Running title: Variation of WSC content in cocksfoot

Short communication

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

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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 perene* 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_2O_5 and 0.4 g K_2O / 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_2O_5 and 4.0 g K_2O /m^2) 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 P_2O_5 and 7.0 g K_2O /m^2 was applied in April; 0.4 g N, 0.3 g P_2O_5 and 0.4 g K_2O /m^2 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℃ 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 (σ^2_g) and the error variance (σ^2_e) were used to estimate the broad-sense heritability (h^2) as:

$$h^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_e)$$

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.

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Table 1. Cutting date of experiment.

Cultivar group	2001				2002		
	1st	2nd	3rd	4th	1st	2nd	3rd
Early maturity group	28 May	19 July	17 September	16 October	3 June	17 July	5 September
Medium maturity group	5 June	23 July	17 September	16 October	11 June	23 July	18 September
Late maturity group	12 June	31 July	17 September	16 October	18 June	29 July	26 September

Table 2. Water soluble carbohydrate (WSC) content of cockfoot accessions at second cut in 2001 and 2002.
Aus,Australia;Can,Canada;CIS,formerly USSR;Cze,Czech Repibric;Dnk,Denmark;Deu,Germany;Fin,Finland;Fra,France;Grc,Greece;Hok,Hokkaido region of Japan;Jpn,Japan(except for Hakkaido);Kor,Korea;NARCH,National Agricultural Reseach Center for Hokkaido Region;Nld,Netherlands;Nor,Norway;NZ,New Zealand;Pol,Poland;Rom,Rumania;Swe,Sweden;Swi,Swizerland.

Early maturity group					Medium maturity group					Late maturity group				
Cultivar / Strains	Origin	WSC content (%)			Cultivar / Strains	Origin	WSC content (%)			Cultivar / Strains	Origin	WSC content (%)		
		2001	2002	mean			2001	2002	mean			2001	2002	mean
Asta	CIS	3.5	4.0	3.8	Arctic	Can	3.2	4.8	4.0	Porto	Aus	5.3	7.5	6.4
Dzhegutinskaya	CIS	3.3	4.4	3.8	Kay	Can	1.8	3.7	2.8	Dedinovskaya	CIS	7.5	8.1	7.8
Khutorskaya	CIS	2.8	3.5	3.2	Lidacta	Can	3.1	3.7	3.4	Sverdlovskaya	CIS	5.5	7.6	6.5
Magutnaya	CIS	4.5	4.7	4.6	Mobite	Can	1.8	4.2	3.0	Filippa	Dnk	3.7	4.9	4.3
Poltavskaya	CIS	4.9	5.9	5.4	Leningradskaya	CIS	2.3	4.0	3.1	Leigestra	Deu	3.8	6.0	4.9
Vik 61	CIS	4.6	5.1	4.8	Neva	CIS	1.0	2.9	1.9	Lidaglo	Deu	5.1	5.4	5.3
Zyryanovskaya	CIS	4.2	3.1	3.6	Milona	Cze	2.2	3.6	2.9	Amply	Fra	3.6	5.5	4.5
Amba	Dnk	3.2	4.4	3.8	Niva	Cze	3.3	4.6	4.0	Arly	Fra	4.0	4.8	4.4
Fesper	Dnk	4.2	2.9	3.6	Jesper	Dnk	3.0	4.6	3.8	Athos	Fra	4.2	6.0	5.1
Perrevia	Grc	4.5	3.7	4.1	Rano Trifolium	Dnk	2.0	4.3	3.1	Cambria	UK	5.2	6.7	6.0
Akimidori	Jpn	4.8	4.9	4.8	Haka	Fin	2.4	3.6	3.0	Lucyle	Fra	6.0	6.5	6.3
Korea Zairai	Kor	3.4	4.3	3.9	Prairial	Fra	1.7	4.5	3.1	Lude	Fra	5.1	6.0	5.5
Goliat	Rom	4.0	3.7	3.9	Hokuto	Hok	2.3	4.1	3.2	Lully	Fra	3.6	5.2	4.4
Gorom	Rom	3.3	2.8	3.0	Frontier	Jpn	3.3	4.7	4.0	Lupre	Fra	5.2	6.5	5.9
Able	USA	3.4	4.4	3.9	Bar.H.DGL 051	Nld	2.9	5.1	4.0	Lutetia	Fra	5.7	7.0	6.3
Ambassador	USA	4.2	4.0	4.1	Barata	Nld	1.6	3.7	2.6	Hayking	Hok	4.2	5.0	4.6
Crown	USA	3.3	4.4	3.8	Baraula	Nld	2.1	4.2	3.1	Hayking 2	Hok	6.1	9.0	7.6
Hallmark	USA	3.6	4.3	3.9	Bartyle	Nld	2.1	3.0	2.6	Alma	Nld	5.2	7.3	6.3
Justus	USA	2.6	3.6	3.1	Dorise	Nld	1.1	3.9	2.5	Pizza	Nld	5.1	6.7	5.9
Potomac	USA	2.7	4.8	3.7	Apelsvoll	Nor	2.5	3.9	3.2	Grassland's Kara	NZ	3.4	4.4	3.9
Wasemidori	NARCH	4.7	6.3	5.5	Hattfjelldal	Nor	1.9	3.8	2.8	Grassland's Wana	NZ	4.7	6.1	5.4
Kitamidori	NARCH	3.8	5.5	4.7	Olimp	Rom	2.0	3.9	2.9	Baza	Pol	4.6	6.3	5.4
Hokuiku 23	NARCH	4.2	5.2	4.7	Dactus	Swe	2.7	4.5	3.6	Intensiv	Rom	5.8	7.5	6.6
Hokuiku 50	NARCH	5.3	7.4	6.3	Comet	USA	2.3	3.9	3.1	Poiana	Rom	4.2	5.6	4.9
Hokuiku 51	NARCH	4.1	5.5	4.8	ISI(81-1)	USA	2.1	3.3	2.7	Glorus	Swe	8.3	9.1	8.7
Hokuiku 52	NARCH	3.8	4.9	4.4	Napier	USA	1.6	4.1	2.9	Loke	Swe	5.2	5.8	5.5
Hokuiku 63	NARCH	2.1	3.5	2.8	Pennlate	USA	3.6	4.8	4.2	Prato	Swi	3.6	5.3	4.5
Hokuiku 64	NARCH	2.8	3.7	3.3	Okamidori	NARCH	4.1	4.6	4.3	Toyomidori	NARCH	5.0	7.4	6.2
Hokuiku 65	NARCH	4.7	6.5	5.6	Harujiman	NARCH	3.1	5.3	4.2	Hokuiku 14	NARCH	6.1	9.0	7.5
Hokuiku 74	NARCH	3.9	7.7	5.8	Hokuiku 13	NARCH	2.4	3.8	3.1	Hokuiku 68	NARCH	5.5	7.8	6.7
Average		3.8	4.6	4.2	Hokuiku 49	NARCH	2.1	3.0	2.5	Hokuiku 70	NARCH	4.5	7.3	5.9
Max.		5.3	7.7	6.3	Hokuiku 75	NARCH	4.0	4.5	4.3	Hokuiku 76	NARCH	7.0	7.8	7.4
Min.		2.1	2.8	2.8	Hokuiku 66	NARCH	3.8	4.4	4.1	Hokuiku 77	NARCH	4.5	6.6	5.6
STD		0.8	1.2	0.9	Hokuiku 69	NARCH	4.0	5.0	4.5	Average		5.0	6.6	5.8
CV		20.5	26.3	20.8	Average		2.5	4.1	3.3	Max.		8.3	9.1	8.7
L.S.D.(*P*=0.05)		1.6	1.8	1.7	Max.		4.1	5.3	4.5	Min.		3.4	4.4	3.9
					Min.		1.0	2.9	1.9	STD		1.1	1.3	1.2
					STD		0.8	0.6	0.6	CV		22.8	19.1	19.8
					CV		32.7	14.7	19.6	L.S.D.(*P*=0.05)		1.6	1.8	1.7
					L.S.D.(*P*=0.05)		1.5	1.2	1.3					

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 .

Characteristics selected as independent variables	Partial regression coefficient	Standrad regression coefficient	F-value
Dry matter content	0.51	0.48	32.2
Thickness of stems	0.35	0.30	13.1
Heading date	0.32	0.34	10.2
Resistance to brown stripe	0.17	0.18	2.8
R=0.713, R2=0.509, Standard error=1.166, F=23.84, P<0.0001			