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Opportunities for exploiting variations in haulm fodder traits of intermittent drought tolerant lines in a reference collection of groundnut (*Arachis hypogaea* L.)

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ABSTRACT

Groundnut haulm has a great value as feed stock in the semi-arid tropics. Two-hundred-two and 194 cultivars of groundnut grown under intermittent water stress and fully irrigated treatment for two consecutive years at ICRISAT (2008/2009 and 2009/2010) in Patancheru in India were investigated for haulm fodder quality traits and for potential trade-offs between pod or haulm yield and haulm fodder traits. Highly significant ($P < 0.0001$) cultivars-dependent variations were found for a range of laboratory haulm fodder quality traits. Haulm nitrogen contents ranged from 1.94 to 2.88% and from 1.81 to 2.66% while *in vitro* digestibility ranged from 57.3 to 64.3% and from 59.5 to 64.2% under water restriction and fully irrigated conditions, respectively. Under fully irrigated conditions haulm nitrogen content and *in vitro* digestibility were mildly, but significantly inversely, related to pod yields with the two haulms traits accounting for 5 and 4% of the variations in pod yields. However, potential trade-offs between haulm traits and pod yields became more pronounced under water stress where variations in haulm nitrogen content and *in vitro* digestibility accounted for 40 and 28% of the variations in pod yields, respectively. For haulm nitrogen content and *in vitro* digestibility no significant interactions were observed between cultivar and treatment suggesting stability of haulm fodder traits across poorer and better water management practices. These results demonstrate that breeding for fodder traits in groundnut can be parallel to breeding for productivity traits, although careful choice of cultivars with high fodder trait value would be needed under water stress conditions.

1. Introduction

Groundnut is an important food-feed crop in mixed crop-livestock systems of the semi-arid tropics (SAT) with pods providing food for humans and the haulms fodder for livestock (Larbi et al., 1999; Omokanye et al., 2001). Farmers do pay attention to haulm fodder traits as it was shown that superior haulm fodder quantity and quality traits positively affected the adoption and dissemination of new groundnut cultivars (ICRISAT, 2008; Birthal et al., 2011.). There is good reason for farmers to pay attention to haulm fodder traits since it was shown that livestock productivity could be influenced substantially by choice of cultivar. For example in male sheep fed exclusively on groundnut haulms harvested from 10 different cultivars (Prasad et al., 2010) observed high daily intake levels, superior to 4% of the live weight, and showed cultivar-dependent variation in daily live weight gain between 65 and 137 g/day. Etela and Dung (2011) feeding haulms from six different cultivars as sole feed to West Africa dwarf sheep observed even greater variation in daily live weight changes, i.e. from −6 to 46 g/day.

While choice of cultivar will have implications for livestock productivity where haulms contribute significantly to feed resources, farmers will unlikely sacrifice pod yield for haulm fodder traits. However this does not need to be the case. As observed by Nigam and Blümmel (2010), who investigated a wide range of groundnut cultivars and breeding lines, such trade-offs might not be required as no inverse relationships were observed between haulm fodder quality traits and pod and haulm yields. However these observations were based on on-station trials under very good agronomic conditions in breeding materials with a likely limited range of genetic variation, and trade-off effects between pod and haulms traits might exist either with a larger genetic range of variation and where conditions are less perfect such as under water stress. This study was then undertaken to assess the range of variation in fodder quality trait in a reference collection of groundnut, i.e. a set of lines representing most of the genetic variation in the entire groundnut collection (Upadhyaya et al., 2002), and possible trade-off effects between fodder quality traits and productivity (haulm and pod yield) parameters. Since haulm nitrogen content is both an important quality trait and that groundnut yield depends on

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remobilization of nitrogen resources to the pods, the relationship between pod yield under water stress and haulm nitrogen content are especially looked at in relation to putative trade-offs.

2. Material and methods

2.1. Field experiments

Two experiments were conducted at ICRISAT headquarters (Patancheru, AP, India, 17° 30′ N; 78° 16′ E; altitude 549 m) between November 2008 and April 2009 and November 2009–April 2010 and are described in detail in the companion paper (Hamidou et al., submitted for publication, FCR). In short, the soil used was a sandy-clay loam Alfisol, with a pH of about 7.0. Two water regimes were imposed, i.e. a fully irrigated control that received irrigation every 7–10 days, and an intermittent stress treatment that was fully irrigated until flowering (approximately 45 days after sowing) and then was exposed to an intermittent drought stress by skipping every other of the irrigation to the fully irrigated control. Seeds were hand-planted in 2-row plots of four meters long with 33 cm between rows and 10 cm between plants. The experimental design was an Alpha-lattice design with water treatment as the main factor and genotypes as sub-factors in three replications. At harvest, after removing the pods from the plants, the haulm of five randomly chosen plants per plot were collected and dried at 70 °C for three days in a forced air oven. After drying the weight was taken and the samples were grinded for subsequent Near Infrared Spectroscopy (NIRS) analysis (see below).

2.2. Groundnut haulm fodder quality analysis

The NIRS instrument used was a FOSS Forage Analyzer 5000 with software package WinISI II. For conventional laboratory analysis nitrogen was determined by auto-analyzer method, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) by Goering and Van Soest 1970 and *in vitro* digestibility and metabolizable energy content were estimated based on sample incubation in rumen microbial inoculum using the *in vitro* gas production technique and the associated equations described by Menke and Steingass (1988). NIRS validations were based on blind-predictions, in other words the predicted samples were not part of the set from which the NIRS equations were developed. Relationships between blind-predicted and measured variables were described by R^2 and standard error of prediction (SEP). There was good agreement between conventionally analyzed and NIRS predicted values (Table 1) over a wide range of groundnut cultivars.

3. Results

3.1. Variations in groundnut pod and haulm traits

Out of 202 and 194 cultivars a total of 172 were common to both years. Means and ranges across two years in haulm nitrogen contents and *in vitro* digestibilities of these are reported in Table 2.Observations were based on a total of 202 groundnut cultivars grown under water restriction and 194 cultivars grown under control conditions in Rabi season (off season) 2008/2009 and 2009/2010. Cultivars-dependent variations within treatments were substantial and significant for all traits. Notably pod yields varied by more than five-fold and haulm yields varied by more than three-fold (Hamidou et al., submitted for publication, companion paper). For haulm fodder quality traits, nitrogen contents varied by 0.94 and 0.85 percentage units under water stress and control conditions, respectively, while *in vitro* digestibilities varied by 7.0 and 4.7 percentage units under water stress and control conditions, respectively. Regardless of treatment strong heritabilities ($h^2 \geq 0.7$) were observed for haulm nitrogen content while weaker h^2 were found for haulm *in vitro* digestibilities (≥0.24).

Table 1
Comparisons of NIRS blind-predicted nitrogen, neutral (NDF) and acid detergent fiber (ADF), acid detergent lignin (ADL), *in vitro* digestibility (IVOMD) and metabolizable energy content (ME) with conventionally measured traits based on coefficient of variation (R^2) and standard error of prediction.

Haulm trait	N	R^2	SEP
Haulm nitrogen	220	0.99	0.09
Haulm NDF	77	0.99	1.8
Haulm ADF	79	0.98	2.0
Haulm ADL	80	0.85	0.9
Haulm IVOMD	218	0.97	1.18
Haulm ME	221	0.96	0.20

Table 2
Means, ranges and statistical variations in pod yields (kg/ha), haulm yields (kg/ha) and haulm nitrogen contents (%) and *in vitro* digestibilities (%) in groundnut cultivars grown under stress (N = 202) and control (N = 194) condition at Patancheru, India in 2009 and 2010.

Variable	Means	Ranges	$P < F$	h^2
Pod yield				
Stress	988	316–1951	0.0001	0.77
Control	1753	589–3283	0.0001	0.70
Haulm yield				
Stress	2916	1232–4622	0.0001	0.73
Control	3840	1777–6045	0.0001	0.70
Haulm nitrogen				
Stress	2.41	1.94–2.88	0.0001	0.77
Control)	2.23	1.81–2.66	0.0001	0.70
Haulm digestibility				
Stress	60.9	57.3–64.3	0.0001	0.26
Control	61.6	59.5–64.2	0.0001	0.44

Pod and haulm yield data were obtained from the accompanying work of Hamidou et al. (submitted for publication).

3.2. Relationships between groundnut pod and haulm traits

Relationships between haulm nitrogen content and *in vitro* digestibility and pod and haulm yield are presented in Fig. 1a–d. These haulm fodder quality traits were significantly inversely related to grain yields (Fig. 1a and c). Generally, relationships between traits were consistently stronger under water stress than under control conditions. Thus, while the significant inverse relationship between haulm nitrogen content and haulm *in vitro* digestibility and pod yield accounted for 5 and 4% of the variations in pod yields under control conditions, these haulm fodder quality traits accounted for 40 and 28% of the variations in pod yields under water stress conditions (Fig. 1a and c). Relationships between haulm nitrogen content and *in vitro* digestibility and haulm yields were consistently and significantly positive and the treatment effect on these relationships was smaller than observed for pod yields (Fig. 1b and d).

Highly significant cultivars-dependent variations were observed for all four traits with similar trait ranges and trait heritabilities than observed for the 202 and 194 cultivars when compared within their respective treatment (Table 3).

When the data were combined across years and treatments, haulm nitrogen content and *in vitro* digestibility were significantly inversely related to pod yield and were significantly positively associated with haulm yield (Table 4). However, the tightness of the relationships with pod yield was less than when water treatments were kept separate, and in particular lower than under water stress (Fig. 1a and c) indicating that the water treatment was responsible for this tight relationship. Pod and haulm yield were weakly (P = 0.08) negatively associated.

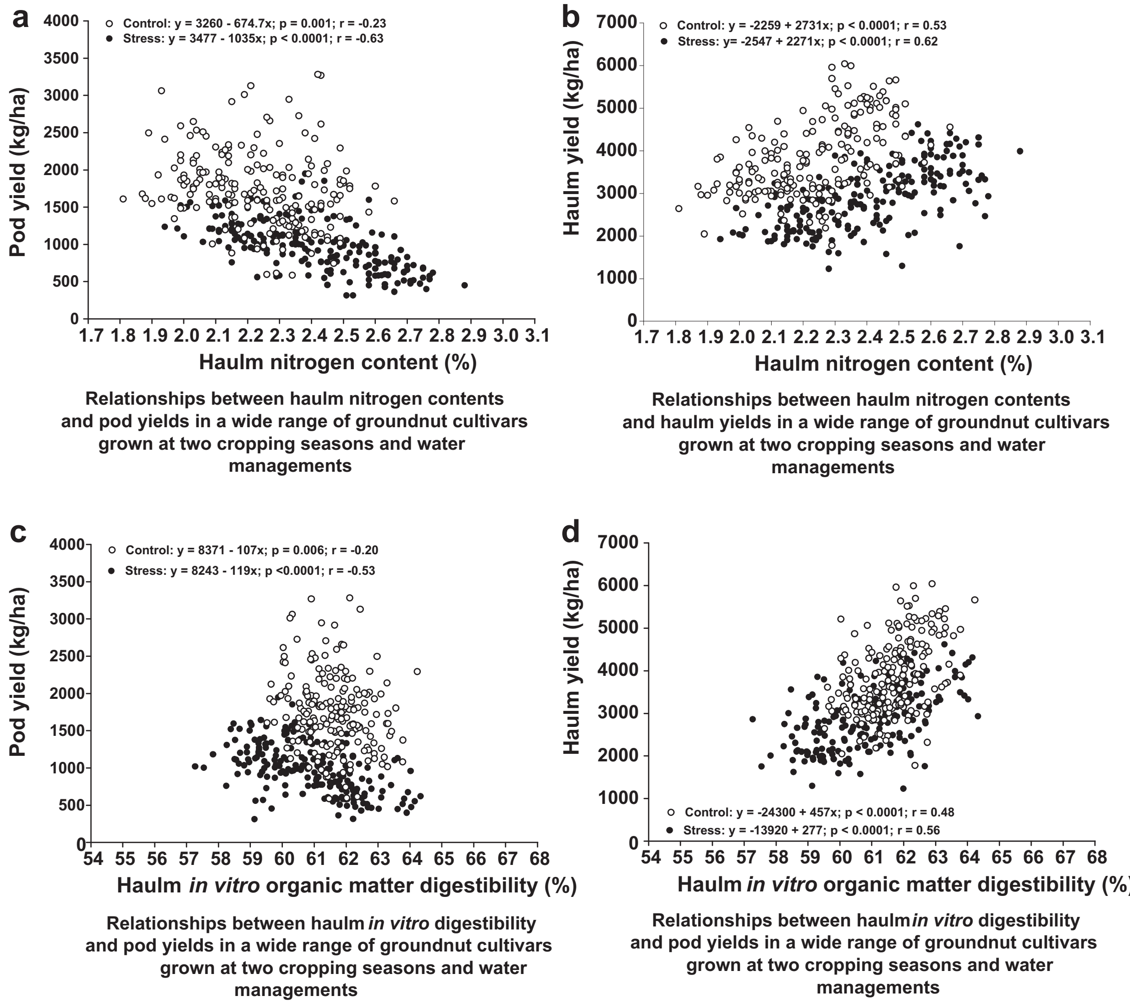

Fig. 1. a: Relationships between haulm nitrogen contents and pod yields in a wide range of groundnut cultivars grown at two cropping seasons and water managements, b: Relationships between haulm nitrogen contents and haulm yields in a wide range of groundnut cultivars grown at two cropping seasons and water managements. c: Relationships between haulm *in vitro* digestibility and pod yields in a wide range of groundnut cultivars grown at two cropping seasons and water managements. d: Relationships between haulm *in vitro* digestibility and pod yields in a wide range of groundnut cultivars grown at two cropping seasons and water managements.

Table 3
Across treatment (control and water-stressed) and years (2009 and 2010) means, ranges and statistical variations in pod yields (kg/ha), haulm yields (kg/ha) and haulm nitrogen contents (%) and *in vitro* digestibilities (%) in 172 groundnut cultivars grown at Patancheru, India.

Variable	Means	Ranges	$P<F$	h^2
Pod yield	1353	466–2488	0.0001	0.72
Haulm yield	3461	1539–5178	0.0001	0.75
Haulm nitrogen	2.34	1.96–2.70	0.0001	0.81
Haulm digestibility	61.4	59.0–63.9	0.0001	0.49

3.3. Effects of environment on groundnut haulm traits

Cultivar, treatment and year had all significant effects on haulm nitrogen and *in vitro* digestibility (Table 5) with year generally having the strongest effect. Significant interactions existed between treatment and year and between cultivar and year for the haulm nitrogen and haulm *in vitro* digestibility. No interactions were observed for cultivar × treatment interactions for haulm nitrogen and *in vitro* digestibility.

A reasonably good agreement existed between traits measured under water restriction and control condition, see Fig. 2a–d. Water stress decreased pod and haulm yield but over-all ranking for these two traits largely persisted across treatment (Fig. 2a/b). In most

Table 4
Intercorrelations between pod yields (kg/ha), haulm yields (kg/ha), haulm nitrogen contents (%) and *in vitro* digestibilities (%) across treatment (control and water stressed) and yeard (2009 and 2010) in 172 groundnut cultivars grown at Patancheru, India.

Variable	Pod yield	Haulm yield	Haulm nitrogen	Haulm digestibility
Pod yield				
Haulm yield	−0.13 [0.08]			
Haulm nitrogen	−0.45 [0.0001]	0.65 [0.0001]		
Haulm digestibility	−0.44 [0.0001]	0.59 [0.0001]	0.77 [0.0001]	

Table 5
Effect of cultivar, year of planting and treatment (control and water-stressed) on haulm nitrogen content and *in vitro* digestibility of 172 cultivars of groundnut grown at Patancheru, India in 2009 and 2010.

Source	*F*-value	*P* > *F*
Variable haulm nitrogen content		
Cultivar	8.1	0.0001
Treatment	372.5	0.0001
Year	2835.9	0.0001
Treatment × year	356.7	0.0001
Cultivar × treatment	1.0	0.620
Cultivar × year	1.7	0.0001
Cultivar × treatment × year	1.0	0.627
Variable haulm *in vitro* digestibility		
Cultivar	2.7	0.0001
Treatment	38.4	0.0001
Year	1368.3	0.0001
Treatment × year	213.1	0.0001
Cultivar × treatment	0.9	0.84
Cultivar × year	1.3	0.008
Cultivar × treatment × year	1	0.49

cases haulm nitrogen contents were higher under water stress than under control conditions but haulm *in vitro* digestibility was less affected by treatment (Fig. 2c/d).

3.4. Opportunities for concomitant improvement of pod yield and haulm fodder quality traits

The average haulm nitrogen content across the 20 cultivars presented in Fig. 3a was 2.51 and 2.73% in the control and water stress treatment with associated average pod yields of 1595 kg/ha (control) and 605 kg/ha (water stress). In contrast, the average haulm nitrogen content across the 20 cultivars presented in Fig. 3b was 2.2 and 2.27% in the control and water stress treatment with associated average pod yields of 2745 kg/ha (control) and 1558 kg/ha (water stress), indicating that selecting for the highest pod yield genotypes would not select the highest haulm nitrogen content genotypes but would still provide germplasm with relatively high haulm *N* content. Similarly average haulm *in vitro* digestibility across the 20 cultivars presented in Fig. 3c was 63.3 and 63.5% in the control and water stress treatment with associated average pod yields of 1575 kg/ha (control) and 697 kg/ha (water stress). In contrast, the average haulm *in vitro* digestibility across the 20 cultivars presented in Fig. 3d was 61.2 and 60.1% in the control and water stress treatment with and again associated average pod yields of 2745 kg/ha (control) and 1558 kg/ha (water stress). While selecting highest ranking pod yielders will not result in highest haulm nitrogen and *in vitro* digestibilities there is still considerable variation in these haulm quality traits to be exploited (Fig. 3a–d).

4. Discussion

4.1. Variations in groundnut haulm fodder quality traits

Farmers in mixed crop-livestock systems are often resource poor with regards to land and water, which severely limits their options for mitigating feed scarcity. Fodder is sourced mainly from crop production in the form of crop residues. In India, for example, crop residues is providing about 44% of the country's fodder resources at the turn of the century (NIANP, 2003) but their

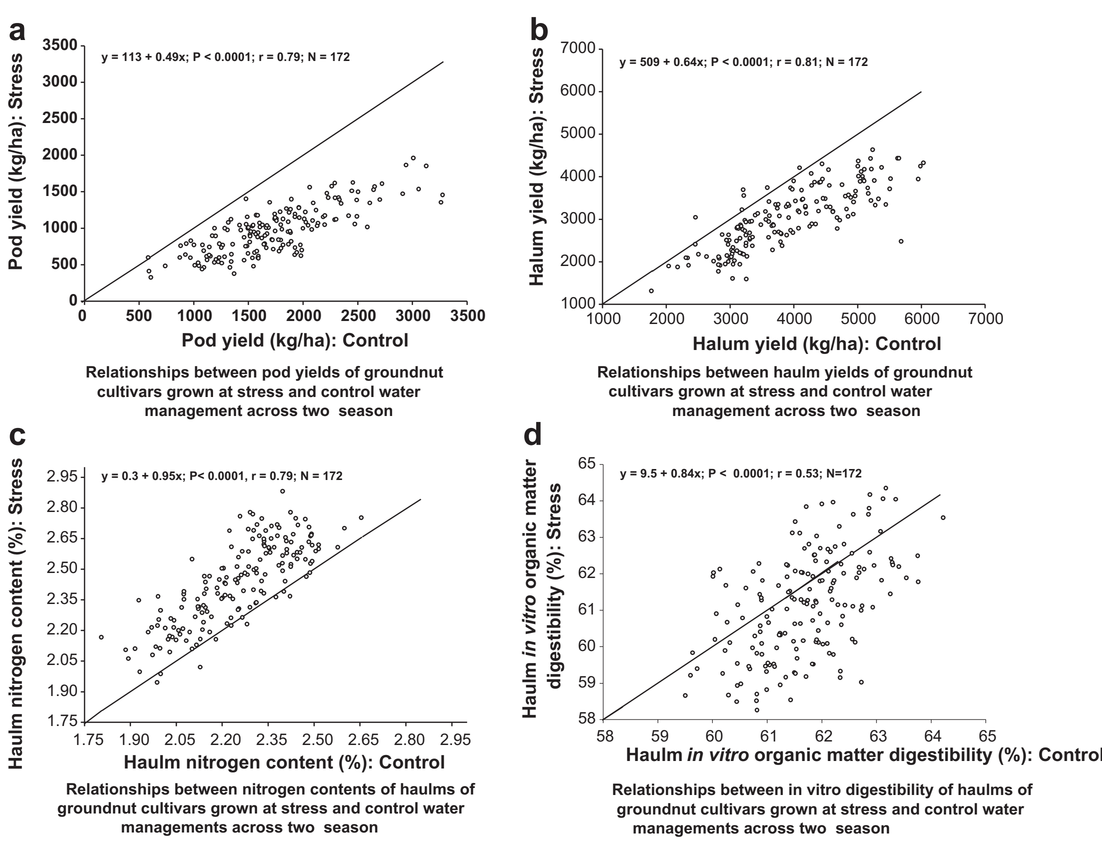

Fig. 2. a: Relationships between pod yields of groundnut cultivars grown at stress and control water management across two season. b: Relationships between haulm yields of groundnut cultivars grown at stress and control water management across two season. c: Relationships between nitrogen contents of haulms of groundnut cultivars grown at stress and control water managements across two season. d: Relationships between *in vitro* digestibility of haulms of groundnut cultivars grown at stress and control water managements across two season.

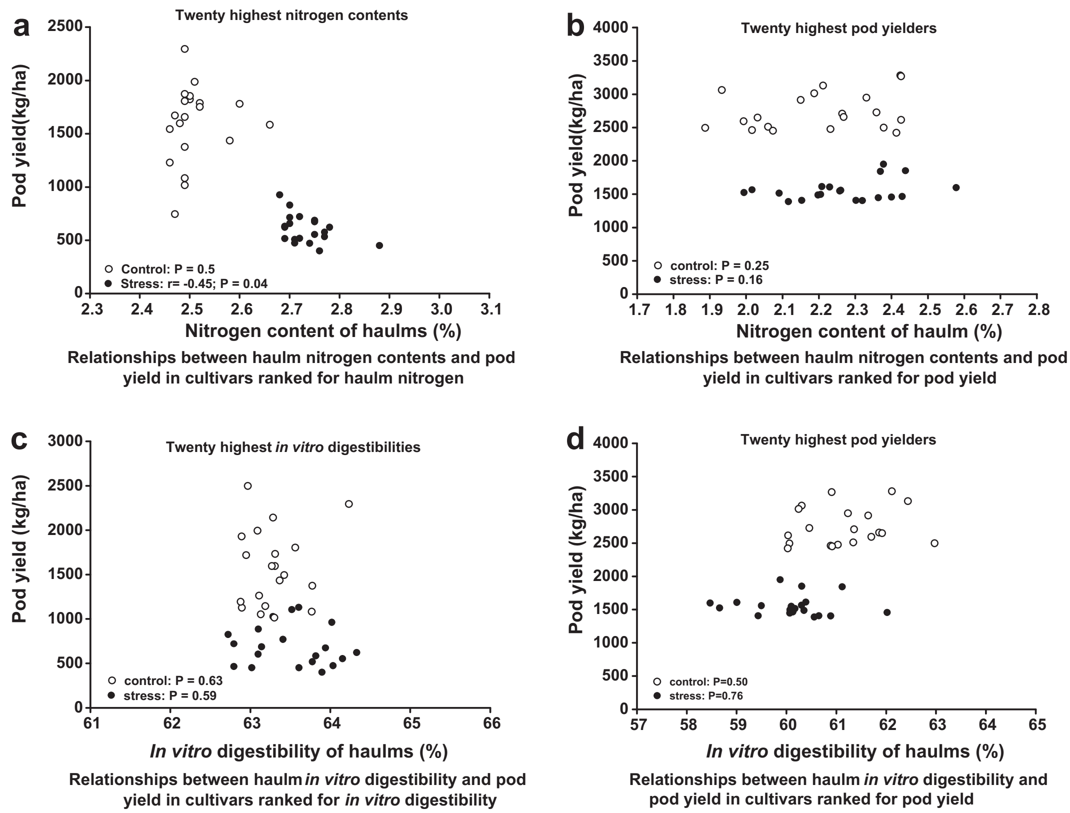

Fig. 3. a: Relationships between haulm nitrogen contents and pod yield in cultivars ranked for haulm nitrogen. b: Relationships between haulm nitrogen contents and pod yield in cultivars ranked for pod yield. c: Relationships between haulm *in vitro* digestibility and pod yield in cultivars ranked for *in vitro* digestibility. d: Relationships between haulm *in vitro* digestibility and pod yield in cultivars ranked for pod yield.

contribution is predicted to increase to almost 70% by the 2020 (Ramachandra et al., 2007). The bulk of these crop residues are from cereals and only about 10% from legumes, to which groundnut haulms contributing more than 30% (NIANP, 2003). So, looking at fodder trait in an important food and cash crop such as groundnut is important. A distinguishing feature of cereal and leguminous crop residues affecting their fodder value is the low nitrogen content of the former. Ruminant livestock, or more correctly their rumen microbial populations, require a minimum threshold level of about 1–1.2% of nitrogen in the feed for efficient feed digestion (Van Soest, 1994). Cereal crop residues provide on average only about half of this requirement while leguminous crop residues provide usually more than minimum nitrogen requirements (Sundstøl and Owen, 1984). In the present work, independent of treatment, haulms of all groundnut cultivars would supply feed nitrogen well above this threshold level as the lowest nitrogen contents were 1.94 and 1.81% in the water restricted and control treatment, respectively (see Table 2). Groundnut haulms are therefore very suitable as nitrogen supplement to cereal crop residues to provide for at least the minimum nitrogen content of the overall feed ration of 1–1.2%. The ranges in haulm nitrogen content observed were considerable (water stress: 1.94–2.88% and control: 1.81–2.66%). When used as a supplement to an average cereal crop residue targeting an overall feed ration nitrogen content of 1.2%, groundnut haulms with a nitrogen content of 1.94% would need to contribute about 45% to the feed ration but only about 26% if the haulms contains 2.88% of nitrogen. Since groundnut haulms are generally in short supply relative to cereal crop residues, cultivar with higher nitrogen content in the haulms would make substantial contributions to improved crop residue based feed resources.

In vitro digestibility yields a close estimate of the feed energy available to the livestock and is therefore an important quality trait in feeds (McDonald et al., 1988). Digestibility was the key variable in ex-ante impact assessments of the genetic enhancement of sorghum and pearl miller stover as livestock fodder (Kristjianson and Zerbini, 1999). These authors calculated that a one-percentage unit increase in digestibility would result in increases in milk, meat and draught power outputs ranging from 6 to 8%. In sorghum stover and rice straw a cultivar-dependent difference of 3 to 5 percentage units in *in vitro* digestibility resulted in a 20% and higher price (Blümmel and Rao, 2006). For grasses, a 3–4 percent difference in digestibility was associated with 17–24 percent differences in animal performance (Vogel and Sleper, 1994). Thus the observed cultivars dependent ranges in haulm *in vitro* digestibility of 4.7–7 percentage units in water stressed and control treatments, respectively (Table 2), indicate substantially different potentials in supporting livestock productivity.

This can be further corroborated using data from Prasad et al. (2010) who tested haulms from 10 different cultivars as sole feed with sheep and observed cultivars-dependent variation in daily live weight gain (LWG) of 65–137. Live weight gain was reasonably well predicted by multiple regressions using laboratory traits such as acid detergent lignin (ADL) and metabolizable energy (ME) which is very closely associated with *in vitro* digestibility. While ADL and ME were not specifically reported on in the present paper they were measured, see also Table 1. Based on the Prasad et al. (2010) regression equation (LWG = −0.57 to 25.1 ADL + 25.7 ME, $R^2 = 0.92$) and actual ADL and ME values determined in the present work predicted LWG ranged cultivar dependent from 69 to 149 g per day when fed with haulm from water stress management and from 93 to 160 g when fed with haulm from fully irrigated plots. In other words choice of cultivar will have substantial effects on livestock productivity in feeding systems where groundnut haulms provide the major part of the feed.

4.2. Trade-offs between pod and haulm yield and haulm fodder quality traits

Farmer will rarely sacrifice pod yield for haulm yield and fodder quality even though there is anecdotal evidence that occasionally farmer can make more money from groundnut haulms than from pods (Waliyar, personal communication). In a preliminary investigation Nigam and Blümmel (2010) phenotyped more than 800 groundnut cultivars for haulm fodder quality traits and did not find any significant inverse relationship between haulm traits and pod yields, however, their trials were conducted under optimal on-station agronomic conditions. Absence of trade-offs between haulm fodder traits and pod yield was also reported by Larbi et al. (1999) and Omokanye et al. (2001) using limited number of cultivars from West Africa. In the present work significant inverse relations were observed between haulm nitrogen content and *in vitro* digestibility and pod yields (Fig. 1a/c). However, trade-offs between haulm nitrogen content and *in vitro* digestibility and pod yields under fully irrigated conditions were weak and accounted for only 5 and 4% of the variation in pod yield. By contrast, water stress clearly aggravated potential trade-offs and in this case variations in haulm nitrogen content and *in vitro* digestibility accounted for 40 and 28% of the variation in pod yield under those conditions (Fig. 1a/c). In other words, a higher haulm nitrogen content and higher haulm *in vitro* digestibility was highly significantly related to lower pod yields. Nevertheless, even under such circumstances, i.e. at high level of pod yield under water stress, variations in haulm nitrogen and *in vitro* digestibility exist that can be exploited without detriment to pod yield (see Fig. 3a–d below).

The fact that strong trade-offs between haulm nitrogen content and pod yield were specifically found under water stress clearly suggests a causal role of water stress in eliciting these trade-offs. Groundnut pod containing about 25% protein, i.e. approximately 4% nitrogen, and groundnut pods are demanding sinks for nitrogen. In the companion paper (Hamidou et al., submitted for publication, FCR), it is also reported that the harvest index decreased under water stress in these trials, indicating that water stress had an effect on plant reproduction. We then discussed that part of the groundnut tolerance to water stress may be tolerance of the reproductive stages to water stress. These two facts are likely related. Under water stress, genotypes being the most tolerant (higher pod yield under stress), maintain a larger number of pods, which become active sinks for nitrogen and therefore deplete the haulm of nitrogen relatively more than in sensitive genotypes, leading to lower haulm nitrogen. By contrast, the most sensitive genotypes have a lesser number of pods and consequently a lower sink for nitrogen, which likely lead to leaving a larger proportion of the nitrogen in the stems and leaves. We may even speculate that dual purpose groundnut germplasm (high pod yield and fodder quality under drought stress), might be lines having the capacity to sustain biological nitrogen fixation at high level under water stress.

In the case of the relationship between haulm yield and fodder quality traits, these were positive in all conditions, meaning that the most haulm productive genotypes were also those having the highest fodder quality values. With regards to haulm nitrogen content, the higher fodder quality genotypes could be explained by a higher symbiotic nitrogen fixation ability, leading to higher *N* status in the plant canopy. This itself would be able to drive photosynthesis up, which could lead to enhanced sugar levels in the shoot and then higher levels of digestibility.

4.3. Opportunities for concomitant improvement of pod yield and haulm fodder quality traits

Increasing pod yields will not invariably result in reduced haulm fodder quality traits, rather a concomitant improvement of pod yields and haulm fodder traits seems feasible since considerable independence exists between those traits. It is indeed possible to identify genotypes having high pod yields and relatively high haulm nitrogen content and *in vitro* digestibility under both water regimes. As outlined in Fig. 3a–d suitable lines/cultivars could be chosen based on high haulms quality traits or high pod yields. The latter approach appeared to be more suitable even though cultivars/lines with highest haulm nitrogen and *in vitro* digestibility will unlikely be selected. Indeed, the selection of the highest pod yields genotypes gave an average haulm nitrogen content of 2.2 and 2.27% in the control and water stress treatment, which was within 10 and 20% of the average values of the 20 genotypes with the highest haulm nitrogen content. Similarly, the selection of the highest pod yields genotypes had an average *in vitro* digestibility of 61.2 and 60.1% in the control and water stress treatment, which was within 2–3 units of the average values of the 20 genotypes with the highest *in vitro* digestibility values. Therefore, while selecting highest ranking pod yielders will not result in highest haulm nitrogen and *in vitro* digestibilities, there is still considerable variation in these haulm quality traits to be exploited (Fig. 3a–d).

The selection should probably be done under water stress where the trade-offs between quality traits and pod yield where the largest. Maintaining high haulm nitrogen content might also imply selecting genotypes in which symbiotic nitrogen fixation is tolerant to water stress. There has been recent report of genotypic variation for that trait (Devi et al., 2010). Crosses are currently being made between lines contrasting for pod yield under water stress and some crosses will also combine a contrast for productivity traits and for quality traits.

5. Conclusion

Livestock nutritionally significant variations in haulm nitrogen content and in *in vitro* digestibility were found in this representative set of groundnut germplasm. Under fully irrigated conditions there was virtually no trade-off between these quality traits and the pod and haulm yield productivity. By contrast, under water stress conditions, high quality traits tended to be negatively related to pod yield, suggesting an indirect effect of drought on groundnut reproduction with consequences on the source-sink relationship for nitrogen. However, even at high levels of pod yield under stress, it was possible to identify genotypes with haulm nitrogen contents and *in vitro* digestibility value that were close to the maximum values. Therefore, these results open great opportunities for breeding in parallel for high productivity and high fodder quality under drought stress in groundnut.

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