



# INFLUENCE OF GENOTYPE, SITE, AND YEAR ON MAIZE NUTRITIVE VALUE – YIELD RELATIONSHIPS\*

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Maize genotype selection represents a practical tool influencing forage yield and quality. The main objective was to investigate the contribution of genotype, site, and year to variability of maize yield and quality in the environment of Central Europe. Totally 63 maize genotypes at 11 sites over a 7-year period were evaluated for dry matter yield (DMY), dry matter content (DM), starch, cob, neutral detergent fibre (NDF), *in situ* digestibility of stover NDF (NDFD), and organic matter (OMD). The genotype showed the highest variability from all factors where stover NDFD varied from 261 to 529 g kg<sup>-1</sup> and stover OMD from 376 to 609 g kg<sup>-1</sup>. In contrast to the whole-plant, variability of stover traits was more closely related to NDF than the DM content. Under standardized plant DM, all tested factors were significant and allowed interpretation of 70 and 60% of total variation of yield and quality for stover and whole plant, respectively. The average contributions of genotype, site, and year were 30, 7, and 5%, respectively. For variability in plant productivity and nutritive value, the importance of maize genotype selection was more than two times higher than the contribution of environment.

breeding, hybrid, digestibility, multivariate method



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## INTRODUCTION

Maize is one of the most important forage sources for livestock. Consequently, maize nutritional value has been continually investigated, mainly in studies related to genotype selection (Loučka et al., 2015b), fertilizing efficiency (Liang et al., 2015), timing of harvesting (Lynch et al., 2012) or weather conditions (Krusé et al., 2008).

Maize forage quality can be evaluated through many nutritive traits including starch, neutral detergent fibre (NDF), and other cell wall components (Krusé et al., 2008). However, digestibility of organic nutrients is generally considered as the most important factor, and therefore is evaluated frequently in many studies (Lynch et al., 2012; Masoero et al., 2013). Due to the strong relationship between maize morphology and quality, changes in proportions between cob and

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stover in the whole maize plant significantly influenced silage digestibility and conservation characteristic (Lynch et al., 2012). While starch content (driven by cob weight ratio) is fixed, organic matter digestibility (OMD) was found closely related to neutral detergent fibre digestibility (NDFD) as was reported by Barrère et al. (2004). Genetic progress in maize feeding value was directly related to NDFD improvement (Givens, Deville, 2001). Stover contains crucial amount of plant NDF; therefore its yield and nutritional quality is an important factor influencing whole plant yield as well as nutritional quality (Wolf et al., 1993). Moreover, maize stover left after grain harvest constitutes an important source for livestock feed in some parts of Africa (Ertiro et al., 2013) or China (Liang et al., 2015) which also highlights the importance of separate evaluation of maize stover quality.

Little attention has been paid to quantifying the contribution of genotypes, site factor, and seasonal variation to maize quality. Most studies focusing on maize genotype quality were conducted in a single location and/or season (Krusé et al., 2008; Masoero et al., 2013). The genotype and external factor contribution should be assessed based on studies with a large pool of genotypes conducted across different locations and years. The evaluation may include a multivariate analysis to investigate the complexity of relationships among various factors (Hu, Yang, 2013). Prior investigations of maize quality relationships were mainly focused on simple correlation between specific quality parameters (Barrère et al., 2004; Ertiro et al., 2013); however, simple regression approach gives no causative reasoning to these complex processes. Regarding the year effect and silage nutritive value, Loučka et al. (2015a) revealed that the year contribution to trait variability effect was significant and as much as two times higher for fermentation traits (12.6%) than for silage nutritive value (6.1%). According to Krusé et al. (2008), the use of an appropriate model can facilitate quantification of environmental impacts on maize nutritive value in relation to weather changes (one-site study). It documents a lower but significant effect of environment on maize quality; however the contribution of maize genotype selection on variability in forage quality in relation to environment has not yet been quantified. The main objective of our research was therefore to investigate data from seven-year field experiments in Central Europe by multivariate analysis to assess the contribution of dry matter, harvesting year, site specifics, and genotypes to nutritive quality value of maize stover and whole plant. A secondary aim was to investigate the relationships between stover and whole plant nutritive quality. These results help better understand the importance of suitable maize hybrid selection and evaluation regarding to plant productivity and nutritive value.

## MATERIAL AND METHODS

### Field experiments

In 2006–2012, the yield and quality of 59 maize hybrids (registered from 1991 to 2012) and 4 cultivar candidates were evaluated at 11 sites in the Czech Republic. In this large experiment, numbers of genotypes and sites varied naturally among years which resulted in an unbalanced design. Silage maize was grown on commercial farms in pilot plant experiments with three replicates in a block design. Each plot was represented by 8 rows with row spacing 70 cm and length of at least 20 m. These farms used the following baseline technological parameters: crop sowing from late April until mid-May, pre-emergent or early post-emergent weed control, no irrigation and mineral N-fertilization at 150 kg N ha<sup>-1</sup>. The number of genotypes and sites, with range of maturity according to FAO, and altitude of sites for specific years are summarized in Table 1.

Harvest proceeded in silage maturity with one-half to two-third milk line with respect to year, site, and specific genotype. At each treatment, 10 consecutive plants in random section were hand-clipped with a stubble height of 10 cm in three replicates. Weight of whole-plants and plant parts (stover and cob) were determined for each sample. Stover and cob were chopped separately and dry matter content (DM) was collected from 2 kg fresh sample of each plant part under 55 ± 5°C. Consequently, the average yield per plant (DMY, g) and cob ratio (g kg<sup>-1</sup>, without husk) were expressed based on DM.

### Forage analyses

Dried material was subsequently milled to pass through a 1-mm sieve for laboratory analyses. In the cob, the starch content was measured by Ewer's polarimetric method. In the stover, content of NDF (S NDF, g kg<sup>-1</sup>) was determined according to AOAC Official Method 973.18 (AOAC, 2005). The stover digestibilities of NDF (S NDFD, g kg<sup>-1</sup>) and OMD (S OMD, g kg<sup>-1</sup>) were assessed by *in situ* incubation in cow's rumen during 24 h. In the cob, the contents of NDF (283 g kg<sup>-1</sup>), NDFD (700 g kg<sup>-1</sup>), and OMD (779 g kg<sup>-1</sup>) were considered to be constant values. The whole-plant digestibility of NDF (P NDFD, g kg<sup>-1</sup>) and OMD (P OMD, g kg<sup>-1</sup>) were calculated from DMY, cob ratio, and digestibility of both cob and stover. The weight of stover or plant NDFD (wNDFD) and OMD (wOMD) was calculated from stover or plant weight, NDFD and OMD, respectively.

### Statistical analyses

The influence of year, site, and genotype on yield and quality was evaluated by the main effect ANOVA

Table 1. Number (*n*) of observations, sites, genotypes and their descriptions within evaluated years

Year	Observation <i>n</i>	Site <i>n</i>	AMSL (m)	Genotype <i>n</i>	FAO
2006	123	7	180–600	35	220–400
2007	114	7	180–600	33	220–400
2008	42	2	240–600	14	220–400
2009	63	5	180–600	18	130–370
2010	42	4	240–440	12	245–360
2011	21	1	440	7	130–260
2012	24	3	240–440	8	235–340

AMSL = above mean sea level, FAO = values of maturity classes of maize genotypes

due to the unbalanced design. These statistical procedures were performed using STATISTICA 9.1 (StatSoft, Tulsa, USA). Redundancy Analysis (RDA) was applied to four main analyses ( $A_{1-4}$ ) for variance partitioning procedure (ter Braak, Šmilauer, 2002) with the assessment of variability proportion of the tested variables which could be explained by explanatory variables. Covariates can be used to exclude some effects by standardizing them to average value. Parameters included as stover or whole-plant yield and quality are shown in Fig. 1. Standardization by parameters (dependent variables) was used because the analyzed data were of various types and units. The statistical significance of the first and all of the other constrained canonical axes was determined by the Monte Carlo permutation test (199 permutations). All ordination analyses were performed in the CANOCO program (ter Braak, Šmilauer, 2002). An ordination diagram was created in CanoDraw for graphical visualization of the results.

## RESULTS

The variability of tested variables as well as results yielded by the main effect ANOVA are summarized separately for stover and whole plant in Table 2 and Table 3, respectively. The tested factors significantly influenced all variables of stover and whole plant traits. Among 63 genotypes in this study, the plant NDF, NDFD, and OMD varied from 387 to 509, 402 to 591, and 615 to 708 g kg<sup>-1</sup>, respectively. These variables varied more in stover than in whole plant. For year, the range of minimum and maximum of the mean was the lowest for most traits in comparison with the effect of site or genotype.

The multivariate analyses ( $A_1$ , Table 4) investigated the contribution of DM to stover or whole-plant yield and quality factors within ranges in Tables 2, 3. DM significantly influenced both stover and whole-plant traits, however this contribution was considerably lower for stover (3.7%) in comparison with the whole-plant (13.5%). The obvious effect of DM was excluded from following analyses as a covariate which is hereinafter referred to as standardized DM.

In the second analyses ( $A_2$ , Table 4), the year, site, and genotype explained 70.5% of variability of stover yield and quality (all canonical axes). After separation of individual factors, variation in genotype, site, and year explained 31.0, 8.2, and 4.7% of variability whilst 26.6% represented an overlap of their respective influence. All of these analyses were statistically significant.

In contrast to  $A_2$ , the year, site, and genotype explained 59.5% of variability of whole-plant yield and quality ( $A_3$ , Table 4). In this case, genotype, site, and year explained 28.4, 5.6, and 5.1% of variability whilst 20.4% represented an overlap of their respective influences. Similarly to  $A_2$ , all these analyses were statistically significant.

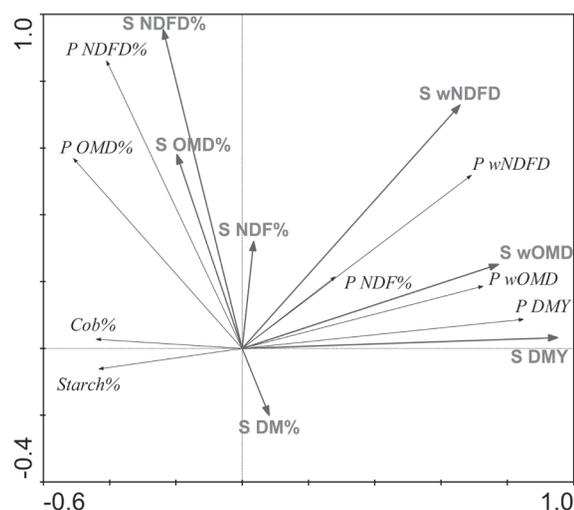


Fig. 1. Ordination biplot showing relation between stover (S, explanatory variables, arrows in bold) and whole plant yield and quality (P, dependent variables) of maize (63.6% of variability explained by all canonical axes;  $P = 0.005$ ; 199 permutations)

NDF = neutral detergent fibre, NDFD = *in situ* NDF digestibility, wNDFD = weight of digestible NDF, OMD = *in situ* organic matter digestibility, wOMD = weight of OMD per whole plant, Starch = content of starch, Cob = weight of cob per plant, DM = dry matter, DMY = dry matter yield per plant

Table 2. Mean values, variability of evaluated variables and their ranges within evaluated years, sites, and genotypes for stover yield and qualitative traits ( $n = 429$ )

		DM (g kg <sup>-1</sup> )	DMY (g)	NDF (g kg <sup>-1</sup> )	NDFD (g kg <sup>-1</sup> )	OMD (g kg <sup>-1</sup> )	wOMD (g per plant)
Mean		229	1030	653	388	490	431
SD		29	274	64	75	57	129
CV (%)		12.7	26.6	9.8	19.3	11.6	29.9
Year	min	208	902	619	346	456	377
	max	238	1162	732	489	541	534
	<i>P</i>	< 0.01	< 0.001	< 0.01	< 0.01	< 0.01	< 0.01
Site	min	198	678	578	351	443	265
	max	268	1319	749	476	562	609
	<i>P</i>	< 0.01	< 0.001	< 0.01	< 0.01	< 0.01	< 0.01
Genotype	min	186	521	524	261	376	211
	max	289	1795	843	529	609	747
	<i>P</i>	< 0.01	< 0.001	< 0.01	< 0.01	< 0.01	< 0.01

SD = standard deviation, CV = coefficient of variation, DM = dry matter content, DMY = dry matter yield, NDF = content of neutral detergent fibre, NDFD = *in situ* NDF digestibility, OMD = *in situ* organic matter digestibility, wOMD = weight of OMD per plant stover; min, max = minimum and maximum of the mean

Table 3. Mean values, variability of evaluated variables, and their ranges within evaluated years, sites, and genotypes for whole plant yield and qualitative traits ( $n = 429$ )

		DM (g kg <sup>-1</sup> )	DMY (g)	Cob (g kg <sup>-1</sup> )	Starch (g kg <sup>-1</sup> )	NDF (g kg <sup>-1</sup> )	NDFD (g kg <sup>-1</sup> )	OMD (g kg <sup>-1</sup> )	wOMD (g)
Mean		346	2393	570	323	441	503	660	1381
SD		42	523	62	42	38	50	25	304
CV (%)		12.1	21.9	10.9	13.0	8.6	9.9	3.8	22.0
Year	min	318	2307	520	260	429	462	639	1322
	max	353	2550	624	352	470	572	679	1516
	<i>P</i>	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Site	min	286	1897	471	279	405	462	641	1067
	max	423	2727	673	404	479	555	687	1595
	<i>P</i>	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Genotype	min	270	1327	440	220	387	402	615	776
	max	419	3635	658	375	509	591	708	2036
	<i>P</i>	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

SD = standard deviation, CV = coefficient of variation, DM = dry matter content, NDF = content of neutral detergent fibre, NDFD = *in situ* NDF digestibility, OMD = *in situ* organic matter digestibility, wOMD = weight of OMD per whole plant, Starch = content of starch, Cob = weight of cob per plant, DMY = dry matter yield per plant; min, max = minimum and maximum of the mean

In  $A_4$ , the contribution of stover to whole plant yield and quality was investigated. This contribution was significant and represented 63.6% of variability of whole-plant yield and quality. The relations among the evaluated traits are shown by an ordination biplot in Fig. 1. The most important first canonical axis (horizontal) represents the forage yield, the second axis (vertical) represents mainly the forage digestibility. When the effect of DM was excluded, stover explained 57.4% of variability.

## DISCUSSION

In respect to large data set ( $n = 429$ ) of 63 maize genotypes on 11 sites over a 7-year period, results show significant effects of genotype, site, and year for all evaluated traits. Obtained ranges of plant NDF and OMD were comparable with those in the studies by Barrère et al. (2004) and Lynch et al. (2012), respectively. In spite of later harvest, Ertiro et al. (2013) reported ranges for stover *in vitro* OMD from

Table 4. Results of redundancy analyses (A) investigating the effect of explanatory variables on stover and whole-plant yield and quality\*

A	Tested variables	Explanatory variables	Covariate	% ax. 1 (all) <sup>1</sup>	F 1 (all) <sup>2</sup>	P 1 (all) <sup>3</sup>
A <sub>1</sub>	stover yield and quality	S DM	–	3.7	16.3	0.005
	plant yield and quality	P DM	–	13.5	66.5	0.005
A <sub>2</sub>	stover yield and quality	Y, S, G	S DM	32.4 (70.5)	176.6 (12.2)	0.005 (0.005)
		Y	S DM, S, G	2.4 (4.7)	30.2 (10.7)	0.005 (0.005)
		S	S DM, Y, G	5.5 (8.2)	67.0 (11.1)	0.005 (0.005)
		G	S.DM, Y, S	16.0 (31.0)	136.2 (6.8)	0.005 (0.005)
A <sub>3</sub>	plant yield and quality	Y, S, G	P DM	25.8 (59.5)	148.5 (9.8)	0.005 (0.005)
		Y	P DM, S, G	2.4 (5.1)	23.3 (9.1)	0.005 (0.005)
		S	P DM, Y, G	2.7 (5.6)	30.9 (12.0)	0.005 (0.005)
		G	P.DM, Y, S	14.6 (28.4)	97.6 (4.6)	0.005 (0.005)
A <sub>4</sub>	plant yield and quality	stover yield and quality, S DM	–	32.7 (63.6)	204.5 (105.2)	0.005 (0.005)
		stover yield and quality	P DM, S DM	32.1 (57.4)	328.5 (257.0)	0.005 (0.005)

S DM = dry matter content in stover, P DM = DM in whole-plant, Y = year, S = site, G = genotype

<sup>1</sup>variability of maize yield and quality explained by canonical axis 1 or by all axes in brackets

<sup>2</sup>F-statistics for the test of axis 1 or all axes in brackets

<sup>3</sup>corresponding probability value obtained by the Monte Carlo permutation test (199 permutations) for the test of axis 1 or all axes in brackets

\*traits included as stover or plant yield and quality are shown in Tables 2 and 3, respectively

605 to 704 g kg<sup>-1</sup>, which was a higher mean than *in situ* values from 376 to 609 g kg<sup>-1</sup> in the present experiment. This highlights the impact of various methods of digestibility assessment. From this reason, the higher emphasis should be given to relative comparisons than to absolute values. Regarding NDFD, the variability among tested genotypes was large with a higher range for stover in contrast to the whole plant. Variation indicators were lower for OMD in comparison with NDFD which is consistent with our findings for both stover and whole plant (see coefficient of variation in Tables 2, 3). Barrière et al. (2004) explained this by the diluting effect of the grain, which is more digestible than rest of the plant. This may be the reason for results obtained by Lynch et al. (2012) who reported significant differences between 6 hybrids for stover OMD but not for the plant OMD.

#### Maize maturity

Maturing of maize is generally accompanied by the increase of DM (Kruse et al., 2008), therefore changes in DM content were significantly related to yield and maize quality (A<sub>1</sub>, Table 4). The effect of stover DM was significant; however it explained only 3.7% of variability of both the stover yield and quality in contrast to 13.5% for plant DM vs plant yield and quality. The DM was significantly increased in both stover and whole-plant over the harvest time, however, these changes were more apparent in whole plant (Lynch et al., 2012), which corresponds with a lower range of stover DM vs plant DM. This lower stover DM range

may be one of the reasons for the lower contribution of stover DM to stover yield and quality. In accordance to Givens, Deville (2001), *in vivo* NDFD was more dependent on NDF than on DM, giving rise to the possibility that NDF is a better index of maturity than DM. When the stover or plant NDF was used as an explanatory variable for stover or plant yield and quality, the explained variabilities were 12.7 and 6.3%, respectively (data not shown). This result reveals that the contribution of stover NDF to variability in the stover yield and quality was three times higher that of DM, therefore it could be proposed as better predictor for stover maturity. Boon et al. (2012) also considered cell wall components (expressed as g per kg NDF) as a more appropriate maturity indicator than the contents based on dry matter. In spite of it, this relationship with NDF was not observed for the whole plant in our study.

#### Contribution of genotypes and external factors

Under standardized DM, the average contribution of genotype towards the total variation of stover or both plant yield and quality was around 30%, of site from 6 to 8%, and of year around 5%. Under presented conditions, the maize genotype was found as a factor with the highest contribution to overall variation in yield and nutritive value, but the design did not allow the evaluation of the genotype × environment interaction. It is in line with a range of studies, where significant differences were observed in forage quality among maize genotypes (Lynch et al., 2012; Ertiro

et al., 2013; Loučka et al., 2015b). Barrière et al. (2004) recorded a significant effect of genotype variation in respect to NDF, NDFD, OMD, and forage yield regardless if 477 or just 27 genotypes were compared. Differences in quality among maize hybrids were more apparent in studies with a large genotype pool (e.g. Ertiro et al., 2013) but a significant effect of genotype on forage quality is often reported in one or few-site experiments with lower number of hybrids, both for the stem (Liang et al., 2015), and whole plant qualitative traits (Lynch et al., 2012; Loučka et al., 2015b).

On the other hand, the differences in forage quality among maize genotypes did not always occur in field experiments. In spite of contrast hybrid selection, Darby, Lauer (2002) described no important differences in forage yield and quality for 5 hybrids (two sites, two years). In the study of Kruse et al. (2008), the genotypes represented the spectra of German silage maize genotypes; however they did not find any impact of genotype on fibre components at silage maturity. These contrast results support the assumption that other factors such as variation of site and/or weather condition could be partly responsible for the ascertained differences among maize genotypes. This is in line with Loučka et al. (2015b) that differences among forage quality of maize genotypes could be diminished by variation between sites and years. Ertiro et al. (2013) also pointed that variation of some maize quality traits depended on the environment. Kruse et al. (2008) reported that variation of fibre content was more strongly influenced by environmental than by genotypic factor. They assumed that the intensity of change in fibre components was strongly associated with temperature and solar radiation. Similarly, Loučka et al. (2015a) reported that explanation of the year contribution could be attributed to differences in temperature and precipitation in individual months. Colder and humid conditions earlier in the maize growing season and warmer and drier conditions later in the season tend to produce higher fibre and lower crude protein contents in silage. They observed the year contribution to variability of maize silage nutritive value about 6% under standardized DM, which is comparable with 5% in the present study. Regarding to water regime, the impact of soil water availability on maize fibre was negligible in the study of Kruse et al. (2008), which was in line with Masoero et al. (2013) who observed no significant impact of irrigation on maize forage digestibility.

In respect to explained variation in  $A_2$  and  $A_3$ , genotype, year, and site (and also maturity) remain the most important factors significantly affecting both yield and maize quality. Maize genotype selection represented practical tool responsible for 30% of variation in yield and quality. In spite of the lower contribution of site and/or weather condition (together about half of the genotype contribution), results clearly show

that external factors were responsible for significant changes in maize forage quality. Contribution of these factors to variability in particular traits should be further investigated in detail.

#### Relationship between plant and stover traits

Stover parameters were able to interpret about 60% of variability for the whole-plant yield and quality, i.e. much more than the stover weight ratio, which emphasizes the importance of stover in accordance with Wolf et al. (1993). It is known that the content of nutrients varies significantly with height of stover (Liang et al., 2015) which corresponds with the negative relationship between stover yield and digestibility in Fig. 1. Contrast to it, the study of Ertiro et al. (2013) indicates weak correlation between stover yield and forage quality. According to Barrière et al. (2004), both plant OMD and NDFD were negatively correlated with the whole-plant yield. This is also in accordance with our results presented in Fig. 1. DMY on the first axis was negatively correlated with plant digestibility as well as with starch and cob ratio. After excluding the DM effect, plant and stover NDFD became independent in respect to the first axis, which presented the relation between DMY or NDF vs starch, cob ratio, and plant OMD (figure not presented). It seems that the discrepancy with Ertiro et al. (2013) can be explained by the differences in DM or NDF content of maize stover, which were not clearly presented in their study. Barrière et al. (2004) also reported that the correlation between NDF and NDFD was close to zero when harvest was at a similar maturity stage, which is in accordance with our findings. Therefore, we conclude that evaluation of maize genotypes in NDFD should be realized only under standardized plant DM or stover NDF content. This kind of independence between NDFD and DMY is supported by the results of Frey et al. (2004) that it is feasible to develop a silage maize hybrid with both high whole-plant yield and excellent quality. Since the negative relations between stover or whole-plant yield and starch were stable across all analyses, the improving of stem NDFD seems to be critical for better maize quality while avoiding the negative effect to DMY.

#### CONCLUSION

The presented results show a large variability in forage quality among maize genotypes cultivated in Central Europe over the last years. Under standardized plant DM, maize genotypes contributed by 30% to variability in plant nutritive value and productivity, in contrast to 5–8% for site or year. Therefore, the selection of suitable maize genotype represents an effective tool for influencing plant productivity and forage quality in the field condition. However, effec-

tive evaluation of maize genotypes should be realized under standardized stover NDF or plant DM content.

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