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Citation	Euphytica, 153(1-2), 267-280 https://doi.org/10.1007/s10681-006-9262-9
Issue Date	2007-01
Doc URL	http://hdl.handle.net/2115/17208
Rights	The original publication is available at www.springerlink.com
Type	article (author version)
File Information	Euphytica153-1-2.pdf

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Ecotypic variation of water-soluble carbohydrate concentration and winter hardiness in cocksfoot (*Dactylis glomerata* L.)

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Key words: *Dactylis glomerata*, ecotype, fructan, growth habit, water-soluble carbohydrate, winter hardiness.

Summary

The variation of the water-soluble carbohydrate (WSC) concentration in foliage and stubble and winter hardiness among cocksfoot (*Dactylis glomerata* L.) ecotypes originated from diverse countries were studied in the field in order to find a breeding material for the improvement of WSC. The total WSC concentration of foliage was found to be highest in Japanese cultivars and lowest in ecotypes from northern Europe in summer and autumn. The variation of fructan concentration in stubble among ecotypes was larger than the variation of mono and disaccharide concentrations in stubble and that of total WSC concentration in foliage in both winters over which this study was conducted. The total WSC and fructan concentrations in the stubble of ecotypes from Spain and southern Europe, derived from the Mediterranean region, were highest in summer. Ecotypes from Japan showed the highest concentration of total WSC in stubble, and were especially high in fructan concentration in autumn. Ecotypes from France, central Europe and central Asia, like Japanese cultivars, also showed a high fructan concentration. The latitude of origin was negatively correlated with WSC concentration and plant vigor in autumn. The altitude of origin was negatively correlated with plant vigor in autumn and positively correlated with fructan concentration in stubble, winter hardiness and snow endurance. Typhula snow blight resistance was related with the amount of the increase in fructan in the plants and with their growth habits in autumn. The fructan concentration of foliage and stubble in autumn was found to be closely related with winter hardiness in cocksfoot ecotypes. A breeding material which has a high WSC concentration in the foliage could not be found among the ecotypes in this study. However, some ecotypes that showed disease resistance, good winter hardiness and plant vigor would be useful as breeding materials for Hokkaido.

Abbreviations: IDSM, index of damage by snow mold

Introduction

Dactylis glomerata L. (cocksfoot) is cultivated for hay making and grazing in temperate zones, because it has good regrowth and adaptability to various environmental conditions. Improvement of the forage quality is a major breeding objective in cocksfoot, because its forage quality is lower than that of other temperate grasses, for example timothy (*Phleum pratense* L.), especially in vegetative growth that is harvested in summer in Japan (Masuko et al. 1994). Grass breeders have focused on the improvement of water-soluble carbohydrate (WSC) concentration (Smith et al. 1997; Casler 2001), which is related to palatability and digestibility (Mayland et al. 2001), as well as the fermentation quality of silage. For perennial ryegrass, high WSC cultivars have been developed in the UK (Humphreys 1989; Miller et al. 2001), and the effectiveness of high WSC cultivars for milk production has been reported (Miller et al. 2001).

A breeding program for the improvement of the WSC concentration in cocksfoot has been initiated by the National Agricultural Research Center for Hokkaido Region (NARCH) at Sapporo. At the beginning of this breeding program, we evaluated the variation of WSC concentration among cultivars and strains at the vegetative growth stage in order to select breeding materials for the improvement of WSC. Wide genetic variation was found among these cultivars (Sanada et al. 2005). It is well known that some ecotypes show resistance to diseases and environmental stresses that cultivars do not possess, and those ecotypes are utilized for the improvement of disease resistances and environmental stresses in cultivars.

The ecotypes of cocksfoot are widely distributed in Europe, north Africa and Asia (Borrill 1978; Lumaret 1988). Cocksfoot was introduced to Hokkaido about 140 years ago, and an ecotype highly adapted to Hokkaido has been cultivated since then (Sugiyama & Nakashima 1999). The ecotypes of cocksfoot may have a large genetic variation in WSC concentration that is related to different environmental conditions, because cocksfoot was adapted to various climatic conditions including cold and drought regions.

The WSC concentration and its composition in grass have been correlated with regrowth (Donaghy & Fulkerson 1997) and drought tolerance (Volaire et al. 1998). The Mediterranean germplasm in the cocksfoot showed high drought tolerance and they accumulated WSC to stubble corresponding to the drought in summer (Volaire & Lelièvre 1997; Piano et al. 2004).

The temperate grasses accumulate WSC as an energy reserve in correspondence with low temperatures (Eagles 1967; Moriyama et al. 2003), and a relationship between WSC and winter stress factors such as freezing tolerance and snow mold resistance has been reported (Matsumoto & Sato 1983; Shibata & Shimada 1986; Tronsmo 1993). A variation of winter hardiness among European and Japanese cultivars of cocksfoot has been reported in Hokkaido (Nakayama & Abe 1996; Nakayama et al. 1997). However, the variation of winter hardiness among ecotypes collected in diverse countries and the relationship between WSC concentration and the geographic origins of these ecotypes have not been reported in cocksfoot. Snow mold resistance in cereals was closely correlated with WSC in autumn and was especially related to fructan concentration (Gaudet et al. 1999). It was well known that the winter hardiness of cocksfoot was lower than that of timothy and meadow fescue (*Festuca pratensis* Huds.), which were mainly cultivated in the northern region (Gudleifsson et al. 1986).

The Hokkaido region of Japan is covered with snow for more than four months out of the year, which is longer than other cultivated areas in the world between N 42° to 45°. Winter survival is a very important factor in animal production and in the agricultural economics of livestock farming in the northern region (Larsen 1994). The winter hardiness of grass is dependent on various stress factors, such as snow mold resistance, snow endurance, icing tolerance and freezing tolerances, which are related with each other (Tronsmo 1993; Hofgaard et al. 2003). In Hokkaido, snow mold damage was the main factor in determining winter survival in cocksfoot (Abe 1986). Snow endurance is another important factor in determining survival under the snow, characterized by low temperature, anaerobic conditions and darkness, and it is related to WSC concentration (Tamura & Hoshino 1979; Bertrand et al. 2003).

The objectives of this study were to identify genetic variation of WSC in foliage and stubble among cocksfoot ecotypes derived from diverse countries in order to find breeding material for the improvement of WSC and winter hardiness, and to determine whether WSC is correlated with winter hardiness and growth habits in relation to geographical origins. To clarify the relationship among winter stress factors, the WSC concentration and their geographical origins, snow mold resistance and snow endurance were evaluated under natural infection and with chemical control of snow mold in the field.

Materials and methods

The experiment was carried out on volcanic ash soil at the National Agricultural Research Center for Hokkaido Region (NARCH) in Sapporo (N 43˚ 00', E 141˚ 24'). A total of 65 accessions of cocksfoot were used for the evaluation of winter hardiness: 43 accessions of ecotypes introduced from the Institute of Grassland and Environmental Research (IGER) in the UK, 11 accessions of ecotypes introduced from the N.I. Vavilov Institute of Plant

Industry (VIR) in Russia, 4 accessions of ecotypes collected in Hokkaido, and 2 cultivars developed in Russia. Four standard cultivars developed at NARCH were used for the determination of maturity: ‘Wasemidori’ is an early maturing cultivar; ‘Okamidori’ and ‘Harujiman’ are medium maturing cultivars; and ‘Toyomidori’ is a very late maturing cultivar. Ecotypes originated from 11 areas outside of Japan (Table 1).

Seeds were sown on 16 May 2001 in paper pots filled with volcanic ash soil containing 4.0 g N, 1.5 g P_2O_5 and 4.0 K_2O kg^{-1} soil, and seedlings were grown in a greenhouse. Six seedlings of each accession were transplanted into a field at a spacing of 40 × 80 cm on 7 August in a randomized block design with four replications. Compound fertilizer (4.0 g N, 5.5 g P_2O_5 and 4.0 g K_2O m^{-2}) was applied at transplanting. Plots were harvested on 4 July, 3 September and 15 October in 2002 and on 9 July, 9 September and 21 October in 2003 by the flail type forage harvester (Star Noki, Chitose, Japan). After the melting of the snow in 2002 and 2003, 7.0 g N, 9.6 g P_2O_5 and 7.0 g K_2O m^{-2} were applied in April; 4.0 g N, 3.0 g P_2O_5 and 4.0 g K_2O m^{-2} were applied after harvesting.

Evaluation of water-soluble carbohydrate

Foliage samples including all tissues, i.e. leaves and leaf sheaths, were harvested at an 8cm height from the ground surface; they were collected from all plots in autumn, on 20 October 2001 and at the end of summer, on 2 September 2002. Plant samples above this height were termed ‘foliage’ and those from below this height and down to the ground surface were termed ‘stubble.’ Stubble samples of 5 tillers per plant from the ground surface to an 8cm height were collected at defoliation on 2 September 2002. Five tillers per plant were defoliated at an 8cm height, and stubble samples from these plants were collected on 2 November 2002 at the end of autumn. All samples were immediately transferred to an oven and dried at 70C° for 48 h. Dried samples were ground through a 0.5 mm screen using a cyclone mill following coarse grinding using a Wiley type mill. Total water-soluble carbohydrate was extracted from a 0.25 g ground sample by boiling de-ionized water containing 1mg ml^{-1} propylene glycol as the internal standard for 1 hr. The extract was passed through a 0.45 μ m pore filter and analyzed using high performance liquid chromatography (HPLC). Mono and disaccharides (fructose, glucose and sucrose) and fructan (degree of polymerization: DP≥3) in the extract were separated using gel permeation HPLC columns (Shodex KS-802 and KS-803 combined, Showa Denko, Tokyo, Japan) with a flow rate of 0.8 ml min^{-1} of HPLC grade water at 50 °C, and were detected using a refractive index detector (L-2490, Hitachi, Tokyo, Japan) (Yoshida et al., 1998). Carbohydrates were identified using fructose, glucose and sucrose as the external standards and quantified using propylene glycol as the internal standard.

Climatic conditions during the experiment

In the first winter (2001/2002), continuous snow cover started on 28 November 2001, which was one week earlier than in the average year. The snow melted on 27 March 2002, which was two weeks earlier than in the average year. The average monthly temperatures were -5.3°C for December 2001 and -4.5, -2.7 and 0.7°C for January, February and March

2002, respectively. The average temperature in March 2002 was 2.0°C higher than in the average year.

From summer to autumn, the average monthly temperatures in 2002 were 18.8, 16.3, 10.1 and 1.5 °C for August, September, October and November, respectively. Monthly precipitation in 2002 was 95, 112, 152 and 156 mm for August, September, October, and November, respectively.

In the second winter (2002/2003), continuous snow cover started on 11 December 2002, which was 6 days later than the average year. The snow melted on 10 April 2003, which was the same as in the average year. The average monthly temperatures were -4.8 °C for December 2002 and -5.6, -5.8 and -1.2 °C for January, February and March 2003, respectively. Those temperatures were almost the same as in the average year. The maximum snow depth was about 100 cm in both winters, which was the same as in the average year.

Evaluation of winter hardiness

Two fungicides were applied for the control of snow mold before the start of the continuous snow cover (Takai et al. 2004). A 500× solution of iminoctadine-triacetate and tolclofosmethyl was sprayed over two replications on 26 November 2001 and on 4 December 2002. Iminoctadine-triacetate controls ascomycetous snow mold, such as pink snow mold caused by *Monographella nivalis* (Schaffnit) Müller and Sclerotinia snow blight caused by *Myriosclerotinia bolearis* (Bubák & Vleugel) Kohn, and tolclofosmethyl controls basidiomycetus *Typhula* spp. The fungicides were not applied in the other two replications for the purpose of the observation of winter hardiness and snow mold. The winter hardiness of each plot was scored on a scale of 1-9 (1, very poor; 9, very good) based on the vigor of the sprout after the snow melted on 11 April 2002 and on 23 April 2003, respectively. The index of damage by snow mold (IDSM) was calculated to evaluate the snow mold resistance based on an equation (IDSM = 100 x (1-winter hardiness in untreated control plot / winter hardiness in treated plot)). Values of IDSM that were negative were replaced with the value zero. High IDSM scores indicated susceptibility to snow mold. Snow endurance, which indicates tolerance to the low temperature, dark and anaerobic condition under the snow, was evaluated based on winter hardiness in the treated plot.

Evaluation of growth habits and other measured traits

Plant vigor was scored on a scale of 1-9 (1, very poor; 9, very vigorous) on 18 October 2001, 30 August 2002, and 12 October 2002. Plant vigor was evaluated by defining the standard cultivar ‘Okamidori’ as 5 (intermediate) in each season. The dry matter contents in foliage and stubble were measured on 2 September 2002 after drying in an oven at 70 C° for 48 h. Brown stripe caused by *Cercosporidium graminis* (Fuckel) Deighton occurred in August 2001, and stem rust caused by *Puccinia graminis* Pers.: Pers. subsp. *graminicola* Urban. occurred on October 2001 and 2002. The severity of the stem rust and brown stripe were scored on a scale of 1-9 (1, extremely low; 9, extremely high). Regrowth after cutting was scored on a scale of 1-9 (1, very poor; 9, very good) on 11 September 2002. Heading date was scored as the number of days after 1 May in 2002 and 2003.

Statistical analysis

Data were statistically analyzed using analysis of variance, and the significance of mean squares was then estimated. The ecotype from Morocco was dead after the first winter and was removed from statistical analysis. Where significant differences were found, least significant differences (LSD) were calculated. Simple correlation coefficients were calculated among WSC concentrations in foliage and stubble; winter hardiness and related traits; other measured traits, i.e. plant vigor, dry matter content, severity to disease, heading date and regrowth; and geographic origins of ecotypes, i.e. latitude and altitude of the collection point. The collection point and town for each of the 58 accessions was recorded, and their latitudes were used for the analysis. The altitude of the collection point was recorded for 25 accessions, mainly those from Europe. The altitude data were square-root transformed before the analysis in order to achieve a normal distribution. Stepwise multiple regression analysis, with winter hardiness, snow endurance and IDSM in 2003 as the dependent variable, was carried out using WSC in stubble and related traits, plant vigor, and severity to stem rust in autumn 2002 as independent variables.

Results

Variation of WSC concentration among ecotypes

The concentrations of WSC in foliage for each geographic area of origin are shown in Table 2. The total WSC concentration of foliage was highest in Japanese cultivars and lowest in ecotypes from northern Europe in both years. Other ecotypes, except for those from Russia, showed similar levels of total WSC concentration in October 2001. The concentration and proportion of fructan in total WSC was higher in Japanese cultivars and ecotypes from Ireland and lower in ecotypes from Russia, northern Europe and western Asia in October 2001. The total WSC concentration in September 2002 was lower than that in October 2001. Mono and disaccharides, which made up the main proportion of WSC, were present at higher levels in Japanese cultivars and in ecotypes from Japan and were at lower levels in ecotypes from the UK and northern Europe in September 2002.

Concentrations of WSC in stubble, measured in September 2001 and November 2002, are shown in Table 3 for each geographic area of origin. The variation of fructan concentration among ecotypes was larger than that of mono and disaccharide concentration in both years. The total WSC and fructan concentrations of ecotypes from Spain and southern Europe, which originated around the Mediterranean Sea, were highest in September 2002. The total WSC and fructan concentrations of the ecotypes, except for those from Ireland, U.K., Spain, and southern Europe, were twice as high in November 2002 as in September 2002. Ecotypes from Japan showed their highest concentration of total WSC in stubble, and showed especially high fructan concentrations in November 2002. Ecotypes from France, central Europe and central Asia showed fructan concentrations as high as those of Japanese cultivars.

The amount of increase in WSC in autumn, which was calculated from the difference in the WSC concentration in stubble between September and November in 2002, was large for ecotypes from central Asia and Japan and was small for ecotypes from Ireland, Spain and southern Europe (Table 3).

The correlation coefficients for total WSC concentration between foliage and stubble in September 2002 were not significant ($r=0.11$, $P>0.05$, $n=63$). On the other hand, the correlation coefficients for total WSC concentration between foliage in October 2001 and stubble in November 2002 were significant ($r=0.34$, $P<0.01$, $n=63$).

Variation of other measured traits among ecotypes

Ecotypes from France, middle Europe and Japan were early maturing (Table 4), just like standard cultivar ‘Wasemidori’ which headed on 23 days after 1 May (data not shown). Ecotypes from Russia and northern Europe were late maturing between standard cultivar ‘Okamidori’ and ‘Toyomidori’, which headed on 31 and 37 after 1 May, respectively (data not shown). The plant vigor of ecotypes from Ireland, UK, Spain and France, as well as of Japanese cultivars, was high in summer, but these European ecotypes were more vigorous in autumn than the Japanese cultivars. Ecotypes from Ireland, UK, Spain and southern Europe were more resistant to brown stripe and stem rust than Japanese cultivars. On the other hand, ecotypes from Russia and northern Europe were susceptible to both diseases. The dry matter content in foliage was highest for ecotypes from northern Europe, and dry matter content in stubble was highest for ecotypes from Spain.

Variation of winter hardiness among ecotypes

Typhula snow blight caused by *T. ishikariensis* Imai var. ishikariensis was observed in all accessions in untreated control plots in both winters. Pink snow mold was not observed in untreated control plots in either winter. According to these results, the winter damage observed in this experiment was caused by Typhula snow blight.

The winter hardiness, snow endurance and index of damage by snow mold (IDSM) of each ecotype over the two winters are shown in Table 5. Ecotypes from Hokkaido showed the highest winter hardiness of all ecotypes in both winters. Ecotypes from France, middle Europe and central Asia, as well as Japanese cultivars, also showed good winter hardiness in both winters. The winter hardiness of ecotypes from Spain and southern Europe was lower than that of other ecotypes in the second winter.

The snow endurance of ecotypes from France and central Europe was higher than that of Japanese cultivars. Ecotypes from the UK, Ireland and Spain showed good snow endurance in the first season, in which the snow cover period was shorter, but their snow endurance decreased in the second season, in which the snow cover period was longer.

The values of IDSM were lowest for ecotypes from northern Europe, Russia, central Asia and Japan, indicating that they had the highest levels of resistance to Typhula snow blight. The values of IDSM for ecotypes from Europe, except for northern Europe, were higher than those for other ecotypes in both years, and were especially high in the second year.

Correlation of WSC concentrations with other measured traits

The simple correlation coefficients between WSC concentrations and other measured traits in autumn are shown in Tables 6 and 7. Plant vigor in autumn was significantly correlated with the WSC concentrations of foliage in autumn and was not correlated with those of stubble in autumn. The severity of stem rust was negatively correlated with the WSC concentration of foliage, but was not correlated with the WSC of stubble. Stem rust infection reduced the WSC concentrations of foliage, but did not affect the WSC of stubble in autumn. The latitude of origin was negatively correlated with the WSC concentration and plant vigor in autumn in 2001. The altitude of origin was negatively correlated with plant vigor in autumn in both years and was positively correlated with fructan concentration in stubble, winter hardiness and snow endurance in 2002.

Plant vigor in summer was not correlated with the WSC concentrations of foliage and stubble (Table 6). The severity of brown stripe was negatively correlated with the mono and disaccharide concentration in foliage and the WSC concentrations in stubble. Regrowth after cutting was not correlated with the WSC concentration of foliage and stubble in summer. The WSC concentrations of stubble were negatively correlated with the dry matter content of stubble and foliage. The latitude of origin was positively correlated with the WSC concentrations in stubble and negatively correlated with dry matter content in stubble.

Total WSC and fructan concentrations in foliage and stubble showed positive correlations with winter hardiness and snow endurance ($P<0.05$, Table 7). Ecotypes which have high fructan concentration in autumn tend to show high winter hardiness and snow endurance. The amount of the increase in WSC in autumn, which was measured as the difference in the WSC concentration in stubble between September and November in 2002, was positively correlated with winter hardiness and negatively correlated with IDSM in the second season. Plant vigor in autumn was positively correlated with winter hardiness and the WSC concentration in the first season, but was not correlated with these variables in the second winter. The severity of stem rust was negatively correlated with the WSC concentration in foliage, winter hardiness and plant vigor in autumn. The latitude of origin was positively correlated with the severity of stem rust in both winters. A relationship between the altitude of origin and winter hardiness was clearly found in the second season. The altitude of origin was positively correlated with the fructan concentration, winter hardiness, and severity of stem rust, and was negatively correlated with plant vigor in autumn. This result indicates that the fructan concentration and winter hardiness of the ecotypes increased with increasing altitude, whereas their plant vigor in autumn and resistance to stem rust decreased.

Based on multiple regression analysis with winter hardiness, snow endurance and IDSM in 2003 as the dependent variables, the amount of increase in fructan and plant vigor in autumn were selected as independent variables. The amount of increase in fructan, which explained 30 % of the variation in winter hardiness, was most closely correlated with winter hardiness (Table 8). The increases in fructan and plant vigor in autumn were correlated with snow endurance and IDSM, which explained 19 and 50 % of the variation in snow endurance and IDSM, respectively. The increases in fructan and plant vigor were more closely correlated with IDSM than snow endurance, because the coefficient of determination (R^2) for them was larger in IDSM than in snow endurance. The amount of increase in fructan had a negative correlation with IDSM. Therefore, ecotypes that showed a greater increase in fructan and lower plant vigor in autumn tended to have higher snow mold resistance because of their lower IDSM.

From these results, the fructan concentrations of foliage and stubble in autumn were found to be closely related with winter hardiness in cocksfoot ecotypes. Fructan concentrations were related with plant vigor in autumn, disease resistance, and geographic origin, all of which were related with each other. Snow mold resistance was related with the amount of increase in fructan and the growth habits in autumn. The ecotypes collected at high altitudes or high latitudes showed susceptibility to stem rust and lower growth in autumn.

Discussion

Most of the European ecotypes of cocksfoot showed lower WSC concentrations in foliage, and these concentrations were less than that of Japanese cultivars in our experiment. The variation of the WSC concentration in foliage among the ecotypes in summer was narrower than that found in cultivars in our previous study (Sanada et al. 2005). Therefore, a new breeding material to improve the WSC concentration in cocksfoot could not be found in these accessions. Although the correlation between the WSC concentration in foliage and the geographic origin of the cultivars has not been clear in our previous study, no correlation between WSC concentration in foliage and the latitude of origin in ecotypes was found in this study, except in the case of mono and disaccharides in autumn ($r=-0.27$, $P<0.05$). Disease resistance related to the WSC concentration in foliage might complicate this relation. Negative effects of infection with stem rust and brown stripe on forage quality have been reported in cocksfoot cultivars (Isawa et al. 1984; Sanada et al. 2005). Though ecotypes from the UK and Ireland that showed lower WSC concentrations were more resistant to stem rust and brown stripe than Japanese cultivars, they could be used as breeding materials for the improvement of disease resistance. Some ecotypes that have not undergone breeding were more susceptible to diseases than bred and introduced cultivars that have been selected on the basis of disease resistance. Some ecotypes, especially from Russia and northern Europe, which were susceptible to brown stripe and stem rust and showed poor plant vigor in summer and autumn, showed lower WSC concentrations in their foliage. Crown rust infection was negatively correlated with growth in autumn and winter in perennial ryegrass (Humphreys 1989). Although those ecotypes showed high snow mold resistance, they could not be used directly as breeding materials for the improvement of WSC and winter hardiness. It might be necessary to improve the resistance to disease in order to increase the WSC concentration of foliage both in summer and autumn.

Unlike the observations of WSC concentration in foliage, a wide variation of WSC concentration in stubble was found in both years. Ecotypes from Spain and southern Europe, around the Mediterranean Sea where the summer drought can be severe, showed the highest fructan concentration in stubble in summer. A positive correlation between drought tolerance and fructan content in stem and stubble in the Mediterranean ecotypes has been reported in the Mediterranean environment (Volaire & Lelièvre 1997; Piano et al. 2004). The temperature and precipitation at Sapporo in summer were less severe than under the Mediterranean climatic conditions. However, those ecotypes showed high fructan concentrations in Sapporo under somewhat dry conditions, which was quite different from the conditions in the Mediterranean region. Ecotypes from Spain showed no summer dormancy in this study, although summer dormancy was found in Mediterranean ecotypes collected from areas with dry climatic conditions (Knight 1973; Volaire & Lelièvre 1997). These Spanish ecotypes also had more vigorous growth in summer and autumn than did Japanese cultivars and ecotypes. Because they were very sensitive to dry conditions, they might express a strong ability to produce and accumulate fructans in the stubble, in correspondence with the summer climate in Sapporo. The higher dry matter content in stubble in summer observed in ecotypes collected from lower latitudes may be related with the sensitivity to drought of Spanish ecotypes. This correlation by which southern ecotypes showed higher dry matter content in the leaves and stems has been observed in a controlled environment (Ryser & Aeschlimann 1999). Drought-tolerant germplasm showed a higher fructan concentration and dry matter content and had an elevated osmotic pressure in order to protect plants from desiccation (Volaire & Thomas, 1995; Volaire et al. 1998).

No correlation between the WSC concentration and dry matter content of foliage in summer was found in this experiment. Infection with brown stripe might affect this correlation, which has been found in a controlled environment (Volaire & Lelièvre 1997; Ryser & Aeschlimann 1999). A negative correlation between WSC concentration and the severity of brown stripe was found in summer. Though the WSC was synthesized in leaves by photosynthesis and then translocated to the leaf sheath and stem (Pollock & Cairns 1991), infection with brown stripe would inhibit the sugar synthesis in leaves and the translocation to stubble, and would thus reduce the WSC concentration in stubble. However, no correlation between the WSC concentration in foliage and stubble and the plant growth in summer, which was related with dormancy, was found in this study. To elucidate this relationship, further study using physiological and genetic analysis is required.

On the other hand, the Mediterranean ecotypes did not increase their fructan concentration in autumn in correspondence with low temperatures, while other ecotypes increased this concentration. This result may be related to the lack of dormancy in autumn and winter in the Mediterranean ecotypes (Knight 1973). The increase of fructan in autumn, which would be related to low-temperature response, was positively correlated with growth in autumn and winter hardiness in this study. An ecotype from Norway was found to have low growth (Eagles 1967; Thomas & Stoddart 1995) and to accumulate fructan in leaf sheaths during exposure to low temperature (Eagles 1967) under a controlled environment. Although ecotypes from the northern region showed high growth under high and moderate temperatures in a controlled environment (Eagles 1967; Ryser & Aeschlimann 1999), they showed low growth in summer in the field in this study. This growth was affected by environmental conditions such as rainfall and photo period.

Ecotypes from the UK, Ireland, Spain and southern Europe, which showed a lower increase of fructan concentration and more vigorous growth in autumn than the northern ecotypes, might have a low sensitivity to low temperatures. Ecotypes from the southern region have been less sensitive to low temperatures under a controlled environment (Eagles 1967; Thomas & Stoddart 1995). The fructan concentration of foliage and stubble in autumn showed a positive correlation with winter hardiness and snow endurance but no correlation with snow mold resistance. The WSC in grasses and cereals has been found to be related with freezing tolerance and snow mold resistance during autumn and winter (Shibata & Shimada 1986; Suzuki 1989; Gaudet et al. 1999). The fructan concentrations have been more closely related with snow mold resistance than the mono and disaccharide concentrations in wheat (*Triticum aestivum* L.) (Yoshida et al. 1998). The fructan concentration in autumn was related with snow endurance rather than with snow mold resistance in this study. Temperate grasses accumulate fructans at low temperatures (Chatterton et al. 1989) and during hardening in autumn to act as energy reserves (Pollock & Cairns 1991; Moriyama et al. 2003), thus increasing their snow mold resistance (Abe 1986). Some ecotypes which had high fructan concentration in autumn showed susceptibility to Typhula snow blight in this study. Though cocksfoot has reduced WSC under low O_2 concentrations at low temperatures (Bertrand et al. 2003), the fructans in cocksfoot are important as an energy reserve so that the plants can maintain their metabolism and survive anaerobic and dark conditions under the snow. Although cocksfoot ecotypes would have the same mechanism for snow mold resistance as other grasses and cereals concerning with fructan metabolism, a different mechanism like antifungal activity might be expressed for resistance to snow mold under the snow regardless of fructan concentration.

Ecotypes from France that were collected from the Alpine area at altitudes ranging from 700 to 1300m showed good winter hardiness. A positive correlation between the altitude of origin and winter hardiness has been found in Turkish populations of cocksfoot (Abe 1980). Ecotypes derived from mountainous areas in south France showed winter dormancy and lower drought tolerance in South Australia (Knight 1973). Those ecotypes showed less dormancy in autumn and more snow endurance than the Japanese cultivars in our experiment, and they accumulated more fructan in stubble in autumn than did the ecotypes derived from southern European plains in autumn. Those ecotypes might be naturally selected for winter hardiness and increased fructan concentration with increasing altitude. A negative correlation between plant vigor in autumn and winter hardiness has been reported in cocksfoot cultivars (Nakayama et al 1997). Some ecotypes, which had lower fall dormancy and better winter hardiness, might be useful as breeding materials to break the negative correlation between dormancy in autumn and winter hardiness. Dormancy in autumn has been related with forage yield in autumn in cocksfoot (Abe 1986) and must be decreased to increase the yield in autumn. Those ecotypes which showed lower snow mold resistance than Japanese cultivars should be crossed with Japanese cultivars and ecotypes to be used as breeding materials for the improvement of yield in autumn, but this objective should be carried out with concomitant selection for snow mold resistance.

No breeding material that has high WSC concentration in foliage could be found among the ecotypes in this study. However, large variations were found in fructan concentration, plant vigor, disease resistance and winter hardiness among ecotypes that were adapted to varying climate conditions. Some ecotypes that showed disease resistance, good winter hardiness and plant vigor would be useful as breeding materials for the Hokkaido area. Further studies involving physiological and genetic analysis of WSC and the growth habits of the ecotypes are necessary to identify and characterize the complicated interactions among climatic factors that account for their differential geographic origins.

Acknowledgments

We thank the Institute of Grassland and Environment Research in the U.K. and the N.I. Vavilov Institute of Plant Industry in Russia for supplying the seed of the ecotypes used in this study. We thank Dr M. D. Casler, USDA-ARS, U.S. Dairy Forage Research Center, for a critical reading of the manuscript and Dr M. Yoshida, National Agricultural Research

Center for the Hokkaido Region, for technical advice on the analysis of WSC by HPLC. We thank H. Morishita for field management and Dr R. A. B. M. Golam and M. Fujimori for the analysis of WSC.

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Table 1. Origins of ecotypes collected in Europe and Asia.

Region	Numer of accessions	Origin (No. of accessions) and cultivar	Latitude
Ireland	3	Ireland	N51°34´- N52°05´
UK	8	U.K.	N50°48´- N52°29´
Spain	6	Spain	N42°49´- N43°23´
France	7	France	N44°17´- N44°36´
Russia	7	Russia	N51°38´- N64°34´
Northern Europe	3	Norway (2), Sweden (1)	N57°47´- N60°
Southern Europe	6	Portugal (2),Italy (2), Greece (2)	N37°52´- N44°21´
Middle Europe	6	Hungary (2), Switzerland(1),Czech (1), Germany (1), Rumania (1)	N45° - N52°07´
Western Asia	4	Armenia (3), Turkey (1)	N40°10´- N40°19´
Central Asia	4	Pakistan (1), Kazafstan (1), Tajikistan (1), China (1)	N38° - N43°43´
Morocco	1	Morocco	
Japan	4	Hokkaido	N43°28´- N45°25´
Japanese cultivar	4	Wasemidori, Okamidori, Harujiman, Toyomidori	
Russian cultivar	2	VIK61, Grujinskaya	

Table 2. Regional means of water-soluble carbohydrate concentrations of foliage in cocksfoot ecotypes.

	October 20, 2001			September 2, 2002		
Region	Mono and disaccharide (mg g^{-1} DW)	Fructan (mg g^{-1} DW)	Total WSC (mg g^{-1} DW)	Mono and disaccharide (mg g^{-1} DW)	Fructan (mg g^{-1} DW)	Total WSC (mg g^{-1} DW)
Ireland	88.7	100.8	189.5	21.7	3.6	25.3
UK	85.5	72.8	158.3	18.9	4.4	23.3
Spain	90.8	75.6	166.4	28.6	11.3	39.9
France	72.1	63.8	135.8	22.8	5.8	28.6
Russia	59.5	29.3	88.7	32.0	10.3	42.3
Northern Europe	41.0	28.3	69.4	16.4	7.5	23.9
Southern Europe	99.2	50.6	149.8	33.3	4.9	38.3
Middle Europe	81.9	58.8	140.7	28.3	11.5	39.8
Western Asia	84.2	32.9	117.1	23.9	6.9	30.8
Central Asia	68.1	68.7	136.8	27.8	6.5	34.3
Morocco	152.6	46.9	199.5			
Japan	94.2	54.0	148.2	42.1	9.0	51.0
Japanese cultivar	123.9	131.3	255.2	54.3	9.2	63.5
Russian cultivar	80.7	73.0	153.7	31.6	9.5	41.1
Mean	82.3	64.6	146.9	29.4	7.7	37.1
SD	29.0	39.5	55.7	15.1	6.5	17.4
LSD(0.05)	55.7	21.4	43.9	2.5	2.2	1.7

SD, standard deviation ; LSD, least significant difference.

Table 3. Regional means of water-soluble carbohydrate concentrations of stubble in cocksfoot ecotypes.

Region	September 2, 2002			November 2, 2002			Amount of increase in autumn [a]		
	Mono and disaccharide (mg g^{-1} DW)	Fructan (mg g^{-1} DW)	Total WSC (mg g^{-1} DW)	Mono and disaccharide (mg g^{-1} DW)	Fructan (mg g^{-1} DW)	Total WSC (mg g^{-1} DW)	Mono and disaccharide (mg g^{-1} DW)	Fructan (mg g^{-1} DW)	Total WSC (mg g^{-1} DW)
Ireland	39.6	82.8	122.4	59.1	71.3	130.4	19.4	-11.5	8.0
UK	39.8	73.6	113.4	52.8	86.4	139.2	13.0	12.8	25.8
Spain	70.5	150.9	221.4	49.2	104.0	153.2	-21.3	-46.9	-68.2
France	29.9	71.5	101.4	62.1	152.5	214.6	32.2	80.9	113.1
Russia	26.7	36.7	63.4	48.7	130.2	178.9	22.1	93.5	115.5
Northern Europe	35.3	33.5	68.9	57.3	78.2	135.4	21.9	44.6	66.6
Southern Europe	58.0	126.7	184.7	64.9	121.7	186.6	6.9	-5.0	1.9
Middle Europe	44.6	73.9	118.5	67.3	180.0	247.3	22.7	106.0	128.7
Western Asia	35.4	41.9	77.3	54.3	95.8	150.1	18.9	53.9	72.8
Central Asia	23.6	26.5	50.0	67.8	146.6	214.4	44.2	120.1	164.3
Japan	37.4	98.4	135.7	67.1	274.9	342.1	29.8	176.6	206.3
Japanese cultivar	40.7	85.2	126.0	66.7	152.1	218.8	26.0	66.9	92.8
Russian cultivar	52.6	74.7	127.3	53.6	157.8	211.5	1.0	83.1	84.2
Mean	41.1	75.1	116.2	59.3	134.7	194.0	18.2	59.6	77.8
SD	19.4	46.5	79.3	9.9	51.9	56.9	21.4	67.7	99.2
LSD(0.05)	27.8	58.4	93.0	ns	79.0	82.9	33.2	108.7	137.2

Note. [a] difference of WSC concentration between September and November in 2002; SD, standard deviation ; LSD, least significant difference ;ns, not significant.

Table 4. Regional means of variables in cocksfoot ecotypes collected in Europe and Asia.

Region	Heading date[a]	Plant vigor in summer[b]	Plant vigor in autumn[b]	Severity to brown stripe[c]	Severity to stem rust[c]	Regrowth after cutting[d]	Dry matter content in foliage (%)	Dry matter content in stubble (%)
	2002-2003	2002	2001-2002	2002	2001-2002	2002	2002	2002
Ireland	32.5	8.0	7.3	3.8	1.8	6.5	23.8	17.0
U.K.	29.8	7.7	7.1	3.9	2.1	7.6	23.4	17.8
Spain	27.8	7.9	7.2	3.4	1.8	8.4	23.5	21.9
France	24.8	7.5	6.7	5.1	2.8	8.4	25.4	17.2
Russia	33.1	5.0	3.1	4.7	6.7	5.2	27.2	15.0
Northern Europe	34.1	5.1	4.5	6.0	5.7	6.2	28.3	16.1
Southern Europe	28.4	2.9	4.8	3.1	2.0	4.5	22.7	16.9
Middle Europe	24.6	6.9	5.8	4.9	3.8	7.5	26.3	17.0
Western Asia	31.4	5.1	4.3	4.5	4.8	5.9	26.6	16.8
Central Asia	31.6	6.0	4.2	4.6	5.1	4.5	27.0	16.1
Japan	24.6	6.9	5.3	4.9	4.1	6.4	23.7	21.0
Japanese cultivar	30.4	7.5	5.9	4.4	4.0	6.6	24.1	18.2
Russian cultivar	31.1	5.3	5.6	3.9	2.7	6.4	25.1	18.9
Mean	29.6	6.3	5.5	4.4	3.6	6.5	25.2	17.7
SD	7.6	2.1	1.7	1.2	1.9	2.1	3.9	5.0
LSD(0.05)	1.2	0.3	0.3	0.4	0.3	0.3	1.5	1.7

Note. [a] days after 1 May, mean for 2 years ; [b] scored on a scale of 1-9 (1, very poor; 9, very vigorous), mean for 2 years in autumn ; [c] scored on a scale of 1-9 (1, extremely low; 9, extremely high), mean for 2 years in stem rust; [d] scored on a scale of 1-9 (1, very poor; 9, very good); SD, standard deviation ; LSD, least significant difference.

Table 5. Regional means of winter hardiness and related traits in cocksfoot ecotypes collected in Europe and Asia.

Region	Winter hardiness[a]			Snow endurance[b]			Index of damage by snow mold[c] (%)		
	2001/2002	2002/2003	Mean	2001/2002	2002/2003	Mean	2001/2002	2002/2003	Mean
Ireland	5.7	3.1	4.4	7.6	5.0	6.3	24.3	37.9	31.1
U.K.	5.8	3.4	4.6	6.8	5.0	5.9	14.2	30.7	22.5
Spain	5.2	2.8	4.0	6.9	5.3	6.1	24.5	48.3	36.4
France	6.2	5.5	5.8	6.4	7.5	7.0	4.0	28.0	16.0
Russia	4.6	4.7	4.7	4.2	4.7	4.4	0.7	2.9	1.8
Northern Europe	4.0	4.5	4.3	4.4	5.0	4.7	6.9	7.1	7.0
Southern Europe	2.9	2.4	2.6	3.1	2.8	2.9	9.7	9.7	9.7
Middle Europe	5.3	5.8	5.5	6.4	7.1	6.8	17.8	17.8	17.8
Western Asia	4.9	4.7	4.8	4.5	6.1	5.3	8.5	22.7	15.6
Central Asia	5.1	5.9	5.5	4.8	6.4	5.6	0.0	2.7	1.4
Japan	5.9	6.1	6.0	5.3	6.1	5.7	0.0	5.3	2.6
Japanese cultivar	5.5	5.5	5.5	5.9	6.0	5.9	7.2	8.7	7.9
Russian cultivar	5.7	4.2	4.9	5.6	6.3	5.9	4.7	34.8	19.7
Mean	5.1	4.5	4.8	5.5	5.6	5.6	9.4	19.7	14.6
SD	1.1	1.5	1.3	1.5	1.6	1.5	11.5	17.9	14.6
LSD(0.05)	1.6	1.5	1.5	1.5	1.5	1.5	4.7	4.0	4.4

Note. [a] scored on a scale of 1-9 (1, very poor; 9, very good) in untreated plot ; [b] scored on a scale of 1-9 (1, very poor; 9, very good) in treated plot ; [c] 100 × (1-winter hardiness / snow endurance), negative value was described as 0 % ; SD, standard deviation ; LSD, least significant difference.

Table 6. Simple correlation coefficients among WSC concentrations in foliage and stubble, variables and geographic origin for cocksfoot ecotypes on 2 September 2002.

Variables		Heading date	Plant vigor in summer	Severity to brown stripe	Dry matter content in foliage	Dry matter content in stubble	Regrowth	Latitude	Altitude
		n=64	n=64	n=64	n=64	n=64	n=64	n=58	n=25
Foliage in September 2002	Mono and disaccharide	0.23	-0.02	-0.25*	-0.11	0.13	-0.17	-0.17	-0.07
	Fructan	0.18	-0.03	-0.02	-0.06	0.11	-0.03	-0.01	0.07
	Total WSC	0.26*	-0.02	-0.22	-0.11	0.15	-0.16	-0.15	-0.04
Stubble in September 2002	Mono and disaccharide	-0.14	0.11	-0.41***	-0.30*	0.52***	0.22	-0.26*	-0.10
	Fructan	-0.13	0.17	-0.42***	-0.32*	0.63***	0.21	-0.29*	0.10
	Total WSC	-0.14	0.17	-0.44***	-0.33**	0.64***	0.22	-0.30*	0.04
Origin	Latitude	0.28	-0.08	0.20	0.17	-0.36**	-0.12		
	Altitude	-0.35	-0.33	0.16	0.39	0.07	-0.27		

*,** and *** , significant at $P \leq 0.05$, ≤ 0.01 and ≤ 0.001, respectively.

Table 7. Simple correlation coefficients among WSC concentrations in foliage and stubble, winter hardiness and related traits, and geographic origin for cocksfoot ecotypes collected in Europe and Asia.

Variables	WSC	Winter hardiness	Snow endurance	IDSM^{a}	Plant vigor in autumn	Severity to stem rust	Latitude	Altitude
		n=64	n=64	n=64	n=64	n=64	n=58	n=25
2001/2002								
Foliage in October 2001	Mono and disaccharide	0.11	0.22	0.22	0.27*	-0.46***	-0.27*	0.06
	Fructan	0.35**	0.41***	0.16	0.49***	-0.38**	-0.15	-0.17
	Total WSC	0.30*	0.40**	0.23	0.47***	-0.50***	-0.25	-0.07
Winter hardiness		-	-	-	0.38**	-0.28*	-0.02	0.01
Snow endurance		0.72**	-	-	0.63***	-0.59***	-0.01	-0.13
IDSM^{a}		-0.11	0.57**	-	0.39**	-0.54***	-0.10	-0.13
Plant vigor in autumn		-	-	-	-	-0.72***	-0.26*	-0.35
Severity to stem rust		-	-	-	-	-	0.28*	0.24
2002/2003								
Stubble in November 2002	Mono and disaccharide	0.25	0.20	0.04	0.09	-0.10	-0.22	0.01
	Fructan	0.40**	0.25*	0.20	-0.04	0.03	-0.13	0.41*
	Total WSC	0.41***	0.26*	0.18	-0.02	0.01	-0.16	0.37
Amount of increase in autumn^{a}	Mono and disaccharide	0.45***	0.26*	-0.36**	-0.27*	0.32*	0.06	0.07
	Fructan	0.55***	0.30*	-0.44***	-0.30*	0.34**	0.07	0.21
	Total WSC	0.55***	0.31*	-0.45***	-0.30*	0.35**	0.07	0.20
Winter hardiness		-	-	-	-0.24	0.39**	-0.14	0.54**
Snow endurance		0.75***	-	-	0.21	-0.06	-0.24	0.54**
IDSM^{b}		-0.51**	0.16	-	0.66***	-0.65***	-0.13	-0.10
Plant vigor in autumn		-	-	-	-	-0.91***	-0.20	-0.56**
Severity to stem rust		-	-	-	-	-	0.26*	0.56**

Note.^{a} difference of WSC concentration in stubble between September and November in 2002; ^{b} index of damage by snow mold; *,** and *** , significant at P≤0.05, ≤0.01 and ≤0.001, respectively.

Table 8. Linear and multiple regression equations for the means of cocksfoot ecotypes data with winter hardiness as an independent variable using physiological traits in autumn as dependent variables.

		Regression coefficient of dependent variables			
Independent variables	Intercept	Increase of fructan[a]	Plant vigor in autumn	R^2	P-value
Winter hardiness	3.96	0.01	-	0.30	<0.001
Snow endurance	3.82	0.01	0.23	0.19	0.002
IDSM[b]	-3.40	-0.06	4.49	0.50	<0.001

Note. [a] difference of WSC concentration in stubble between September and November in 2002; [b] index of damage by snow mold.