

5th International Conference "Agriculture for Life, Life for Agriculture"

## Variation of nutritional values in leaves and stalks of different maize genotypes having high protein and high oil during vegetation

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### Abstract

This study was aimed to determine the variations into the nutrient contents found different plant parts of maize genotypes that containing high level of oil and protein at their different vegetative stages. In this study, six different maize genotypes were used as plant materials that have been generated by crossing of two special types of maize namely, IHO and IHP with two normal elite inbred lines (B73 and Mo17). Field trials have been conducted by using randomized complete block design along with three replications in the region of northwest of Turkey. Samplings were designed in accordance to plant sowing date and they were taken from the field at 40th, 60th, 82nd, 100th and 122nd days after sowing. Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), crude ash (CA), organic matter digestibility (OMD) and dry matter digestibility (DMD) have been investigated during this study work. The results of this research showed that the significant differences were found among genotypes and some sorts of significant variations have been observed into the cell wall components. Genotypes, having high level of protein in their stalks, have been found superior in terms of nutrient content while the genotypes, having high level of oil in their leaves, were found best for nutrient values.

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Peer-review under responsibility of the University of Agronomic Sciences and Veterinary Medicine Bucharest

*Keywords:* genotypes; crude protein; crude oil; NDF; ADF.

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## 1. Introduction

Maize, one of the most important crop for feeding the animals. Generally, animal rations contain 15-20% of maize according to energy value of used maize varieties in preparation of rations and animal breed (Emeklier, 2002; Özcan, 2009). Maize is a hot season loving cereal crop, mostly used in silage production due to its higher dry matter yield as compared to other species and having delicious taste and high nutritive value. Maize is mostly used in human nutrition and agricultural-based industry, however, it has become the most important forage produced in agricultural fields as silage used for animal feeding in last 30 years (Allen and Kilkeny, 1986). Currently, maize produced in the world is mostly used as animal feed (65-70%) followed by human nutrition (20-25%) and industrial purposes (8-10%). In recent years, it has been used in biofuel production to compensate for rising oil prices (Özcan, 2009). Maize is ranked as second cereal in the world in terms of plantation area. According to 2014 statistics, maize planted in 4,015,913 acres and 18,563,390 tones maize for silage obtained from this plantation. In recent years, a significant increase in maize growing areas has been observed encouraged by the Ministry of Food, Agriculture and Livestock in Turkey. There is a considerable effort for developing of high yielding maize varieties for silage production (Özata et al., 2012). Maize silage widely used in animal feeding as delicious forage source and its production is also profitable. The major factors influencing the quality of maize silage as forage source are: variety, harvesting time and mechanization techniques in silage production (Keleş and Cibik, 2014). In general, it is possible to provide a delicious and high quality forage, which lovingly renovated by animals, without use additives in maize silage production (Sarıççek et al., 2001). Acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), dry matter digestibility (DMD) and organic matter digestibility (OMD) were considered to determine the nutritive value of forage that affected the silage quality. The feeding values of different maize cultivars were determined by considering those parameters in various studies conducted to investigate the potential use of different maize varieties for silage production (Akdeniz et al., 2004; Özata, 2012). The effect of harvesting time on silage fermentation and quality characteristics were also evaluated by using those parameters in different studies (Filya, 2004; Özdüven et al., 2009; Yıldız et al., 2011). Normal maize cultivars were used almost in all of these studies and measurements/analyses were made on subsequent periods after maize ear development (such as at milk stage and dough etc.). The use of maize genotypes with different characteristics and be set to the sampling period of include early stage of vegetation may provide additional information on the available scientific literature. Maize silage for animal feeding has high energy value, however; its protein and mineral content considerably low for animal feeding requirements (Alçıçek et al., 1997). It is reported that this problem can be solved by using protein-rich legumes and forage crops or adding urea during ensiling of maize. On the other hand, special types of maize can be alternative for solving this problem; because of they are different from the normal genotypes. To our best knowledge, there are limited studies in scientific literature that concluding of using potential of special types of maize in animal feeding. Main examples of special types of maize are high oil maize, high protein maize and opaque type maize genotypes (Lambert, 2001). Those had important advantages in term of their nutritive contents and energy values compared to normal genotypes (Dado, 1999). Determination of using potential and forage values of special types of maize genotypes as forage source can provide significant benefits in practice for animal feeding. Indeed, breeder idea that silage yield and quality depend on the grain yield only began to change, a new idea also that efficient types of grain corn varieties can be used as high-quality silage quality have spread (Dwyer et al., 2006). From this standpoint, investigation of changes in nutritive values of specialty maize genotypes, which are generally bred for grain production, may reveal opportunities for use of these maize types in both grazing and silage production.

This study was conducted to determine that how changes the nutritional values, such as ADF, NDF, ADL, SOM, total protein and total fiber content, in the stalk and leaf samples taken from specialty types of maize lines and hybrids during the vegetation.

## 2. Materials and Methods

Field trails have been arranged by using the randomized complete block design (RCBD) with three replications during the period of two years i.e., 2011 and 2012 in the experimental area of Canakkale Onsekiz Mart University, 'Dardanos Research and Application Center' situated in northwest of Turkey. A total of eight different maize genotypes have been used as experimental materials, which were already developed in 2010 by crossing them with

special maize genotypes namely, IHO and IHP along with two well-known inbred lines (B73 and Mo17), shown in Table 1.

Table 1. List of the plant materials used in study during the years 2011 and 2012.

Parental lines	Crosses
B73 (Male line)	IHO x B73
Mo17 (Male line)	IHO x Mo17
IHO (Female line with high level of oil)	IHP x B73
IHP (Female line with high level of protein)	IHP x Mo17

Sowing practice has been done by using a mechanical drill at 18 May and 13 May during the first and second sowing seasons in both of the years, respectively. Each genotype has been sown in two-rowed plots having 70cm of row to row distance and each row was consisted of the length of 2 m. Drip irrigation has been applied to the plants throughout their growing season. The amount of water supplied to plants was recorded as 422.6 mm/ha and 420.2 mm/ha in first and second year, respectively. Irrigation has been terminated in the last week of August for both of the years. ENTEC®Perfect (14% N, 7% P<sub>2</sub>O<sub>5</sub>, 17% K<sub>2</sub>O, 2% MgO, 9% S, 0.002% B and 0.001% Zn) and ENTEC®26 (26% N and 13% S) were applied in account of 17 kg/ha as fertilizers in the shape of pure nitrogen. The soil of the experimental area was loamy, having low organic matter contents, slightly alkaline and calcareous (pH: 7.82-7.93; E.C: 0.60-0.62 mS/cm; lime: 11.10-13.69%; organic matter: 1.26-1.28%; phosphorus: 3.74-3.82 kg/ha and potassium: 52.41-55.78 kg/ha). Data regarding to the climatic conditions of the experimental area for the growing seasons in 2011–2012 are given in Figure 1. The highest values regarding to the total amount of precipitation have been recorded in the month of December while the lowest were observed in the month of July during both of the years. Monthly mean temperature values of second year (2012) have been noticed slightly higher than that of the first year (2011). However, the similar trend has been continued in both of the years (Figure 1).

Three plants were taken randomly from each plot for the purpose of sampling at the interval of 40, 60, 82, 100 and 122 days after sowing. Leaf and stalk portions of the obtained samples were separated after harvesting. The collected samples were kept under 70 °C for the period of 48 hours aimed to make them dry and then grinded in a laboratory mill (Fritsch pulverisette 14, Germany), finally sieved them by using 0.5 mm sieve. ADF, NFD, ADL, OMD, DMD, total fiber content and total protein content have been determined by using NIR instruments (Spectrastar 2400D, Unity Scientific, USA). For this purpose, grinded samples were put into the sampling cup of instrument and scanned them between the intervals of 1200 nm to 2400 nm. After that, the obtained spectra have been applied to INGOT calibration model (Maize Silage and Forage) and then the predicted values of ADF, NFD, ADL, OMD, DMD, total fiber content and total protein content of the samples have been obtained. Finally, the data have been analyzed in SAS V8 software (SAS Ins., 1999). Variance of analyses were performed according to the model appropriate to randomized complete block design. Tukey multiple comparison test has been applied to compare the differences between mean values.

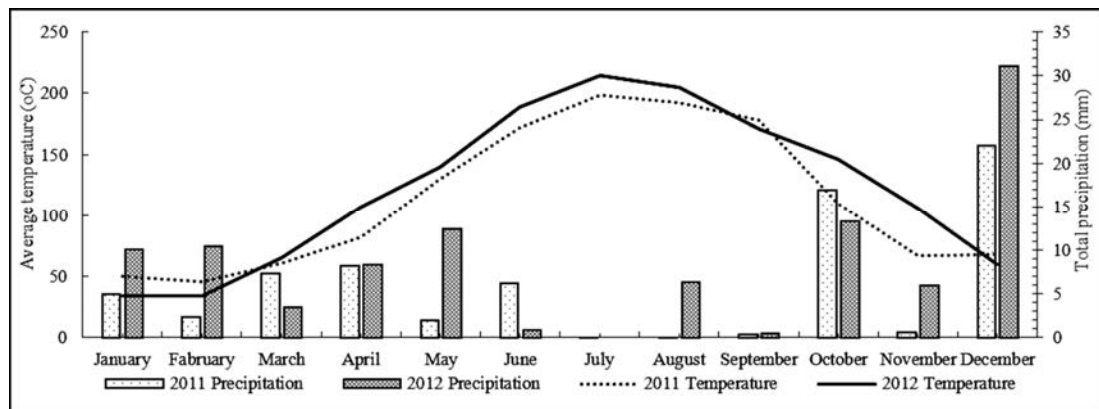


Fig. 1. Mean temperature and precipitation values of the experimental seasons (2011-2012).

### 3. Results and Discussions

Results, regarding to variance of analyses, showed that the main effects i.e., year, genotype and DAS were significantly affected the for all investigated traits. Significant Three way interactions have been found for stalk protein content, stalk ADL, leaf fiber content, leaf ADF and leaf SOM content. In case of leaf traits, except year\*genotype for leaf ADL content, All of the other two way interactions have been significantly affected (Table 1). Our focus was mainly on the main effect of the DAS and interaction effect of DAS x Genotype during this study.

Table 2. Mean squares in variance of analyses by the stalk and leaf parts.

		Stalk							
SV	DF	Protein	Ash	Fiber	NDF	ADF	ADL	DMD	OMD
Rep	4	1.65**	0.29	3.49	7.64	4.01	0.15	6.14	9.39
Year	1	11.6**	9.57**	51.99**	254.71**	139.68**	18.26**	461.51**	581.94**
Genotype	7	12.3**	3.34**	57.98**	74.50**	86.56**	1.58**	111.73**	190.12**
DAS	4	141.7**	66.58**	230.10**	539.62**	499.15**	41.83**	1365.79**	2097.51**
Y x DAS	4	12.5**	3.50**	78.97**	215.22**	126.34**	3.38**	193.97**	406.45**
Y x G	7	0.76	0.49**	7.32**	13.51*	10.69**	0.24*	17.40*	22.12
D x G	28	0.57	0.38**	6.92**	13.02**	9.32**	0.38**	17.95**	22.48**
Y x G x D	28	0.88**	0.168	3.41	6.39	5.56	0.26**	11.43	16.62
Error	156	0.41	0.18	2.52	5.99	3.59	0.09	8.35	11.08
		Leaf							
SV	DF	Protein	Ash	Fiber	NDF	ADF	ADL	DMD	OMD
Rep	4	3.38**	1.33**	4.94**	15.65**	6.79**	0.23**	14.97**	13.90**
Year	1	8.55**	13.02**	6.38**	3.95	32.81**	0.65**	21.40**	235.16**
Genotype	7	7.48**	11.49**	21.02**	38.16**	27.69**	0.81**	62.34**	84.25**
DAS	4	203.8**	39.8**	179.23**	374.61**	222.65**	2.31**	449.32**	525.18**
Y x DAS	4	28.9**	3.65**	60.03**	68.60**	67.19**	0.66**	73.86**	139.01**
Y x G	7	2.34**	0.34	3.92**	15.08**	4.81**	0.08	7.30**	11.15**
D x G	28	1.88**	1.09**	2.11**	5.20**	2.28**	0.10**	5.13**	4.71**
Y x G x D	28	0.69	0.22	1.09*	2.91	1.53**	0.03	2.66	4.13*
Error	156	0.74	0.26	0.66	2.46	0.78	0.04	1.79	2.38

Protein content found in the parts of leaf and stalk was declined by the plant growth. The highest protein content values were observed in DAS40 for leaf (9.93%). The highest percentage of protein in stalk was determined in DAS40 (14.1%) and DAS82 (14.3%) while the lowest values for protein content (stalk: 5.76%, leaf: 9.30%) have been measured in DAS122 as already expected. There was significant variation among genotypes for protein content (Table 1). IHP had higher protein content in its stalk (7.9%) and leaf (12.9%) than that of the others. In general, genotypes having high protein in seeds had also have higher percent of protein in their leaves and stalks (Figure 2). One of the most important factor is the maturity condition of plant in its harvesting time. A reduction in forage quality has been observed with the progress of plant maturation (Cherney, 1990). Maturation causes decreases in protein and its digestibility but increases in crude ash content (Koç, 1991; Bakoğlu et al., 1999). It has been shown in a previous study that the cellulose content increased from 2.6% to 36.1% while the protein content decreased from 30.4% to 6.4% in crested wheatgrass (Gökkuş et al., 1997). Our results were in agreement with the previous findings. Inorganic matter content in stalk has been decreased by the plant growth but it was found with an increase in leaf by the maturation. The highest ash contents were recorded as 8.20% and 10.55% in stalk at DAS40 and in leaf at DAS122, respectively. The lowest ash content has been determined as 5.45% in DAS82 for leaf and 8.27% in DAS60 for stalk. Generally, genotypes having high protein content, had high ash content, too. However, the genotypes already have high level of oil had lower ash content. Alatürk (2012) reported that the inorganic matter content of plants was decreased along with the maturity of plant. The requirement of mineral matter of plants is generally high at their fast growing stages. Most of the minerals were found in the protoplasm where physiological activity is in abundance while less in cell wall (Spears, 1994). With the progress of plant growth, ash content have

decreased due to increasing of the ratio of total organic matter to mineral matters increases depending on cell wall compounds. Gokkus et al. (2012) noticed that the mineral matters, including macro and micro elements, were found high in spring months as compared to other months.

An increase into the fiber content of all genotypes has been observed along with the plant growth. The lowest values of fiber content were observed for leaf (24.5%) and stalk (25.7%) in DAS40 while the highest values have been determined for stalk in DAS122 (30.2%) and for leaf in DAS60 (30.6%). IHPxMo17 cross and B73 parent had the highest fiber content as compared to others. High protein genotypes had higher fiber content in their leaves and stalks than those having high level of oil. NDF content, found in stalk and leaf parts, has been increased in parallel with plant growth. Indeed, the highest value for NDF content was observed in DAS122 both for leaf (61.0%) as well as stalk (62.9%) while the lowest values (stalk: 53.9% and leaf: 54.5%) have been observed for NDF content in DAS40. All genotypes had high NDF values (>50%) along with the vegetation and they reached the highest values in DAS122. NDF content of stalk reached the highest value in DAS122 (34.8%) while the highest value (34.5%) for this trait is observed in DAS60 for leaf part. It was identified that the lowest ADF values (26.2% in stalk and 28.7% in leaf) found in DAS40.

It has been observed that the crosses had higher NDF content as compared to their parental lines. ADL content has been increased along with the growth of plant in all genotypes. ADL content, found in stalk parts, reached the highest value at DAS122 while ADL content of leaf in case of DAS82. Changes in ADL content varied by the genotypes and the lowest value for ADL content has been observed in B73 parental lines. NDF content of genotypes were also increased along with the increase in plant growth. Deniz et al., (2001) mentioned that no significant differences were found in between three different maize varieties, however, the developmental stages i.e.; silking, milky and dough stage, had significant effects on NDF contents of all varieties. This study revealed that NDF value reduced in maize silage that prepared by the samples of post developmental stages, although it has been expected to increase. This case has been explained by the inclusion of the kernels having very low NDF values in post developmental stages of plant (Johnson et al., 1966). The lowest NDF values have been observed at flowering stages in all genotypes. However, our results showed that the high protein genotypes had higher NDF content as compared to those having high levels of oil. Increasing in cell wall compounds has been associated with the presence of mature cells rather than young cells. It was also determined that the special maize parents, along with their crosses, had high ADF values in their stalks. Deniz et al., (2001) observed that the ADF content increased by the progress towards the grain filling period, however, cultivars may have different responses. But in case of our study, ADF content was increased along with the growth of plant, as with most of the cultivated crops. Özdüven et al., (2009) reported that ADL content in different varieties also increased by the progress of plant development. Indeed, it has been described in several reports that the increase in cell wall material depending on the maturity level of plant (Griffin and Jung, 1983; Nelson and Mooser, 1994; Akyıldız, 1966; Johnson et al., 1966; Lyons et al., 1999; Açıkgöz, 2001; Frost et al., 2008). Our results were in consistent with these findings. DMD value reduced in the progress of plant maturation. The lowest as well as the highest values were observed in DAS40 (stalk: 64.7% and leaf: 63.5%) and DAS122 (stalk: 50.5% and leaf: 57.4%), respectively. Crosses of high protein parent had the lower values, however, normal inbred (B73 and Mo17) had higher values as compared to other genotypes. In general, DMD value was found to be high while crosses had lower values than their parents. OMD values of genotypes reduced in course of plant development. The highest OMD values were observed in the time of first sampling (stalk: 69.6% and leaf: 60.9%), but the lowest DMD values have been noticed in the date of last sampling. Normal parents (B73 and Mo17) had higher DMD values as compared to other genotypes.

The DMD values have been found more than 80% in cool seasoned grasses after the first 2–3 weeks as compared to their active growth period. Thereafter, dry matter digestibility reduces nearly with  $\frac{1}{2}$  or  $\frac{1}{3}$  ratio in each day until it reaches below 50%. Reason of this that the amount of cell wall structural components, viz NDF, ADF and ADL, are difficult to digest by the animals which increased in matured plants (Ball et al., 2001). The ratio of digestible organic matter in plants is correlated with cell wall components, but increases in cell wall components are resulted in decreases in the amount of digestible organic matter content (Castle, 1982; Holechek et al., 1989; Huston and Pinchak, 1991; Steen, 1992; Gonzalez-Andres and Ceresuela, 1998; Ventura and ark., 1999; Ventura et al., 2004; Pecetti et al., 2007; Frost et al., 2008; Bouazza et al., 2012; Kökten et al., 2012).

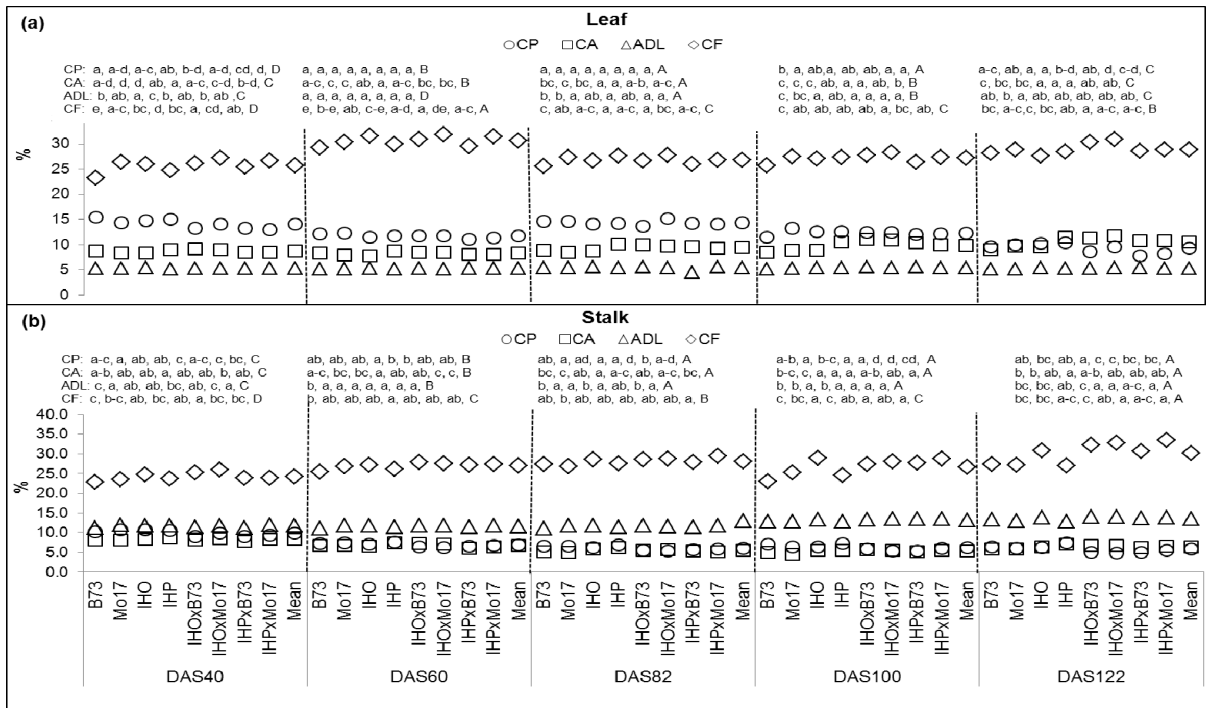


Fig. 2. CP, CA, ADL and CF in leaf (a) and stalk (b) parts of genotypes along with sampling dates. Different small letters show the significant differences between genotypes within DAS. Different upper-case letters indicate significant differences between means of DAS.

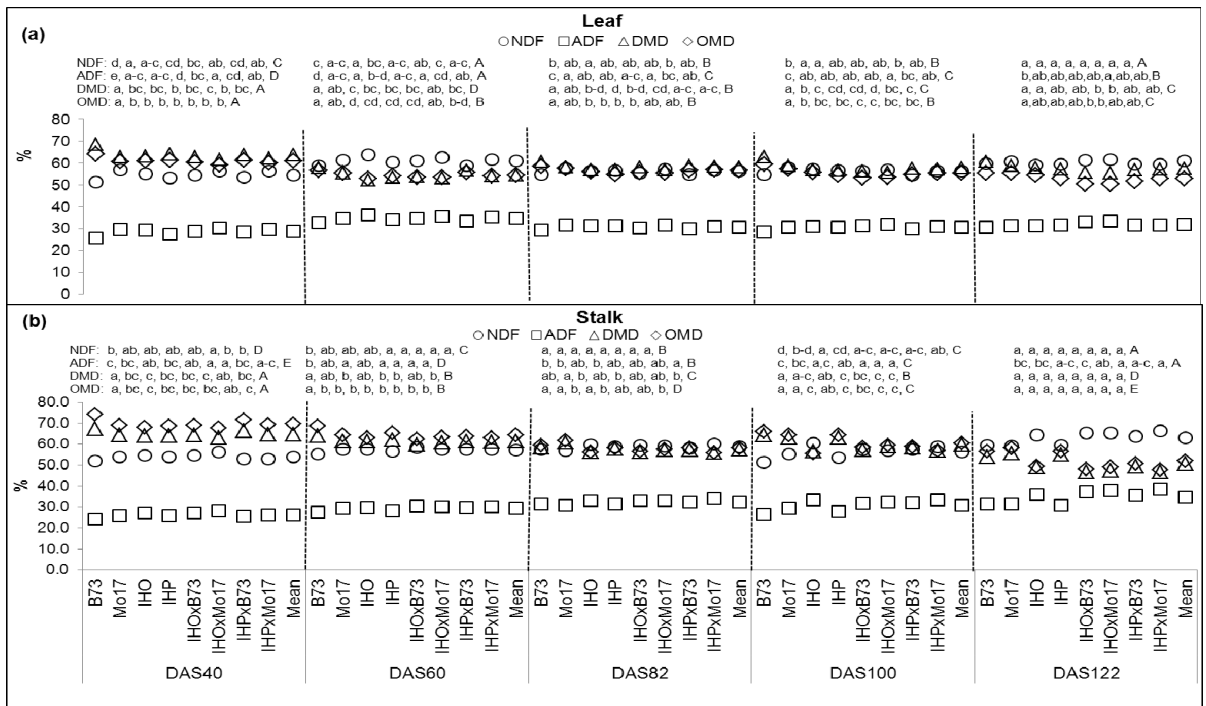


Fig. 3. NDF, ADF, DMD and OMD content in leaf (a) and stalk (b) parts of genotypes along with sampling dates. Different small letters show the significant differences between mean genotypes within DAS. Different upper-case letters indicate significant differences between means of DAS.

#### 4. Conclusions

Results of this study showed that the significant differences were found among genotypes for all investigated traits in all sampling dates. ADF, NDF, ADL and fiber content has been increased along with increasing of plant growth. Crude ash content had increased in stalk parts in accordance to plant age while it was decreased in leaf parts. In case of DMD and OMD contents, they showed a decline along with the growth of plant in both of stalk as well as leaf parts. When considering the sampling times, it has been understood that the blooming time is the most appropriate time for using the genotypes as forage. Finally, it has been identified that the 80<sup>th</sup> and 100<sup>th</sup> day of intervals were the most appropriate dates for this purpose. IHP, B73 and Mo17 were found having the lowest values for stalk fiber, NDF, ADF and ADL contents. Moreover; IHP, B73, Mo17 parents and IHPxMo17 cross have been noticed with their highest values for stalk DMD and OMD contents. Leaf fiber content, NDF, ADF and ADL content were lowest in IHOxB73 and B73 genotypes. Leaf DMD and OMD values were found highest in IHOxB73, IHOxMo17, B73 and Mo17. In the light of this research work, our results revealed that the stalks of high protein genotypes and leaves of high oil ones had higher nutritional values as compared to normal maize genotypes. In conclusion, genotypes used herein had high nutritive value in flowering stage; however, they had moderate level in grain production.

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