系統間および系統内の変動係数からみた北海道の異なる気象地帯から収集されたシロクローバ系統の特徴

奥村 健治・高田 寛之・廣井 清貞・磯部 祥子*

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目 次

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次へ進む 研究報告第191号目次へ

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農業・食品産業技術総合研究機構 北海道農業研究センター

Features of the white clover lines collected from two distinct climate regions in Hokkaido revealed by coefficients of variation between and within lines

Kenji Okumura, Hiroyuki Takada, Kiyosada Hiroi and Sachiko Isobe*

Summary

There are two types of farming areas in Hokkaido which are classified based on their climate in winter: a soil-freezing area, mainly located the eastern part of the island, and a snowy area, mainly located in the southern, central and northern parts. There are different factors in these two areas which contribute to the overwintering ability of forage crops. In this study, we conducted a field evaluation to detect the differences in phenotypic character among white clover （Trifolium repens L.） lines collected from various regions in Hokkaido. Seventy-eight collected lines and 6 control cultivars were evaluated for 32 traits, including morphology, overwintering and disease damage, for two years. The process of analysis was 1） detection of the traits with statistically significant difference among lines, 2） comparison of coefficients of variation （CVs） between and within lines for each trait, 3） comparison of the mean values of traits exhibitng lower CVs than those of the cultivars, and 4） grouping the lines by principal component analysis using those traits. Significant differences were found in thirty of 32 traits among lines and 22 traits showed lower CVs in collected lines than in the cultivars. In five traits, plant size, leaf density, vigor in the seeding year, budding and vigor in the spring of the 2nd year, differences were found between two groups based on the whether the collecting site was in a soil-freezing or snowy area. The results can be applied to a breeding program of white clover, especially to improve adaptation to soil-freezing areas.

次へ進む　前に戻る　目次に戻る

Features of the white clover lines collected from two distinct climate regions in Hokkaido revealed by coefficients of variation between and within lines

Introduction

Hokkaido is the northernmost and largest prefecture in Japan. Its cool climate and vast land areas have made dairy production by grassland farming an important industry. Recently the use of clover in Hokkaido has been re-evaluation to reduce the environmental loads as well as the economic costs of livestock production.

Introductions of forage species including legumes and grasses to Hokkaido from foreign countries especially European and North American countries were started about a hundred year ago and then the agronomists were selecting superior species and cultivars. Because of the harsh winters in Hokkaido, however, these forage species had lower yield and persistence. In 1964 the first cultivars of red clover （Trifolium pratense L.） were developed, and their productivity and persistence was remarkably improved compared to introduced cultivars （Matsu-ura, 1987）. The main breeding materials of these cultivars were collected plants which were selected, repeatedly regenerated, and adapted to the Hokkaido environment, i.e., ecotypes. After that cultivars of red clover and alfalfa （Medicago sativa L.） were mainly developed from ecotypes or surviving plants in Hokkaido （Yamaguchi et al., 1995a,b）.

White clover （T. repens L.） is one of the most important forage legumes in Hokkaido, mixed with grasses in pasture and meadow. In spite of the recently policy on promotion of grazing we did not developed any white clover cultivars which were selected in Hokkaido and had superior adaptability to this environment. Large variations and usefulness of ecotypes and local populations for improving the adaptability have been reported in cold tolerance and spring growth （Annicchiarico et al., 2001）, tolerance to freezing （Collins and Rhodes, 1995） and adaptation to low phosphorus （Caradus, 1994）. In Japan Yonemaru et al . （2004） found a superior local population in winter survival and plant spreading from the collection of local populations in northern Tohoku region. So we began a new breeding program for white clover in 2001, and first collected the seed from various regions in Hokkaido for incorporation into a basic breeding population.

The objective of this study was to investigate whether any phenotypic tendencies occurred in white clover lines collected from Hokkaido compared to cultivars. For this purpose, we carried out a field evaluation for two years and followed the three step analysis process: 1） coefficients of variation （CVs） between and within lines were compared between collected lines and cultivars, 2） traits with lower CVs in collected lines were compared between the two collection areas, soil-freezing （hereinafter freezing） and snowy, and 3） and further traits with different values were statistically analyzed based on leaf size and the two areas, and the ecotypic differentiation discussed.

Features of the white clover lines collected from two distinct climate regions in Hokkaido revealed by coefficients of variation between and within lines

Materials and Methods

Seventy-eight seed accessions （collected lines） from Hokkaido （Fig 1） and six cultivars as controls, which were Japanese or introduced, were used in this study. Table 1 shows their leaf size group, collection areas and sites, classification into two climatic areas （freezing and snowy） based on the 75cm deepest snow fall lines （Toyooka et al., 1983）, and passport number of the NIAS （National Institute of Agrobiological Sciences, Japan） Genebank. The collections were carried out to gather at least 20 mature heads in one site, and the land usages were road sides, parks, meadows where alterations of generation by seed were expected （personal information from Dr. Mitsuru Gau）.

The seeds from the collection lines and cultivars were sown in a greenhouse for nursery on April 14, 2004. The seedlings were transplanted to a field of the National Agricultural Research Center for Hokkaido region, Sapporo, Hokkaido on June 10 and 22, 2004. Individual plants were spaced at 50cm intervals in each ways, and ten plants per plot and 2 blocks for each line were arrayed for randomized block design with two replications. Before the transplanting 20 kg/a of calcium carbonate and 0.21, 1.02, 0.33 kg/a of N, P2O5 and K2O, respectively, were applied into the field. Plants were cut and trimmed at the height of 5cm from ground surface and the diameter of 16cm for evaluation of regrowth in mid August 2004.

Thirty-two traits were evaluated for two years, 2004 and 2005 （Table 2）. These traits were first analyzed to detect the differences among lines by analysis of variance （ANOVA）. Same traits in different years were treated as different traits for the analysis. The lines were also classified into groups based on leaf size by cluster analysis. For comparison of variations between and within lines, the coefficients of variation （CVs） were calculated and compared among both the collected lines and standard cultivars. Finally ANOVA and principal component analysis was applied the traits whether there were differences between two groups based on collecting sites, freezing or snowy areas. All statistical analyses were carried out using SAS and R statistical packages.

Results

Evaluation of traits among lines

A total of thirty-two traits were evaluated for two years （Table 2） and analyses of variance for them were carried out among lines. Statistically significant differences （0.05 > p） among the lines were found in 30 of them, except for internode length in the first summer （LIt-1m） and density of leaves in the first summer （DLf-1m）.

次へ進む　前に戻る　目次に戻る

Results

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次へ進む　前に戻る　目次に戻る

Results

Coefficients of variation between and within lines

The mean, maximum and minimum values of the traits among the lines are shown in Table 3 for the collected lines and in Table 4 for the cultivars. Although similar values were observed between the collected lines and the cultivars in most of the traits, flowering date, FlD-2s, of the cultivars were a little earlier （15.7 on average） than those of the collected lines （17.2 on average）. In addition, the cultivars had more of heads, NHd-2m, （5.3 on average） than the collected lines （4.4 on average）.

The coefficients of variation （CVs） between the collected lines averaged out to 17% and varied from 4 to 48% （Table 3）. Higher CVs were found in traits of degree of flowering in the first fall （DFl-1f, 48%） and number of stolons in the first summer （NSl-1m, 42%）. In contrast, lower CVs were found in traits of leaf color in the second spring （CLf-2s） and leaf density and number （DLf-1m, DLf-1f and NLf-2s）, size of plant in the first summer （SzP-1m）, and plant vigor in the first fall （PVg-1f）, these values were less than 10%. Comparing CVs within lines to CVs between lines, they showed greater values （27% vs. 17%） and ranged between 8 and 96% on average. The mean of the maximum CVs （65%） was almost 6 times more than that of the minimum （9.6%）. The mean ratio of CVs of the within/ between lines was 165%, which means that the CVs of within lines were much higher than those of between lines. The CVs of the traits between cultivars varied from 3 to 64%. Higher CVs were found in two traits of the number of stolons （NSl-1m, 64%） and the degree of flowering （DFl-1f, 58%） in the first year. In contrast, lower CVs were found in traits of the number of leaves （NLf-2s） and leaf color （CLf-2s） in the second spring, traits relating to overwintering in the second spring （WHd-2s and Bd-2s） and leaf density in the first summer （DLf-1m）, these values were less than 6%. The CVs within cultivars ranged from 10 to 125% and the mean CV of within cultivars was 27%. The mean CVs of between and within in both the collected lines and cultivars were very close, but the mean CV of the maximum CVs in cultivars was 20% lower than that of the collected lines.

We next compared the CVs between collected line and cultivars for both between and within the lines. We focused on 26 of 32 traits, which were excluded leaflet length, width and petiole length in both of the first and second years. Ten of the traits in both between and within lines showed lower CVs （less than 100%） in collected lines than those in the cultivars. We chose 17 traits （Table 5） including traits with the lower CVs in collected lines and two traits with respect to diseases symptoms, caused by Sclerotinia trifoliorum and mosaic virus.

Results

Leaf size classification

The collected lines and cultivars were classified into four groups （Fig 2 and Table 1） by cluster analysis based on leaf size measurement including leaflet length and width, and petiole length in both the seeding year and the second growing year. The large and medium-large leaf groups included cv. California Ladino and 7 collected lines, and cv. Makibashiro and 17 collected lines, respectively. Two cultivars, Sonja and Rivendel, and 29 collected lines were classified into medium leaf group, and two cultivars, North White and Tahora, and 25 collected lines were classified as the small size group. The percentages of each group per all collected lines are 9, 22, 37 and 32 % for the large, medium-large, medium and small groups, respectively.

次へ進む　前に戻る　目次に戻る

Features of the white clover lines collected from two distinct climate regions in Hokkaido revealed by coefficients of variation between and within lines

Results

Relationship among collection site, climate, and leaf size

We compared the mean values of the traits between the two climatic areas, freezing and snowy, for each of the three types of leaf size （Table 5）. In this analysis the large and medium-large groups were grouped together because they were few in number. Five of the17 traits, budding （Bd-2s） and date of flowering （FlD-2s） in the second spring showed statistically significant differences （P<0.05） between the two groups of lines collected from the freezing and snowy areas. The size of the plant in the first summer （SzP-1m）, degrees of flowering （DFl-1f） and plant vigor（PVg-1f） in the first fall were also showed different tendencies （P<0.1）.

次へ進む　前に戻る　目次に戻る

Features of the white clover lines collected from two distinct climate regions in Hokkaido revealed by coefficients of variation between and within lines

Results

Principal component analysis

Finally, we analyzed the collected lines and cultivars for these five traits by principal component analysis. In this analysis we used 53 collected lines from the eastern area for freezing and the central or south areas for snowy. The 1st principal component showed positive correlations strongly with flowering date in the second spring, FlD-2s, and weakly with DFl-1f. The 2nd component were related to the two flowering traits and size of plant in the 1st summer （SzP-1m） and the 3rd components was negatively related to SzP-1m, plant vigor in the first fall （PVg-1f） and budding in the second spring （Bd-2s）. The proportions of variance for the components were 0.782 for the 1st, 0.134 for 2nd and 0.052 for 3rd, respectively. Fig 3 shows the distribution of collected lines and cultivars. The 1st principal component （horizontal-axis） divided the lines into late flowering and large leaf group from others, as well as the lines in freezing from those of snowy areas in small leaf group. The 3rd principal component （vertical-axis） classified the lines into freezing and snowy areas in large leaf and small leaf groups. There was a tendency for the lines from the freezing area to show lower values in size, vigor, budding, and late flowering, but to have a slightly higher degree of flowering in the first fall..

次へ進む　前に戻る　目次に戻る

Discussion

Taking ecotypes into breeding programs has been successfully practiced to develop new cultivars in Japan as well as in the countries growing forage legumes. Okumura （2005） reviewed the breeding history of forage legumes in Hokkaido, where pioneer cultivars of red clover and alfalfa were mainly developed from ecotypes in Hokkaido. Woodfield and Caradus （1994） suggested the maintenance of a wide genetic base through incorporation of ecotype material adapted to various environmental and edaphic stresses in white clover. Taylor （2008） suggested the importance of farmer strains well adapted locally for the development of cultivars for the breeding progress of clover in the United States.

The term “ecotype” was first proposed by Turesson （1922） as an ecological unit to cover the product arising as a result of the genotypical response of an ecospecies to a particular habitat. Hokkaido is the northernmost region in Japan and is characterized by a severe winter cold habitat to plants species compared to other parts of Japan. Some plant species have been known to develop specific characteristics adapted to this specific climate condition. For example, the natural populations of Miyakogusa （Lotus japonicus, wild relatives of birdsfoot trefoil, L. corniculatus） in Hokkaido showed later flowering than those from the southern part of Japan （Suginobu et al., 1988）. Shimada （1984） reviewed that geographic distribution of wild plant species was restricted to the contour line of snow depth and that the two areas divided by the line also had the specific two features needed for survival of forage plants to survive, by tolerance to freezing damage in the low snow depth （freezing） areas and resistance to snow mold in the high snow depth （snowy） area. Surviving alfalfa plants from old fields in East-Hokkaido showed a higher percentage of the prostrate type, which was thought to be adaptation to the freezing area （Suginobu et al., 1980）. These reports indicated that Hokkaido is a specific enough habitat for the formation of ecotypes in forage legumes and that further ecotypes could be developed for the two habitats, snowy and freezing. However, there have been few reports on the formation of ecotypes of white clover adapted to Hokkaido environment yet.

The relationship between leaf size and cold tolerance was revealed to be the tendency for small leaved lines to show higher cold tolerance than large leaved ones in white clover （Davies and Young, 1967, Caradus et al., 1989a, b）. We firstly classified the collected lines and cultivars into 4 groups based on leaf size related traits by cluster analysis （Table 1）. The percentages of each group were 9, 22, 37 and 32 % for the large, medium-large, medium and small groups, respectively and these percentages coincided with the recent trend in commercial cultivars in Hokkaido （personal information from the Japan Grassland Agriculture and Forage Seed Association）.

There were differences in five traits of budding （Bd-2s）, date of flowering （FlD-2s） in the second spring, size of plant in the first summer （SzP-1m）, degrees of flowering （DFl-1f） and plant vigor （PVg-1f） in the first fall （Table 5）. The lines collected from the freezing areas tended to show slow growth from the seeding year to the spring of the second year and were a little late in flowering in the spring. In contrast, these lines showed a higher degree of flowering in the first fall than those from the snowy areas. These results indicate that plants with slow growth during late fall and early spring might be able to avoid damage in the freezing areas due to unfavorable conditions. Similarly, the highly degree of flowering in fall in the lines from the freezing areas resulted from the promotion of flowering in the short day condition and regeneration from the seed in late summer to fall, since these seasons in the freezing area are stable and appropriate for seed maturation. The principal component analysis （Fig 3） also shows the clear distinction between the two areas, freezing and snowy, in each leaf size group. The directions of differences also corresponded in the three leaf size groups. We did not have any accurate information about how many years and generations passed after seeding in the collection sites, although we chose sites without any evidence of recent renovation or seeding of white clover. Our results suggested that the difference of the traits between the two areas was caused by the selection of plants that were adapted to the two distinct environmental conditions.

Eastern Hokkaido, which accounts for more than a third of the total grassland area, 0.56 million hectares, in Hokkaido is the most important target area for forage breeding. The development of new cultivars for this area is needed to improve cold tolerance against severe winter freezing. The tendencies for lower production in the collected lines from the freezing areas seem to indicate a less than optimal yield, but these tendencies are linked cold hardiness and small leaves. To advance a breeding program in the freezing area, the plants from the lines collected from freezing areas with superior spreading, high leaf density and plant vigor, as well as those which showed larger CVs compared to cultivars, need to be selected （Table 4）. Burdon （1980a） described how it was important for genotypes well adapted to a particular environment to have not only a suitable set of general fitness, but also a set of local fitness. It is necessary to control the variability for general fitness related characters, such as dry matter and seed yield, and preserve the variability for the characters for local fitness, such as winter hardiness within a line.

In this study, we did not take the following three traits, hydrocyanic acid （HCN）, freezing tolerance and snow mold resistance into account. However, these traits are quite important for local adaptability and future improvement of persistence in white clover. Daday （1954a,b） found the correlations between decreasing frequency glucoside genes and January temperature from the Mediterranean region to northern-eastern Europe and low to high altitudes in the Central European Alps. Daday （1958） also showed a decrease of the gene frequency of lotaustralin from southern areas, Kyusyu and Shikoku, to the northern and cold area of Hokkaido. This tendency was thought to result from natural selection against the temperature and to be common to the populations of North America. Collins and Rhodes （1995） found that the Swiss populations from high altitudes contained the highest levels of carbohydrate and had the lowest LT50 compared to a control variety, Grassland Huia. In our observation, Sclerotinia disease was not conspicuous, but in red clover its resistance affected the adaptability of cultivars in Hokkaido （Yamaguchi et al., 2004）. In addition, Matsumoto et al. （2000） reported the differentiation of Typhula ishikariensis Biotype A, and the differentiated groups had different distributions in Hokkaido as well as a difference in the damage to plants. For further consideration of the adaptation of white clover in Hokkaido it would be useful to add the information on these traits. We will develop and apply methods using artificial inoculation of snow mold as well as a test of freezing tolerance and detection of HCN to evaluate the germplasms of white clover in the future.

次へ進む　前に戻る　目次に戻る

Features of the white clover lines collected from two distinct climate regions in Hokkaido revealed by coefficients of variation between and within lines

Acknowledgments

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次へ進む　前に戻る　目次に戻る

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次へ進む　前に戻る　目次に戻る

系統間および系統内の変動係数からみた北海道の異なる気象地帯から収集されたシロクローバ系統の特徴

奥村 健治・高田 寛之・廣井 清貞・磯部 祥子*

摘 要

北海道は冬期の気象条件により道東を中心とする土壌凍結地域とそれ以外の積雪地域に大きく分けることができ、これらの２地域ではクローバ類を含む牧草類の越冬性の要因が異なることが知られている。そこで、本研究は土壌凍結地域と積雪地域の地域適応性に関わり差異が現れる形質の検討を行うことを目的に、島嶼部を除く北海道全域から収集された78地点のシロクローバ種子をそれぞれ収集系統として、代表的な市販６品種を加えて系統の特性調査を実施した。先ず系統間に有意な差が認められる形質を特定し、続いて形質に自然選択がかかる場合に系統間および系統内の変異が小さくなることを想定して、変動係数を収集系統と品種を比較して、小さくなった形質の絞り込みを行い、これらの形質について地域間の差を検討した。その結果、小葉長などの形態特性や開花日など延べ32形質のうち、節間長と１年目夏期の葉の密度の２形質を除き系統間に有意な差が認められた。これらの形質を収集系統と品種に分けて、系統間と系統内の変異を変動係数で比較したところ、系統間の方が変異の大きかった形質は収集系統で４形質と品種の10形質より少なかった。さらに、品種と収集系統の変動係数を比較したところ、系統間あるいは系統内のいずれかで収集系統の方が低くなった形質は22形質であった。これらの形質のうち葉の大きさに関わる形質を除く15形質、および収集系統の方が高い変動係数を示したものの地域適応性に強く関わると考えられる病害程度の２形質の計17形質について、葉の大きさグループ毎に土壌凍結および積雪地域収集の２グループ間に差が認められるかどうかを検定した。その結果、土壌凍結地域からの収集系統は１年目の夏の株の広がり、秋の葉の密度および秋の草勢、２年目の早春の萌芽および春の草勢の計５形質が積雪地域からの収集系統より低い傾向が認められた。これらの情報はそれぞれの地域に適した今後の品種育成に活かすことができると考えられた。

次へ進む　前に戻る　目次に戻る

The breeding and characteristics of a common buckwheat cultivar, “Kitanomashu”

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Hideyuki FUNATSUKI 5）, Kiyoshi SEKIMURA 6）, Shinjiro KATO 6） and Masamichi AGATSUMA 6）

Summary

A new common buckwheat cultivar, “Kitanomashu”, was developed at National Agricultural Research Center for the Hokkaido Region, Sapporo, Japan. This cultivar was registered as “Buckwheat Norin No.4”, released by the Ministry of Agriculture, Forestry and Fisheries and recommended by the Hokkaido prefectural government.
"Kitanomashu" was a deteminate type of variety derived from a plant of a population of about 9,000 plants by Kitawasesoba.
The characteristics of "Kitanomasyu" are summarized as follows.
（1） Lodging is less than 'Kitawasesoba'.
（2） Taste is better than 'Kitawasesoba'.
（3） It must be cultured in an isolated area as a population
（4） The appropriate amount of fertilizer and sowing time is recommended for high yield and against lodging.
（5） Shattering is the same as 'Kitawasesoba'.
"Kitanomashu" is suitable for all area of Hokkaido.

Present address
1）National Agricurural Research Center for Tohoku Region
2）Local Crop Breeding Research Team
3）Crop Functionality and Utilization Research Subteam （Hokkaido Region）
4）Independent Researcher （Rice Quality Research Team, Hokkaido Region）
5）Crop Cold Tolerance Research Team
6）Retired

Fig 1. Collection sites of white clover accessions in Hokaido.

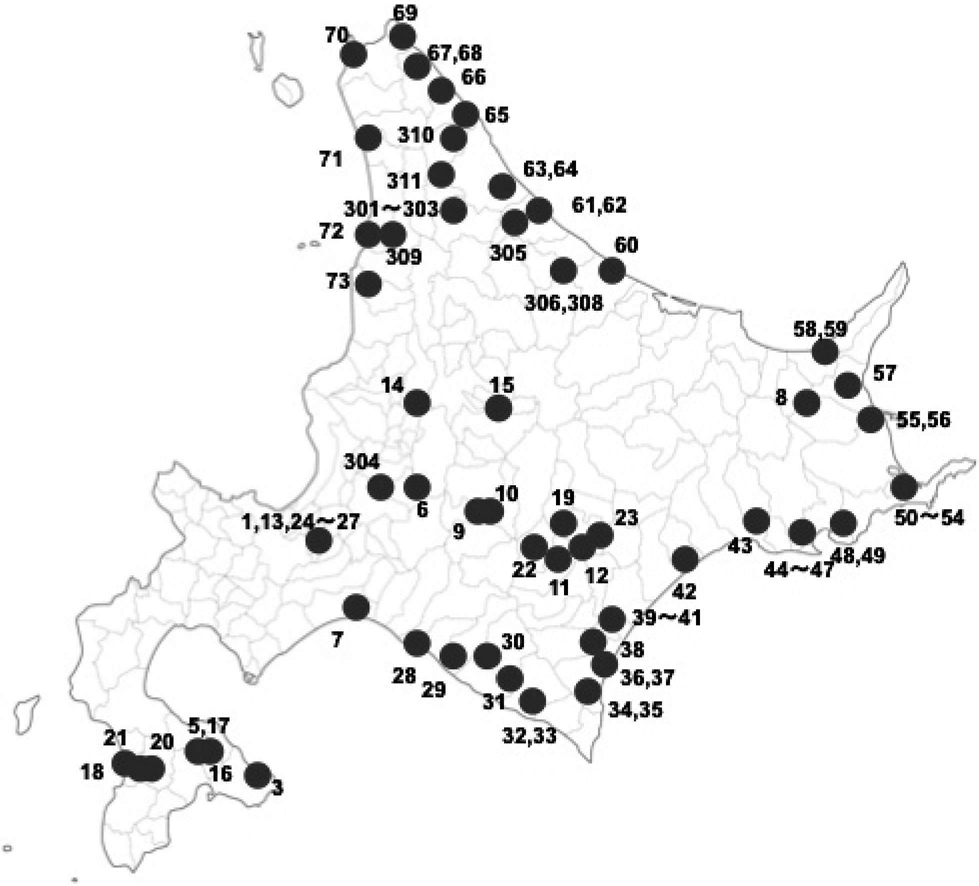

Table 1. Accessions and cultivars of white clovre used in this study

Accession	Leaf Size[1)]	Area in Hokkaido[2)]	Snowy / Freezing[3)]	Collection site/cultivar name and origin	Passport No[4)]
1	S	C	S	Sappro City , NARCH[5)]	30018538
3	S	SM	F	Hakodate City (Esan Town)[6)] , Esan mountain	30018540
5	M	S	S	Nanae Town , Gunkawa	30018542
6	S	CM	F	Furano City , Furano-dake mountain	30018543
7	S	C	F	Tomakomai City , Tomakomai East Port	30018544
8	M	E	F	Nakashibetsu Town , Konsen[7)]	30018545
9	S	E	S	Shintoku Town , Shintoku Station	30018546
10	L	E	S	Shikaoi Town , Biman	30018547
11	M	E	F	Memuro Town , Tokachi[7)]	30018548
12	M	E	F	Otofuke Town , Tokachigawa Hot Spring	30018549
13	M	C	S	Sappro City , Moiwa mountain	30018550
14	S	C	S	Pippu Town , Kamikawa[7)]	30018551
15	S	CM	F	Higasikawa Town , Asahi-dake mountain	30018552
16	S	S	F	Oono Town , Hachiro-numa	30018553
17	S	SM	F	Nanae Town , Yokotsu-dake mountain	30018554
18	S	S	S	Esashi Town , Kamomejima	30018555
19	S	E	S	Shikaoi Town , *[8)]	30018556
20	M	S	S	Assabu Town , Sinei	30018557
21	ML	S	S	Kitahiyama Town , Wakamatsu	30018558
22	S	EM	S	Shimizu Town , Nissyo Pass	30018559
23	ML	E	F	Honbetsu Town , Biribetsu	30018560
24	M	C	S	Sappro City , NARCH	30018561
25	S	C	S	Sappro City , NARCH	30018562
27	M	C	S	Sappro City , Tukisamu Park	30018564
28	M	C	F	Mukawa Town , *	30018565
29	M	C	F	Hidaka Town (Monbetsu Town) , *	30018566
30	ML	C	F	Nikappu Town , Thoroughbred Road	30018567
31	M	C	F	Mitsuishi Town , Keishuba-Ikushu Center	30018568
32	ML	C	F	Urakawa Town , Nishihorobetsu	30018569
33	M	C	F	Urakawa Town , Kineusu	30018570
34	S	E	F	Hiro Town , Toyoni	30018571
35	L	E	F	Hiro Town , Kamitoyo	30018572
36	ML	E	F	Taiki Town , Kouku	30018573
37	ML	E	F	Taiki Town , Seika	30018574
38	S	E	F	Toyokoro Town , Chyoubushi	30018575
39	S	E	F	Urahoro Town , Tokachi River	30018576
40	M	E	F	Urahoro Town , Ougon Fall	30018577
41	S	E	F	Urahoro Town , Atsunai	30018578
42	S	E	F	Kushiro City (Onbetsu Town) , Onbetsu	30018579
43	M	E	F	Kushiro Town , *	30018580
44	S	E	F	Akkeshi Town , Kamioboro	30018581
45	M	E	F	Akkeshi Town , Kamioboro	30018582
46	M	E	F	Akkeshi Town , Oboro Station	30018583
47	S	E	F	Akkeshi Town , Itoizawa Station	30018584
48	ML	E	F	Hamanaka Town , *	30018585
49	ML	E	F	Hamanaka Town , Hokuto	30018586
50	L	E	F	Nemuro City , Attoko	30018587
51	S	E	F	Nemuro City , Hattaushi	30018588
52	S	E	F	Nemuro City , Ochi-ishi cape	30018589
53	S	E	F	Nemuro City , Ochi-ishi	30018590
54	ML	E	F	Nemuro City , Hanasaki	30018591
55	M	E	F	Betsukai Town , Furen	30018592
56	M	E	F	Betsukai Town , Shiretoko	30018593
57	ML	E	F	Shibetsu Town , Kitashibetsu	30018594
59	M	E	S	Shari Town , *	30018596
60	ML	E	F	Monbetsu City , *	30018597

Table 2. List of traits in this studty

Trait	Character	Method	Rank or Unit	Date of observation	F significance[2)]
LPt-1m	Petiole length in the 1st[1)] summer	Measurement	mm	7/29	*
LLf-1m	Leaflet length in the 1st summer	Measurement	mm	7/29	**
LLw-1m	Leaflet width in the 1st summer	Measurement	mm	7/29	*
LPt-2m	Petiole length in the 2nd[1)] summer	Measurement	mm	7/14	**
LLf-2m	Leaflet length in the 2nd summer	Measurement	mm	7/14	**
LLw-2m	Leaflet width in the 2nd summer	Measurement	mm	7/14	**
LPd-2m	Peduncle length in the 2nd summer	Measurement	cm	7/14	**
LIt-1m	Internode length in the 1st summer	Measurement	mm	7/29	NS
TSt-1m	Stem thickness in the 1st summer	Measurement	mm	7/29	*
FlD-2s	Flowering date in the 2nd year	Date when 3 heads per plant had begun to flower	dates from June 1st		**
APl-1m	Area of plant in the 1st summer	Measurement	square cm(long diameter x short diameter)	8/9	**
SzP-1m	Size of plant in the 1st summer	Obsevational score rated from 1 to 9	1:Extremely small, 3:Low, 5:Intermediate, 7:large, 9:Extremely	9/10	**
SzP-1f	Size of plant in the 1st fall	Obsevational score rated from 1 to 9	1:Extremely small, 3:Low, 5:Intermediate, 7:large, 9:Extremely	11/19	**
DLf-1m	Density of leaves in the 1st summer	Obsevational score rated from 1 to 9	1:Extremely low, 3:Low, 5:Intermediate, 7:High, 9:Extremely	8/9	NS
DLf-1f	Density of leaves in the 1st fall	Obsevational score rated from 1 to 9	1:Extremely low, 3:Low, 5:Intermediate, 7:High, 9:Extremely	9/10	**
NSl-1m	Number of Stolons in the 1st summer	Obsevational score rated from 1 to 9	1:Almost none, 3:Very few, 5:Intermediate, 7:Many, 9:Extremely	7/18	**
PCv-1f	Plant cover in the 1st fall	Obsevationan	Percentage of plant covre to 50 x50 cm plot	9/10	**
NLf-2s	Number of leaves in the 2nd spring	Obsevational score rated from 1 to 9	1:Almost noe, 3:Very few, 5:Intermediate, 7:Many, 9:Extremely	6/30	**
NFl-2m	Number of florets in the 2nd spring	Measurement	1:Almost none, 3:Very few, 5:Intermediate, 7:Many, 9:Extremely	7/3	**
NHd-2m	Number of heads in the 2nd spring	Obsevational score rated from 1 to 9	1:Almost none, 3:Very few, 5:Intermediate, 7:Many, 9:Extremely	7/3	**
PHb-1m	Plant habit in the 1st summer	Angles that plants make with the ground at the flowering	1:Erect, 3: Semi-erect, 5:Intermediate, 7:Semi-prostrate, 9:Prostrage	8/9	**
PHb-2s	Plant habit in the 2nd spring	Obsevational score rated from 1 to 9	1:Erect, 3: Semi-erect, 5:Intermediate, 7:Semi-prostrate, 9:Prostrage	6/30	**
DFl-1f	Degree of flowering in the 1st fall	Obsevational score rated from 1 to 9	1:Extremely low, 3:Low, 5:Intermediate, 7:High, 9:Extremely	9/10	**
CLf-2s	Leaf color in the 2nd spring	Obsevational score rated from 1 to 9	1:Extermely light green, 3:Light green, 5:Intermediate, 7:Green, 9:Extremely	6/30	**
FCl-2s	Flower color in the 2nd spring	Obsevational score rated from 1 to 9	1:White, 2:Pale red, 3:Light Red, 4:Red	6/30	**
LMr-2s	Clearness of leaf V-mark in the 2nd spring	Obsevational score rated from 1 to 9	0:None, 1:Extremely vague, 3:Vague, 5:Intemediate, 7:Clear, 9:Extremely	6/22	**
PVg-1f	Plant vigor in the 1st fall	Obsevational score rated from 1 to 9	1:Extremely poor, 3:Poor, 5:Intermediate, 7:Good, 9:Excellent	11/24	**
WHd-2s	Overwintering ability in the 2nd spring	Obsevational score rated from 1 to 9	1:Extremely poor, 3:Poor, 5:Intermediate, 7:Good, 9:Excellent	4/16	**
Bd-2s	Budding in 2nd[1)] spring in the 2nd spring	Obsevational score rated from 1 to 9	1:Extremely poor, 3:Poor, 5:Intermediate, 7:Good, 9:Excellent	4/30	**
PVg-2s	Plant vigor in spring in the 2nd spring	Obsevational score rated from 1 to 9	1:Extremely poor, 3:Poor, 5:Intermediate, 7:Good, 9:Excellent	6/13	**
Scl-2s	Sclerotinia root and crown rot damage in the 2nd spring	Obsevational score rated from 1 to 9	1:Extremely low, 3:Low, 5:Intermediate, 7:High, 9:Extremely	4/30	**
Vir-2s	Virus symptom in the 2nd spring	Obsevational score rated from 1 to 9	1:Almost none, 3:Low, 5:Intermediate, 7:High, 9:Extremely high	6/30	**

1) 1st: seeding year,2004, 2nd: second growing year, 2005

2) *, **, ns: significantly different among lines at 5, 1% level and not different, respectively, based on ANOVA.

Table 4 Mean ,maximum, minimum values of the traits and their coefficient of variation (CVs) in the 6 cultivars.

Trait	Value			CV-between[1)]	CV-within[1)]			CV[2)] within /between	COL/CV between[3)]	COL/CV within[3)]
	average	max	min		average	max	min			
NSl-1m	2.7	4.9	0.4	64.1	86.9	170.7	44.1	135	65	89
TSt-1m	1.9	2.4	1.7	14.6	10.5	17.9	4.7	72	135	120
LIt-1m	26.4	29.4	22.6	10.1	20.6	27.6	10.7	204	143	115
LPt-1m	78.9	109.0	57.2	24.8	24.0	32.8	14.7	97	87	95
LLf-1m	19.1	25.6	14.2	24.5	13.9	18.5	8.8	57	69	106
LLw-1m	15.8	24.1	11.5	30.6	23.6	74.1	10.1	77	48	59
PHb-1m	4.2	5.0	3.6	12.1	17.3	27.0	10.9	143	105	96
DLf-1m	4.3	4.6	4.0	4.7	12.2	15.9	9.7	260	162	126
APl-1m	0.2	0.3	0.2	21.6	46.1	71.5	35.0	214	144	103
SzP-1m	5.0	5.7	3.9	16.0	19.8	35.1	8.8	124	53	96
DFl-1f	2.0	3.4	0.8	57.5	124.5	202.4	48.9	216	84	77
DLf-1f	4.6	5.2	3.7	12.7	26.9	46.6	12.2	211	72	80
PCv-1f	66.4	83.0	47.7	19.0	27.3	47.2	11.8	144	54	90
PVg-1f	4.9	5.3	4.6	6.0	23.6	43.7	12.1	393	141	74
SzP-1f	21.7	23.5	19.0	8.2	14.6	21.3	5.8	177	160	104
WHd-2s	3.6	3.8	3.3	6.3	30.7	41.0	17.2	484	199	77
Bd-2s	3.9	4.2	3.7	4.2	31.9	42.6	21.6	760	281	77
Scl-2s	1.0	1.0	1.0	0.0	0.0	0.0	0.0	–	–	–
PVg-2s	3.5	4.0	3.2	10.5	23.4	28.3	17.2	223	133	97
FlD-2s	15.7	19.6	13.4	14.5	13.2	16.8	7.4	91	77	126
Lm-2s	3.1	3.9	2.5	15.0	55.9	88.9	30.9	372	143	101
FCl-2s	1.4	1.7	1.2	13.0	26.4	38.4	15.9	203	97	131
PHb-2s	5.5	6.1	5.0	8.5	11.0	13.5	7.2	130	113	132
NLf-2s	4.4	4.5	4.2	3.2	18.8	23.0	16.6	587	308	103
CLf-2s	4.5	4.8	4.2	4.0	10.4	12.2	7.9	261	90	114
Vir-2s	2.1	3.6	1.2	41.2	45.7	73.2	28.9	111	101	123
NHd-2m	5.3	6.1	4.2	13.9	18.8	31.2	13.2	135	98	115
NFl-2m	4.3	5.3	3.4	15.8	16.8	19.6	12.3	106	90	105
LPt-2m	14.1	20.1	9.4	26.4	25.1	34.2	12.1	95	94	113
LLf-2m	27.1	37.6	19.1	25.6	16.4	18.4	13.7	64	68	108
LLw-2m	22.8	30.0	17.1	22.2	14.9	17.1	12.7	67	73	117
LPd-2m	21.6	27.1	15.2	19.3	19.4	26.0	15.9	100	77	108
mean				17.8	27.2	43.0	15.6	203.7	115.0	102.5

1) CV-between and -within show coefficents of variation between and within lines, respectively

2) CV-within/between shows percentage between averages of CV-within and CV-between.
3)COL/CV between and within show percentages between mean values of coefficent of variation in the collection lines and the cultivars

Table 3 Mean ,maximum, minimum values of traits and their coefficient of variation (CVs) in the 78 lines collected from Hokkaido

Trait	Value			CV-between[1)]	CV-within[1)]			CV[2)] within /between
	average	max	min		average	max	min	
NSl-1m	2.1	4.0	0.1	41.8	77.1	308.1	30.8	185
TSt-1m	2.0	4.5	1.5	19.6	12.6	84.3	5.4	64
LIt-1m	27.9	36.3	18.9	14.5	23.7	74.1	10.3	164
LPt-1m	80.9	131.3	50.1	21.6	22.7	74.0	12.7	105
LLf-1m	20.4	29.2	14.7	17.0	14.7	65.9	5.2	87
LLw-1m	15.5	21.6	11.3	14.7	14.0	65.7	7.1	95
PHb-1m	4.4	6.0	3.0	12.8	16.7	29.5	7.5	131
DLf-1m	4.5	5.3	3.6	7.6	15.4	23.3	5.8	202
APl-1m	0.3	0.5	0.1	31.1	47.6	122.7	26.3	153
SzP-1m	5.2	6.1	3.9	8.4	19.1	38.4	10.3	228
DFl-1f	2.3	5.6	0.4	48.4	95.5	227.1	29.1	197
DLf-1f	4.7	5.5	3.4	9.1	21.5	43.0	10.3	236
PCv-1f	67.9	82.4	50.2	10.3	24.7	52.0	6.9	240
PVg-1f	5.0	6.2	4.1	8.5	17.5	41.9	0.0	206
SzP-1f	22.9	34.4	17.9	13.1	15.1	28.2	6.0	115
WHd-2s	3.5	4.7	2.5	12.6	23.6	49.0	10.8	188
Bd-2s	3.9	5.1	2.8	11.8	24.4	44.6	10.4	207
Scl-2s	1.1	1.7	1.0	11.4	8.0	57.1	0.0	70
PVg-2s	3.8	5.7	2.7	14.0	22.7	38.0	7.3	162
FlD-2s	17.2	22.6	13.3	11.2	16.7	25.6	5.3	149
Lm-2s	2.9	4.8	1.8	21.4	56.4	104.2	22.8	263
FCl-2s	1.4	1.8	1.0	12.6	34.6	62.2	0.0	274
PHb-2s	5.2	6.5	3.6	9.6	14.5	22.9	4.7	151
NLf-2s	4.5	5.8	3.6	9.9	19.4	35.5	8.3	197
CLf-2s	4.7	5.0	4.3	3.6	11.9	21.6	3.4	330
Vir-2s	2.2	5.2	1.0	41.6	56.3	104.0	0.0	135
NHd-2m	4.4	5.6	2.8	13.6	21.5	35.2	9.7	159
NFl-2m	4.4	6.0	3.2	14.2	17.7	28.6	5.9	124
LPt-2m	15.1	27.3	7.4	24.8	28.3	73.3	16.6	114
LLf-2m	28.1	42.1	19.9	17.4	17.8	28.3	9.2	102
LLw-2m	23.8	34.7	16.6	16.3	17.5	26.5	10.7	107
LPd-2m	22.0	29.8	14.1	14.8	20.9	35.5	9.6	141
mean				16.8	26.6	64.7	9.6	165.1

1) CV-between and -within show coefficents of variation between and within lines, respectively

2) CV-within/between shows percentage between averages of CV-within and CV-between.

Table 5 Mean values of selected traits in lines collected from Freezing and Snowy areas

Leaf Size(LS)	L-ML[1)]		M		S		P value of ANOVA in CS
Collection Site(CS)	F[2)]	S[2)]	F	S	F	S	
NSl-1m	1.7	2.3	2.1	2.2	2.8	2.5	＞0.1
PHb-1m	5.1	5.1	4.5	4.2	4.0	4.1	＞0.1
SzP-1m	5.2	5.6	5.2	5.3	5.1	5.3	0.09
DFl-1f	3.7	3.1	2.2	1.7	2.0	1.8	0.06
DLf-1f	4.4	4.7	4.9	4.8	4.8	4.8	＞0.1
PCv-1f	66	72	72	69	67	65	＞0.1
PVg-1f	5.2	5.6	5.1	5.0	4.7	4.9	0.07
WHd-2s	3.7	3.9	3.5	3.5	3.2	3.3	＞0.1
Bd-2s	4.1	4.4	3.9	4.0	3.4	3.7	＜0.01
Scl-2s	1.0	1.1	1.1	1.0	1.0	1.1	＞0.1
PVg-2s	4.1	4.6	4.0	3.7	3.4	3.4	＞0.1
FlD-2s	19.6	19.3	16.5	16.1	17.0	15.6	＜0.05
FCl-2s	1.6	1.4	1.3	1.3	1.4	1.4	＞0.1
CLf-2s	4.7	4.7	4.8	4.8	4.7	4.6	＞0.1
Vir-2s	1.9	2.1	2.1	1.8	2.9	2.5	＞0.1
NHd-2m	3.6	4.0	4.7	4.6	4.7	4.8	＞0.1
NFl-2m	5.1	5.2	4.6	4.2	4.0	3.7	＞0.1

1)Leaf size,L:large, ML:medium large, M:medium, S:small

2)F:lines collected from freezing areas, S:lines collected from snowy areas

Fig 2. Cluster Dendrogram of the lines based on leaf size measurements, leaflet length and width, and petiole length. Collected lines and cultivars are shown as accession number or, C: Calfornia Ladino, M: Makibashiro, S:Sonja, R:Rivendel, T:Tahora, N:North White.

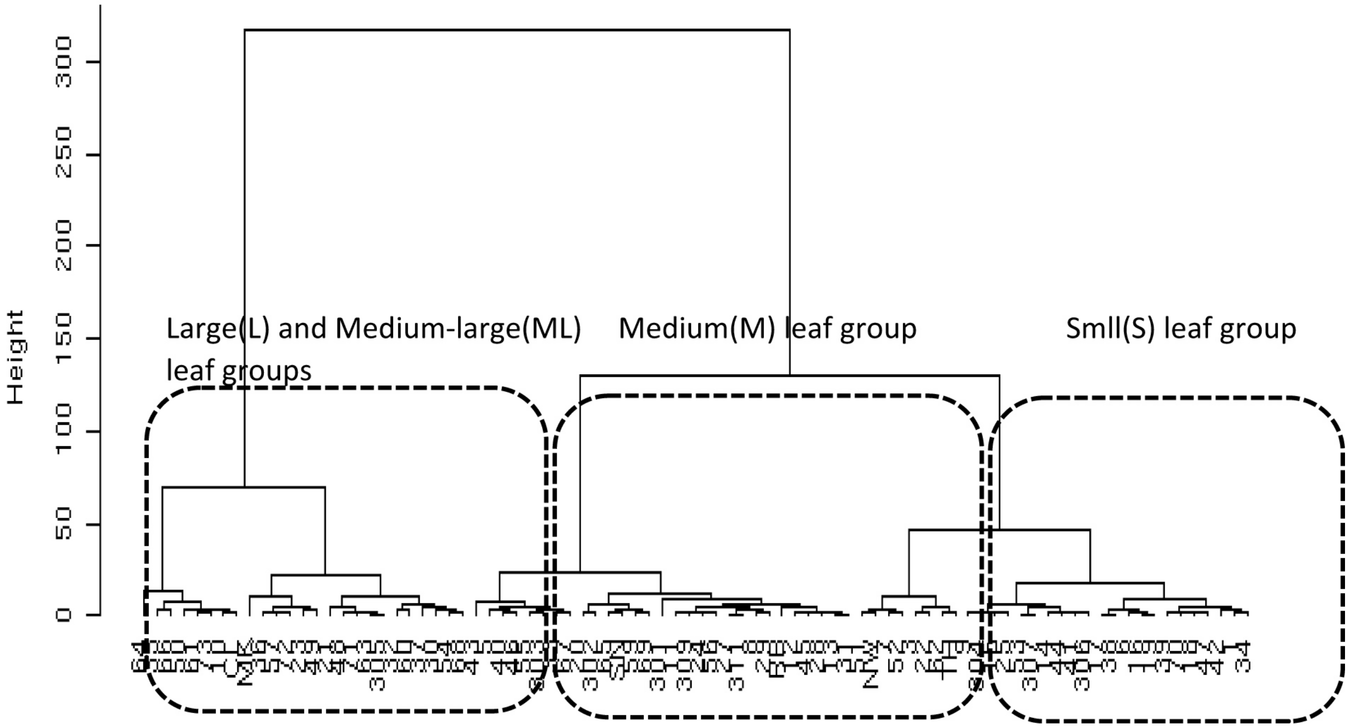

Fig 3. Distiribution of collected lines and cultivars on the first and third pricipal components based on the five traits, SzPÅi1mÅj, DFlÅi1fÅj, PVgÅi1fÅj, BdÅi2sÅj and PVgÅi2sÅj. The marks and alphabets are shown as follows; Åõ,Åú :collected lines with large or larege-medium size leaf , Åû,Åü: collected lines with medium size leaf, Å¢,Å£: collected lines with small size leaf, Åú,Åü,Å£: collected from freezing area, , Åõ,Åû,Å¢: collected from snowing area C: Calfornia Ladino, M: Makibashiro, S:Sonja, R:Rivendel, T:Tahora, N:North White.