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Inheritance of the concentration of water-soluble carbohydrates and its relationship with the concentrations of fibre and crude protein in herbage of cocksfoot (*Dactylis glomerata* L.)

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Abstract

The concentration of water-soluble carbohydrates (WSC) of cocksfoot is lower than that of other temperate grasses. Increasing in the WSC concentration in cocksfoot is important in increasing its digestibility and preference by ruminants. The genetic variation in mono- and disaccharides, fructans and total WSC concentrations and their interrelationships with crude protein (CP) and fibre concentrations of cocksfoot (*Dactylis glomerata* L.) at the vegetative growth stage in half-sib cross populations were assessed in two experiments conducted under spaced-planting and sward conditions. There was a wide range in the means for concentrations of fructan, WSC and neutral-detergent fibre (NDF) in parents and progeny whereas there was a narrow range of the means for concentrations of mono- and disaccharides, CP and acid-detergent fibre (ADF). Mean concentrations of mono- and disaccharides showed the smallest range. Mean squares of entries in parents and progenies were significant for mono- and disaccharides, fructan and total WSC concentrations in all populations. The entry × year interactions for fructan and total WSC concentrations were significant in parents and half-sib progeny. However, the entry × year interactions for mono- and disaccharide concentrations in progeny were not significant. Concentration of WSC was under genetic control since mean squares of the concentration values were significant and variance components for all traits were significantly larger than zero. There were negative genetic correlations between WSC and ADF and NDF concentrations. Narrow sense heritabilities (h_n) estimated from variance components of progeny for total WSC concentrations were 0.59 and 0.53 in sward and space-planting conditions, respectively. The h_n of fructan and WSC concentrations were similar in both sward and spaced-planting conditions, whereas that of mono- and disaccharide concentrations varied from 0.20 to 0.69. It was found that the genetic variation of total WSC concentration in cocksfoot depended mainly on genetic variation in fructan concentration. These results suggested that the forage quality of cocksfoot at the vegetative growth stage was influenced by an additive gene effect and could be improved genetically by recurrent selection.

Keywords: fructan, genetic variation, heritability, mono- and disaccharides, water-soluble carbohydrates

Introduction

Cocksfoot (*Dactylis glomerata* L) is cultivated for hay-making and grazing in temperate zones because it has good regrowth characteristics and adaptability to various environmental conditions. Improvement in forage quality is a major breeding objective in temperate grasses such as cocksfoot. Increases in *in vitro* dry matter digestibility (IVDMD) have been documented in new cultivars, including legumes and temperate and tropical grasses, and a 0.01 increase in IVDMD due to plant breeding on average has led to a proportionate increase of 0.032 in average daily liveweight gains (Casler and Vogel, 1999). Forage quality of cocksfoot is lower than that of other temperate grasses, such as timothy (*Phleum pratense* L.), grown in Hokkaido, especially vegetative growth harvested in summer (Masuko *et al*., 1994). Genetic variation in dry matter digestibility (DMD), fibre and crude protein (CP) concentration and their interrelationships have been reported in cocksfoot (Stratton *et al*., 1979; Shenk and Westerhaus, 1982). It is important to estimate the heritability of forage quality for the purpose of efficient and reliable selection for forage quality. Previous research has indicated that the heritability of DMD, acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) were moderate in half-sib families in cocksfoot (Stratton *et al*., 1979; Shenk and Westerhaus, 1982), and correlations among those forage quality traits were variable at first cut (Stratton *et al*., 1979; Saiga, 1981). Therefore, the selection for forage quality traits should be performed at the vegetative growth stage when forage quality is not affected by the presence of reproductive stems, which have a negative relationship with DMD (Saiga, 1981).

Attention has been paid to the concentration of water-soluble carbohydrates (WSC) as part of considerable breeding efforts in perennial ryegrass (*Lolium perenne* L.) (Humphreys, 1989a). Water-soluble carbohydrates are a total of components of non-structural carbohydrates and their concentration is an important trait for the nutritive value of forages for ruminants, because it is related to preference (Mayland *et al*., 2001) and digestibility (Humphreys, 1989b) as well as to the fermentation quality of silage (Smith *et al*., 1997; Wilkins and Humphreys, 2003). Feeding of perennial ryegrass with a high WSC concentration has been shown to increase growth rates of lambs (Lee *et al*., 2001) and the absorption of amino acids from the small intestine in beef steers (Lee *et al*., 2002). Water-soluble carbohydrates are also related to regrowth of grass after defoliation and persistence of grazing pasture (Fulkerson and Donaghy, 2001). Water-soluble carbohydrates of temperate grasses consist of mono- and disaccharides and fructan, which are made in the cytoplasm during periods of photosynthesis. Fructan, a polymer of fructose, is accumulated as an energy reserve mainly in the leaf sheaths of temperate grasses (Pollock and Cairns, 1991). The WSC concentration of cocksfoot is lower than that of other temperate grasses (Cooper, 1962; Masuko *et al*., 1994), and the improvement of WSC concentration is important to increase the digestibility of and preference for cocksfoot, and could increase the persistency and efficiency of ruminant livestock production. Information on the genetic variation in WSC concentration and its relationship to concentrations of fibre and CP is required to improve breeding for WSC concentration. Heritability estimates and the genetic correlation among mono- and disaccharides, fructan and total WSC have not been described for cocksfoot.

Earlier research indicated that the correlation of yield with IVDMD was negative in cocksfoot (Stratton *et al*., 1979). It is important also to clarify the relationship between WSC concentration and forage yield. Changes in the proportion of mono- and disaccharides and fructan on increasing WSC concentration have not been clear in other temperate grasses. Mono- and disaccharides are more important than fructan in the primary stages of silage fermentation because fructan is degraded slowly during the early stage of ensilaging (Merry *et al.*, 1995). The proportion and concentration of mono- and disaccharides and fructan needed for the improvement of silage quality in cocksfoot must be clarified.

The objectives of this study were to assess the genetic variation of mono- and disaccharides, fructan and total WSC concentration and their interrelationship to fibre and CP concentration, and yield, during the vegetative growth stage in cocksfoot to determine the phenotypic and genetic correlations among them, and to estimate broad- and narrow- sense heritability of WSC concentration using two breeding populations under two different growing regimes.

Materials and methods

All experiments were carried out on volcanic ash soil at the National Agricultural Research Center for the Hokkaido Region (NARCH) in Sapporo (N 43˚ 00’, E 141˚ 24’).

Experiment 1

Eleven Russian cultivars, three ecotypes collected in Hokkaido and cultivars developed at ‘Wasemidori’ and ‘Kitamidori’ were used as a basic population. Heading time of all accessions was early maturity. Seeds were sown on 8 August 1997 in paper pots containing 4.0 g N, 1.5 g P_2O_5 and 4.0 g K_2O kg $^{-1}$ soil, and seedlings were grown in a greenhouse. Fifty seedlings of each accession were transplanted into a field with a spacing of 0.8 m × 0.8 m on 16 September 1997 in a randomized block design with two replications. Plants were harvested in June, August and October in 1998 using a flail type forage harvester. Thirty plants showing good winter hardiness were selected in April 1999. Fifteen of the plants were derived from the Russian cultivars, and others were derived from Japanese ecotypes and cultivars. Those plants were divided into three clones and transplanted to a greenhouse for isolation and then polycrossed. Seeds were collected from the three replications of each clone in a polycross nursery and bulked. Thirty half-sib progeny were used with standard cultivar 'Wasemidoi' and Russian cultivar 'Kievskaya' for the progeny trial. Seeds were sown in single 4.0 m rows, spaced 0.6 m apart, at 15 kg ha^{-1}, on 20 August 1999. Plots were harvested on 2 June, 2 August and 30 September in 2000, and on 28 May, 10 July, 23 August and 25 September in 2001 using a flail type plot harvester (Swift Machine, Saskatchewan, Canada) at a height of 8 cm. Forage yield was measured at harvesting. Compound fertilizer (40 kg N, 55 kg P_2O_5 and 40 kg K_2O ha^{-1}) was applied at the seeding. After snow melt of 2000 and 2001, 56 kg N, 77 kg P_2O_5 and 56 kg K_2O ha^{-1} were applied in April; 32 kg N, 24 kg P_2O_5 and 32 kg K_2O ha^{-1} were applied after each harvesting. Forage samples were collected on 2 August in 2000 and 23 August in 2001 for the measurement of concentrations of WSC, CP, ADF and NDF. About 0.5 kg of fresh grass was sampled at each harvesting 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. Samples were stored in a refrigerator before the analysis of forage quality.

Experiment 2

Two hundred and forty elite clones preserved vegetatively as genetic resources at NARCH were measured for WSC concentration in August 2000. Fifteen clones with high or medium WSC concentration and of medium maturity were selected in 2001. Those clones were divided into three clones, and transplanted to a greenhouse for isolation and then polycrossed. Seeds were collected from the three replications of each clone in the polycross nursery and bulked. Fifteen half-sib progeny and three standard Japanese cultivars, 'Wasemidori,' 'Okamidori' and 'Harujiman,' were used for the progeny trial. Seeds were sown on 9 May 2002 in paper pots filled with volcanic ash soil containing fertilizer at rate of 4.0 g N, 1.5 g P_2O_5 and 4.0 g K_2O kg^{-1} soil, and seedlings were grown in a greenhouse.

Twenty seedlings of 15 half-sib progeny and three cultivars were transplanted into a field at a spacing of 0.4 m × 0.8 m on 12 June 2002 in a randomized block design with four replications. The parental clones were transplanted into a field at a spacing of 0.8 m × 0.8 m on 12 July 2002 in a randomized block design with two replications separated from half-sib progeny. All plots were harvested on 29 August and 21 October in 2002, and 18 June, 18 August and 15 October in 2003 by a plot harvester (Swift Machine, Saskatchewan, Canada) or flail type forage harvester (Star Noki, Chitose, Japan) at a height of 10 cm. Compound fertilizer (40 kg N, 55 kg P_2O_5 and 40 kg K_2O ha^{-1}) was applied at seeding. After snow melt in 2003, 42 kg N, 58 kg P_2O_5 and 42 kg K_2O ha^{-1} was applied in April; 23 kg N, 17 kg P_2O_5 and 23 kg K_2O ha^{-1} were applied after each harvesting. Forage samples were collected on 29 August in 2002 and 18 August in 2003 from each plot and clone. The method of sample collection and preparation for the analysis of forage quality were the same as in Experiment 1.

Analysis of forage quality

Mono- and disaccharide and fructan, CP, ADF and NDF concentrations were estimated by near-infrared reflectance spectroscopy (NIRS) (NIRSytems Model 6500, MD, USA). The WSC concentration was expressed as total concentration of mono- and disaccharides, and fructan. Crude protein, ADF and NDF concentrations were measured using calibration equations described by Takai and Nakayama (2000). A total of 152 samples were selected as a calibration data set for chemical analysis of WSC using high-performance liquid chromatography (HPLC). Thirty-eight of the samples were used for validation. Partial least-square regression was used to develop predictive evaluation for WSC concentration. Total water-soluble carbohydrates were extracted from a 0.25 g ground sample by boiling de-ionized water containing 1 mg ml^{-1} propylene glycol as the internal standard for 1 h. The extract was passed through a 0.45-μm pore filter and analyzed using 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). Carbohydrates were identified using fructose, glucose and sucrose as the external standards and quantified using propylene glycol as the internal standard. The standard error of prediction by NIRS was 4.9 g kg^{-1} for mono- and disaccharides and 7.9 g kg^{-1} for fructan. Calibration coefficients of determination were 0.78 for mono- and disaccharides and 0.91 for fructan.

Statistical analysis

Analyses of variance were computed as a split plot in time for each experiment. Years, entries and replications were considered as random effects. Standard cultivars were removed from the analysis of variance. Variance components were estimated from the linear function of the mean square. Broad-sense (h_b) and narrow-sense (h_n) heritabilities in Experiments 1 and 2 were estimated on a phenotypic-mean and plot-mean basis averaged across years and replications (Nguyen and Sleper, 1983) as follows:

$$h_b = \sigma^2_p / \sigma^2_{pm} = \sigma^2_p / (\sigma^2_p+\sigma^2_{py}/y+\sigma^2_{pr}/r+\sigma^2_e/ry)$$

and

$h_n = \sigma^2_f / \sigma^2_{pfm} = \sigma^2_f / (\sigma^2_f + \sigma^2_{fy}/y + \sigma^2_{fr}/r + \sigma^2_e/ry)$,

where σ^2_p and σ^2_f are the variance components due to parents and half-sib progeny, respectively; σ^2_{pm} and σ^2_{pfm} are the total phenotypic variance of parents and half-sib progeny estimated from variance components plot means averaged across years and replications, respectively; σ^2_{py} and σ^2_{fy} are the variance components due to parents × years and half-sib progeny × years, respectively; σ^2_{pr} and σ^2_{fr} are the variance components due to parents × replications and half-sib progeny × replications, respectively; σ^2_e is the variance component due to genotype × years × replications; and y and r are the number of years and replications, respectively. Heritability estimates were obtained assuming a diploid inheritance model without epitasis and non-inbred parent. Negative variance components were equated to zero in the heritability calculation. Narrow-sense heritabilities were estimated by doubling the regression of half-sib progeny on the parent mean (h_{po}) in Experiment 2 (Falconer and Mackay, 1996).

Phenotypic and genetic correlation coefficients were calculated between forage quality traits from plot means averaged over replications and years in all experiments. Genetic correlations were estimated from genetic covariance and variance of each trait using progeny data in Experiments 1 and 2 (Falconer and Mackay, 1996).

Results

Genetic variation and heritabilities of WSC concentration and forage quality traits, and yield, under spaced planting for parents and sward conditions for

progeny

Ranges of fructan, total WSC, ADF and NDF concentrations were larger than that of mono- and disaccharides and CP concentrations in parents and progeny under spaced planting and sward conditions, respectively, in Experiment 1 (Table 1). Mono- and disaccharides, fructan and total WSC concentrations of parents and progeny were lower in 2000 than in 2001, while CP, ADF and NDF concentrations were higher in 2000 than in 2001. The mean squares of entry and year were significant for forage quality traits and yield in progeny under sward conditions for the unselected population (Table 2). Mean squares for entry × year interactions in progenies were significant in all traits except for mono- and disaccharide concentrations of progeny (Table 2). Variance components of entries for forage quality traits were larger than that of entry × year in progeny, whereas they were smaller than those of entry × year in parents (Table 2).

Phenotypic and genetic correlations between WSC concentration and forage quality traits in half-sib progeny under sward conditions are shown in Table 3. Mono- and disaccharide, fructan and WSC concentrations were negatively correlated with CP, ADF and NDF concentrations in half-sib progeny under sward conditions. Magnitudes of correlation coefficients for mono- and disaccharide, fructan and total WSC concentrations with CP concentrations were lower than those for ADF and NDF concentrations. Mono- and disaccharides, fructan, WSC and CP concentrations were not correlated with yield, whereas ADF and NDF concentrations were positively correlated with yield.

The h_b estimates of all traits in parents were lower than h_n estimates of these traits in progeny and were not significant taking the standard error into account, except for mono- and disaccharide and WSC concentrations in Experiment 1 (Table 4). The h_n of mono- and disaccharide, CP and ADF concentrations, and yield in half-sib progenies under sward conditions were similar to one another, while that of fructan concentration was lower. Correlation coefficients between parents and progeny for all traits were significantly positive.

Genetic variation and heritabilities of WSC concentration and forage quality traits under spaced planting for parents and progeny

The range of each forage quality trait for means of year and entry in parents was larger than that in progeny under spaced planting for the selected population in Experiment 2 (Table 5). Mean squares of entry were significant for all traits in parents and progeny (Table 6). Mean squares of years in progeny were significant for all traits while they were not significant in parents for forage quality traits except for CP concentration. On the other hand, entries × year interactions in progeny were not significant in forage quality traits except for CP concentration while they were significant in parents for all traits. Variance components of entries for all forage quality traits were smaller than those of entry × year in parents (Table 6). Variance components of entries for fructan, WSC, ADF and NDF concentrations were larger than those of entry × year in progeny under spaced planting.

Phenotypic and genetic correlations between WSC and forage quality traits in half-sib progeny under the spaced-planting condition were shown in Table 3. Mono- and disaccharide, fructan and WSC concentrations were negatively correlated with ADF and NDF concentrations in half-sib progenies under spaced planting. The magnitude of correlation coefficients for fructan and total WSC concentrations with CP concentration was higher than that for mono- and disaccharide concentration with CP concentration. Plant vigour that was represented by plant biomass was not correlated with forage quality traits under spaced planting.

The h_b estimates of all traits in parents were lower than h_n estimates in progeny under spaced planting in Experiment 2 (Table 4). Magnitudes of h_b estimates in parents were similar to each other except for plant vigour. The h_n estimates of fructan, WSC, ADF and NDF concentrations in progeny, which ranged from 0.53 to 0.71, were moderate, whereas those of mono- and disaccharide concentrations, and CP concentration, were 0.20 and 0.29 respectively. The h_{po} estimates were the highest for fructan concentration and the lowest for ADF concentration. Although h_{po} of forage quality traits estimated from parent-progeny regression, which ranged from 0.20 to 0.97, were low to high, standard errors were large in all traits.

Discussion

Most cocksfoot cultivars are developed by phenotypic and genotypic selection based on a set of polycross progenies in sward plots. Because significant heritabilities and correlations between parent and progeny for WSC concentration were found in Experiment 1, which was carried out under sward conditions for progeny, this selection method would be useful in increasing the WSC concentration of cocksfoot. Walters and Evans (1974) reported that the difference in digestibility found in spaced planting in cocksfoot disappeared under sward conditions. Genetic variations for WSC concentration and forage quality traits were found under both spaced planting for parents and under sward conditions for progeny. Therefore, phenotypic and genotypic selection based on a set of polycross progenies in sward plots may be useful for the selection of WSC concentration and yield in cocksfoot.

A significant entry × year interaction under sward conditions, except for mono- and disaccharide concentrations, indicated a differential response of some accessions to varying environmental conditions. This result showed that evaluation of forage quality for the selection should be carried out under different environmental conditions. Although the range of mono- and disaccharide concentrations was the smallest in forage quality traits, entry × year interaction of mono- and disaccharide concentrations was not significant whereas those of fructan and total WSC were significant. This result showed that mono- and disaccharides concentrations in swards were more stable than fructan and total WSC concentrations across years.

Genetic and phenotypic correlations of yield with forage quality traits have been reported in cocksfoot (Stratton *et al*., 1979; Shenk and Westerhous, 1982). A correlation of yield with NDF concentration was positive (Stratton *et al*., 1979) or inconsistent (Shenk and Westerhous, 1981) whereas correlation of yield with IVDMD was negative (Stratton *et al*., 1979). The magnitude of the correlation coefficient between WSC and yield under sward conditions was low, while a positive correlation of ADF and NDF concentrations with yield was found in Experiment 1. Stratton *et al*. (1979) suggested that it may be difficult to either increase IVDMD or lower fibre concentration without a corresponding decrease in dry matter yield in cocksfoot. However, Casler *et al*. (2002) reported that the selection for high forage yield in cocksfoot did not decrease forage quality. The present result, showing a low correlation between WSC concentration and yield, suggests that it may be possible to increase WSC concentration at the vegetative growth stage without a decrease in dry matter yield.

All forage quality traits were under genetic control, since the mean squares of these traits were significant, and variance components for all traits were significantly larger than zero for Experiment 2 under spaced planting. Because the population in Experiment 2 showed wide genetic variation in total WSC concentration, it should be possible to select and develop high WSC-concentration strains at the vegetative growth stage. The use of a between- and within-family selection method has been effective for the breeding of grasses (Vogel and Pedersen, 1993). Casler *et al*. (1989) proposed recurrent progeny-test selection for sward-plot forage yield, combined with within-family spaced-plant selection for forage quality traits as an effective breeding scheme in grasses. Because genetic variation for total WSC concentration between families was found under spaced planting in Experiment 2, a combination of within-family and progeny-test selection will be effective in increasing WSC concentration and forage yield.

The h_b estimates of all traits in parents were lower than h_n in Experiment 2 which was carried out under spaced planting. Bughrara *et al*. (1991) found that the h_n estimates for ADF and NDF concentration were higher than h_b in tall fescue and suggested that epistatic linkage or multiple allelic effects, or both, were probably involved. The h_n of fructan and total WSC concentrations under spaced planting in Experiment 2 was similar to that under sward conditions in Experiment 1 whereas that of mono- and disaccharide concentrations under spaced planting was lower than that under sward conditions. This result may have been due to the small number of clones and progeny and the narrow genetic variation for mono- and disaccharide concentrations in this population which was selected for WSC concentration. This reason is supported by the high h_{po} estimates, calculated from parent-progeny regression, in all traits except for mono- and disaccharide, and CP, concentrations although the standard error for these estimates was large in Experiment 2. Therefore, it will be necessary to evaluate large number of clones and half-sib families in selection for mono- and disaccharide concentrations.

There were significant entry × year interactions for all forage quality traits in parents in both experiments. These results indicate that phenotypic-genotypic selection based on the performance of the progeny should be carried out in the selection for forage quality traits in cocksfoot. However, this polycross family evaluation is not highly effective because of the mild selection intensities in a small number of families (Casler *et al*. 2002). Casler *et al*. (2002) suggested that half-sib family selection method is effective for the selection of forage yield and quality under hay management in cocksfoot. The half-sib family selection method may be effective for the selection of forage quality traits using a large number of half-sib families.

There were negative genetic correlations between WSC concentration and fibre traits, such as ADF and NDF concentrations, in both experiments. These correlations indicate that fibre concentrations may be decreased by the selection for high WSC concentration in cocksfoot. Plant carbohydrates are mainly produced by photosynthesis. The proportion of structural carbohydrates in cocksfoot may decrease by increasing the non-structural carbohydrate relatively, if it is assumed that photosynthetic ability is not different among genotypes.

Genetic variation and heritability for total WSC concentration in this study were stable in both sward and spaced-planting conditions. These results suggest that the forage quality of cocksfoot at the vegetative growth stage is influenced by an additive gene effect and could be improved genetically by selection. Humphreys (1989a) suggested that genetic control of WSC in perennial ryegrass was complex and might be influenced by non-additive gene effects. Cooper (1962) suggested that the heritability of WSC concentration, estimated by pair-crossing of four cultivars of cocksfoot, was 0.78 and 0.56 in July and August cuts, respectively, and genetic improvement would be expected. However, the difference in yearly means for fructan concentration was larger than that for mono- and disaccharide concentrations in both experiments. There was a wide range in the means of fructan, total WSC and NDF concentrations in parents and progenies, whereas the range of mono- and disaccharide, CP and ADF concentrations was narrower in both experiments. Because mono- and disaccharide, and CP concentrations, which accumulate in vacuoles, depend on the photosynthetic and nitrogen-uptake capacity of each genotype, their genetic variation may be smaller than that for fibre traits. On the other hand, large genetic variation in fructan and total WSC concentrations was found in all populations, and fructan concentrations varied in each population and year. A genotype × environment interaction for WSC concentration has been found in tall fescue (Burner *et al*., 1983). Total WSC concentrations changed corresponding to changes in fructan concentrations. Therefore, the genetic variation in total WSC concentration was mainly consistent with the genetic variation in fructan concentration. As a consequence, crossing between various breeding materials may be necessary to increase mono- and disaccharides concentration. For stability of a high concentration of WSC under various environments, the selection for WSC concentration should be carried out not only for fructan concentration, which varied with environment conditions, but also for mono- and disaccharide concentrations which were more stable than fructan concentrations. Although both mono- and disaccharides, and fructan, are digestible in the rumen, mono- and disaccharides make a greater contribution to the primary stage of silage fermentation than does fructan (Merry *et al.*, 1995). The results of these experiments indicate that cocksfoot strains with high mono- and disaccharide, and fructan, concentrations should be developed for use both in grazing and for making silage.

Conclusions

The data, presented on the significant genetic variation and moderate h_n estimates, indicate that selection for WSC, CP and fibre concentrations in cocksfoot could be successful. The results suggest that forage quality of cocksfoot at the vegetative growth stage was influenced by an additive gene effect and could be improved genetically by recurrent and phenotypic-genotypic selection. A significant genotype × year interaction suggests that the evaluation of forage quality traits should be carried out by divergent selection under multiple environments, especially for the evaluation of parental clones. Due to the presence of significant genetic variation for mono- and disaccharide and fructan concentrations in both experiments, selection for these traits would be successful. It was found that the genetic variation of fructan concentration was larger than that of mono- and disaccharide concentrations and that the genetic variation of total WSC concentration in cocksfoot depended mainly on genetic variation in fructan concentration. From these results, fructan concentration would increase more than mono- and disaccharide concentrations by the recurrent selection for WSC concentration in cocksfoot.

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References

BUGHRARA S.S., SLEPER D.A. and KRAUSE G.F. (1991) Genetic variation in tall fescue digestibility estimated using a prepared cellulase solution. *Crop Science*, **31**, 883-889.

BURNER D.M., BALASKO J.A. and THAYNE W.V. (1983) Genetic and environmental variance of water soluble carbohydrate concentration, yield and disease in tall fescue. *Crop Science*, **23**, 760-763.

CASLER M.D., CARPENTER J.A. and ATTEWELL J.R. (1989) Selection strategies for developing smooth bromegrass cell wall ideotypes. *Theoretical and Applied Genetics*, **78**, 775-782.

CASLER M.D. and VOGEL K.P. (1999) Accomplishments and impact from breeding for increased forage nutritional value. *Crop Science*, **39**, 12-20.

CASLER M.D., FALES F.D., MCELROY A.R., HALL M.H., HOFFMAN L.D., UNDERSANDER D.J. and LEATH K.T. (2002) Half-sib family selection for forage yield in orchardgrass. *Plant Breeding*, **121**, 43-48.

COOPER J.P. (1962) Selection for nutritive value. In: *Report of the Welsh Plant Breeding Station for 1961, Welsh Plant Breeding Station, Aberystwyth*, U.K., pp. 145-156.

FALCONER D.S. and MACKAY T.F.C. (1996) *Introduction to Quantitative Genetics*, 4th edition, pp. 145-183. New York, USA: Prentice Hall.

FULKERSON W.J. and DONAGHY D.J. (2001) Plant-soluble carbohydrate and senescence – key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental Agriculture*, **41**, 261-275.

HUMPHREYS M.O. (1989a) Water-soluble carbohydrates in perennial ryegrass breeding. I. Genetic differences among cultivars and hybrid progeny grown as spaced plants. *Grass and Forage Science*, **44**, 231-236.

HUMPHREYS M.O. (1989b) Water-soluble carbohydrates in perennial ryegrass breeding. III. Relationships with herbage production, digestibility and crude protein content. *Grass and Forage Science*, **44**, 423-430.

LEE M.R.F., JONES E.L., MOORBY J.M., HUMPHREYS M.O., THEODOROU M.K., MACRAE

J.C. and SCOLLAN N.D. (2001) Production response from lambs grazed on *Lolium perenne* selected for an elevated water-soluble carbohydrate concentration. *Animal Research*, **50**, 441-449.

LEE M.R.F., HARRIS L.J., MOORBY J.M., HUMPHREYS M.O., THEODOROU M.K., MACRAE J.C. and SCOLLAN N.D. (2002) Rumen metabolism and nitrogen flow to the small intestine in steers offered *Lolium perenne* containing different levels of water-soluble carbohydrate. *Animal Science*, **74**, 587-596.

MASUKO T., ONO A., FURUKAWA N. and OTANI T. (1994) Changes in crude protein, and water soluble carbohydrate (WSC) contents of temperate grass cut at three stages of growth in Hokkaido. *Journal of Japanese Grassland Science*, **40**, 227-229.

MAYLAND H.F., SHEWMAKER G.F., HARRISON P.A. and CHATTERTON N.J. (2001) Nonstructural carbohydrate in tall fescue cultivars; relationship to animal preference. *Agronomy Journal*, **92**, 1203-1206.

MERRY R.J., WINTERS A.L., THOMAS P.I., MÜLLER M. and MÜLLER T. (1995) Degradation of fructans by epiphytic and inoculated lactic acid and by plant enzymes during ensilaging of normal and sterile hybrid ryegrass. *Journal of Applied Bacteriology*, **79**, 583-591.

NGUYEN H.T. and SLEPER D.A. (1983) Theory and application of half-sib mating in forage grass breeding. *Theoretical and Applied Genetics*, **64**, 187-196.

POLLOCK C.J. and CAIRNS A.J. (1991) Fructan metabolism in grasses and cereals. *Annual*

Review of Plant Physiology and Plant Molecular Biology, **42**, 77-101.

SHENK J.S. and WESTERHAUS M.O. (1982) Selection for yield and quality in orchardgrass. *Crop Science*, **22**, 422-425.

STRATTON S.D., SLEPER D.A. and MATCHES A.G. (1979) Genetic variation and interrelationships of *in vitro* dry matter disappearance and fiber content in orchardgrass herbage. *Crop Science*, **19**, 329-333.

SAIGA S. (1981) Studies on breeding for improvement of forage quality of orchardgrass (*Dactylis glomerata* L.). *Research Bulletin of Hokkaido National Agricultural Experiment Station*, **129**, 25-92.

SMITH K.F., REED K.F.M. and FOOT J.Z. (1997) An assessment of the relative importance of specific traits for the genetic improvement of nutritive value in dairy pasture. *Grass and Forage Science*, **52**, 167-175.

TAKAI T. and NAKAYAMA S. (2000) Relationship between ADF contents in seeding year in the second year of after math growth of orchardgrass (*Dactylis glomerata* L.). *Grassland Science*, **46**, 77-78.

VOGEL K.P. AND J.F. PEDERSEN J.F. (1993) Breeding systems for cross-pollinated perennial grasses. *Plant Breeding Reviews*, **11**, 251-274.

WALTERS R.J.K. and EVANS E.M. (1974) Herbage quality. In: *Report of the Welsh Plant Breeding Station for 1973 Welsh Plant Breeding Station, Aberystwyth*, U.K., pp. 142-144.

WILKINS P.W. and HUMPHREYS M.O. (2003) Progress in breeding perennial forage grasses for temperate agriculture. *Journal of Agricultural Science, Cambridge*, **140**, 129-150.

Table 1 Means, standard errors (in parentheses) and ranges of parents and half-sib progeny in cocksfoot evaluated across two years for concentrations of forage quality traits and yield at the vegetative growth stage under spaced planting for parents and sward conditions for progeny in Experiment 1.

Traits	Parents						Progeny					
	Year		Mean	Max.	Min.	Range	Year		Mean	Max.	Min.	Range
	2001	2002					2001	2002				
Mono- and disaccharides (g kg^{-1}DM)	29 (1)	60 (2)	44 (1)	58	33	25	29 (1)	44 (1)	36 (1)	45	31	14
Fructan (g kg^{-1}DM)	13 (1)	54 (6)	33 (4)	75	0	75	16 (1)	73 (3)	45 2)	72	26	46
Total water-soluble carbohydrates (g kg^{-1}DM)	42 (2)	113 (8)	78 (5)	133	39	94	45 (2)	117 (3)	81 (2)	117	58	59
Crude protein (g kg^{-1}DM)	153 (2)	107 (2)	130 (2)	143	100	43	118 (1)	87 (1)	103 (1)	115	93	22
Acid-detergent fibre (g kg^{-1}DM)	346 (2)	331(4)	339 (3)	380	315	65	363 (1)	325 (2)	344 (1)	361	330	31
Neutral-detergent fibre (g kg^{-1}DM)	597(3)	586 (4)	591 (3)	626	558	68	624 (2)	554 (3)	589 (2)	612	567	45
Dry matter yield (kg ha^{-1})	-	-	-	-	-	-	2260 (60)	2390 (30)	2330 (40)	2850	1750	1100

Table 2 Mean squares and variance components estimated from analysis of variance in parents and half-sib progeny of cocksfoot evaluated across two years for concentrations of forage quality traits and yield at the vegetative growth stage under spaced planting for parents and sward conditions for progeny in Experiment 1.

Trait	Parents							
	Mean squares				Variance components			
	Entry (E)	Year (Y)	E x Y	Error	Entry (E)	Year (Y)	E x Y	Error
Mono- and disaccharides (g kg^{-1}DM)	183.6**	27640.9**	59.17**	19.5	21.2	307.3	12.8	19.5
Fructan (g kg^{-1}DM)	1561.9**	49910.9**	916.6**	112.6	106.8	553.1	267.8	112.6
Total water-soluble carbohydrates (g kg^{-1}DM)	2709.5**	151837.3**	1365.9**	211.5	226.3	1685.2	382.7	211.5
Crude protein (g kg^{-1}DM)	358.0**	63186.9**	211.6**	47.4	24.1	702.4	55.0	47.4
Acid-detergent fibre (g kg^{-1}DM)	817.6**	7343.5**	447.3**	72.6	57.2	81.3	129.0	72.6
Neutral-detergent fibre (g kg^{-1}DM)	1055.6**	3505.6**	552.5**	89.4	79.1	38.0	158.8	89.4
Dry matter yield (kg a^{-1})	-	-	-	-	-	-	-	-

Trait	Progeny							
	Mean squares				Variance components			
	Entry (E)	Year (Y)	E x Y	Error	Entry (E)	Year (Y)	E x Y	Error
Mono- and disaccharides (g kg^{-1}DM)	60.2**	9758.4**	26.4	27.1	7.4	108.1	27.1	27.1
Fructan (g kg^{-1}DM)	503.1**	147239.0**	264.5**	109.5	42.6	1633.1	51.7	109.5
Total water-soluble carbohydrates (g kg^{-1}DM)	853.8**	232808.2**	416.1**	180.2	75.7	2582.1	78.6	180.2
Crude protein (g kg^{-1}DM)	203.7**	43316.6**	54.8**	18.4	20.7	48.1	12.2	18.4
Acid-detergent fibre (g kg^{-1}DM)	284.8**	62388.2**	108.8**	51.9	28.1	69.2	19.0	51.9

Neutral-detergent fibre (g kg^{-1}DM)	656.7**	220129.2**	229.8**	116.9	67.0	2443.3	37.6	116.9
Dry matter yield (kg a^{-1})	36.6**	88.2**	9.0**	2.6	3.8	0.9	2.1	2.6

***, $P<0.01$; **, $P<0.01$; *, $P<0.05$ respectively.

Table 3 Means, standard errors (in parentheses) and ranges of parents and half-sib progeny in cocksfoot evaluated across two years for concentrations of forage quality traits and yield at the vegetative growth stage under spaced planting for parents and sward conditions for progeny in Experiment 2.

Traits	**Parents**						**Progeny**					
	Year		**Mean**	**Max.**	**Min.**	**Range**	**Year**		**Mean**	**Max.**	**Min.**	**Range**
	2002	**2003**					**2002**	**2003**				
Mono- and disaccharides (g kg^{-1}DM)	62 (3)	54 (4)	58 (3)	75	33	42	27 (1)	60 (2)	43 (1)	50	37	13
Fructan (g kg^{-1}DM)	92 (11)	71 (8)	71 (8)	141	34	107	14 (1)	58 (3)	36 (2)	53	26	27
Total water-soluble carbohydrates (g kg^{-1}DM)	153 (14)	124 (12)	124 (12)	216	67	149	40 (2)	118 (5)	79 (3)	104	64	40
Crude protein (g kg^{-1}DM)	81 (3)	127 (5)	127 (5)	131	92	39	184 (2)	141 (2)	163 (1)	172	157	15
Acid-detergent fibre (g kg^{-1}DM)	325 (8)	299 (7)	299 (7)	357	276	81	309 (2)	293 (2)	301 (2)	309	291	18
Neutral-detergent fibre (g kg^{-1}DM)	586 (11)	544 (11)	544 (11)	628	504	124	544 (3)	518 (3)	531 (2)	543	511	32
Plant vigour	5.1 (0.3)	5.5 (0.4)	5.5 (0.4)	7.1	3.4	3.7	6.4 (0.1)	6.2 (0.1)	6.3 (0.1)	7.0	5.8	1.2

Plant vigour; 1 = very poor to 9 = very vigorous.

Table 4 Mean squares and variance components estimated form analysis of variance in parents and half-sib progeny of cocksfoot evaluated across 2 years for concentrations of forage quality traits at the vegetative growth stage under spaced planting in Experiment 2.

Trait	Parents							
	Mean squares				Variance components			
	Entry (E)	Year (Y)	E x Y	Error	Entry (E)	Year (Y)	E x Y	Error
Mono- and disaccharides (g kg^{-1}DM)	377.3**	765.9	333.1**	38.5	2.1	14.4	147.3	38.5
Fructan (g kg^{-1}DM)	2980.1**	6709.7	2608.5**	150.9	13.7	136.7	1228.8	150.9
Total water-soluble carbohydrates (g kg^{-1}DM)	5141.9**	12584.3	4674.4**	278.7	18.3	263.6	2197.9	278.7
Crude protein (g kg^{-1}DM)	500.3**	31569.6**	411.6**	38.4	2.8	1038.6	186.6	38.4
Acid-detergent fibre (g kg^{-1}DM)	1501.8**	9895.9	1700.6**	91.5	-5.4	273.2	804.5	91.5
Neutral-detergent fibre (g kg^{-1}DM)	3717.4**	2637.9	3071.8**	1800	23.7	775.5	1445.9	180.0
Plant vigour	10.9**	4.0	2.9**	0.2	46.0	2.0	34.0	16.0

Trait	Progeny							
	Mean squares				Variance components			
	Entry (E)	Year (Y)	E x Y	Error	Entry (E)	Year (Y)	E x Y	Error
Mono- and disaccharides (g kg^{-1}DM)	114.6**	32178.1**	115.8	43.9	3.5	714.1	12.9	43.9
Fructan (g kg^{-1}DM)	511.4**	59181.2**	234.4	162.8	43.7	1311.5	-0.6	162.8
Total water-soluble carbohydrates (g kg^{-1}DM)	1004.5**	178637.0**	620.0	334.1	68.0	3962.3	39.0	334.2
Crude protein (g kg^{-1}DM)	193.5**	55152.5**	173.8*	80.9	7.7	1223.8	17.3	80.9
Acid-detergent fibre (g kg^{-1}DM)	292.1**	8050.8**	124.8	100.4	26.4	176.7	-11.1	100.4
Neutral-detergent fibre (g kg^{-1}DM)	720.6**	20385.2**	210.5	260.3	78.3	447.2	-42.3	260.3

Plant vigour	0.86*	2.5*	0.4	0.4	0.1	0.1	0.0	0.2

**, *P*<0.01; *, *P*<0.05, respectively.

Table 5 Phenotypic (above diagonal) and genetic correlations (below diagonal) between concentrations of forage quality traits and yield in half-sib progenies of cocksfoot at the vegetative growth stage under sward and spaced-planting conditions.

Trait	**Mono- and disaccharides**	**Fructan**	**Total water-soluble carbohydrates**	**Crude protein**	**Acid-detergent fibre**	**Neutral-detergent fibre**	**Yield**	**Plant vigour**
Sward condition (Experiment 1)								
Mono- and disaccharides	-	0.85**	0.92**	-0.23	-0.35	-0.50**	-0.02	-
Fructan	0.82	-	0.99**	-0.36*	-0.49**	-0.66**	-0.13	-
Total water-soluble carbohydrates	0.89	0.96	-	-0.34	-0.47**	-0.64**	-0.11	-
Crude protein	-0.22	-0.35	-0.33	-	-0.50**	-0.24	-0.15	-
Acid-detergent fibre	-0.34	-0.48	-0.45	-0.48	-	0.93**	0.35	-
Neutral-detergent fibre	-0.48	-0.64	-0.62	-0.23	0.90	-	0.40**	-
Yield	-0.02	-0.13	-0.10	-0.15	0.34	0.38	-	-
Spaced planting (Experiment 2)								
Mono- and disaccharides	-	0.78**	0.90**	-0.32	-0.49	-0.60*	-	0.16
Fructan	0.96	-	0.98**	-0.04	-0.75**	-0.76**	-	0.14
Total water-soluble carbohydrates	1.23	0.75	-	-0.14	-0.70**	-0.75**	-	0.15
Crude protein	-0.58	-0.04	-0.16	-	-0.58*	-0.48	-	-0.47
Acid-detergent fibre	-0.58	-0.49	-0.52	-0.56	-	0.96**	-	0.18
Neutral-detergent fibre	-0.65	-0.46	-0.51	-0.43	0.56	-	-	0.26
Plant vigour	0.19	0.10	0.12	-0.48	0.12	0.14	-	-

**, *P*<0.01; *, *P*<0.05, respectively.

Table 6 Broad and narrow sense heritabilities and correlation coefficients between concentrations of forage quality traits and yield in parents and half-sib progeny of cocksfoot.

Traits	Spaced planting and sward conditions (Experiment 1)			Spaced planting (Experiment 2)		
	h_b	**h_n**	**r**	**h_b**	**h_n**	**h_{po}**
Mono- and disaccharides	0.68 (0.32)	0.69 (0.29)	0.43*	0.16 (0.17)	0.20 (0.18)	0.40 (0.71)
Fructan	0.42 (0.24)	0.55 (0.30)	0.55**	0.14 (0.14)	0.68 (0.19)	0.97 (0.94)
Total water-soluble carbohydrates	0.50 (0.07)	0.59 (0.17)	0.52**	0.10 (0.11)	0.53 (0.22)	0.78 (0.88)
Crude protein	0.41 (0.21)	0.70 (0.27)	0.46**	0.17 (0.15)	0.29 (0.21)	0.73 (0.62)
Acid-detergent fibre	0.42 (0.22)	0.67 (0.28)	0.45*	0.00	0.68 (0.19)	0.22 (0.89)
Neutral-detergent fibre	0.45 (0.23)	0.70 (0.26)	0.39*	0.18 (0.19)	0.71 (0.18)	0.96 (0.88)
Yield	-	0.71 (0.30)	-	-	-	-
Plant vigour	-	-	-	0.68 (0.3)	0.76 (0.31)	1.20 (0.60)

h_b; broad sense heritability of parent clones.

h_n; narrow sense heritability of half-sib progenies in Experiments 1and 2.

r; simple correlation coefficient between parents and half-sib progenies.

h_{po}; narrow sense heritability calculated from regression between parents and half-sib progenies.

**; *P*<0.01; * , *P*<0.05, respectively.