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1   Running title: Variation of WSC content in cocksfoot

2

3   Short communication

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5   Genetic variation in water soluble carbohydrate concentration in diverse cultivars

6   of *Dactylis glomerata* L. during vegetative growth

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13

1 *Abstract.*

2   The water-soluble carbohydrate (WSC) content of *Dactylis glomerata* L.

3 (cocksfoot) during vegetative growth is lower than that of other temperate grasses.

4 Variation in the WSC content of vegetative growth among cultivars was assessed in

5 order to assess the potential to improve forage quality. There were significant

6 differences among cultivars for WSC content ($P<0.01$) in all maturity groups.

7 Average WSC content of the late (L) maturity group was higher than that of the

8 early (E) and medium (M) maturity groups. Variation in the WSC content was

9 largest among group L cultivars and smallest in group M. The WSC content of

10 cultivars in groups M and L was consistent across 2 years (interactions between

11 cultivars and years were not significant). Brown stripe infection was shown to

12 reduce WSC content in susceptible cultivars. As a result of multiple regression

13 analysis with WSC content as the dependent variable, dry matter content, heading

14 date, thickness of stems and resistance to brown stripe were selected as

15 independent variables that were correlated with WSC. Therefore, variation for

16 these traits must also be monitored in any program to increase WSC content in

17 orchardgrass.

18 *Additional keywords*: Germplasm, Orchardgrass.

19

20 **Introduction**

21   *Dactylis glomerata* L. (cocksfoot) is cultivated for hay making and grazing in

22 temperate zones, because it has good regrowth and adaptability to various

23 environmental conditions. Improvement of forage quality is a major breeding

24 objective in cocksfoot, because its forage quality is lower than that of other

25 temperate grasses, for example timothy (*Phleum pratense* L.), especially in

1   vegetative growth harvested in summer in Japan (Masuko *et al.* 1994). Variation in

2   dry matter digestibility (DMD) among cultivars and clones in cocksfoot has been

3   measured previously and broad and narrow sense heritability for DMD in this

4   germplasm were high (Saiga 1981; Shenk and Westerhaus 1982). However,

5   selection for DMD should be performed at the vegetative growth stage when DMD is

6   not affected by the presence of reproductive stems, which have a negative

7   relationship with DMD (Saiga 1981). The improvement of DMD was conducted in

8   some temperate grasses and several highly digestible cultivars have been released

9   (Casler 2001).

10   Water-soluble carbohydrate (WSC) content of perennial ryegrass (*Lolium perene*

11   L.) has been the subject of considerable breeding efforts (Humphreys 1989). WSC is

12   an important trait for the nutritive value of forages for animals, because it is related

13   to palatability and digestibility, as well as the fermentation quality of silage (Smith

14   *et al.* 1997; Mayland *et al.* 2001). For perennial ryegrass, high WSC cultivars have

15   been developed in the UK (Humphreys 1989; Miller *et al.* 2001) and the

16   effectiveness of high WSC cultivars for milk production has been reported (Miller *et*

17   *al.* 2001).

18   In cocksfoot, improvement of the WSC content could increase forage quality and

19   palatability, both for grazing and silage making, and thus aid efficient animal

20   production. A breeding program for improvement of the WSC content in cocksfoot

21   has been initiated by the National Agricultural Research Center for Hokkaido

22   Region (NARCH) in Sapporo. Ideally, breeding material with high WSC content

23   should be selected at the beginning of this breeding program. However, there are no

24   known sources of high WSC among cultivars of cocksfoot. Therefore, it is necessary

25   to measure the WSC content in a broad range of germplasm in order to improve the

1   WSC content in a breeding program.

2   Recently, a variety of *Lolium multiflorum* Lam. with high WSC content was

3   developed by recurrent selection for high dry matter content (Marais *et al.* 2003).

4   Therefore, dry matter content may be an important index for high WSC selection in

5   cocksfoot also.

6   Time and effort for breeding could be saved in initial screenings if the breeder had

7   better information on the relationship between WSC content and morphological and

8   agronomic traits, for example, dry matter content and plant type. The relationship

9   between morphological and agronomic traits and WSC content has not been

10   reported in cocksfoot. Therefore, this study was also conducted to quantify these

11   relationships.

12

13   **Materials and methods**

14   The experiment was carried out on volcanic ash soil at the National Agricultural

15   Research Center for Hokkaido Region (NARCH) in Sapporo (N 43° 00', E 141° 24'). A

16   total of 97 accessions of cocksfoot cultivars bred in Japan, USA, Australia, New

17   Zealand and European countries were divided into 3 groups based on the maturity

18   time determined by our previous introduction test (unpublished) : 30 accessions in

19   the early maturity (E) group, 34 accessions in the medium maturity (M) group and

20   33 accessions in the late maturity (L) group. Seeds were sown on 9 May 2000 in

21   paper pots filled with volcanic ash soil containing 0.4 g N, 1.5 g P_2O_5 and 0.4 g K_2O /

22   kg soil and seedlings were grown in a greenhouse. Ten seedlings of each cultivar

23   were transplanted into a field at a spacing of 20 $\times$ 80 cm on 19 June in a

24   randomized block design with 3 replications. Compound fertilizer (4.0 g N, 5.5 g

25   P_2O_5 and 4.0 g K_2O /m^2) was applied at transplanting. The first cut of each group

1 occurred at their respective heading dates in 2001 and 2002. Cutting dates are

2 presented in Table 1. After the melting of snow in 2001 and 2002, 7.0 g N, 9.6 g P_2O_5

3 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

4 applied after harvesting.

5   The evaluation of the WSC content was carried out at the second cut in each year

6 when fewer reproductive stems were present compared with the first cut. The

7 samples were harvested between 10 00 a.m. and 11 30 a.m. in order to minimize the

8 effect of environmental conditions such as temperature and solar radiation on the

9 WSC content (Masaki *et al.* 1978). About 500 g of fresh grass was collected by a

10 small plot harvester (Rem, Saskatchewan, Canada) and dried in an oven at 70℃ for

11 48 h. Dried samples were ground through a 1.0 mm screen using a Cyclone Mill

12 following coarse grinding using a Wiley type mill.

13   WSC content was analyzed by near infrared reflectance spectroscopy (FOSS

14 NIRSytems Model 6500, U.S.A.) with reflectance data calibrated against total WSC

15 contents measured using the anthrone method (Yemm and Willis 1954). Dry matter

16 yield, dry matter content, plant height, leaf length and width of each cultivar were

17 measured at harvest. Morphological traits were scored on a scale of 1-9 as follows:

18 plant type (1, erect; 9, prostrate), number of stems (1, few; 9, abundant), thickness

19 of stems (1, thin; 9, thick), and number of reproductive stems (1, none; 9, abundant).

20 Stems at vegetative stage except for reproductive stems mean pseudostems, which

21 is predominantly leaf sheath material in this experiment. Resistance to brown

22 stripe (*Cercosporidium graminis* (Fuckel) Deighton) was scored on a scale of 1-9 (1,

23 susceptible; 9, resistant).Heading date was scored as days after 1 May.

24   Data was statistically analyzed using analysis of variance within each maturity

25 group. The significance of mean squares for cultivar, year and cultivar × year

1   interactions was then estimated. Where significant differences were found, least

2   significant difference (l.s.d.) were calculated. Heritability estimates were calculated

3   by using variance components from analysis of variance. The genotypic variance

4   among the cultivars (σ^2_g) and the error variance (σ^2_e) were used to estimate the

5   broad-sense heritability (h^2) as:

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

7   Stepwise multiple regression analysis, with WSC content in all cultivars as the

8   dependent variable, was carried out using morphological and agronomic traits

9   investigated in 2002 as independent variables.

10

11   **Results and Discussion**

12   Variation in the WSC content among the cocksfoot accessions at the time of the

13   second cut is shown in Table 2. There was significant variation ($P<0.05$) in the WSC

14   content among the cultivars in each group. The average WSC content in group L

15   was higher than that in group E or M in both years. The WSC content in group L

16   ranged from 3.9 to 8.7%. The variation of WSC content in group M was smaller

17   than the other 2 groups, although it ranged from 1.9 to 4.5% on average across 2

18   years. Hayking 2 and Glorus, which have been registered as recommended cultivars

19   in the Hokkaido region of Japan, and strains bred by NARCH for Hokkaido showed

20   higher WSC content than other cultivars. Those cultivars that combine high WSC

21   with adaptation to the environmental conditions of Hokkaido were considered as

22   good breeding materials for improvement of the WSC content. The relationship

23   between WSC content and origin of cultivars was not clear.

24   Main effects associated with cultivars and years were significant for all 3 groups

25   as shown by the result of analysis of variance. Interactions between cultivars and

6

1   year in groups M and L were not significant. On the other hand, there was a

2   significant interaction between cultivars and years for group E.

3   Broad sense heritability was high in all groups. For group L, it was estimated

4   0.91, while for group E and M it was 0.65 and 0.75, respectively.

5   Correlation coefficients between the WSC content across the two years were

6   significant overall (r=0.82, P<0.001) but also within each of the maturity groups.

7   The correlation coefficient of group L was the highest at 0.83 (P<0.001), while that

8   of group M was 0.63 (P<0.01). Several cultivars in group E such as Hokuiku 50 and

9   Poltavskaya showed high WSC content in both years (Table 2), although the

10   correlation coefficient of group E was lowest at 0.53 (P<0.05).

11   From these results, the WSC content of cocksfoot cultivars during vegetative

12   growth was consistent across all maturity groups in both years. It is known that the

13   WSC content is affected by environmental conditions such as solar radiation and it

14   varies according to the time of sampling (Fisher *et al.* 1999; Smith *et al.* 2001).

15   Despite this, this result shows that effective selection for high WSC content can be

16   carried out in the presence of these environmental effects. Therefore, a new cultivar

17   that shows stable high WSC expression in multiple environments can be bred in

18   cocksfoot. It is necessary to clarify the narrow sense heritability of WSC content in

19   order to evaluate selection effectiveness accurately in future.

20   As the result of stepwise multiple regression analysis, dry matter content,

21   heading date, thickness of stems and resistance to brown stripe were selected as

22   independent variables and their contribution ratio was about 50% (Table 3). The

23   standardized partial regression coefficient associated with the dry matter content

24   was the highest. From these results, the WSC content in cocksfoot was closely

25   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

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

Early maturity group

Cultivar / Strains	Origin	WSC content (%) 2001	2002	mean
Asta	CIS	3.5	4.0	3.8
Dzhegutinskaya	CIS	3.3	4.4	3.8
Khutorskaya	CIS	2.8	3.5	3.2
Magutnaya	CIS	4.5	4.6	4.6
Poltavskaya	CIS	4.9	5.9	5.4
Vik 61	CIS	4.6	5.1	4.8
Zyryanovskaya	CIS	4.2	3.1	3.6
Amba	Dnk	3.2	4.4	3.8
Fesper	Dnk	4.2	2.9	3.6
Perrevia	Grc	4.5	3.7	4.1
Akimidori	Jpn	4.8	4.9	4.8
Korea Zairai	Kor	3.4	4.3	3.9
Goliat	Rom	4.0	3.7	3.9
Gorom	Rom	3.3	2.8	3.0
Able	USA	3.4	4.4	3.9
Ambassador	USA	4.2	4.0	4.1
Crown	USA	3.3	4.4	3.8
Hallmark	USA	3.6	4.3	3.9
Justus	USA	2.6	3.6	3.1
Potomac	USA	2.7	4.8	3.7
Wasemidori	NARCH	4.7	6.3	5.5
Kitamidori	NARCH	3.8	5.5	4.7
Hokuiku 23	NARCH	4.2	5.2	4.7
Hokuiku 50	NARCH	5.3	7.4	6.3
Hokuiku 51	NARCH	4.1	5.5	4.8
Hokuiku 52	NARCH	3.8	4.9	4.4
Hokuiku 63	NARCH	2.1	3.5	2.8
Hokuiku 64	NARCH	2.8	3.7	3.3
Hokuiku 65	NARCH	4.7	6.5	5.6
Hokuiku 74	NARCH	3.9	7.7	5.8
Average		3.8	4.6	4.2
Max.		5.3	7.7	6.3
Min.		2.1	2.8	2.8
STD		0.8	1.2	0.9
CV		20.5	26.3	20.8
L.S.D.(P=0.05)		1.6	1.8	1.7

Medium maturity group

Cultivar / Strains	Origin	WSC content (%) 2001	2002	mean
Arctic	Can	3.2	4.8	4.0
Kay	Can	1.8	3.7	2.8
Lidacta	Can	3.1	3.7	3.4
Mobite	Can	1.8	4.2	3.0
Leningradskaya	CIS	2.3	4.0	3.1
Neva	CIS	1.0	2.9	1.9
Milona	Cze	2.2	3.6	2.9
Niva	Cze	3.3	4.6	4.0
Jesper	Dnk	3.0	4.6	3.8
Rano Trifolium	Dnk	2.0	4.3	3.1
Haka	Fin	2.4	3.6	3.0
Prairial	Fra	1.7	4.5	3.1
Hokuto	Hok	2.3	4.1	3.2
Frontier	Jpn	3.3	4.7	4.0
Bar.H.DGL 051	Nld	2.9	5.1	4.0
Barata	Nld	1.6	3.7	2.6
Baraula	Nld	2.1	4.2	3.1
Bartyle	Nld	2.1	3.0	2.6
Dorise	Nld	1.1	3.9	2.5
Apelsvoll	Nor	2.5	3.9	3.2
Hattfjelldal	Nor	1.9	3.8	2.8
Olimp	Rom	2.0	3.9	2.9
Dactus	Swe	2.7	4.5	3.6
Comet	USA	2.3	3.9	3.1
ISI(81-1)	USA	2.1	3.3	2.7
Napier	USA	1.6	4.1	2.9
Pennlate	USA	3.6	4.8	4.2
Okamidori	NARCH	4.1	4.6	4.3
Harujiman	NARCH	3.1	5.3	4.2
Hokuiku 13	NARCH	2.4	3.8	3.1
Hokuiku 49	NARCH	2.1	3.0	2.5
Hokuiku 75	NARCH	4.0	4.5	4.3
Hokuiku 66	NARCH	3.8	4.4	4.1
Hokuiku 69	NARCH	4.0	5.0	4.5
Average		2.5	4.1	3.3
Max.		4.1	5.3	4.5
Min.		1.0	2.9	1.9
STD		0.8	0.6	0.6
CV		32.7	14.7	19.6
L.S.D.(P=0.05)		1.5	1.2	1.3

Late maturity group

Cultivar / Strains	Origin	WSC content (%) 2001	2002	mean
Porto	Aus	5.3	7.5	6.4
Dedinovskaya	CIS	7.5	8.1	7.8
Sverdlovskaya	CIS	5.5	7.6	6.5
Filippa	Dnk	3.7	4.9	4.3
Leigestra	Deu	3.8	6.0	4.9
Lidaglo	Deu	5.1	5.4	5.3
Amply	Fra	3.6	5.5	4.5
Arly	Fra	4.0	4.8	4.4
Athos	Fra	4.2	6.0	5.1
Cambria	UK	5.2	6.7	6.0
Lucyle	Fra	6.0	6.5	6.3
Lude	Fra	5.1	6.0	5.5
Lully	Fra	3.6	5.2	4.4
Lupre	Fra	5.2	6.5	5.9
Lutetia	Fra	5.7	7.0	6.3
Hayking	Hok	4.2	5.0	4.6
Hayking 2	Hok	6.1	9.0	7.6
Alma	Nld	5.2	7.3	6.3
Pizza	Nld	5.1	6.7	5.9
Grassland's Kara	NZ	3.4	4.4	3.9
Grassland's Wana	NZ	4.7	6.1	5.4
Baza	Pol	4.6	6.3	5.4
Intensiv	Rom	5.8	7.5	6.6
Poiana	Rom	4.2	5.6	4.9
Glorus	Swe	8.3	9.1	8.7
Loke	Swe	5.2	5.8	5.5
Prato	Swi	3.6	5.3	4.5
Toyomidori	NARCH	5.0	7.4	6.2
Hokuiku 14	NARCH	6.1	9.0	7.5
Hokuiku 68	NARCH	5.5	7.8	6.7
Hokuiku 70	NARCH	4.5	7.3	5.9
Hokuiku 76	NARCH	7.0	7.8	7.4
Hokuiku 77	NARCH	4.5	6.6	5.6
Average		5.0	6.6	5.8
Max.		8.3	9.1	8.7
Min.		3.4	4.4	3.9
STD		1.1	1.3	1.2
CV		22.8	19.1	19.8
L.S.D.(P=0.05)		1.6	1.8	1.7

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 Research Center for Hokkaido Region;Nld,Netherlands;Nor,Norway;NZ,New Zealand;Pol,Poland;Rom,Rumania;Swe,Sweden;Swi,Switzerland.

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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			

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