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Research article

Variation in potential feeding value of triticale forage among plant fraction, maturity stage, growing season and genotype

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ABSTRACT

Cereal forages, such as triticale forage, progressively gain interest as alternative crop for maize. The main study objective was to investigate the variation in potential feeding value of triticale forage among maturity stage, growing season and genotype, using total plant and stem fractions. Therefore, near infrared spectroscopy (NIRS) was evaluated as fast screening tool. The prediction ability was good (ratio of prediction to deviation, RPD $\geq$3.0) for total plant residual moisture, starch, sugars and for stem crude ash (CAsh) and neutral detergent fibre (aNDFom); suitable for screening (2.0 $\leq$ RPD <3.0) for total plant CAsh, acid detergent fibre (ADFom), *in vitro* digestibility of organic matter (IVOMD), *in vitro* digestibility of neutral detergent fibre (IVNDFD) and for stem total lignin (TL) and IVNDFD; poor (1.5 $\leq$ RPD <2.0) for total plant crude protein, crude fat, aNDFom, lignin (sa) and for stem Klason lignin (KL); unreliable (RPD <1.5) for stem residual moisture and acid soluble lignin (ASL). The evolution in potential feeding value of 36 genotypes harvested at the medium and late milk to the early, soft and hard dough stage was followed. The most important changes occurred between the late milk and early dough stage, with little variation in quality after the soft dough stage. During 2 growing seasons, variation in feeding value of 120 genotypes harvested at the soft dough stage was demonstrated. Interestingly, variation in stem IVNDFD is almost twice as high as for the total plant (CV 12.4% versus 6.6%). Furthermore, Spearman correlations show no link between dry matter yield and digestibility of genotypes harvested at the soft dough stage. Based on linear regression models ADFom appears as

Abbreviations: 1-VR, determination coefficient of cross-validation; ADFom, acid detergent fibre expressed exclusive of residual ash; aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; CAsh, crude ash; CELL, cellulose; CFat, crude fat; CP, crude protein; CV, coefficient of variation; DM, dry matter; DMY, dry matter yield; DOMY, digestible organic matter yield; GDD, growing degree days; HCELL, hemicellulose; IVNDFD, *in vitro* digestibilty of neutral detergent fibre; IVOMD, *in vitro* digestibility of organic matter; KL, Klason lignin; Lignin (sa), lignin determined by solubilisation of cellulose with sulphuric acid; MS, maturity stage; MSE, mean squared error; NIRS, near infrared spectroscopy; RPD, ratio of prediction to deviation; SECV, standard error of cross-validation; STA, starch; SUG, sugars; TL, total lignin.

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main predictor of both plant IVOMD and plant IVNDFD. Stem IVNDFD is particularly determined by KL.

1. Introduction

Available weather models for North-West Europe predict higher average temperatures and longer periods of drought which clearly negatively affects crop yield [1]. Maize (*Zea mays* L.), a commonly grown forage crop, will be consequently more exposed to extreme weather conditions during summer. Indeed, a recent study on silage maize in Germany predicted an average yield reduction of -120 to -1050 (kg/ha)/annum for the period 2021–2050 compared to the reference period 1971–2000 due to climate change [2]. The introduction of alternative crops, such as cereal forages, would not only result in more diversification of the crop rotation system. They also offer the advantage to be harvested before summer dryness what allows the cultivation of a second crop [3]. Furthermore, if winter cereals are chosen, soil erosion and nitrate leaching into ground water are reduced because the soil is covered for most of the growing season [3–5]. Nevertheless, the implementation of cereal forages on dairy farms will only be possible if their dry matter yield (DMY) and feeding value are sufficiently high [6].

Triticale (× *Triticosecale* Wittmack), a hybrid cereal combining the quality parameters of wheat and the robustness of rye, shows great potential as alternative roughage crop [7]. Compared to other cereal forages, triticale is a high-yielding crop that can be grown on low-fertility soils [8,9]. Especially under dry conditions, triticale out yields forages of oats, wheat and barley [4,10,11]. Triticale forage is also considered as alternative to maize forage in terms of DMY under limited water supply [12]. Research on the feeding value of triticale likewise confirms its potential as forage crop. Forages of barley and triticale are shown to have higher nutrient content and better digestibility than oats and wheat [13,14]. Compared to maize forage, however, the feeding value of triticale is generally inferior due to a higher neutral detergent fibre (aNDFom) and a lower starch (STA) content and thus a lower digestibility [15]. Nevertheless, different studies indicate the strong influence of maturity stage at harvest, genotype and growing season on the chemical composition and *in vitro* digestibility of triticale forage [3,16–19].

Several strategies have been suggested to improve the feeding value of triticale forage. On the one hand, agronomic characteristics such as a higher grain/straw ratio can significantly improve the feeding value of triticale forage, similarly to what was demonstrated by Walsh et al. [20] in barley and wheat forage. On the other hand, triticale breeders aim to improve the composition and digestibility of specific plant fractions. Higher crude protein (CP) and STA concentrations in the kernels, which are breeding targets in triticale, resulted in enhanced feeding value of the total plant [21]. Moreover, higher digestibility of the stem can further expand the opportunities for triticale forage [22]. The existence of variation in stem digestibility was demonstrated in other forage crops, such as maize and perennial ryegrass (*Lolium perenne* L.) [23,24]. More specifically, van Parijs [24] demonstrated the breeding potential for a higher stem *in vitro* aNDFom digestibility (IVNDFD) in perennial ryegrass. To the best of our knowledge, no single study reports the available variation for stem IVNDFD in triticale.

The main objective of this study was to investigate the variation in potential feeding value of triticale forage among plant fractions. Specific objectives were: (i) to evaluate near infrared spectroscopy for estimating the chemical composition and *in vitro* digestibility of triticale total plant and stem fractions; (ii) to investigate the potential feeding value of triticale forage harvested at five maturity stages; (iii) to explore the variation in total plant and stem *in vitro* digestibility and forage yield among two growing seasons and among genotypes, and; (iv) to identify the most important chemical parameters that contribute to variability in total plant and stem *in vitro* digestibility. More specifically, in order to explore the existing variation in potential feeding value of triticale forage, a diverse set of triticale genotypes needs to be screened for residual moisture, CP, STA, sugars (SUG), crude fat (CFat), crude ash (CAsh), aNDFom, acid detergent fibre (ADFom), lignin (sa), Klason lignin (KL), total lignin (TL), IVNDFD and *in vitro* digestibility of organic matter (IVOMD). Given the large number of samples to screen, near infrared spectroscopy (NIRS), a commonly used method to estimate the chemical characteristics of forages and other feedstuffs, was investigated as fast, reliable and accurate evaluation technique [25]. In this study, NIRS calibrations were used to estimate the potential feeding value of 36 triticale genotypes harvested at five stages of kernel maturity: medium milk, late milk, early dough, soft dough and hard dough, and of 120 genotypes at the soft dough stage during two consecutive growing seasons. In a final step, we identified chemical parameters that can be used as screening criteria for a higher *in vitro* digestibility of triticale total plant and stems.

2. Materials and methods

2.1. Plant material

The study involved total plant and stem samples of winter triticale genotypes harvested at the Ghent University's Research Farm in Bottelare, Belgium over three growing seasons (Supplementary Table 1). This site has a sandy loam soil and according to the Köppen Climate Classification system, it has a marine west coast climate with mild differences between minimum and maximum temperatures and adequate rainfall year-round [26]. During the 2016–2017 season, 36 genotypes of European origin were grown to investigate the effect of maturity stage on chemical composition and *in vitro* digestibility. This information allowed us to determine the optimal harvest stage for maximal feed quality. During seasons 2017–2018 and 2018–2019, 120 genotypes were harvested at the optimal maturity stage to study the available variation in potential feeding value. This set contained triticale genotypes of European, Canadian and American origin. Eight triticale genotypes were common over the two sets.

2.2. Weather conditions

A meteorological station located at the Ghent University's Experimental Farm in Melle, Belgium (latitude 50°58'50", longitude 3°48'56'') automatically provided data of daily temperature and rainfall during the 2016–2017 growing season. For the seasons 2017–2018 and 2018–2019, temperature and rainfall were recorded at a meteorological station located at the Ghent University's Research Farm in Bottelare, Belgium (latitude 50°57'43", longitude 3°45'37''). The temperature sum (growing degree days, GDD) was calculated, following Formula 1:

$$GDD\ (^{\circ}C) = [(T_{max} + T_{min})\ /\ 2] - T_{base} \quad (1)$$

Where $T_{\max}$ is the maximum daily temperature, $T_{\min}$ is the minimum daily temperature and T_{base} is the lower threshold for development (5 °C was used as base temperature in this study). GDD were summed monthly. When the daily GDD was <0, no degree days were accumulated and 0 was assumed for that day [27].

2.3. Experimental design

On October 27, 2016, a field trial with 36 winter triticale genotypes was set up in Melle, Belgium (latitude 50°59'4", longitude 3°49'2'') in a single replicate design. Seeds were sown using a Hege 80 plot seeder (Wintersteiger, Ried, Austria) and the plot size was 7.5 m^2 (5.0 m × 1.5 m). In November 2017, 120 triticales were hand-sown in a field trial in Merelbeke, Belgium (latitude 50°57'46", longitude 3°45'35'') and arranged according to a randomized complete block design in 3 replicates. Every replicate was split into 2 rows containing 60 genotypes (columns) each. Plots consisted of six rows, 0.125 m apart and 1.0 m long. In November 2018, the 120 genotypes were hand-sown in a field trial in Oosterzele, Belgium (latitude 50°57'28", longitude 3°46'6'') to obtain an exact replicate of the 2017–2018 experiment. All plots were sown at a density of 350 kernels/m^2 and standard crop management practices for fertilisation, herbicide, fungicide and insecticide treatments were applied. To maximize biomass yield, no plant growth regulator was used.

Stage of maturity was scored separately for each genotype, thus triticales were harvested at different dates according to maturity. In growing season 2016–2017, the 36 triticales were harvested at five maturity stages: Z75 - medium milk (MS1), Z77 - late milk (MS2), Z83 - early dough (MS3), Z85 - soft dough (MS4) and Z87 - hard dough (MS5), corresponding to the maturation of the kernels (Zadoks Scale) [28]. At harvest, total plant biomass of 0.8 m^2 per plot was cut by hand at 7 cm above ground level. In total 180 total plant samples (36 genotypes × 5 maturity stages) of about 800 g were taken. Taking into account the results obtained in the first experiment, the 120 genotypes were harvested at the soft dough stage during the 2017–2018 and 2018–2019 growing seasons. At harvest, the biomass of the third and fourth row of the plots (0.25 m^2/plot) was weighted and total plant samples of approximately 500 g were collected, resulting in a total set of 720 samples (120 genotypes × 3 repetitions × 2 growing seasons). From triticale plants of the second and fifth row from each plot, the main stem part between the second and the third node was hand cut. Stem and leaf were separated whereby stem samples consisted of the sheaths and true stem together.

Samples were dried in a ventilated Vötsch oven (Weiss Technik, Reiskirchen-Lindenstruth, Germany) at 65 °C to determine dry matter (DM) content at harvest. Total plant samples were subsequently ground in a Retsch mill (Retsch Benelux, Aartselaar, Belgium), equipped with a 1 mm screen. Stem samples were ground in a Fritsch cutting mill (Fritsch International, Idar-Oberstein, Germany), using a 0.5 mm sieve to improve reproducibility of the wet-chemical analysis results. All samples were air-dry preserved in plastic bottles, closed with a push and a screw cap, and stored in a cool, dark place.

2.4. NIRS analysis

NIRS analysis was carried out with a FOSS NIRS DS2500 apparatus on total plant samples and with a FOSS NIRS XDS Rapid Content Analyzer on stem samples, taking the spectrum from 400 to 2500 nm in steps of 2 nm (FOSS Analytics, Hilleroed, Denmark). Spectra were collected using winISI software (version 4.10.0 (total plant samples) and version 4.9.0 (stem samples), Infrasoft International LLC, State College, PA, USA). Based on spectral variation 160 out of 900 total plant samples and 150 out of 720 stem samples were selected for wet-chemical analysis (Supplementary Table 2). The samples originated from the three growing seasons and served as the calibration set.

2.5. Wet-chemical analysis

2.5.1. Total plant samples

Total plant samples were analysed for residual moisture by drying overnight at 103 °C [29]. After incineration in a muffle furnace at 550 °C for 3 h, crude ash (CAsh) content was obtained [30]. Cell wall components were analysed with an ANKOM200 Fiber Analyzer (ANKOM Technology, Macedon, New York). Neutral detergent fibre (aNDFom) was determined after adding α-amylase and sodium sulphite and was expressed on ash-free basis. Acid detergent fibre (ADFom) was also expressed on ash-free basis and the residue was treated with 72% H_2SO_4 to obtain lignin (sa) [31]. Hemicellulose (HCELL) was calculated as the difference between aNDFom and ADFom and cellulose (CELL) as the difference between ADFom and lignin (sa). *In vitro* digestibility of organic matter (IVOMD) was determined with a cellulase preparation of *Trichoderma viride,* according to the procedure of De Boever et al. [32]. *In vitro* digestibility of neutral detergent fibre (IVNDFD) was determined by 48 h incubation in buffered rumen fluid according to Tilley & Terry [33] and by

analysis of aNDFom in feed and residue [31]. The rumen fluid came from three fistulated sheep fed good quality grass hay. For determination of crude fat (CFat), crude protein (CP) and sugars (SUG), subsamples were additionally ground through a 1 mm sieve with a Brabender rotary mill (Brabender GmbH & Co. KG., Duisburg, Germany). For CFat, samples were extracted with light petroleum ether [34]. CP (N x 6.25) was determined following the Dumas combustion principle [35]. SUG were analysed with the Luff Schoorl reagens [36]. Starch (STA) was determined on a 0.75 mm subsample after incubation with amyloglucosidase [37].

2.5.2. Stem samples

Stem samples were analysed for residual moisture by drying 1 g of sample at 103 °C (modification of ISO 6496 [29]). The same sample was then ashed at 550 °C to obtain CAsh (modification of ISO 5984 [30]). Subsequently, aNDFom was determined with the filter bag method in an ANKOM apparatus and after adding heat stable α-amylase [31]. Total lignin (TL) was calculated as the proportion of Klason lignin (KL) + acid soluble lignin (ASL) on aNDFom basis. TL is expressed on aNDFom as better NIRS predictions were generally obtained than on DM (data not shown). The KL method was based on the NREL/TP-510-42618 standard with modification for ANKOM use [38] and followed according to van Parijs et al. [39]; without acid insoluble protein determination. ASL was calculated by spectrometrically measuring the absorbance of the KL filtrate at 320 nm and using 30 L/g cm as extinction coefficient [39]. Finally, IVNDFD was determined using the same procedure as for the total plant samples on an ad random selection of 83 out of 150 stem samples.

2.6. Data analysis

2.6.1. NIRS calibrations development

NIRS spectra were subjected to pre-treatment consisting of Standard Normal Variate (SNV) - Detrend, followed by the 1,4,4,1 derivative mathematical method. Subsequently, calibration equations were developed by using modified partial least square (MPLS) regression. Calibrations derived from the calibration set were tested by cross-validation and then applied to the complete sample set. Cross-validation was performed by splitting the calibration set for the total plant samples in four groups, whereas for the stem samples in five groups. NIRS calibrations were evaluated using the determination coefficient of cross-validation (1-variance ratio, 1-VR), the standard error of cross-validation (SECV) and the ratio of prediction to deviation (RPD, calculated as standard deviation (SD) of the calibration set divided by the SECV). According to Minasny & McBratney [40]; there is no statistical or utilitarian basis as to how the RPD thresholds are determined and the interpretation of the values depends on the authors. In the present study, an RPD higher than 3.0 was considered to account for a good calibration, an RPD between 2.0 and 3.0 was considered to be suitable for screening, an RPD between 1.5 and 2.0 was considered poorly calibrated and an RPD lower than 1.5 was considered unreliable.

2.6.2. Variation among maturity stages

All statistical analyses were conducted in R software (version 3.6, [41]). The data for studying the effect of maturity stage were analysed using a Kruskal-Wallis test since the normality and homoscedasticity assumptions of parametric tests were not fulfilled. This test was used to analyse the effect of maturity stage on DM content at harvest, chemical composition and *in vitro* digestibility of triticale forage (significance level $P < 0.05$). When significant effects were detected, the post hoc test function *kruskal* of R package agricolae including Bonferroni correction was run to detect differences among maturity stages [42]. Data were presented as means and standard error of means (SEM).

2.6.3. Variation among growing seasons and genotypes

Digestibility and DMY are the main parameters that will determine the implementation of triticale as forage crop. These parameters are combined in the digestible organic matter yield (DOMY), calculated as organic matter yield multiplied by plant IVOMD. The 120 genotypes grown in growing seasons 2017–2018 and 2018–2019 were considered for exploring the variation in plant IVOMD, plant and stem IVNDFD, DMY and DOMY. Coefficients of variation (CV), expressed in %, were calculated as SD divided by the mean, multiplied by 100. Analyses of variance were conducted across growing seasons with the *aov* function for plant IVOMD, plant and stem IVNDFD, DMY and DOMY. Residuals were tested for assumptions of normality. Next, linear mixed models with random and fixed intercepts were fit using the R package lme4 to obtain stable genetic values for each parameter [43]. To correct the parameters for year and harvest date effects, growing season and DM content at harvest were identified as fixed intercepts while genotype was considered a random intercept. Both row and column were nested in growing season as random intercepts, following Formula 2:

$$Y = \mu + S_j + DM + G_i + SC_{jk} + SR_{jl} + e_{ijkl} \quad (2)$$

Where Y is the corrected parameter, μ is the overall mean, S_j is the effect of the j-th growing season, DM is the effect of the DM content at harvest, G_i is the effect of the i-th genotype, SC_{jk} is the interaction effect of the j-th growing season with the k-th column, SR_{jl} is the interaction effect of the j-th growing season with the l-th row and e_{ijkl} is the residual error term.

2.6.4. Correlations and linear regression models

Spearman analysis was conducted on the genetic values by using the R corrgram package. Simple Spearman correlations were calculated to identify the mutual relationship between plant IVOMD, plant IVNDFD, stem IVNDFD and DMY. As digestibility is a complex parameter for wet-chemical analysis, we assessed the importance of underlying chemical parameters on plant IVOMD, plant IVNDFD and stem IVNDFD. Subsequently linear regression models were built and models that best explained variation in the

digestibility parameters were discussed.

3. Results

3.1. Weather conditions

Triticale forage yield and composition are highly influenced by weather conditions. For these experiments, the monthly temperature sum and precipitation during the growing season were compared to the climatic normals for Ukkel, Belgium during 1991–2020 [44] (Fig. 1). In general terms, the three studied growing seasons were warmer and drier compared to the climatic normals. March, May and June in season 2016–2017 proved to be unusually warm. Comparing precipitation with the climatic normals revealed a low precipitation in May and June. Season 2017–2018 was warmer and drier compared to the other seasons as well as to the climatic normals. Especially October, January, April, May, June and July were remarkably warm months. December was very rainy, while May, June and July were exceptionally dry. Although May 2019 was colder, temperatures were higher than the climatic normals during season 2018–2019. March and June were wet, while July was drier.

3.2. Variation in chemical composition and digestibility among plant fractions and performance of NIRS calibrations

3.2.1. Total plant fraction

The descriptive statistics of the residual moisture content, chemical composition and *in vitro* digestibility of the total plant calibration set as well as the performance of the NIRS calibrations can be found in Table 1. Residual moisture content was relatively constant (on average 81 g/kg). Nutrient composition showed a large variation because triticale forage was harvested at different maturity stages. The main component in total plant triticale were cell walls with aNDFom varying between 396 and 683 g/kg DM, ADFom between 211 and 432 g/kg DM and lignin (sa) between 18 and 58 g/kg DM. Further, SUG and STA varied widely from 6 to 254 g/kg DM and from 19 to 369 g/kg DM, respectively. Total plant triticale was relatively low in CP (on average 76 g/kg DM) and also CFat (17 g/kg DM) and CAsh (49 g/kg DM) were low. The IVNDFD amounted to 0.51 on average, whereas IVOMD averaged some 0.10-units higher. The performance of the NIRS calibrations applied to total plant samples was relatively good. With the exception of CP, CFat and lignin (sa), 1-VR was higher than 0.70. The prediction ability was good for residual moisture, STA and SUG (RPD $\geq$3.0); suitable for screening for CAsh, ADFom, IVOMD and IVNDFD (2.0 $\leq$ RPD $<$3.0) and poor for CP, CFat, aNDFom, and lignin (sa) (1.5 $\leq$ RPD $<$2.0).

3.2.2. Stem fraction

The descriptive statistics of the residual moisture content, chemical composition and IVNDFD of the triticale stem calibration set as well as the performance of the NIRS calibrations are presented in Table 2. Residual moisture fluctuated between 54 and 115 g/kg. Similarly as the total plant samples, the main component in triticale stems was aNDFom, which varied between 819 and 963 g/kg DM, while CAsh varied to a lesser extent. Triticale stems were poor in ASL (28 g/kg aNDFom on average). Due to a higher KL content (139

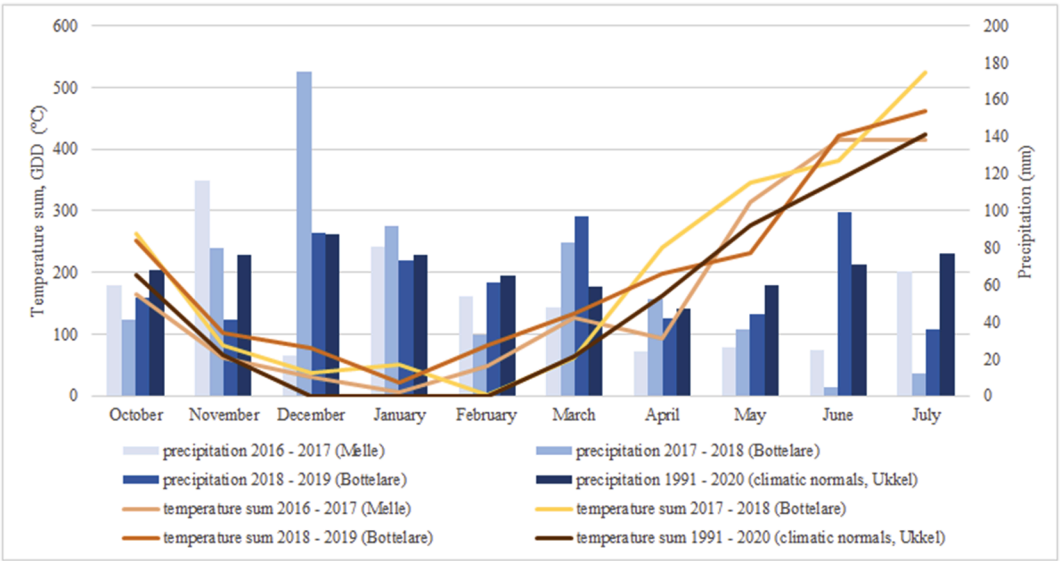

Fig. 1. Temperature sum (growing degree days, GDD, (°C)) and precipitation (mm) at Melle and Bottelare in 2016–2017, 2017–2018 and 2018–2019 during the growing season, compared to the climatic normals from Ukkel, Belgium during 1991–2020 [source: Royal Meteorological Institute of Belgium]. Growing degree days was accumulated monthly, using 5 °C as a base temperature.

Table 1
Descriptive statistics of the residual moisture content (g/kg), chemical composition (g/kg DM) and *in vitro* digestibility of the total plant triticale calibration set (n = 160) and performance of the NIRS calibrations.

Parameters	Mean	SD	MIN	MAX	CV	Lab error[a]	SECV	1-VR	RPD
Residual moisture (g/kg)	81	5.6	68	93	6.9	0.2	1.8	0.90	3.2
Chemical composition (g/kg DM)									
Crude protein	76	12	54	106	16	2.2	7.2	0.63	1.6
Starch	231	98	19	369	42	3.5	25	0.94	4.0
Sugars	63	70	6	254	111	3.3	7.8	0.99	9.1
Crude fat	17	3.2	10	26	19	3.3	2.1	0.58	1.6
Crude ash	49	8.4	31	68	17	1.1	3.7	0.80	2.3
aNDFom	508	52	396	683	10	10	27	0.72	1.9
ADFom	286	37	211	432	13	3.3	19	0.70	2.0
Lignin (sa)	34	8.1	18	58	24	7.6	5.5	0.50	1.5
***In vitro* digestibility**									
IVNDFD	0.51	0.07	0.32	0.62	14	0.012	0.03	0.73	2.0
IVOMD	0.62	0.06	0.41	0.74	10	0.009	0.03	0.80	2.3

SD, standard deviation; MIN, minimum; MAX, maximum; CV, coefficient of variation (%); SECV, standard error of cross-validation; 1 – VR, determination coefficient of cross-validation; RPD, ratio of prediction to deviation; DM, dry matter; aNDFom, NDF assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom, ADF expressed exclusive of residual ash; lignin (sa), lignin determined by solubilisation of cellulose with sulphuric acid; IVNDFD, *in vitro* digestibility of neutral detergent fibre; IVOMD, *in vitro* digestibility of organic matter.

[a] Lab error for starch, aNDFom, IVNDFD and IVOMD was calculated as the mean SD of duplo analyses. The other parameters were analysed in one replicate per sample and lab error was based on the validation procedure of the lab.

g/kg aNDFom), TL averaged 168 g/kg aNDFom. IVNDFD was low (0.38 on average), but ranged from 0.24 to 0.51. The performances of the NIRS calibrations applied to stem samples were acceptable. Apart from residual moisture and ASL, 1-VR exceeded 0.70. The prediction ability was good for CAsh and aNDFom (RPD $\geq$3.0); suitable for screening for TL and IVNDFD (2.0 $\leq$ RPD <3.0); poor for KL (1.5 $\leq$ RPD <2.0) and unreliable for residual moisture and ASL (RPD <1.5).

3.3. Variation among maturity stages

The DM content at harvest, chemical composition and *in vitro* digestibility of triticale forage harvested at the five stages of maturity in growing season 2016–2017 are reported in Table 3. In the maturity range mean DM content increased from 286 to 625 g/kg together with an increase in STA content ($P < 0.05$), while the contents of CP, SUG, CFat, CAsh, aNDFom, ADFom and CELL decreased ($P < 0.05$) and those of lignin (sa) and HCELL remained relative constant. Mean IVOMD increased ($P < 0.05$) from 0.59 to 0.68, whereas IVNDFD hardly changed. Especially for DM content, SUG and STA, the clear shift from MS2 to MS3 shows that triticale forage should be harvested in the dough stages to maximize its potential feeding value. Moreover, little variation in chemical composition and *in vitro* digestibility was observed after MS4, the soft dough stage. Coefficients of variation additionally showed considerable variation in compositional and digestibility parameters within MS4 for the 36 studied triticales (Supplementary Table 3). In order to further explore variation between soft dough triticale forage for *in vitro* digestibility, DMY and DOMY, and the influence of growing season, a larger set of 120 triticales was tested during two consecutive growing seasons.

Table 2
Descriptive statistics of the residual moisture content (g/kg), chemical composition and *in vitro* aNDFom digestibility of the triticale stem calibration set (n = 150) and performance of the NIRS calibrations.

Parameters	Mean	SD	MIN	MAX	CV	Lab error[a]	SECV	1-VR	RPD
Residual moisture (g/kg)	75	11	54	115	15	0.3	8.8	0.34	1.3
Chemical composition									
Crude ash (g/kg DM)	56	12	31	95	21	0.3	2.9	0.94	4.2
aNDFom (g/kg DM)	882	26	819	963	2.9	2.5	8.6	0.87	3.0
KL (g/kg DM)	114	11	80	140	9.6	3.0	6.1	0.72	1.9
KL (g/kg aNDFom)	139	13	103	168	9.4	3.3	7.1	0.71	1.9
ASL (g/kg aNDFom)	28	4.3	16	44	15	1.7	3.1	0.24	1.4
TL (g/kg aNDFom)	168	11	142	191	6.5	1.8	4.6	0.80	2.3
***In vitro* digestibility**									
IVNDFD[b]	0.38	0.07	0.24	0.51	18	0.006	0.02	0.86	2.7

SD, standard deviation; MIN, minimum; MAX, maximum; CV, coefficient of variation (%); SECV, standard error of cross-validation; 1 – VR, determination coefficient of cross-validation; RPD, ratio of prediction to deviation; DM, dry matter; aNDFom: NDF assayed with a heat stable amylase and expressed exclusive of residual ash; KL, Klason lignin; ASL, acid soluble lignin; TL, total lignin; IVNDFD, *in vitro* digestibility of neutral detergent fibre.

[a] Residual moisture and crude ash lab error are calculated based on duplo values of 3 stem samples.

[b] Only 83 stem samples were determined for IVNDFD.

Table 3
DM content at harvest (g/kg), chemical composition (g/kg DM) and *in vitro* digestibility of triticale forage harvested at the medium milk (MS1), late milk (MS2), early dough (MS3), soft dough (MS4) and hard dough (MS5) maturity stage in growing season 2016–2017 (n = 36).

Parameters	Maturity stages[a]						
	MS1	MS2	MS3	MS4	MS5	SEM	P-value
n	36	36	36	36	36		
DM content at harvest (g/kg)	286^{e}	303^{d}	499^{c}	559^{b}	625^{a}	10.6	<0.001
Chemical composition (g/kg DM)							
Crude protein	92^{a}	87^{a}	71^{c}	74^{b}	73^{bc}	0.7	<0.001
Starch	39^{d}	81^{c}	270^{b}	284^{a}	295^{a}	8.4	<0.001
Sugars	210^{a}	191^{b}	36^{c}	20^{d}	15^{d}	6.6	<0.001
Crude fat	20^{a}	21^{a}	19^{b}	17^{c}	16^{d}	0.2	<0.001
Crude ash	59^{a}	57^{a}	52^{b}	51^{b}	50^{b}	0.4	<0.001
aNDFom	530^{a}	514^{b}	489^{c}	492^{c}	489^{c}	1.9	<0.001
ADFom	308^{a}	297^{b}	272^{c}	269^{cd}	265^{d}	1.5	<0.001
Lignin (sa)	33^{a}	31^{c}	28^{d}	31^{bc}	32^{ab}	0.2	<0.001
Hemicellulose	221^{ab}	217^{ab}	216^{b}	223^{ab}	224^{a}	0.9	0.008
Cellulose	276^{a}	266^{b}	245^{c}	238^{cd}	233^{d}	1.5	<0.001
***In vitro* digestibility**							
IVNDFD	0.53^{b}	0.55^{a}	0.55^{a}	0.54^{a}	0.54^{a}	0.001	<0.001
IVOMD	0.59^{c}	0.62^{b}	0.67^{a}	0.67^{a}	0.68^{a}	0.003	<0.001

MS, maturity stage; SEM, standard error of the means; DM, dry matter content determined at harvest; aNDFom, NDF assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom, ADF expressed exclusive of residual ash; lignin (sa), lignin determined by solubilisation of cellulose with sulphuric acid; IVNDFD, *in vitro* digestibility of neutral detergent fibre; IVOMD, *in vitro* digestibility of organic matter. Means with different superscript letters in the same row differed at $P < 0.05$.

3.4. Variation among growing seasons and genotypes

The variation in plant IVOMD, plant and stem IVNDFD, DMY and DOMY in the set of 120 triticales, harvested at the soft dough stage, is shown in Table 5 (ANOVA results in Table 4). Overall, the genotype × growing season interaction was significant ($P < 0.001$). Although the significant interaction, it was observed that on average total plant IVOMD and IVNDFD were respectively 0.02 and 0.03 units higher in season 2017–2018 than in 2018–2019. Conversely, DMY and DOMY were considerably higher in 2018–2019. In addition, the variation in IVNDFD for the stem was almost twice as high as for the total plant (CV 12.4% versus 6.6%). The analysis of variance further revealed significant differences among genotypes for all parameters (in 2017–2018: $P < 0.001$, in 2018–2019: $P < 0.001$). Although the mean squares for genotype were much lower than for growing season, stem IVNDFD shows a high breeding potential. Lastly, significant differences ($P < 0.001$) were obtained among DM content at harvest for all parameters, except for stem IVNDFD. Based on the results obtained in Table 4, an experimental correction for year and harvest date effects is needed. This allows to correctly evaluate correlations between yield, chemical and digestibility parameters, without the influence of fixed factors.

3.5. Correlations and linear regression models

The Spearman correlation matrix shows the mutual relationships between DMY, plant IVOMD, plant IVNDFD and stem IVNDFD (Supplementary Figure 1). Regarding DMY, no strong correlations were found with the digestibility parameters. Nevertheless, strong correlations were detected between the digestibility parameters. Plant IVOMD was significantly positively correlated with plant IVNDFD ($\rho = 0.93$) ($P < 0.01$) and with stem IVNDFD ($\rho = 0.72$) ($P < 0.01$) and plant IVNDFD had a positive correlation with stem IVNDFD ($\rho = 0.79$) ($P < 0.01$).

The extent to which underlying chemical parameters explain the digestibility parameters was determined by using linear regression models. Plant IVOMD was highly significantly negatively correlated with ADFom ($\rho = -0.97$), CELL ($\rho = -0.96$), aNDFom ($\rho = -0.94$)

Table 4
Mean squares from the analysis of variance for plant IVOMD, plant IVNDFD, stem IVNDFD, DMY and DOMY of the 120 triticale genotypes evaluated in the 2017–2018 and 2018–2019 growing seasons.

Source	DF	Plant IVOMD	Plant IVNDFD	Stem IVNDFD	DMY	DOMY
Genotype (G)	119	0.0043***	0.0041***	0.0103***	25***	12***
Growing season (GS)	1	0.0674***	0.0703***	0.0069***	17289***	5528***
G × GS	118	0.0006***	0.0005***	0.0011***	17***	6***
Row × GS	10	0.0005	0.0006**	0.0009*	34***	11***
Column × GS	118	0.0004	0.0003**	0.0006	13***	5**
DM content at harvest	1	0.0032**	0.0092***	0.0001	177***	79***
Residual	346	0.0004	0.0002	0.0005	8	3

DF, degrees of freedom; plant IVOMD: *in vitro* organic matter digestibility of the total plant; plant IVNDFD, *in vitro* neutral detergent fibre digestibility of the total plant; stem IVNDFD, *in vitro* neutral detergent fibre of the stem; DMY, dry matter yield; DOMY, digestible organic matter yield (***, significant at the 0.001 probability level; **, significant at the 0.01 probability level; *, significant at the 0.05 probability level).

Table 5
Descriptive statistics of plant IVOMD, plant IVNDFD, stem IVNDFD, DMY and DOMY in growing season 2017–2018, 2018–2019 and both growing seasons averaged.

Parameter	Growing season	Mean	SD	MIN	MAX	CV
Plant IVOMD	2017–2018	0.64	0.03	0.56	0.73	5.2
	2018–2019	0.62	0.03	0.54	0.70	5.2
	overall	0.63	0.03	0.54	0.73	5.4
Plant IVNDFD	2017–2018	0.51	0.03	0.43	0.58	6.0
	2018–2019	0.48	0.03	0.41	0.55	6.4
	overall	0.49	0.03	0.41	0.58	6.6
Stem IVNDFD	2017–2018	0.38	0.05	0.27	0.48	11.8
	2018–2019	0.39	0.05	0.27	0.50	12.8
	overall	0.39	0.05	0.27	0.50	12.4
DMY (t/ha)	2017–2018	16.5	2.5	9.9	23.2	15.4
	2018–2019	26.5	4.6	13.3	38.9	17.6
	overall	21.6	6.3	9.9	38.9	28.9
DOMY (t/ha)	2017–2018	10.1	1.7	5.6	14.1	17.2
	2018–2019	15.7	2.8	8.1	23.0	17.9
	overall	12.9	3.7	5.6	23.0	28.5

SD, standard deviation; MIN, minimum; MAX, maximum; CV, coefficient of variation (%); plant IVOMD: *in vitro* organic matter digestibility of the total plant; plant IVNDFD, *in vitro* neutral detergent fibre digestibility of the total plant; stem IVNDFD, *in vitro* neutral detergent fibre of the stem; DMY, dry matter yield; DOMY, digestible organic matter yield.

and lignin (sa) ($\rho = -0.89$) ($P < 0.01$); and highly significantly positively correlated with plant IVNDFD ($\rho = 0.93$) and STA ($\rho = 0.84$) ($P < 0.01$) (Fig. 2). The linear regression equation containing ADFom best explained the relationship with plant IVOMD ($R^2 = 0.91$, $P < 0.001$, MSE = 0.00006) (Fig. 4 **A.**) (Supplementary Table 4). The graph indicates that an ADFom content of 250 g/kg DM leads to a plant IVOMD of 0.68, while 300 g/kg ADFom decreases it to 0.61. As plant IVNDFD and plant IVOMD were highly correlated, we tried to explain the variation in plant IVNDFD as well. The highest negative correlation coefficient for plant IVNDFD was observed with ADFom ($\rho = -0.89$), followed by CELL ($\rho = -0.88$), lignin (sa) ($\rho = -0.87$) and aNDFom ($\rho = -0.81$) ($P < 0.01$) (Fig. 2). The regression model for ADFom best explained the relationship with plant IVNDFD ($R^2 = 0.81$, $P < 0.001$, MSE = 0.00012) (Supplementary Table 5) and showed that 250 g/kg ADFom results in a plant IVNDFD of 0.55, while 300 g/kg ADFom decreases it to 0.48 (Fig. 4. **B.**). Regarding stem IVNDFD, it was negatively correlated with KL ($\rho = -0.94$ and -0.93) and TL ($\rho = -0.93$) ($P < 0.01$); and positively correlated with ASL ($\rho = 0.87$) ($P < 0.01$) (Fig. 3). KL explained most of the variation in stem IVNDFD ($R^2 = 0.92$, $P < 0.001$, MSE = 0.00013) (Supplementary Table 6). A linear regression curve was added to the graph (Fig. 4. **C.**) which indicates that a KL content of 120 g/kg aNDFom results in a stem IVNDFD of 0.41, while 130 g/kg KL reduces stem IVNDFD to 0.36.

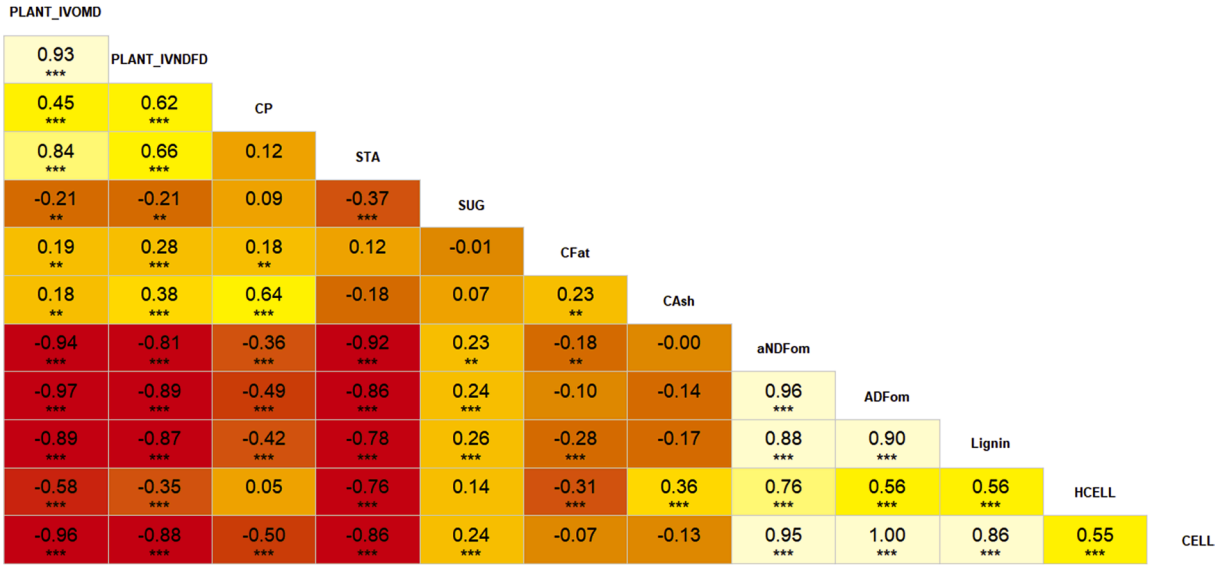

Fig. 2. Spearman correlation matrix for triticale total plant samples: *in vitro* organic matter digestibility of the total plant (plant IVOMD), *in vitro* neutral detergent fibre digestibility of the total plant (plant IVNDFD), CP (crude protein), STA (starch), SUG (sugars), CFat (crude fat), CAsh (crude ash), aNDFom (NDF assayed with a heat stable amylase and expressed exclusive of residual ash), ADFom (ADF expressed exclusive of residual ash), lignin (sa) (lignin determined by solubilisation of cellulose with sulphuric acid), HCELL (hemicellulose) and CELL (cellulose), using genetic values averaged over growing seasons 2017–2018 and 2018–2019. Significant correlations were indicated by stars ($P < 0.01$,***; $0.01 < P < 0.05$, **).

Fig. 3. Spearman correlation matrix for triticale stem samples: *in vitro* neutral detergent fibre digestibility of the stem (stem IVNDFD), CAsh (crude ash), aNDFom (NDF assayed with a heat stable amylase and expressed exclusive of residual ash), KL (Klason lignin), ASL (acid soluble lignin) and TL (total lignin), using genetic values averaged over growing seasons 2017–2018 and 2018–2019. Significant correlations were indicated by stars ($P < 0.01$,***).

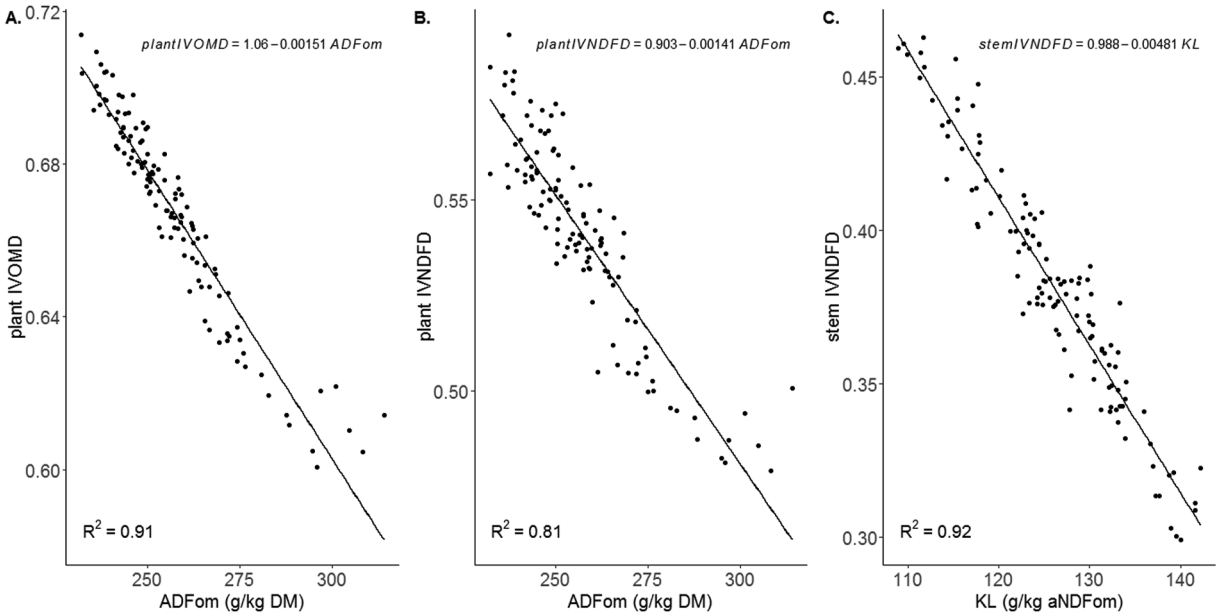

Fig. 4. A. Relationship between ADFom (g/kg DM) and plant IVOMD: plant IVOMD = 1.06–0.00151 ADFom [$R^2 = 0.91$, $P < 0.001$, MSE = 0.00006] B. Relationship between ADFom (g/kg DM) and plant IVNDFD: plant IVNDFD = 0.903–0.00141 ADFom [$R^2 = 0.81$, $P < 0.001$, MSE = 0.00012] C. Relationship between KL (g/kg aNDFom) and stem IVNDFD: stem IVNDFD = 0.988–0.00481 KL [$R^2 = 0.92$, $P < 0.001$, MSE = 0.00013] ADFom, ADF expressed exclusive of residual ash; Plant IVOMD, *in vitro* digestibility of organic matter for total plant samples; Plant IVNDFD, *in vitro* digestibility of neutral detergent fibre for total plant samples; KL, Klason lignin; aNDFom, NDF assayed with a heat stable amylase and expressed exclusive of residual ash; stem IVNDFD, *in vitro* digestibility of neutral detergent fibre for stem samples.

4. Discussion

4.1. Variation in chemical composition and digestibility among plant fractions and performance of NIRS calibrations

In this study, cross-validation results indicated good predictive NIRS performance for total plant residual moisture, STA, SUG and stem CAsh and aNDFom (RPD $\geq$3.0). Good screening is possible for total plant CAsh, ADFom, IVOMD, IVNDFD and stem TL and IVNDFD, showing an RPD between 2.0 and 3.0. Unfortunately, the NIRS calibrations for CP, CFat and lignin (sa) performed less well ($1.5 \leq$ RPD <2.0). Their low RPD values could be explained by the small variation in the total plant calibration set. In addition, the total plant triticale samples in our study were analysed in one replicate per sample for CP, CFat and lignin (sa) what resulted in high lab

errors. In future studies, the enlargement of the calibration set with more diverse triticale samples from other origins and grown under different field practises will probably enhance the NIRS calibrations by increasing the reference values range [25]. The unreliable calibration for stem residual moisture (RPD = 1.3) can be attributed to the re-absorption of moisture from the environment between scanning and reference analysis. Several authors [45,46] previously reported the loss of accuracy of NIRS calibrations due to changes in sample moisture content, even when dried samples had been stored in airtight containers. NIRS analysis immediately followed by reference analysis will not only enhance stem residual moisture prediction but general performance as well. NIRS can accurately predict organic macro-components that are composed of combinations of molecular O–H, C–H, C–O and N–H bonds [47]. Lignin consists of complicated phenolic polymers what explains the bad performance of the ASL calibration (RPD = 1.4) [48]. Another concern is that the 1-VR values for total plant CP, CFat and lignin (sa); and stem residual moisture and ASL are too low for future estimations.

In forage research NIRS has been most commonly used to analyse the protein and fibre content. Reports by Bruno-Soares et al. [49]; Dale et al. [50]; Foskolos et al. [51] and Lyu et al. [52] demonstrated that the NIRS prediction of CP, aNDFom, ADFom and lignin (sa) in cereal, grass, maize and sorghum forages was useful (2.0 $\leq$ RPD <3.0) to good (RPD $\geq$3.0). This is not consistent with the poor cross-validation results we obtained (1.5 $\leq$ RPD $\leq$2.0). Little is known about the development of NIRS calibrations to predict the *in vitro* digestibility of triticale forage. For IVOMD, CRA-W Gembloux, Belgium developed NIRS equations for grass-hay, tropical, maize and (mixed) grass forages [50,53,54]. RPD values were almost twice as high as the values obtained in the present study. Anyway, in the cited studies, the incorporation of forage samples from different plant species probably explains the larger range in the calibration set characteristics. More variability in chemical and digestibility parameters within the calibration set will consequently lead to more robust NIRS calibrations [25]. NIRS is also used as screening tool for *in vitro* fibre digestibility for 30 h (IVFD-30) on triticale forage lines in the breeding program at the Field Crop Development Centre in Lacombe, Canada [55], but NIRS statistics were not reported.

The uniqueness of this study is the development of useful NIRS calibrations for aNDFom, TL and IVNDFD in triticale stem samples (2.0 $\leq$ RPD $\leq$3), which enabled us to screen for variability on stem level. Van Parijs [24] previously demonstrated that NIRS is an appropriate screening tool to predict aNDFom and TL in ryegrass stems, which confirms our findings. NIRS calibrations in maize forage were already developed for IVNDFD [23,56]. But, to our knowledge, no prior research examined the NIRS potential to predict IVNDFD of triticale stems.

4.2. Variation among maturity stages

As triticale forage is mostly conserved as silage in our regions, DM content at harvest is crucial. According to literature data, DM content of triticale forage is variable between 350 and 416 g/kg for MS3 and 329–380 g/kg for MS4 (Table 6). While these values are lower than the ones obtained in this study, the different maturity stages could be clearly separated on DM basis. DM content has been shown to increase rapidly one to three weeks before MS4 [13]. Similarly, our results showed a fast increase in DM content starting from MS3. It is widely known that DM accumulation becomes much more rapid with relatively high temperatures [16]. The weather data for growing season 2016–2017 indeed proved a drier and warmer June compared to the climatic normals, which can explain the higher obtained DM contents. Although a DM concentration between 300 and 400 g/kg is recommended to minimize oxidative losses of silages [57], nutritional value is not optimal in this DM range. STA accumulation during the dough stages corroborates the dilution effect of CP, SUG and fibre concentrations, which results in a general enhancement of potential feeding value from milk to dough stages.

Contrary to literature findings considering early and soft dough triticale forage, our study revealed lower CP, SUG and fibre contents while STA content was higher (Table 6). Concerning *in vitro* digestibility, lower IVOMD coefficients were demonstrated for triticale forage in MS3 (0.59–0.61) and in MS4 (0.63–0.64) [3,19,58]. Our results also differ from Lyu et al. [52] who reported IVNDFD coefficients of triticale forage around 0.40. Differences in chemical composition and *in vitro* digestibility between forage studies may be related to several factors, e.g. growing season, soil type, the triticale genotypes in study, DM range within the harvest stage and used reference method.

To compete with other forages, triticale should be harvested at the stage where fibre content is lowest and CP, STA, SUG contents

Table 6
DM content (g/kg) and chemical composition (g/kg DM) of triticale forage harvested at the early dough and soft dough maturity stage.

Dry matter (g/kg)	Crude protein (g/kg DM)	Starch (g/kg DM)	Sugars (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	ADL (g/kg/DM)	Growing season	Number of triticale genotypes in the study	Reference
Early dough stage (MS3)									
416	124	NA	NA	540	326	41	1990	1	[6]
370	72	NA	NA	576	346	47	1993–1994	9	[16]
350	83	157	178	447	NA	NA	2001–2002, 2002–2003	1	[14]
Soft dough stage (MS4)									
329	73	NA	NA	NA	NA	NA	1999–2000	12	[3]
380	85	NA	NA	605	328	40	2003–2004, 2004–2005	29	[17]
354	59	NA	NA	565	345	45	2015–2016	1	[18]
360	57	NA	NA	595	369	41	2016–2017	1	[18]

NA, not available.

and *in vitro* digestibility are highest. Our results clearly showed significant differences ($P < 0.05$) for DM content at harvest and STA between the dough ripe stages. In contrast, no further increase in *in vitro* digestibility was observed from MS3 onwards. This can be explained by the high proportion of aNDFom compared with STA + SUG content. CP and SUG are two important chemical attributes of cereal forages for silage production [59]. Nevertheless, triticale forage is low in CP. Its content was found to drop in MS3 and tended to stabilise in the later stages. In cereal forages, this CP decline has been reported previously and is caused by ear development during advancing maturity [60]. SUG are other crucial components because it is used by lactic acid bacteria for the spontaneous acidification in ensiled feed [61]. But, the concentration of SUG was shown to further decrease ($P < 0.05$) within the dough stages due to starch accumulation in the ear. Furthermore, close to complete starch digestion from unprocessed grains in wheat forages harvested up to MS4 and with DM contents up to 510 g/kg is shown in cattle [62]. MS5 or later, with DM contents above 650 g/kg, may give poor starch digestibility. Compared to MS5, cereal forages harvested in MS4 will also show better ensilability characteristics and better aerobic stability due to a lower DM content [59]. Therefore, MS4 (the soft dough maturity stage) is considered as the optimal harvest stage for triticale forage. At this MS, further screening on variation in growing season and genotypes was performed.

4.3. Variation among growing seasons and genotypes

The analysis of variance identified a significant interaction between growing season and genotype for *in vitro* digestibility and DMY. This was also shown for DMY in soft dough triticale by Bilgili et al. [22]. The effect of growing season on DMY of triticale forage has also been proven. Lekgari et al. [17]; Bilgili et al. [22] and Coblentz et al. [18] previously reported about varying weather conditions across growing seasons which resulted in significant DMY differences for soft dough triticale forage. Gathered GDD and precipitation data revealed a remarkably warmer and wetter June during 2018–2019 compared to 2017–2018. More biomass accumulation in 2018–2019 leads to higher plant lengths (data not reported) and consequently higher DMY and DOMY. The variance analysis further showed that the digestibility parameters were influenced by growing season effects as well. Indeed, it is widely known that the nutritional value of forages is dependent largely on seasonal factors such as temperature, light and rainfall trends [63]. In addition, the mean squares in our study indicated that growing season was relatively important compared to genotype. Based on these findings, a correction for growing season effect in the linear mixed models seems appropriate.

On the other hand, a smaller, but still significant influence of the triticale genotype was proved for DMY and plant IVOMD ($P < 0.001$). Similarly, Bilgili et al. [22] identified significant differences in DMY and Baron et al. [58] observed large differences in DMY and IVOMD between triticale lines harvested in the soft dough stage. Although triticale forage was harvested when each genotype reached the soft dough stage, DM content at harvest is added in the linear mixed models as fixed factor to correct for harvest date effects. Haesaert et al. [3] reported a high correlation between DM content at harvest and plant IVOMD ($r = 0.755$) which confirms the necessary correction for DM content.

4.4. Correlations and linear regression models

Similarly as reported by Haesaert et al. [3] for late soft dough triticale, our study revealed no significant correlation between DMY and plant IVOMD. As DMY and digestibility are not linked in the soft dough stage, it is possible to screen triticale genotypes on either DMY or either digestibility. Nevertheless, to discover improved triticale varieties suitable as forage crop both DMY and digestibility should be taken into account.

A main strength of the current work is the detection of underlying chemical parameters that greatly influence *in vitro* digestibility of soft dough triticale forage. Our results revealed that plant IVOMD and both plant and stem IVNDFD are heavily negatively influenced by the cell wall components ADFom and KL. De Boever et al. [64] demonstrated the same link between ADFom and IVOMD of maize silage and van Parijs [24] showed the negative correlation between KL and IVNDFD of ryegrass stems. Future investigations are needed to unravel the plant characteristics and genetic mechanisms of triticales low in ADFom content. Likewise, ADFom has been used as a screening criterion for triticale forage digestibility in the FCDC breeding program, Canada [55,58]. Our dataset showed considerable variation in IVNDFD of the triticale stem too, what makes breeding for this trait possible. In addition, KL can be used as screening criterion in order to improve the stem IVNDFD of triticale forage.

5. Conclusions

This research indicated the variation in potential feeding value of triticale forage among total plant and stem fractions. The cross-validation statistics highlighted the potential of NIRS as screening tool to predict chemical composition and *in vitro* digestibility of triticale forage. Important changes in potential feeding value between the milk and dough maturity stages were revealed. This study also demonstrated the important influence of growing season and genotype on *in vitro* digestibility and forage yield. Interestingly, at the soft dough maturity stage, triticale forage yield and *in vitro* digestibility seem not to be linked. Furthermore, larger variation in neutral detergent fibre digestibility was found for stem compared to total plant. Lastly, it was shown that acid detergent fibre and Klason lignin are the most important parameters that determine *in vitro* digestibility of triticale forage.

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Appendix. ASupplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2022.e12760.

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