Short communication

Genetic variation in water soluble carbohydrate concentration in diverse cultivars of *Dactylis glomerata* L. during vegetative growth

Y. Sanada^A^B^, T. Takai^A^ and T. Yamada^A^

^A^ National Agricultural Research Center for Hokkaido Region, Hitsujigaoka 1, Toyohira, Sapporo, 062-8555, Japan.

^B^ Corresponding author: email:ysanada@affrc.go.jp
Abstract.

The water-soluble carbohydrate (WSC) content of Dactylis glomerata L. (cocksfoot) during vegetative growth is lower than that of other temperate grasses. Variation in the WSC content of vegetative growth among cultivars was assessed in order to assess the potential to improve forage quality. There were significant differences among cultivars for WSC content ($P<0.01$) in all maturity groups. Average WSC content of the late (L) maturity group was higher than that of the early (E) and medium (M) maturity groups. Variation in the WSC content was largest among group L cultivars and smallest in group M. The WSC content of cultivars in groups M and L was consistent across 2 years (interactions between cultivars and years were not significant). Brown stripe infection was shown to reduce WSC content in susceptible cultivars. As a result of multiple regression analysis with WSC content as the dependent variable, dry matter content, heading date, thickness of stems and resistance to brown stripe were selected as independent variables that were correlated with WSC. Therefore, variation for these traits must also be monitored in any program to increase WSC content in orchardgrass.

Additional keywords: Germplasm, Orchardgrass.

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 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 harvested in summer in Japan (Masuko et al. 1994). Variation in
dry matter digestibility (DMD) among cultivars and clones in cocksfoot has been
measured previously and broad and narrow sense heritability for DMD in this
germplasm were high (Saiga 1981; Shenk and Westerhaus 1982). However,
selection for DMD should be performed at the vegetative growth stage when DMD is
not affected by the presence of reproductive stems, which have a negative
relationship with DMD (Saiga 1981). The improvement of DMD was conducted in
some temperate grasses and several highly digestible cultivars have been released
(Casler 2001).

Water-soluble carbohydrate (WSC) content of perennial ryegrass (Lolium perenne
L.) has been the subject of considerable breeding efforts (Humphreys 1989). WSC is
an important trait for the nutritive value of forages for animals, because it is related
to palatability and digestibility, as well as the fermentation quality of silage (Smith
et al. 1997; Mayland et al. 2001). 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).

In cocksfoot, improvement of the WSC content could increase forage quality and
palatability, both for grazing and silage making, and thus aid efficient animal
production. A breeding program for improvement of the WSC content in cocksfoot
has been initiated by the National Agricultural Research Center for Hokkaido
Region (NARCH) in Sapporo. Ideally, breeding material with high WSC content
should be selected at the beginning of this breeding program. However, there are no
known sources of high WSC among cultivars of cocksfoot. Therefore, it is necessary
to measure the WSC content in a broad range of germplasm in order to improve the
WSC content in a breeding program.

Recently, a variety of Lolium multiflorum Lam. with high WSC content was developed by recurrent selection for high dry matter content (Marais et al. 2003). Therefore, dry matter content may be an important index for high WSC selection in cocksfoot also.

Time and effort for breeding could be saved in initial screenings if the breeder had better information on the relationship between WSC content and morphological and agronomic traits, for example, dry matter content and plant type. The relationship between morphological and agronomic traits and WSC content has not been reported in cocksfoot. Therefore, this study was also conducted to quantify these relationships.

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 97 accessions of cocksfoot cultivars bred in Japan, USA, Australia, New Zealand and European countries were divided into 3 groups based on the maturity time determined by our previous introduction test (unpublished): 30 accessions in the early maturity (E) group, 34 accessions in the medium maturity (M) group and 33 accessions in the late maturity (L) group. Seeds were sown on 9 May 2000 in paper pots filled with volcanic ash soil containing 0.4 g N, 1.5 g P$_2$O$_5$ and 0.4 g K$_2$O/kg soil and seedlings were grown in a greenhouse. Ten seedlings of each cultivar were transplanted into a field at a spacing of 20 × 80 cm on 19 June in a randomized block design with 3 replications. Compound fertilizer (4.0 g N, 5.5 g P$_2$O$_5$ and 4.0 g K$_2$O/m$^3$) was applied at transplanting. The first cut of each group
occurred at their respective heading dates in 2001 and 2002. Cutting dates are presented in Table 1. After the melting of snow in 2001 and 2002, 7.0 g N, 9.6 g P₂O₅ and 7.0 g K₂O /m² was applied in April; 0.4 g N, 0.3 g P₂O₅ and 0.4 g K₂O /m² was applied after harvesting.

The evaluation of the WSC content was carried out at the second cut in each year when fewer reproductive stems were present compared with the first cut. The samples were harvested between 10 00 a.m. and 11 30 a.m. in order to minimize the effect of environmental conditions such as temperature and solar radiation on the WSC content (Masaki et al. 1978). About 500 g of fresh grass was collected by a small plot harvester (Rem, Saskatchewan, Canada) and dried in an oven at 70°C for 48 h. Dried samples were ground through a 1.0 mm screen using a Cyclone Mill following coarse grinding using a Wiley type mill.

WSC content was analyzed by near infrared reflectance spectroscopy (FOSS NIRSytems Model 6500, U.S.A.) with reflectance data calibrated against total WSC contents measured using the anthrone method (Yemm and Willis 1954). Dry matter yield, dry matter content, plant height, leaf length and width of each cultivar were measured at harvest. Morphological traits were scored on a scale of 1-9 as follows: plant type (1, erect; 9, prostrate), number of stems (1, few; 9, abundant), thickness of stems (1, thin; 9, thick), and number of reproductive stems (1, none; 9, abundant). Stems at vegetative stage except for reproductive stems mean pseudostems, which is predominantly leaf sheath material in this experiment. Resistance to brown stripe (Cercosporidium graminis (Fuckel) Deighton) was scored on a scale of 1-9 (1, susceptible; 9, resistant). Heading date was scored as days after 1 May.

Data was statistically analyzed using analysis of variance within each maturity group. The significance of mean squares for cultivar, year and cultivar × year
interactions was then estimated. Where significant differences were found, least significant difference (l.s.d.) were calculated. Heritability estimates were calculated by using variance components from analysis of variance. The genotypic variance among the cultivars ($\sigma^2_g$) and the error variance ($\sigma^2_e$) were used to estimate the broad-sense heritability ($h^2$) as:

$$h^2 = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_e}$$

Stepwise multiple regression analysis, with WSC content in all cultivars as the dependent variable, was carried out using morphological and agronomic traits investigated in 2002 as independent variables.

Results and Discussion

Variation in the WSC content among the cocksfoot accessions at the time of the second cut is shown in Table 2. There was significant variation ($P<0.05$) in the WSC content among the cultivars in each group. The average WSC content in group L was higher than that in group E or M in both years. The WSC content in group L ranged from 3.9 to 8.7%. The variation of WSC content in group M was smaller than the other 2 groups, although it ranged from 1.9 to 4.5% on average across 2 years. Hayking 2 and Glorus, which have been registered as recommended cultivars in the Hokkaido region of Japan, and strains bred by NARCH for Hokkaido showed higher WSC content than other cultivars. Those cultivars that combine high WSC with adaptation to the environmental conditions of Hokkaido were considered as good breeding materials for improvement of the WSC content. The relationship between WSC content and origin of cultivars was not clear.

Main effects associated with cultivars and years were significant for all 3 groups as shown by the result of analysis of variance. Interactions between cultivars and
year in groups M and L were not significant. On the other hand, there was a
significant interaction between cultivars and years for group E.

Broad sense heritability was high in all groups. For group L, it was estimated
0.91, while for group E and M it was 0.65 and 0.75, respectively.

Correlation coefficients between the WSC content across the two years were
significant overall (r=0.82, P<0.001) but also within each of the maturity groups.
The correlation coefficient of group L was the highest at 0.83 (P<0.001), while that
of group M was 0.63 (P<0.01). Several cultivars in group E such as Hokuiku 50 and
Poltavskaya showed high WSC content in both years (Table 2), although the
correlation coefficient of group E was lowest at 0.53 (P<0.05).

From these results, the WSC content of cocksfoot cultivars during vegetative
growth was consistent across all maturity groups in both years. It is known that the
WSC content is affected by environmental conditions such as solar radiation and it
varies according to the time of sampling (Fisher et al. 1999; Smith et al. 2001).
Despite this, this result shows that effective selection for high WSC content can be
carried out in the presence of these environmental effects. Therefore, a new cultivar
that shows stable high WSC expression in multiple environments can be bred in
cocksfoot. It is necessary to clarify the narrow sense heritability of WSC content in
order to evaluate selection effectiveness accurately in future.

As the result of stepwise multiple regression analysis, dry matter content,
heading date, thickness of stems and resistance to brown stripe were selected as
independent variables and their contribution ratio was about 50% (Table 3). The
standardized partial regression coefficient associated with the dry matter content
was the highest. From these results, the WSC content in cocksfoot was closely
related with the dry matter content. This is similar to the results reported for \( L \).
multiflorum, in which dry matter content was closely correlated with WSC content (Marais et al. 2003). It seems that cultivars in which the dry matter content was high also showed a higher WSC content, since WSC in the vacuole of the plant cell was a larger component as the water content of the plant decreased (Sugawara 1983).

However, it is difficult to evaluate large numbers of individuals for dry matter content because it requires destructive sampling. Estimation of forage quality from morphological traits for initial screening has been attempted using many individuals, and high correlation of in vitro dry matter digestibility with leaf width and late maturity was reported (Lenz and Buxton 1991). The thickness of stems and resistance to brown stripe, which can be evaluated easily, seem to be important indices for high WSC selection, through which it is possible to evaluate a large number of individuals. It is well known that disease may reduce the WSC content and other feed components (Isawa 1983; Smith et al. 1998). Since plant diseases that decrease the forage quality affect not only preference of domestic animal but also yield, disease resistance is one of the most important objectives in grass breeding.

Evaluation for disease resistance may efficiently advance the selection of a high WSC content in the initial stage of breeding when large numbers of individuals are evaluated. However, the genetic correlations between all of these traits need to be quantified before they can be used in a breeding program. After initial screening, direct selection of WSC is more reliable than indirect selection based on morphological and agronomic traits, because WSC content estimated from these traits contains somewhat error. To select the individual with high WSC correctly, direct and genetic selection of WSC content in cocksfoot should be carried out.
following the case of Lolium spp (Humphreys 1989; Marais et al. 2003).

Heading date in 2002 was closely correlated with the WSC content \( (r=0.56, P<0.001) \). It was also correlated with resistance to brown stripe \( (r=0.64, P<0.001) \) and thickness of stems \( (r=0.31, P<0.01) \). Breeding material with a high WSC content should be identified in each maturity group, because selection for high WSC strain may be biased towards later maturity if the selection is based on these morphological and agronomic traits. One breeding strategy to overcome the correlation between WSC content and heading date is to develop a new breeding material by crossing early and medium heading cultivars with late heading cultivars whilst selecting for uniform flowering time. It is necessary to improve the WSC content of medium maturity cultivars, which are mainly cultivated in the Hokkaido region, even though their WSC content of them is lower than other maturity groups. For improvement of the WSC content in the medium maturity group, the individual with a high WSC content should be carefully selected by the direct evaluation of WSC. Then, the WSC content of cocksfoot may be improved by the recurrent selection through direct and indirect evaluation also in each maturity group.

**Acknowledgments**

The authors would like to express their sincere thanks to Dr. K.F. Smith, Plant Genetics and Genomics, Department of Primary Industries, Hamilton, Victoria, Australia and Dr. H. Yamaguchi, NARCH, for critical reading of the manuscript. The authors would like to thank to H. Morishita for field management and Dr. R. A. B. M. Golam for NIR analysis.
References


Table 1. Cutting date of experiment.

<table>
<thead>
<tr>
<th>Cultivar group</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early maturity group</td>
<td>28 May</td>
<td>19 July</td>
<td>17 September</td>
<td>16 October</td>
<td>3 June</td>
<td>17 July</td>
<td>5 September</td>
</tr>
<tr>
<td>Medium maturity group</td>
<td>5 June</td>
<td>23 July</td>
<td>17 September</td>
<td>16 October</td>
<td>11 June</td>
<td>23 July</td>
<td>18 September</td>
</tr>
<tr>
<td>Late maturity group</td>
<td>12 June</td>
<td>31 July</td>
<td>17 September</td>
<td>16 October</td>
<td>18 June</td>
<td>29 July</td>
<td>26 September</td>
</tr>
</tbody>
</table>


Table 2. Water soluble carbohydrate (WSC) content of cocksfoot accessions at second cut in 2001 and 2002.

<table>
<thead>
<tr>
<th>Cultivar / Strains Origin</th>
<th>WSC content (%) 2001 2002 mean</th>
<th>Cultivar / Strains Origin</th>
<th>WSC content (%) 2001 2002 mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asta CIS</td>
<td>3.5  4.0  3.8</td>
<td>Arctic Can</td>
<td>3.2  4.8  4.0</td>
</tr>
<tr>
<td>Dzhegyutinska CIS</td>
<td>3.3  4.4  3.8</td>
<td>Ky Can</td>
<td>1.8  3.7  2.8</td>
</tr>
<tr>
<td>Khutorska CIS</td>
<td>2.8  3.5  3.2</td>
<td>Dedinovska CIS</td>
<td>7.5  8.1  7.8</td>
</tr>
<tr>
<td>Magutyta CIS</td>
<td>4.5  4.7  4.6</td>
<td>Leningradska CIS</td>
<td>2.3  4.0  3.1</td>
</tr>
<tr>
<td>Poltavska CIS</td>
<td>4.9  5.9  5.4</td>
<td>Lida Vik</td>
<td>5.1  5.4  5.3</td>
</tr>
<tr>
<td>Zyrzanovska CIS</td>
<td>4.2  3.1  3.6</td>
<td>Milona Cze</td>
<td>2.2  3.6  2.9</td>
</tr>
<tr>
<td>Fespef Dnk</td>
<td>4.2  2.9  3.6</td>
<td>Amaly Fra</td>
<td>3.6  5.5  4.5</td>
</tr>
<tr>
<td>Jespef Dnk</td>
<td>3.0  4.6  3.8</td>
<td>Athos Fra</td>
<td>4.2  6.0  5.1</td>
</tr>
<tr>
<td>Rano Trifolium Dnk</td>
<td>2.0  4.3  3.1</td>
<td>Cambria UK</td>
<td>5.2  6.7  6.0</td>
</tr>
<tr>
<td>Akimidori Jpn</td>
<td>4.8  4.9  4.8</td>
<td>Haka Fin</td>
<td>2.4  3.6  3.0</td>
</tr>
<tr>
<td>Prairial Fra</td>
<td>1.7  4.5  3.1</td>
<td>Lucyle Fra</td>
<td>6.0  6.5  6.3</td>
</tr>
<tr>
<td>Korea Zairai Kor</td>
<td>3.4  4.3  3.9</td>
<td>Prairial Fra</td>
<td>1.7  4.5  3.1</td>
</tr>
<tr>
<td>Hokuto Hok</td>
<td>2.3  4.1  3.2</td>
<td>Lugolitate Rom</td>
<td>3.6  5.2  4.4</td>
</tr>
<tr>
<td>Able US</td>
<td>3.4  4.4  3.9</td>
<td>Albelsvoll Norg</td>
<td>2.5  3.9  3.2</td>
</tr>
<tr>
<td>Ambassador US</td>
<td>4.2  4.0  4.1</td>
<td>Grassland's Kara NZ</td>
<td>3.4  4.4  3.9</td>
</tr>
<tr>
<td>Hallmark US</td>
<td>3.6  4.3  3.9</td>
<td>Grassland's Wana NZ</td>
<td>4.7  6.1  5.4</td>
</tr>
<tr>
<td>Justus US</td>
<td>2.6  3.6  3.1</td>
<td>Alma Norg</td>
<td>5.2  7.3  6.3</td>
</tr>
<tr>
<td>Potomac US</td>
<td>2.7  4.8  3.7</td>
<td>Pizza Nld</td>
<td>5.1  6.7  5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max.</td>
<td>3.8  4.6  4.2</td>
<td>Min.</td>
<td>1.0  2.9  1.9</td>
</tr>
<tr>
<td>Mean</td>
<td>3.5  4.4  3.8</td>
<td>STD</td>
<td>1.1  1.3  1.2</td>
</tr>
<tr>
<td>CV</td>
<td>22.8  19.1  19.8</td>
<td></td>
<td>20.5  26.3  20.8</td>
</tr>
</tbody>
</table>

Note: Values followed by the same letter do not differ significantly at P = 0.05.
Table 3. Result of multiple regression analysis with WSC content in all cultivars as dependent variable using morphological and agronomi traits in 2002 as independent variables.

<table>
<thead>
<tr>
<th>Characteristics selected as independent variables</th>
<th>Partial regression coefficient</th>
<th>Standard regression coefficient</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter content</td>
<td>0.51</td>
<td>0.48</td>
<td>32.2</td>
</tr>
<tr>
<td>Thickness of stems</td>
<td>0.35</td>
<td>0.30</td>
<td>13.1</td>
</tr>
<tr>
<td>Heading date</td>
<td>0.32</td>
<td>0.34</td>
<td>10.2</td>
</tr>
<tr>
<td>Resistance to brown stripe</td>
<td>0.17</td>
<td>0.18</td>
<td>2.8</td>
</tr>
</tbody>
</table>

R=0.713, R²=0.509, Standard error=1.166, F=23.84, P<0.0001