Use of near infrared reflectance spectroscopy for the determination of silica content in tall fescue

Cougnon M.[1], Van Waes C.[2], Struyf E.[3], Schoelynck J.[3], Reheul D.[1]
[1]*Department of Plant Production, University of Ghent, Proefhoevestraat 22, 9090 Melle, Belgium;* [2]*ILVOPlant, Burg. Van Gansberghelaan 109, 9820 Merelbeke, Belgium;* [3]*Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium; mathias.cougnon@ugent.be*

Abstract

Major disadvantages of using tall fescue as forage grass are its low digestibility and animal voluntary intake. One of the factors explaining this is the high silica content of tall fescue in comparison with other forage grass species. Therefore, we evaluated the possibility to use NIRS on grass samples to predict the silica content. In 2014, we collected 297 samples on four different trials with tall fescue. These samples were analysed by wet chemistry for silica content, the NIRS spectra were recorded and a calibration model was built. In 2015, forage samples of 25 genotypes in a breeding nursery were collected and used for validation of the calibration equation. The mean silica content in this sample was 0.56% with a standard deviation of 0.14%. The validation statistics indicated that prediction of the silica content for this independent validation sample was good (SEP 0.063%; R^2= 82.2%). The large variation in silica content found within the tall fescue genotypes and the ease of prediction using NIRS are a good basis for selecting tall fescue varieties with lower silica content.

Keywords: *Festuca arundinacea,* breeding, animal preference, silicon

Introduction

There is no consensus whether silicon – commonly referred to as silica in ecological research because of its hardly separable bound with two oxygen molecules – is essential or not to plants. It is however certain that silica plays an important role in the tolerance against different kinds of stress (Guntzer *et al.*, 2012). In grass species e.g. silica protects against herbivory: in sheep preference trials, sheep grazed longer, took more bites and had a higher bite rate on grass species with low silica content (Massey *et al.*, 2009).Tall fescue (*Festuca arundinacea* Schreb.) is a forage grass species with interesting properties in the light of climate change such as good drought resistance and high yield potential (Cougnon et al, 2014). Its low voluntary intake and low digestibility however, are damping the use of the species by farmers, and are brought into relation with the high silica content of this species compared to other forage grass species like perennial ryegrass (*Lolium perenne*) and meadow fescue (*Festuca pratensis*) (Hodson *et al.*, 2005). Hence, studying the silica content of tall fescue genotypes can help to elucidate the factors that influence the low animal preference of this species and can eventually lead to varieties with an improved animal preference.

The screening of a large amount of genotypes is hampered by the cumbersome analytical method. near infrared reflectance spectroscopy (NIRS) is a high throughput method that is often used in grassland science to predict a wide range of parameters like crude protein content, digestibility or clover content of swards (Cougnon *et al.*, 2013). Smis *et al.* (2014) developed a NIRS calibration equation that allowed testing the Si content of different forest plant species. We tested whether it was possible to develop a NIRS calibration for silica content specific for tall fescue.

Materials and methods

Both the NIRS spectra and the Si content of 595 dried and ground grass samples from different trials were determined during the years 2014 and 2015. Silica content was analysed using the wet chemistry

method described by the alkaline method of DeMaster (1981). In brief, about 25-30 mg grinded and homogenized plant material was mixed with 25 ml of Na_2CO_3 solution (0.1 M) and incubated in a water bath maintained at 85 °C for 4 hours. After filtration (0.45 µm), the extractions were colorimetrically analysed for Si. NIRS spectra were recorded on a FOSS XDS Rapid Content Analyzer The inverse reflectance [log(1/R)] was measured from 400 to 2,500 nm in steps of 0.5 nm. From the 595 samples, 165 were collected on a trial comparing the sheep preference of 19 genotypes of tall fescue on three occasions in 2014; 57 were collected from plants in a tall fescue clonal nursery on three occasions in 2014; 6 were collected in a tall fescue-perennial ryegrass pasture on one occasion in 2014; 69 samples originated from a trail were the agronomic performance of different varieties of tall fescue, *Festulolium*, meadow fescue and perennial ryegrass was compared in 5 cuts in 2014; finally 298 samples were harvested on two occasions in 2015 in a study of 25 clones and their progeny in three replicates.

The 297 samples collected in 2014 were used to build a calibration equation. The 298 samples harvested in 2015 were used to validate the equation. The validation samples were independent from the calibration samples as they were harvested in a different year and on a different trial than the calibration samples. Calibration equations were developed in WinISI II 1.5 (Infrasoft, Port Mathilda, PA, USA) using the partial least square regression method. A data pre-treatment was performed before regression: the first derivative of the spectra was taken (with gap 4 and smoothing 4) and scatter correction using standard normal variate (SNV) and detrend was applied. Standard errors of calibration, cross validation and prediction (SEC, SECV and SEP) were calculated, and the R^2 of the regression between the lab values and the predicted values was calculated. No outliers were removed from the calibration or the validation sets.

Results and discussion

The silica content in the 297 calibration ranged from 0.21 to 1.46%, with an average content of 0.62% and standard deviation of 0.19%. The mean Si content in the validation set was 0.56% with standard deviation 0.14%. An equation with 15 variables was selected by minimizing the SECV: the SEC and SECV for this equation were 0.056 and 0.067% the R^2 value of the regression was 96.0%. Validation of this equation resulted in an SEP of 0.063% the regression between the predicted values and the lab values had an R^2 of 82.2% (Figure 1).

The values of the statistics are in line with those found by Smis *et al.* (2014) for their equation for the silica content of *graminoid* species sampled in the alpine parts of the northern, sub-arctic zone of Norway: a calibration equation based on 198 samples and validated with 66 samples resulted in an SEP of 0.102, while the slope of the regression between the predicted values and the lab values was 0.97 and the R^2 was 0.95. By narrowing their data set only to 1 grass species (*Deschampia cespitosa*) the statistics were further improved with a slope of 1.02 and a R^2 of 0.93.

These results suggest that NIRS can be used to predict the silica content both between plant species as within plant species, where the variation in silica is generally lower. Although the equation we developed with the available samples is not suited to determine the silica content very precisely of any tall fescue sample, it showed to be capable to discriminate genotypes with consistently high and low silica contents. For screening populations for breeding purposes this is sufficient. Adding supplementary samples from different trails to the calibration set will make the equation more robust and may eventually lead to a calibration that allows a very precise prediction of the silica content in tall fescue.

Conclusions

NIRS can be used to discriminate between the silica content of different tall fescue genotypes.

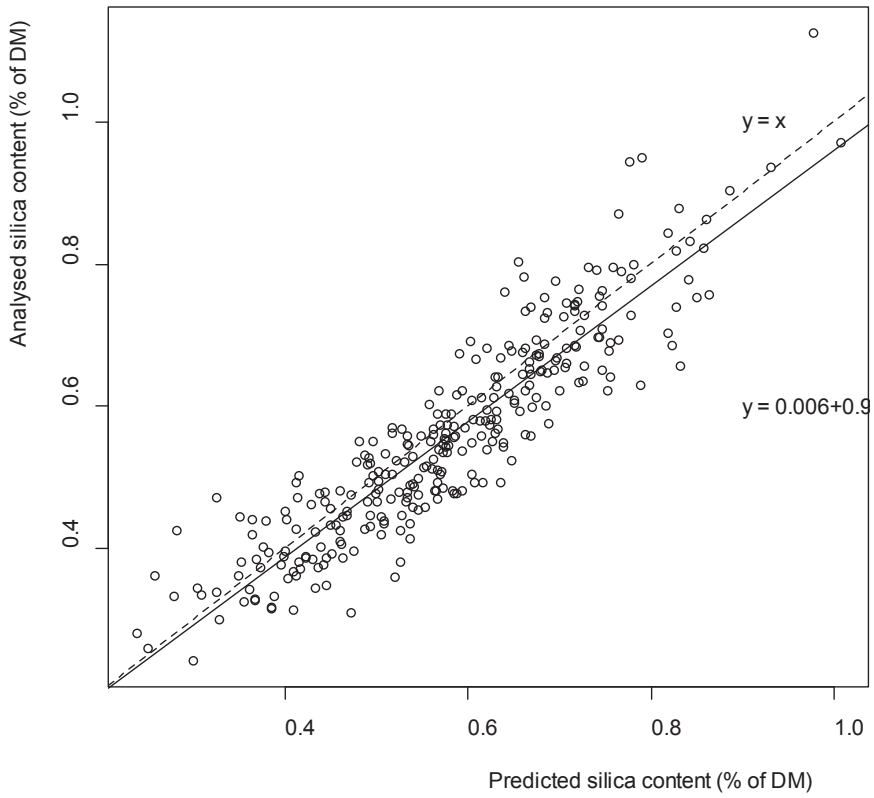

Figure 1. Regression between the silica content of 297 grass samples determined in the lab and predicted using a NIRS calibration equation.

References

Cougnon M., Baert J., Van Waes C.. and Reheul D. (2014) Performance and quality of tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) and mixtures of both species grown with or without white clover (*Trifolium repens* L.) under cutting management. *Grass and Forage Science* 69, 666-677.

Cougnon M, Van Waes C, Dardenne P, Baert J and Reheul D (2014) Comparison of near infrared reflectance spectroscopy calibration strategies for the botanical composition of grass-clover mixtures. *Grass and Forage Science* 69, 167-175.

DeMaster, D.J. (1981) The supply and accumulation of silica in the marine-environment. *Geochimica Et Cosmochimica Acta* 45, 1715-1732.

Guntzer F, Keller C, Meunier J-D (2012) Benefits of plant silicon for crops: a review. *Agronomy for Sustainable Development* 32, 201-213.

Hodson M, White PJ, Mead A and Broadley M (2005) Phylogenetic variation in the silicon composition of plants. *Annals of Botany* 96, 1027-1046

Massey F.P., Massey K., Ennos A.R. and Hartley S.E. (2009) Impacts of silica-based defences in grasses on the feeding preferences of sheep. *Basic and Applied Ecology* 10, 622-630.

Smis A., Murguzur, FJ.A., Struyf E., Soininen E.M., Jusdado J.G..H., Meire P., Bråthen K.A. (2014) Determination of plant silicon content with near infrared reflectance spectroscopy. *Frontiers in plant science* 5, 1-9.