Impact of Abiotic Stress on Quality Traits of Maize Forage at Two Growth Stages

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Abstract. Maize (*Zea mays* L.) forage quality traits are reported to show varying responses to abiotic stress. Four trials were conducted in Sudan (Africa) during the summer and winter seasons (2013 – 2014) at two locations: Shambat (normal soils) and Soba (salt affected soils) to investigate the effects of heat, water, and salt stresses on the nutritive value of maize forage. In each trial nine maize genotypes were studied under two watering regimes arranged in split plot experiment in randomized complete block design. The compound effect of salt, water and heat stresses created by the combination of locations, seasons and watering regimes were used to investigate the effect of abiotic stress on forage quality at silk initiation and dough growth stages. Character associations under stressed and none stressed conditions were studied. NDF, ADF, CP, forage yield and related traits were measured. Abiotic stress significantly lowered the nutritive value in terms of crude protein, digestibility and intake potential. Digestibility under stressed condition was slightly improved as growth stage advanced from silk initiation to dough stage. Correlations under non stress conditions between forage yield and quality traits were either favorable with NDF and weak or insignificant with ADF and CP. Under stress conditions, similar trend generally exists apart from the unfavorable correlation of CP with each of yield and ADF, in addition to earliness with NDF. The compound effect of salt, water and heat stresses have adverse impact on the nutritive value of maize forage. Varieties combining high performance in quality and forage yield could be developed under non-stressed or stressed conditions.

Introduction

Maize (*Zea mays* L.) is the World's primary source for animal feed [1]. It is the only crop amongst non-leguminous combining high quantity of biomass along with better nutritional quality. Forage maize has become a major constituent of ruminant rations in recent years, where its inclusion in dairy cow diets improves forage intake and animal performance [2, 3]

Although the crop has wide adaptability [4] it is the least tolerant to abiotic stresses among cereals. Moisture, salinity and elevated temperatures are among the major abiotic stresses that negatively impact maize performance worldwide [5-8]. Salinity effected soils occur in all continents specially in the arid and semi-arid regions compared to humid regions [9]. The total salt-affected soils in Sudan amounts to 4.8 million hectares [10]. Currently, 50% of all irrigation schemes are affected by salinity. The influence of abiotic stress on the quality of forage crops has been reported by many workers [11-13]. Crop growing season and plant developmental stages are other important factors that influence the forage quality [14]. Incidence of stress at any stage may influence the feed and forage digestibility and reduced nutrient intake [15]. However, varying results on how stress affects the feed quality have been reported. While all ozone studies reported reduced digestibility of plant materials grown under stress, most studies dealing with drought reported an enhanced feed value, while mixed or no effects were seen under salinity, and heat stress [16]. Another factor determining forage quality is the growth stage. Intake potential decreases and NDF concentration increases with advancing maturity. Also, the rate at which fiber is digested slows as plants mature [17]. Associations among characters are of interest to the breeder to point out the possibilities and limitations of simultaneous improvement of desirable characters [18] especially those dealing with agronomic and

quality traits. However, such studies were mostly carried under normal conditions with very few ones considering the nature of associations under stress conditions. The objectives of this study were to investigate the effects of abiotic stress on forage quality and character associations in maize grown under stressed and normal conditions.

Materials and Methods

The study was conducted in Sudan (Khartoum State) during 2013-2014 at two different locations: Shambat (Lat. 15° 39' N; Long. 32° 31' E; Alt 380 masl) and Soba (Lat. 15° 24' N; Long. 32° 32' E; Alt 380 masl). Soba is located about 25 km south of Shambat. In each location the trial was carried out in the Experimental Farm of the Agricultural Research Corporation (ARC).

The plant material

Nine maize genotypes comprising 8 hybrids + 1 open pollinated cultivar were studied (Table 1).

Table 1. Plant material used in the study

S.N.	Genotype	Type/Color	Source
1	PAN-6966	Yellow maize hybrid	South Africa
2	PAN-12	Yellow maize hybrid	South Africa
3	PAN-14	Yellow maize hybrid	South Africa
4	PAN-6P110	Yellow maize hybrid	South Africa
5	Hytech1100	White maize Hybrid	Egypt
6	Hytech2066	Yellow maize hybrid	Egypt
7	Hytech2031	White maize hybrid	Egypt
8	Hytech2055	Yellow maize hybrid	Egypt
9	Hudieba2	Yellow maize (open pollinated)	Sudan

Test-environments

The above maize genotypes were subjected to normal vs stressed environments representing water, heat and salt stresses and their combinations as follows:

1. Normal vs water stress was brought about by adopting watering regimes of one and two week intervals, respectively.
2. Normal temperature vs heat stress was brought about by sowing in winter and summer seasons, respectively.
3. Normal soil vs salt stress was brought about by planting in Shambat and Soba locations, respectively.

The soils at Shambat are well drained loamy clay, non-saline and non-sodic with pH ranging 7.7 - 7.9). The soil at Soba is hazarded by salinity ($EC_e = 12 - 14$ dS/m) and sodicity ($ESP = 24 - 27$), ($SAR = 16 - 23$), with high clay content, low infiltration and permeability, low organic matter and nitrogen and high pH).

The average min-max temperature during the winter season (Nov. –Feb.) ranged 15-20°C and 32-38°C whereas that at summer (April-July) ranged 25.0-28.4°C and 36.9-42.0 °C. The weather is dry in both growing seasons specially during Winter.

The experimental design and cultural practices

To achieve the above treatment combinations 4 trials were conducted 2 at each location (Shambat and Soba) in each season (winter and summer). Each trial was split plot experiment with watering regimes assigned to the main plots and the genotypes to the sub plots in a randomized

complete block design (RCBD) with three replications. The combination of location, season and watering regime (2x2x2) provided 8 stress levels (Table 2) used to test the genotypes at silk initiation and milk stages. Quality traits were studied using material subjected to stress levels No 1 (No stress) and No 8 (Full stress) (Table 2)

Table 2. Abiotic stress levels generated from different combinations of environmental conditions

S.No.	Stress level	location	Season	Water
1	No stress	Shambat	Winter	Normal
2	Water stress	Shambat	Winter	Stressed
3	Heat stress	Shambat	Summer	Normal
4	Water + heat stress	Shambat	Summer	Stressed
5	Salt stress	Soba	Winter	Normal
6	Water + salt stress	Soba	Winter	Stressed
7	Salt + heat stress	Soba	Summer	Normal
8	Full stress	Soba	Summer	Stressed

Unless otherwise indicated, the cultural practices followed were the same in all trials. The land was disc ploughed, disc harrowed and levelled by scraper to obtain fine seed bed. Ridging was done at 0.75 m spacing. The plot consisted of four ridges 4m long. Two seeds were placed in holes spaced at 15 cm on one side of the ridge. The winter sowings were done on the 8th and 12th of Dec. 2013 in Soba and Shambat, respectively. Summer sowings were done on the 13th and 19th of May 2014 in Soba and Shambat, respectively. Nitrogen fertilizer (55 kgN/ha) was applied in a form of urea at the second watering. Weed population was kept to minimum by hand weeding.

Data collection

Proximate analysis for the following quality traits were studied on dry matter basis at silk initiation and dough stages using material from fully normal and fully stressed treatments:

1. Neutral detergent fiber (NDF)
2. Acid detergent fiber (ADF)
3. Crude protein (CP)

The chemical analysis was done following the standard procedure of the AOAC [19]. Crude protein was analyzed using micro-kjeldhal method. The CP in each sample was estimated from total nitrogen using the numerical conversion factor = 6.25. The chemical analysis was carried out in the Laboratory of Animal Production – Shambat - University of Khartoum.

Agronomic traits recorded included: dry matter yield (DMY), days to 50% tasselling, plant height and stem diameter.

Statistical analysis

The data collected were subjected to the standard procedure of analyzing split plot in RCB design [20]. The combined analysis of variance of the genotypes across the stress levels was performed using the statistical package GenStat [21]. Pearson's correlation coefficients between quality and agronomic traits at dough stage were calculated.

Results

Effects of abiotic stress

Table 3 shows mean squares of genotypes, environments, growth stage and their interactions for neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein (CP). The analysis of variance revealed highly significant differences among genotypes, environments and growth stage for the three studied traits. The first and second order interactions of the main effects were also highly significant for the three studied traits.

Table 3. Mean squares of the main and interaction effects for some quality traits of maize forage (2013-2014)

Source of variation	DF	NDF†	ADF††	CP‡
Block	2	0.3220	0.03028	0.21714
Environments (E)	1	1378.8064**	1284.15933**	64.82256**
Growth stages (H)	1	5.7362**	29.77800**	24.64282**
Genotypes (G)	8	37.9792**	163.50119**	10.66437**
E×H	1	8.9039**	155.06433**	0.94285**
E×G	8	16.3440**	81.92183**	11.89984**
G× H	8	21.3543**	43.02976**	1.68034**
G× W× E	8	24.6541**	116.40048**	2.10763**
Residual	70	0.2524	0.01504	0.04994**

† : Neutral detergent fiber (NDF)

†† : Acid detergent fiber (ADF)

‡ : Crude protein (CP)

Genotypic effects

Table 4 shows the performance of genotypes for the three quality traits. The hybrid PAN12 scored the lowest NDF value (65.8%) followed by Hytech2031 (66.6%). PAN6P-110 and PAN6966 scored the highest NDF value (above 70%). The hybrid Hytech2066 exhibited the lowest ADF value (38.4%), followed by PAN12 (38.5%). PAN6966 scored the highest ADF value (46.3%). The highest CP values were scored by PAN12 (12.6%), Hytech2066 and PAN6966 (11.1%) whereas the lowest value was shown by Hytech2055 (9.59%).

Table 4. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein (CP) of maize forage (2013-2014)

Genotypes	NDF (percentage)	ADF (percentage)	CP (percentage)
PAN6966	71.0	46.3	11.1
PAN12	65.8	38.5	12.6
PAN14	68.6	45.7	10.3
PAN6P-110	71.2	48.4	9.66
Hytech1100	68.3	41.3	10.3
Hytech2066	68.2	38.4	11.1
Hytech2031	66.6	38.8	9.95
Hytech2055	69.1	41.3	9.59
Hudeiba2	68.1	41.5	10.4
Mean	68.5	42.3	10.6
SE±	0.1450	0.0354	0.0645
LSD (5%)	0.4091	0.0999	0.2573
CV (%)	0.7	0.3	2.1

Effect of stress levels and growth stage

Tables 5 and 6 show the effect of abiotic stress and growth stage on forage quality traits, respectively. Stressed condition increased NDF (72.1%), ADF (45.7%) and decreased CP (9.79%) as compared to normal condition. The respective values for non-stress condition were 65.0 %, 38.8% and 11.3%. Table 6 shows that harvesting at dough stage significantly reduced CP (10.1%) as compared to silk initiation stage (11.0%). The NDF values at silk initiation and dough stage were 68.3% and 68.7%, respectively. The ADF at dough stage was lower (41.7%) than that of silk initiations (42.8%).

Table 5. Effect of stress level on some quality traits of maize forage (2013-2014)

Environments	NDF†	ADF††	CP‡
Non-stress condition	65.0	38.8	11.3
Stress condition	72.1	45.7	9.79
Mean	68.5	42.3	10.6
SE±	0.0684	0.0167	0.0304
CV (%)	0.7	0.3	2.1

† : Neutral detergent fiber (NDF)

†† : Acid detergent fiber (ADF)

‡ : Crude protein (CP)

Table 6. Effect of growth stage on some quality traits of maize forage (2013-2014)

Growth stage	NDF†	ADF††	CP‡
Silk initiation stage	68.3	42.8	11.0
Dough stage	68.8	41.7	10.1
Mean	68.5	42.3	10.6
SE±	0.0684	0.017	0.0304
CV (%)	0.7	0.3	2.1

† : Neutral detergent fiber (NDF)

†† : Acid detergent fiber (ADF)

‡ : Crude protein (CP)

Interaction effect

Table 7 shows the interaction effect of stress level with growth stage on quality traits. Harvesting at dough stage under normal condition didn't significantly altered NDF value compared to harvesting at silk initiation stage. However, under stressed condition harvesting at dough stage significantly increased NDF (72.7 %) compared to harvesting at silk initiation stage (71.6 %). Harvesting at dough stage under stressed condition significantly decreased ADF value (44.0 %) compared to harvesting at silk initiation stage (47.4 %). Harvesting at dough stage significantly decreased CP percentage under normal (11.0 %) and stressed conditions as well (9.22 %). The respective values for CP obtained by harvesting at silk initiation were 11.7 % and 10.4 %.

Table 7. Interaction effect of stress level with growth stage on percentage neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein (CP) in maize forage (2013-2014)

Growth stage	NDF		ADF		CP	
	Silk initiation	Dough	Silk initiation	Dough	Silk initiation	Dough
Normal condition	65.0	64.9	38.1	39.5	11.7	11.0
Stress condition	71.6	72.6	47.4	44.0	10.4	9.22
Mean	68.5		42.3		10.6	
SE±	0.0967		0.0236		0.0430	
LSD (5%)	0.2727		0.0666		0.1213	
CV (%)	0.7		0.3		2.1	

Associations under non-stress and stress conditions

Table 8 shows correlation among quality and agronomic traits under non-stress conditions. DMY had negative highly significant correlations with ADF ($r = -0.4975$). Correlations were weak and insignificant between DMY with NDF and CP. Plant height had negative significant correlation with ADF ($r = -0.4537$) and insignificant correlation with NDF and CP. Days to tasselling had positive significant correlation with NDF ($r = 0.3579$) and ADF ($r = 0.3592$) but had negative and highly significant correlations with CP ($r = -0.6302$). Correlation between stem diameter and CP was significantly negative ($r = -0.3602$) whereas it was insignificant with NDF and ADF. CP had negative

highly significant correlations with ADF ($r=-0.5336$) but had weak insignificant correlation with NDF. A positive significant correlation was observed between NDF and ADF ($r=0.4121$).

Table 9 shows the correlation among different maize attributes under stressed conditions. DMY had negative highly significant correlation with CP ($r=-0.5288$). Correlations were weak and insignificant between DMY and each of NDF and ADF. Plant height had negative significant correlation with CP ($r=-0.4014$). Correlations were weak and insignificant between plant height and each of NDF and ADF. Days to tasselling had negative significant correlation with NDF ($r=-0.3343$). Correlations were weak and insignificant between days to tasselling and each of CP and ADF. Correlation between stem diameter and CP were positive and highly significant ($r=0.7055$). Correlations were weak and insignificant between stem diameter and each of NDF and ADF. Positive significant correlation was observed between CP and ADF ($r=0.3374$). Correlations between CP and NDF and between NDF and ADF were weak and insignificant.

Table 8. Correlation among different forage maize attributes under non-stressed conditions

Character	1	2	3	4	5	6
NDF	1	-				
Stem diameter	2	0.1503	-			
Plant height	3	-0.2211	0.5160**	-		
Days to tassling	4	0.3579*	0.7995**	0.4556 *	-	
ADF	5	0.4121 *	0.0693	-0.4537*	0.3592 *	-
CP	6	-0.0238	-0.3602*	-0.0708	-0.6302**	-0.5336 **
DMY	7	-0.1591	0.5728**	0.9005**	0.3938*	-0.4975**
						0.1371

*, ** = significantly different from zero at 0.05 and 0.01 probability level, respectively.

Table 9. Correlation among different forage maize attributes under stressed condition

Character	1	2	3	4	5	6
NDF	1	-				
Stem diameter	2	-0.0318	-			
Plant height	3	-0.2034	-0.3001	-		
Days to tassling	4	-0.3343*	0.0119	0.3703*	-	
ADF	5	-0.2853	0.0126	0.1774	-0.2170	-
CP	6	-0.2840	0.7055**	-0.4014*	0.1105	0.3374*
DMY	7	-0.1539	-0.3715*	0.8131**	0.4143*	0.0328
						-0.5288**

*, ** = significantly different from zero at 0.05 and 0.01 probability level, respectively.

Discussion

Forage quality

The highly significant differences noted among genotypes for NDF, ADF, and crude protein (CP) indicated that a considerable portion of the variability observed for these traits was due to genetic factors irrespective of the effects of stressed conditions and growth stage. However, the interaction of genotypes with growth stages and stressed conditions was highly significant indicating that the genotypes performed inconsistently across these factors.

The performance of the maize genotypes for the cell wall fraction and crude protein shown by this study was within the range reported in the literature [22]. However, two hybrids, namely, PAN6966 and PAN6P-110 showed above range values for ADF ($\geq 48\%$). Such results should be cautiously considered as it might be due to sampling error.

The ADF predicts digestibility [14] while the NDF measures the intake potential [23]. Lower values are desirable for each measurement. In the present study stressed conditions (compound effect of heat, salt and water stresses) significantly lowered the nutritive value of forage maize in terms of

crude protein content, digestibility and intake potential. Supporting results were reported by Montgomery [24]. Some disagreeing studies pointed that drought stress has no effect on percentage of crude protein of maize forage [24, 25] while others reported that drought has enhanced feed value, whereas salinity and heat stresses have mixed or no effects [16]. However, we think that drought stress joined with salt and heat stresses (as in this study) will have deleterious effects on the spectrum of nutritive elements since the compound effect of these factors will impede uptake of nutrients reported to play significant role on the content of crude protein [13].

In the present study crude protein percentage significantly decreased as growth stage advanced from silk initiation to the dough stage, however, the dough stage has lower ADF value than silk initiation. This could be explained by the diluting effect of the low ADF content reported for the developed ears and husks in the dough stage [26].

The quality of forage maize at dough stage was reported to be the ideal after which it starts to decline [1]. Also the forage yield and the energy value at dough stage are expected to be better than that at silk initiation due increased plant size and starch formation. At silk initiation, however, 'baby corn' or immature cobs represents additional source of income to the farmer in urban and semi-urban areas.

Characters associations

The correlations between quality traits and forage yield shown by this study pointed to the possibility of developing or identifying cultivars superior in both yield and digestibility, on the other hand the weak and insignificant correlation between yield and each of NDF and protein content may allow for selecting high yielding genotypes with good potential intake and protein content. These results agree with those reported by Silva [27] who concluded that the variability and correlations of traits in forage maize permit selection for quality traits while maintaining yield or vice versa. Disagreeing results were reported in maize [28] and forage sorghum [29]. Correlation among quality traits shown in this study were favorable specially under non-stressed conditions showing the possibility of selecting forage maize genotypes having good levels of protein content, digestibility and potential intake. Similar results, but in forage sorghum were reported [29].

Under stress conditions, the correlation of forage yield with other traits showed no contrasting differences compared to those under non-stressed conditions. This may ease the role of the breeder working on parallel improvement of forage yield and quality traits under stressed conditions since he may make use of the elite materials developed under normal conditions. However, some correlations were quite the opposite to those under non-stress conditions where unfavorable correlation exists between crude protein with each of forage yield ($r = -0.5288$), digestibility ($r = 0.3374$) and plant height ($r = -0.4014$). Similarly, days to tasselling (earliness) had unfavorable correlation with intake potential ($r = -0.3343$). This may partially be attributed to the effect of abiotic stresses reported to affect every aspect of plant growth, which includes anatomical and morphological growth, physiological and biochemical processes [30-32].

Conclusions

The compound effect of salt, water and heat stresses has the greatest adverse effect on the nutritive value of forage maize in terms of crude protein content, digestibility and intake potential. Maize varieties combining high performance in quality and forage yield under non-stressed conditions could be developed. Such materials could also work under stressed conditions

Conflict of Interest

The authors declare that there is no conflict of interest.

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