairy Exp. Sta. (Table 5). Contribution rates of those diseases to the total summer stresses estimated with a multiple regression analysis were approximately 25% in two years, 1982 and 1983. This implies that part of the summer damages was caused by hot temperature and drought. It was recognized that snow mold resistance of the breeding materials was positively correlated with their cold hardiness. It was therefore expected that there would be a possibility of developing widely adapted cultivars, which should have resistance to both summer and winter stresses.

#### Table 3. Relationships of crown rust resistance with yield

| Harvesting time | Coefficient of<br>correlation |
|-----------------|-------------------------------|
| April 30        | 0.16                          |
| May 31          | 0.07                          |
| June 23         | 0.43**                        |
| July 14         | 0.64***                       |
| Aug. 9          | 0.49**                        |
| Sept. 13        | 0.89***                       |
| Oct. 1          | 0.88***                       |
| Nov. 17         | 0.74***                       |
| Total           | 0.68***                       |

\*\*, \*\*\*: Significant at 1 and 0.1% level, respectively.

# 3) Test of cold hardiness

In testing cold hardiness, the following method has been employed. Ryegrass seedlings grown in the fields are trimmed at 1 cm above the ground in mid December and percentages of the surviving plants are measured in the following spring. A large difference in percentages of the surviving plants was observed among the cultivars tested. Those materials from the northern counties such as Canada and Finland showed higher surviving rates, while little differences took place among the cultivars in case where the seedlings were not trimmed (Table 6).

# Genetic variations in resistance to drought and high temperature stresses

Information on genetic variations in resistance to drought and high temperature stresses are required for the further improvement of summer damage resistance of perennial ryegrass. The tetraploid

# Table 5. Correlation coefficients of resistance<sup>a)</sup> among the different stresses and diseases

| Characters                               | Correlation coefficients |
|--|--------------------------|
| Cold hardiness (1982)                    | 0.50***                  |
| Resistance to                            |                          |
| Rhizoctonia rot (recorded                | -0.07~0.10               |
| 4 times in 1982-83)                      |                          |
| Crown rust (1982)                        | 0.23                     |
| Resistance to summer injury (1982, 1983) | 0.03, 0.23               |

Correlations between snow mold resistance measured at Sapporo in 1983 and other stress resistance measured at Nagasaka in 1982 and/or 1983. Number sampled: 64. a): Resistance; 1 (high) to 9 (very low).

Table 4. Classification of perennial ryegrass cultivars by winter damage and susceptibility to crown rust<sup>a)</sup>

|                                      |    |    |    | Suscept | ibility <sup>b)</sup> t | o crown | rust |    |    |    |    | 2224 |
|--------------------------------------|----|----|----|---------|-------------------------|---------|------|----|----|----|----|------|
| Winter damage<br>score <sup>c)</sup> | 1  | 1  |    | 2       |                         | 3       | 2    | 4  |    | 5  | 10 | otal |
|                                      | 2X | 4X | 2X | 4X      | 2X                      | 4X      | 2X   | 4X | 2X | 4X | 2X | 4X   |
| 1                                    | 0  | 2  | 0  | 4       | 1                       | 0       | 0    | 0  | 3  | 0  | 4  | 6    |
| 2                                    | 0  | 7  | 0  | 4       | 1                       | 2       | 5    | 0  | 2  | 0  | 8  | 13   |
| 3                                    | 0  | 2  | 2  | 1       | 15                      | 0       | 16   | 0  | 1  | 0  | 34 | 3    |
| 4                                    | 1  | 0  | 1  | 0       | 11                      | 0       | 21   | 0  | 2  | 1  | 36 | 1    |
| 5                                    | 1  | 0  |    |         | 2                       | 0       | 4    | 0  | 1  | 0  | 8  | 0    |

a): The numerics in each lot in the table show the number of cultivars.

b): Susceptibility; 1 (low) to 5 (high).

c): Winter damage score; 1 (slight) to 5 (severe).

Source: Adachi et al. (1976).

|                    |             | 2323242         |       | Percentage of surviving plants in spring <sup>a)</sup> |       |       |           |  |  |
|--------------------|-------------|-----------------|-------|--|-------|-------|-----------|--|--|
| Cultivar or strain | Origin      | Ploidy<br>level | 1     | 976  | 1977  | 1978  |           |  |  |
|                    |             | level           | Trim. | Non-trim.  | Trim. | Trim. | Non-trim. |  |  |
| Kangaroo Valley    | Australia   | 2X              | 13    | 84   | 74    | 79    | 99        |  |  |
| Norlea             | Canada      | 2X              | 40    | 93   | 92    | 99    | 98        |  |  |
| S 23               | U.K.        | 2X              | 27    | 100  | 87    | 89    | 99        |  |  |
| HJA 0167           | Finland     | 2X              | 58    | 99   | 94    | 99    | 99        |  |  |
| Reveille           | Netherlands | 4X              | 14    | 99   | 63    | 74    | 99        |  |  |

Table 6. Survival rate of plants in cold hardiness test

a): Trimmed at about 1 cm above the ground in mid December.

| Table 7. | Resistance o | f the | two | groups | to | summer | stresses |
|----------|--------------|-------|-----|--------|----|--------|----------|
|----------|--------------|-------|-----|--------|----|--------|----------|

|                       |                   | Summer stre | ess resistance <sup>a)</sup>   |  |  |  |
|-----------------------|-------------------|-------------|--|--|--|--|
| Group                 | No. of accessions | Mean        | Summer stress resistance <sup>a</sup> )   Mean Range   6.9 6.2 - 7.4   6.8 6.4 - 7.4 |  |  |  |
| Cultivars introduced  | 21                | 6.9         | 6.2 - 7.4  |  |  |  |
| Strains bred in Japan | 15                | 6.8         | 6.4 - 7.4  |  |  |  |

a): Resistance; 1 (high) to 9 (very low).

strains and the introduced cultivars, from which the former were derived, were subjected to test on the relevant resistance by protecting the materials from summer diseases with chemicals. That test included some clonal lines as a check to estimate genotypic variabilities within the strains and the cultivars under test. Little difference was observed in average resistance between the strains and the introductions tested (Table 7). It was also recognized, in comparisons of the ranges of resistance to summer stresses within each group, that none of the strains surpassed the introductions. However, as far as genotypic variabilities and heritabilities of summer stress resistance in a broad sense are concerned, those estimates on a plant basis and within-a-strain basis were much greater than those on a strain basis and strain-mean basis, respectively (Table 8). This result suggests that there may be a possibility of breeding cultivars which have high resistance to drought and high temperature stresses, under precise tests and repeated selections of the existing materials through generations.

#### 5) Breeding procedure

Since the breeding procedure with the synthetic variety method requires a longer period in advancing generations as compared with the maternal line selection method, the latter procedure was adopted. In the autumnal season, about 15 seedlings of each

Table 8. Components of variance

|                     | Plant basis | Strain basis |
|---------------------|-------------|--------------|
| Phenotypic variance | 1.28        | 0.21         |
| Genotypic variance  | 0.92        | 0.06         |
| Environ. variance   | 0.36        | 0.15         |
| Heritability        | 0.72        | 0.29         |

material line were planted with a 20 cm spacing within a row. In the second year, green yield and resistance to summer stresses were measured on a row basis for selection. In the spring season of the third year, crossings were made among the selected individuals within the selected lines. These half-sib progenies were planted for the next cycle of selection. Cold hardiness was tested in a row-sown plot of 1 m long with the similar method as described in Section 3). In the second generation through this breeding procedure, 17 lines were selected from 83 lines. Those selected lines showed higher yields as compared with the newest cultivar, Yatsunami; i.e. 8 and 20% higher in annual and after-summer yield, respectively. As shown in Fig. 2, a significant correlation is observed between the first and the second generations; in other words, between parent and offspring. This result means that this procedure is effective for selecting drought and high temperature resistance, because the selections are rarely biased by any summer diseases in both generations. The effectiveness of cold hardiness test is not confirmed yet in breeding program.

#### 6) Screening of new breeding materials

Origins of all the tetraploid materials used in the breeding program of Japan have been limited only

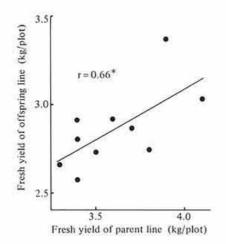


Fig. 2. Relationships of parent and offspring in after-summer yield between the 1st and the 2nd generations

to western Europe. Taking into account that diploid cultivars have also been developed in other countries, breeding materials in Japan may have to be more diversified for further improving their adaptability to warm regions. In fact, some new diploid materials having a potential of high adaptability were identified in the evaluation trials on 157 introductions (Table 9).

#### 7) Use of progenies hybridized with tall fescue

Tall fescue (*Festuca arundinaceae* Schreb.) has greater adaptability than perennial ryegrass, covering the whole areas from northern to southern Japan. An attempt to introduce its relevant genes into tetraploid perennial ryegrass has been presently in progress in the breeding program of Japan, under which a Japanese cultivar of tall fescue Nanryo having high adaptability in warm regions was used for crossing as a pollen parent. No hybrids were obtained from the crossing with tetraploid cultivars, but some progenies were obtained in the crosses with diploid plants (Table 10). Since these progenies were all male-sterile, they were crossed with a tetraploid cultivar, Yatsuboku. All the progenies obtained showed good seed fertility in the second back-crossing

|                                 |                            |                             | 1986                           |                             |                              |                             | 1                            | 987                            | 1                  |
|---------------------------------|----------------------------|-----------------------------|--------------------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|--------------------------------|--------------------|
| Cultivar (origin) <sup>a)</sup> | Rhiz.<br>rot <sup>b)</sup> | Crown<br>rust <sup>b)</sup> | Summer<br>stress <sup>b)</sup> | Fall<br>yield <sup>c)</sup> | Total<br>yield <sup>c)</sup> | Fall<br>yield <sup>c)</sup> | Total<br>yield <sup>c)</sup> | Fall<br>coverage <sup>d)</sup> | Date of<br>heading |
| Barenza (NL)                    | 3.0                        | 1.4                         | 1.8                            | 1.8                         | 3.6                          | 0.7                         | 5.4                          | 85                             | June 8             |
| Verna Pajbjerg (DK)             | 3.0                        | 2.4                         | 2.3                            | 1.6                         | 2.9                          | 0.7                         | 4.6                          | 95                             | May 14             |
| Grasslands Nui (NZ)             | 2.3                        | 2.4                         | 2.3                            | 1.7                         | 3.4                          | 0.7                         | 4.2                          | 95                             | May 12             |
| Pamir (I)                       | 1.3                        | 2.4                         | 1.0                            | 1.6                         | 3.1                          | 0.7                         | 4.9                          | 98                             | Apr. 24            |
| Tarpan (CZ)                     | 2.3                        | 2.6                         | 3.0                            | 1.5                         | 3.4                          | 0.5                         | 4.8                          | 90                             | May 30             |

1.3

2.8

0.6

4.4

87

May 8

Table 9. Resistance to diseases and other characters of the selected diploid cultivars

a): NL; Netherlands, DK; Denmark, NZ; New Zealand, 1; Italy, CZ; Czechoslovakia, J; Japan.

3.3

b): Resistance; 1 (high) to 9 (very low).

1.3

3.0

c): kg/plot (0.8 m<sup>2</sup>).

d): %.

Kiyosato (J)

| Table 10. | Results of the cross | between L. | perenne | as | seed | parent | and |
|-----------|----------------------|------------|---------|----|------|--------|-----|
|           | F. arundenaceae cv.  | Nanryo     |         |    |      |        |     |

| Cultivar        | Ploidy | No. of    | Total no. | Obtaine | d progeny | Hybrid | l/spike |
|-----------------|--------|-----------|-----------|---------|-----------|--------|---------|
| or strain level | cross  | of spikes | Self      | Hybrid  | Ave. Max  |        |         |
| Yatsuboku       | 4X     | 53        | 219       | 70      | 0         | 4      | -       |
| Kiyosato        | 2X     | 28        | 138       | 30      | 112       | 0.81   | 24.3    |
| D-No. 10        | 2X     | 19        | 119       | 28      | 69        | 0.58   | 4.7     |

200

with Yatsuboku. The breeding program with a backcrossing method for selecting resistance to summer stresses has been under way since 1989.

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(Received for publication, Oct. 16, 1990)