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Introgression of staygreen QLT's for concomitant improvement of food and fodder traits in *Sorghum bicolor*

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ABSTRACT

Staygreen quantitative trait loci (QTL) were introgressed into sorghum genetic backgrounds S-35 and R-16 generating 52 and 39 lines, respectively, to investigate the effects of staygreen introgression on stover fodder traits and grain–stover relationships. Trials were conducted in the post-rainy seasons 2008–2009 and 2009–2010 at ICRISAT headquarters using a fully irrigated control and a terminal water stress treatment. Stover fodder quality traits analyzed were nitrogen (N), *in vitro* organic matter digestibility (IVOMD), acid detergent fiber (ADF), acid detergent lignin (ADL) and neutral detergent solubles (NDS = 100 − NDF) using a combination of conventional nutritional laboratory analysis with Near Infrared Spectroscopy (NIRS). Significant ($P < 0.0001$) differences were found among lines for grain and stover yield and all stover fodder quality traits under both water treatments. Water treatment had greater effects on grain and stover yields, which decreased between 20 and 32% under water stress, than on stover fodder quality traits which varied at most by 8% between treatments. Year had the greatest effect (F-value) on all traits followed by water treatment and line. Trade-offs between stover quality traits and grain yields were largely absent in both backgrounds. Year and water treatment had larger effects on stover fodder quality traits than QTL's but QTL's were highly significant ($P < 0.0001$) except for stover N in S-35 background. Significant interactions between QTL, water treatment and year were largely absent. The effect of QTL on selected stover fodder quality traits was background dependent. In S-35 one staygreen QTL (StgB) increased stover IVOMD and grain and stover yield while no concomitant trait improvement was observed in background R-16. It is concluded that staygreen QTL can contribute to improving stover quality and grain and stover yield in a background-dependent manner.

1. Introduction

Crop breeders in the semi-arid tropics internalized that stover fodder traits are important when farmers rejected new cultivars that were improved only for grain yield with no regard to stover traits (Kelley and Parthasarathy Rao, 1994; Kelley et al., 1996). Hall and Yoganand (2000) systematically investigated the roles of sorghum grain and sorghum stover in India and concluded that stover use as fodder contributed significantly to income from cropping and hence overall livelihoods of farmers. This was specifically valid for post rainy (*Rabi*) season sorghum which is an important commodity for about 5 M households in rain-fed India where sorghum stover is particularly important as dry season fodder for livestock. Rainy (*Kharif*) season and *Rabi* season cultivars are differentiated by photosensitivity. Blümmel et al. (2010) investigated new sorghum cultivars submitted for release testing from 2002 to 2008 for variations in food-fodder traits and observed that key stover fodder quality traits were significantly higher in *Rabi* compared to *Kharif* sorghum stover, the former having a mean digestibility of 51.7% compared to 46.5% in *Kharif* stover. Blümmel and Rao (2006) have shown that in sorghum stover-trading price–quality relations are operative with stover *in vitro* digestibility accounting for about 75% of the variation in stover prices. A one percentage point difference in *in vitro* digestibility was associated with a price differentiation of about 5%. *Rabi* sorghum stover commands higher prices than *Kharif* stover because of stover fodder quality and seasonality (Rama Devi et al., 2000; Blümmel and Rao, 2006; Sharma et al., 2010).

However, while there was essentially no relationship between stover digestibility and grain yield in *Kharif* cultivars ($r = 0.17$; $P = 0.05$) these two traits were inversely related in *Rabi* sorghum

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($r = -0.47$; $P < 0.0001$). The authors hypothesized that water stress might have caused, or at least contributed, to these trade-offs by arresting translocation of soluble carbohydrates into the grain (Blümmel et al., 2010). Introgression of so-called staygreen genes may offer opportunities to improve stover fodder quality while maintaining, and even improving, agronomic yield traits, especially under the water – limiting conditions as encountered in *Rabi* sorghum cropping (Van Oosterom et al., 1996). Several quantitative trait loci (QTLs) have been identified, using different breeding populations and staygreen QTL donors, and different types of drought stress (Vadez et al., 2013a; Rama Reddy et al., 2014). Given the potential benefit of the staygreen trait, genotypes displaying this trait have been used to identify the genomic regions responsible for this phenotype. Clearly increasing drought resistance of sorghum and stover digestibility through plant breeding would be very beneficial for mixed crop livestock systems livestock given that other productive crop traits, notably grain and stover yield are not impaired.

2. Material and methods

2.1. Genetic material

The set consisted of introgression lines containing staygreen QTLs from B-35 donor parent in two genetic backgrounds: S-35 and R-16. Staygreen QTL introgression lines in S-35 background contained one of the six staygreen QTLs (Stg1, Stg2, Stg3, Stg4, StgA and StgB) from donor B-35 = BTx642. S-35 is a sweet-stemmed, medium-duration, dual purpose sorghum variety. Introgression lines were generated (29 entries) after 3–4 backcrosses. There were four to five introgression lines (IL) for each of these staygreen QTLs in this genetic background. Similarly, staygreen introgression lines in R-16 backgrounds contained one of the four staygreen QTLs (Stg1, Stg3, Stg4 and StgB) from donor B-35. R-16 is a highly senescent post rainy season adapted cultivar. Introgression lines were generated after 3–4 backcrosses with background selection (Kassahun et al., 2010). The notations attached to the Stg in this line (after colon) indicate particular marker-haplotype for the concerned QTL introgression for progeny families with similar pedigrees (as we had more than one Stg ILs for every recurrent-staygreen QTL combination. *e.g.* Stgb:1C will have same marker-haplotypes for all progenies having this introgression.

2.2. Field experiments

Trials were conducted in the post rainy seasons 2008–2009 and 2009–2010 at ICRISAT headquarters (Patancheru, AP, India, 17° 30′ N; 78° 16′ E; altitude 549 m). In each trial, two water treatments were used: a fully irrigated control and a terminal water stress (WS) treatment. Each group of introgression lines in a given genetic background were considered as independent trials, so that there were two trials in each year. These trials were complete randomized block designs with water treatment as the main factor and genotype as sub-factors randomized within each block three times. Plots were 4-rows, separated by 60 cm, each row measuring 4 meters. Plant-to-plant distance within row was 15–20 cm, giving a density of about 10 plant m^{-2}. For agronomic measurements, only the inner two rows were considered, also leaving a 50-cm border on either end, so that the measurements were made on 2 rows and 3 meters per row. At maturity, panicles of the harvested area were removed first. Stover samples were then harvested and their fresh weight was taken. Sub-samples of the bulk harvests were dried for three days in a forced-air oven at 60 °C. These subsamples were also used for grinding sample for stover fodder quality analysis.

2.3. Stover analysis

Stover nitrogen (N), neutral (NDF) and acid detergent fiber (ADF), acid detergent lignin (ADL) and *in vitro* organic matter digestibility (IVOMD) were investigated using a combination of conventional nutritional laboratory analysis with Near Infrared Spectroscopy (NIRS; Instrument FOSS 5000 Forage Analyzer with WINSI II software package). For conventional analysis, N was determined by auto-analyzer, NDF, ADF and ADL by routine chemical analytical procedures (Van Soest et al., 1991). Neutral detergent solubles (NDS) were calculated as NDS = 100 − NDF. *In vitro* organic matter digestibility was measured in rumen microbial inoculum using the *in vitro* gas production technique and equation described by Menke and Steingass (1988) as modified by Blümmel and Ørskov (1993). For good-of-fitness of the developed NIRS calibrations see Sharma et al. (2010).

2.4. Statistical analysis

SAS 9.2 (2009) statistical package was used for analysis of variance (ANOVA) by general linear model (PROC GLM), comparison of means between treatments using Fisher's least significance difference (LSD) test at 5% level of significance and simple correlations among laboratory traits by PROC CORR. A randomized complete block design $Yijk = \mu + Gi + Ej + Tk + GEij + TEjk + GTik + eijk$ was used were μ is the mean, Gi the effect of ith genotype, Ej the effect of jth environment and Tk is effect of kth treatment, $GEij$ the interaction of ith genotype with jth environment, $TEjk$ the interaction of kth treatment with jth environment, $GTik$ the interaction of ith genotype with kth treatment and $eijk$ the random error. This analysis was carried out for each group of introgression lines in a given genetic background separately (*i.e.* those in R-16 background separately from those in S-35 background) (genetic effect referred to as "line" effect in Table 1). Heritability was estimated based on genotypic variance components divided by phenotypic variance components using a mixed model with restricted maximum likelihood (REML) method with the variance components calculated using PROC VARCOMP.

3. Results

3.1. Line performance and potential trade-offs between grain and stover traits

Mean and ranges in grain and stover yields and in stover N, IVOMD, ADF and NDS of different sorghum lines derived from S-35 and R-16 genetic background, the probability of statistical differences (P value) and heritability (h^2) under water stress and control conditions are summarized in Table 1. Lines differed significantly ($P < 0.0001$) for all traits in both genetic backgrounds and water–stress conditions. Considerable ranges were observed for grain yields (>1.5 fold) and stover yields (>2.0 fold). Similarly stover N contents varied at least 1.6 fold and IVOMD, ADF and NDS differed by at least 5–7 percentage units. Except for lines derived from R-16 background and grown under control conditions, broad sense heritabilities for grain and stover yields were at least 0.43. Heritabilties for stover fodder N were 0.47–0.68, for stover IVOMD 0.62–0.72, for ADF 0.55–0.84 and for NDS 0.52–0.77 (Table 1). NDF and ADL were not specifically reported on here but followed generally the same trend as the other quality traits.

Grain and stover yields were largely independent. Fig. 1a and b presents these relationships for S-35 and R-16 background under control and water–stress conditions. There was no significant relationship between grain and stover yields in any of the treatments.

Table 1
Means, ranges, probability of statistical differences among lines within each genetic background (either S-35 or R-16), and heritability, in grain and stover yields, stover nitrogen (N), *in vitro* organic matter digestibilities (IVOMD), acid detergent fiber (ADF) and neutral detergent solubles (NDS) in staygreen sorghum lines of two backgrounds grown under water stress and control condition at Patancheru, India in 2009 and 2010.

Variable	Means	Ranges	$P < F$	h^2
Grain yield (kg/ha)				
Stress				
S-35	2731	2087–3276	<0.0001	0.52
R-16	2354	1989–2915	<0.0001	0.43
Control				
S-35	3577	2677–4401	<0.0001	0.80
R-16	3476	2619–4040	<0.0001	0.13
Stover yield (kg/ha)				
Stress				
S-35	3217	2376–4644	<0.0001	0.68
R-16	2722	2255–5507	<0.0001	0.88
Control				
S-35	4176	3095–6385	<0.0001	0.64
R-16	3399	2731–7193	<0.0001	0.91
Stover N (%)				
Stress				
S-35	0.68	0.50–0.81	<0.0001	0.63
R-16	0.73	0.47–0.90	<0.0001	0.47
Control				
S-35	0.72	0.49–0.98	<0.0001	0.62
R-16	0.72	0.45–0.96	<0.0001	0.68
Stover IVOMD (%)				
Stress				
S-35	47.4	43.2–51.5	<0.0001	0.71
R-16	47.2	45.1–50.8	<0.0001	0.62
Control				
S-35	48.7	45.1–52.8	<0.0001	0.68
R-16	46.3	44.2–50.1	<0.0001	0.72
Stover ADF (%)				
Stress				
S-35	42.2	37.3–46.7	<0.0001	0.68
R-16	45.2	39.1–47.8	<0.0001	0.71
Control				
S-35	40.7	36.6–45.4	<0.0001	0.55
R-16	46.2	38.1–48.7	<0.0001	0.84
Stover NDS (%)				
Stress				
S-35	33.7	28.4–39.9	<0.0001	0.69
R-16	32.3	29.2–39.2	<0.0001	0.52
Control				
S-35	36.7	32.0–41.7	<0.0001	0.65
R-16	31.5	29.0–38.2	<0.0001	0.77

For potential trade off analysis between stover fodder quality and grain and stover yield, exemplary stover N and IVOMD were chosen. The relationships between stover N and grain and stover yields in S-35 background under control and water stress conditions are presented in Fig. 2a and c. There was no significant relationship between stover N and grain yields under either control or water stress conditions. In contrast, stover N and stover yield were significantly inversely related, with stover N accounting for 54 and 24% of the variation in stover yield under control and water stress conditions, respectively. The relationships between stover IVOMD and grain and stover yields are presented in Fig. 2b and d. There was no significant relationship between stover IVOMD and grain yield. Stover IVOMD was significantly positively correlated with stover yield accounting for 18 and 38% of the variation in stover yield under control and water–stress conditions, respectively.

The relationships between stover N and grain and stover yields in R-16 background under control and water stress conditions are presented in Fig. 3a and c. While no significant ($P > 0.05$) relationship between stover N and grain yield was observed under either control or water stress conditions, the traits nevertheless tended to be negatively ($P = 0.07$–0.11) associated. Like in the case of S-35 background, stover N and stover yield were significantly inversely related in R-16 background, with stover N accounting for 49 and 50% of the variation in stover yield under control and water stress conditions, respectively. Stover IVOMD was significantly inversely related with grain yield under control condition, with stover IVOMD accounting for 18% of the variation in grain yields. This relationship was insignificant under water-stress condition (Fig. 3b and d). Like in the case of S-35 background, stover IVOMD was also significantly positively correlated with stover yield in R-16 background, accounting for 41 and 38% of the variation in stover yield under control and water stress conditions, respectively. However, in the case of R-16, these regressions were mostly driven by a few lines high yields and IVOMD values and the significance of these relationship may not be as relevant as in the case of the S-35 background.

3.2. Effects of line, treatment, year and G × E interactions

The effects of line, treatment, year and their potential interactions on stover N, IVOMD, ADF and NDS are summarized in Table 2. In S-35 background the effects of line, treatment and year were highly significant ($P < 0.0001$) for all four traits. Treatment × year interactions were also significant for all traits. Line × treatment interactions were insignificant while line × year interactions were highly significant for IVOMD, ADF and NDS but only tended to be important for stover N ($P = 0.07$). Line × treatment × year interactions were insignificant. In background R-16, except for stover N where treatment did not have a significant effect ($P = 0.31$), effects of line, treatment and year were highly significant ($P < 0.0001$). Treatment × year effects were highly significant for all four traits. Line × treatment effects were significant for IVOMD, ADF and NDS but not for stover N. Line × year effects were significant for N, ADF and NDS but not for IVOMD. Except for stover N, line × treatment × year effects were not significant. So, in summary, while interaction effects were significant, there was always a strong genetic effects on the different fodder quality traits. Interestingly, the magnitude of the genetic effects (F-value) varied between genetic backgrounds. For instance, the magnitude of the genetic effect for IVOMD was higher in the S-35 than in the R-16 background. By contrast, the magnitude of the genetic effect for stover N was higher in the R-16 than in the S-35 background (Table 2). Here a high F-value for a given trait in a given genetic background would suggest large genetic variation for that trait in that background, for instance for stover N in the lines in R-16 background.

3.3. Effects of QTL, treatment, year and G × E interactions

The effects of QTL, treatment, year and their interactions on stover N, IVOMD, ADF and NDS are summarized in Table 3. In S-35 background QTLs were significant for IVOMD, ADF and NDS but not for N. Treatment and year had highly significant effects on all four traits. The interactions of treatment and year were highly significant for IVOMD, ADF and NDS but not for stover N. QTL interactions with treatment and with year were insignificant as were QTL × treatment × year interactions except for ADF.

In R-16 background QTL were significant for all four traits. It should be noted here that because of low level of polymorphism between QTL donor parent B-35 and R-16, the QTL categories were less properly defined than in the case of S-35. Treatment affected IVOMD, ADF and NDS but not stover N while year affected all four traits. QTL interactions with treatment were all insignificant and QTL interactions with year were generally insignificant except for ADF. QTL × treatment × year interactions were insignificant for all four traits.

The stover N and IVOMD of the different QTL groups are presented in Figs. 4a, b and 5a, b for background S-35 and R-16, respectively. In both backgrounds significant differences for stover

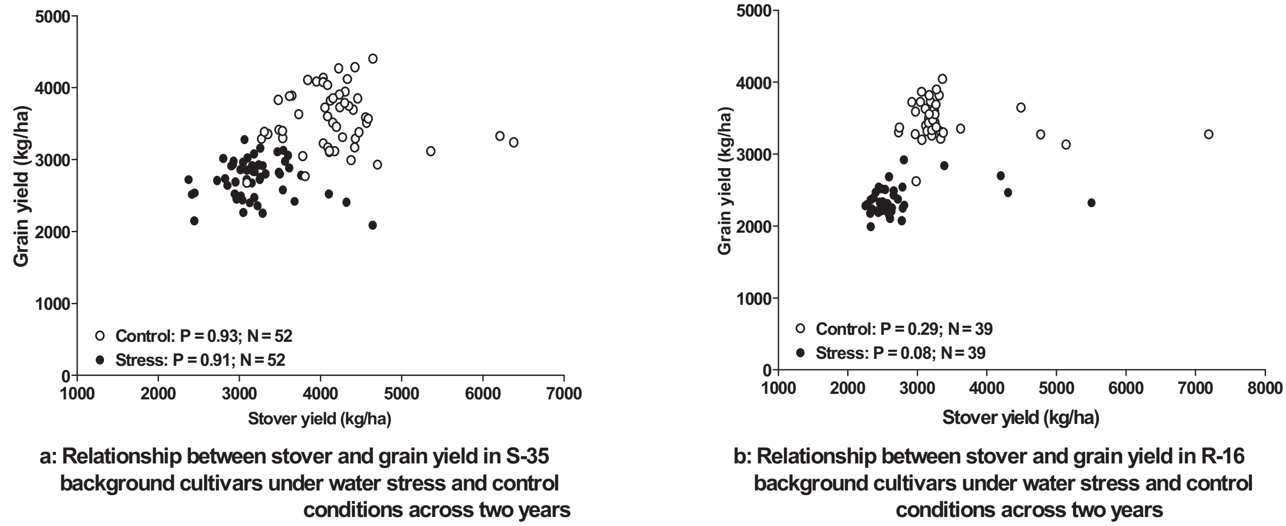

Fig. 1. (a) Relationship between stover and grain yield in S-35 background cultivars under water stress and control conditions across two years. (b) Relationship between stover and grain yield in R-16 background cultivars under water stress and control conditions across two years.

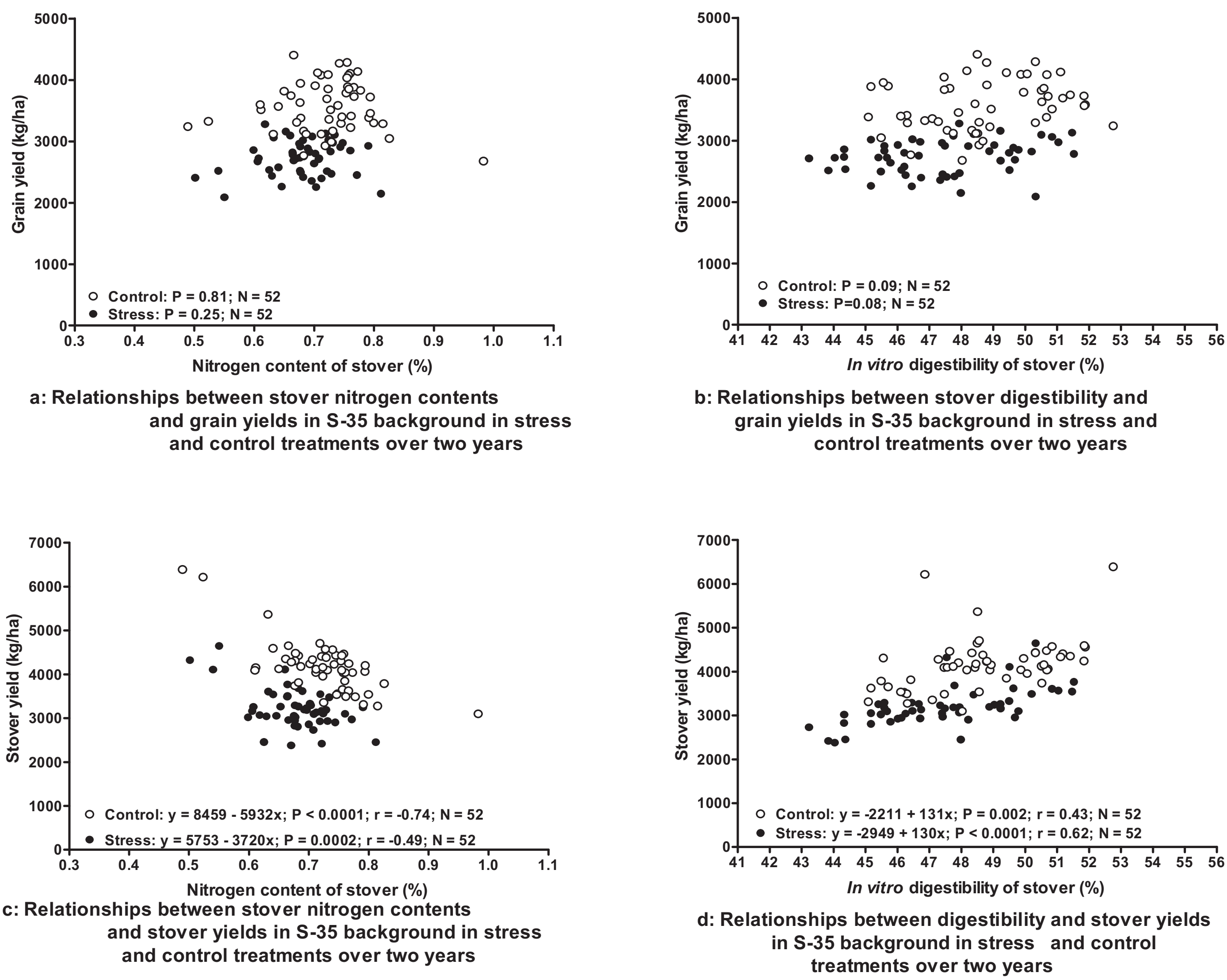

Fig. 2. (a) Relationships between stover nitrogen contents and grain yields in S-35 background in stress and control treatments over two years. (b) Relationships between stover digestibility and grain yields in S-35 background in stress and control treatments over two years. (c) Relationships between stover nitrogen contents and stover yields in S-35 background in stress and control treatments over two years. (d) Relationships between digestibility and stover yields in S-35 background in stress and control treatments over two years.

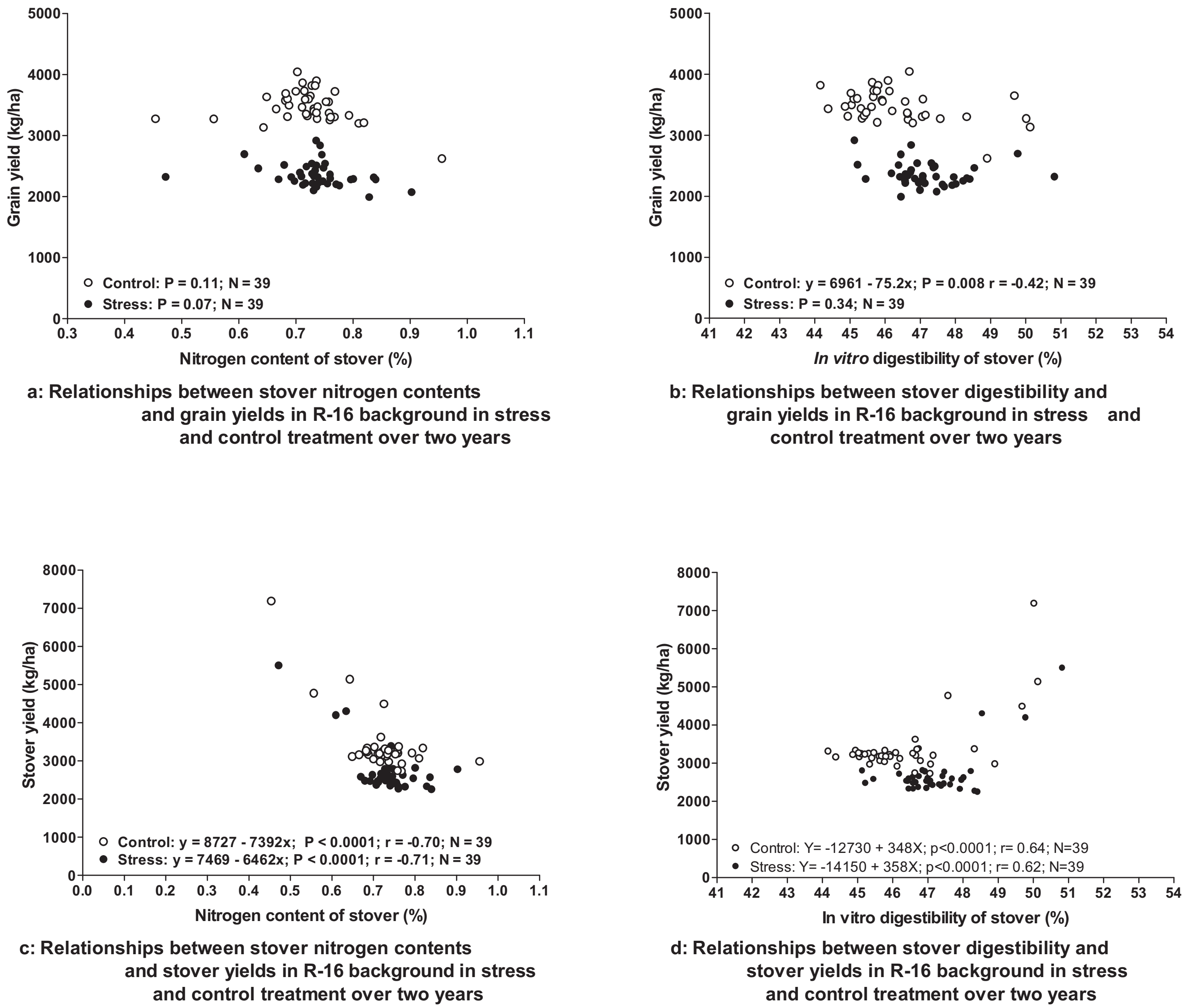

Fig. 3. (a) Relationships between stover nitrogen contents and grain yields in R-16 background in stress and control treatment over two years. (b) Relationships between stover digestibility and grain yields in R-16 background in stress and control treatment over two years. (c) Relationships between stover nitrogen contents and stover yields in R-16 background in stress and control treatment over two years. (d) Relationships between stover digestibility and stover yields in R-16 background in stress and control treatment over two years.

N and IVOMD were observed among different QTLs, indicating that, depending on recurrent background, QTL had significant effects on stover quality traits.

In S-35 background there were no significant relationships between QTL associated stover N and grain and stover yield but IVOMD and grain yield were significantly positively associated, Fig. 6a and d.

In background R-16 stover N and IVOMD and grain yield were not significantly correlated but stover N was inversely associated with stover yield and stover IVOMD was significantly inversely correlated with stover yield, Fig. 7a and d.

4. Discussion

4.1. Variations in grain and stover yields and in stover fodder quality traits

Stover fodder quality is ultimately determined by livestock response in form of meat, milk, draft power and fiber. Laboratory tests are indispensable shortcuts in the assessment of fodder quality since actual livestock productivity trials are unsuitable for routine fodder quality analysis, particularly in crop improvement programs which generate a multitude of samples (Sharma et al., 2010). In the present work we focused on stover N, IVOMD, ADF and NDS because of their relation to animal performance. Nitrogen is an essential nutrient for rumen microbes, and low N (*i.e.* below 1–1.2% N in the feed) content is a key nutritional constraint in cereal straws and stover, unless N is supplemented by legumes, concentrates or non-protein N sources such as urea (Sundstøl and Owen, 1984). Also staygreen QTL introgressions should affect residual greenness in stover and therefore stover N content (Rama Reddy et al., 2014). Acid detergent fiber, an estimate of the cellulose content of plant cell walls, was shown to be related to livestock productivity in sheep fed exclusively on sorghum stover (Ramkrishna Reddy et al., 2010) and ADF is often used as an important negative trait in feed and fodder evaluation (Van Soest, 1994). Neutral detergent solubles estimate plant cell contents, a pool of soluble carbohydrates and amino acids that are metabolically mobile, *i.e.* those available for translocation into the grain (Van Soest, 1994); NDS content should therefore be affected by both staygreen QTL introgression

Table 2
Effects of line, treatment, year and their potential interactions on stover nitrogen (N), *in vitro* organic matter digestibilities (IVOMD), acid detergent fiber (ADF) and neutral detergent solubles (NDS) in staygreen sorghum lines of two backgrounds grown under water stress and control condition at Patancheru, India in 2009 and 2010.

Source	*F*-value	*P* > *F*
Stover N in S-35 background		
Line	4.40	<0.0001
Treatment	17.75	<0.0001
Year	2673.6	<0.0001
Treatment × year	9.87	0.0018
Line × treatment	1.02	0.45
Line × year	1.33	0.07
Line × treatment × year	0.77	0.88
Stover IVOMD in S-35 background		
Line	10.32	<0.0001
Treatment	50.12	<0.0001
Year	50.76	<0.0001
Treatment × year	162.0	<0.0001
Line × treatment	1.29	0.09
Line × year	2.54	<0.0001
Line × treatment × year	0.95	0.58
Stover ADF in S-35 background		
Line	12.4	<0.0001
Treatment	74.7	<0.0001
Year	182	<0.0001
Treatment × year	120.6	<0.0001
Line × treatment	1.12	0.27
Line × year	3.98	<0.0001
Line × treatment × year	1.11	0.29
Stover NDS in S-35 background		
Line	12.2	<0.0001
Treatment	188	<0.0001
Year	198	<0.0001
Treatment × year	8.57	0.003
Line × treatment	1.17	0.21
Line × year	3.54	<0.0001
Line × treatment × year	0.79	0.85
Stover N in R-16 background		
Line	8.8	<0.0001
Treatment	1.03	0.31
Year	1556.2	<0.0001
Treatment × year	58.4	<0.0001
Line × treatment	0.82	0.77
Line × year	2.1	0.0003
Line × treatment × year	1.85	0.003
Stover IVOMD in R-16 background		
Line	5.3	<0.0001
Treatment	34.32	<0.0001
Year	391.5	<0.0001
Treatment × year	89.2	<0.0001
Line × treatment	2.02	0.0007
Line × year	1.10	0.32
Line × treatment × year	1.15	0.25
Stover ADF in R-16 background		
Line	17.2	<0.0001
Treatment	45.2	<0.0001
Year	3.5	0.06
Treatment × year	72.9	<0.0001
Line × treatment	2.2	0.0002
Line × year	2.7	<0.0001
Line × treatment × year	1.26	0.15
Stover NDS in R-16 background		
Line	13.6	<0.0001
Treatment	21.4	<0.0001
Year	759	<0.0001
Treatment × year	36.2	<0.0001
Line × treatment	1.46	0.04
Line × year	3.6	<0.0001
Line × treatment × year	1.28	0.13

Table 3
Effects of QTL, treatment, year and their potential interactions on stover nitrogen (N), *in vitro* organic matter digestibilities (IVOMD), acid detergent fiber (ADF) and neutral detergent soluble (NDS) in staygreen sorghum lines of two backgrounds grown under water stress and control condition at Patancheru, India in 2009 and 2010.

Source	*F*-value	*P* > *F*
Stover N in S-35 background		
QTL	1.2	0.31
Treatment	19.3	<0.0001
Year	2245	<0.0001
Treatment × year	3.6	0.06
QTL × treatment	1.0	0.42
QTL × year	1.8	0.11
QTL × treatment × year	0.69	0.63
Stover IVOMD in S-35 background		
QTL	38.4	<0.0001
Treatment	42.3	<0.0001
Year	17.1	<0.0001
Treatment × year	82.3	<0.0001
QTL × treatment	1.25	0.28
QTL × year	1.0	0.42
QTL × treatment × year	1.8	0.11
Stover ADF in S-35 background		
QTL	43.2	<0.0001
Treatment	41.6	<0.0001
Year	145.5	<0.0001
Treatment × year	64.1	<0.0001
QTL × treatment	0.81	0.55
QTL × year	1.65	0.14
QTL × treatment × year	2.57	0.03
Stover NDS in S-35 background		
QTL	39.3	<0.0001
Treatment	99.9	<0.0001
Year	63.9	<0.0001
Treatment × year	2.85	0.09
QTL × treatment	0.39	0.85
QTL × year	1.66	0.14
QTL × treatment × year	1.64	0.15
Stover N in R-16 background		
QTL	3.2	<0.0001
Treatment	1.5	0.22
Year	997	<0.0001
Treatment × year	73.6	<0.0001
QTL × treatment	0.85	0.97
QTL × year	1.45	0.11
QTL × treatment × year	1.02	0.44
Stover IVOMD in R-16 background		
QTL	2.3	0.002
Treatment	52.7	<0.0001
Year	292.5	<0.0001
Treatment × year	44.5	<0.0001
QTL × treatment	0.59	0.91
QTL × year	0.79	0.71
QTL × treatment × year	1.1	0.35
Stover ADF in R-16 background		
QTL	3.55	<0.0001
Treatment	73.6	<0.0001
Year	8.5	0.004
Treatment × year	32.5	<0.0001
QTL × treatment	0.74	0.77
QTL × year	1.7	0.04
QTL × treatment × year	0.61	0.88
Stover NDS in R-16 background		
QTL	3.9	<0.0001
Treatment	35.5	<0.0001
Year	445.8	<0.0001
Treatment × year	13.8	0.0002
QTL × treatment	0.37	0.9
QTL × year	1.05	0.40
QTL × treatment × year	0.84	0.64

and water stress. *In vitro* organic matter digestibility is a single measurement that summarizes all positive and negative quality traits reflecting overall stover fodder quality; IVOMD was found to be well correlated with sorghum stover pricing in commercial stover trading (Blümmel and Rao, 2006).

Highly significant differences were found for all laboratory quality traits among the lines independent of water treatment and background (Table 1). The ranges in stover quality traits observed among the lines are nutritionally significant for ruminant livestock. Exemplary IVOMD varied by a least 5.7 percentage points, while

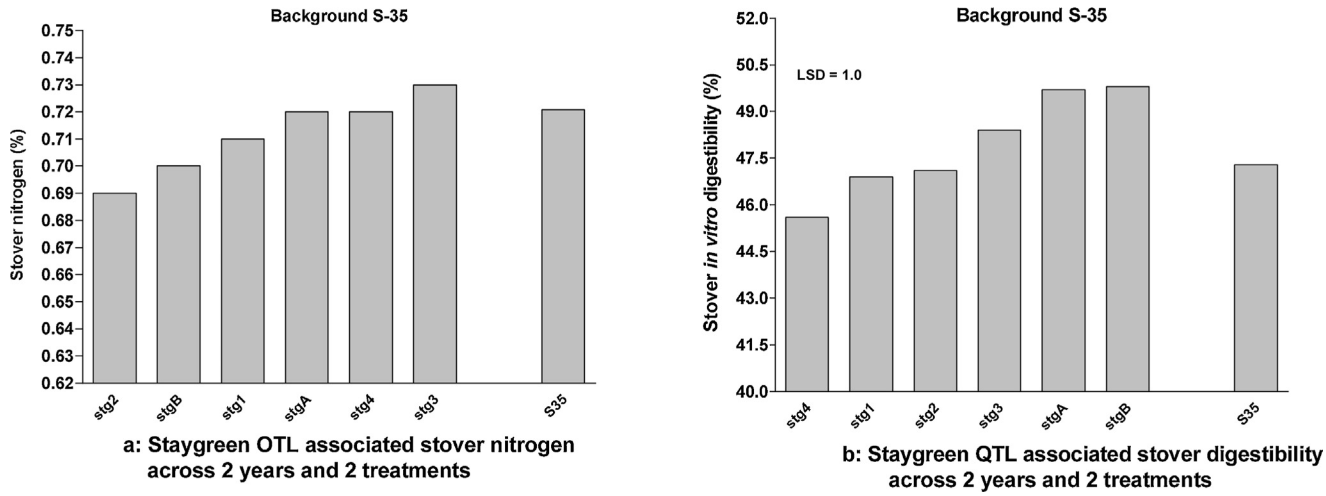

Fig. 4. (a) Staygreen OTL associated stover nitrogen across 2 years and 2 treatments. (b) Staygreen QTL associated stover digestibility across 2 years and 2 treatments.

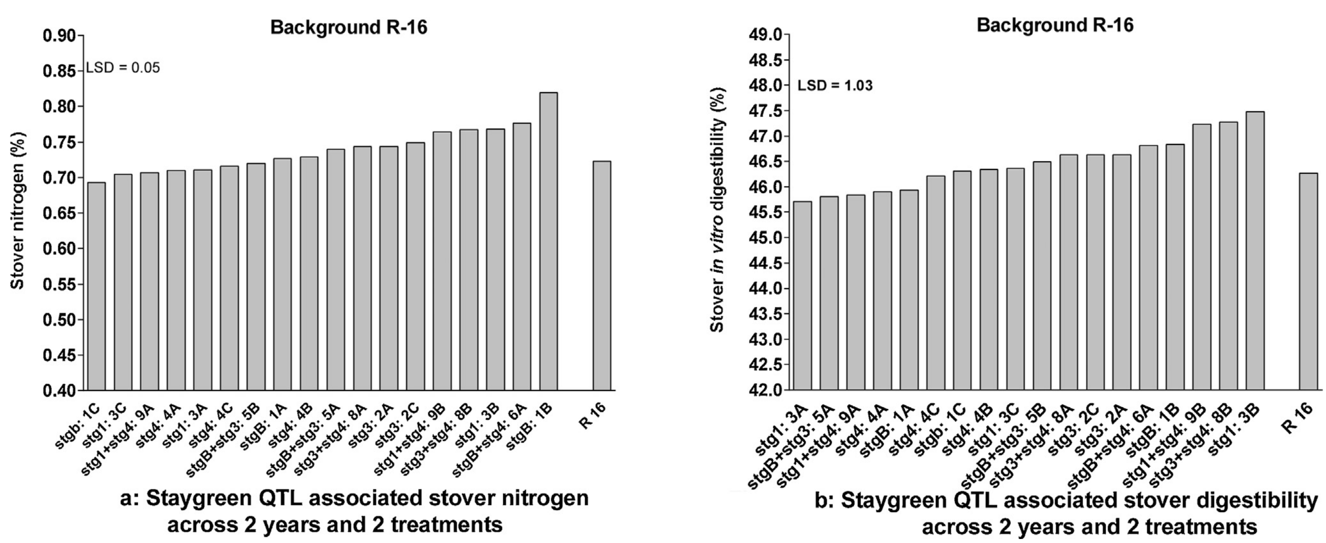

Fig. 5. (a) Staygreen QTL associated stover nitrogen across 2 years and 2 treatments. (b) Staygreen QTL associated stover digestibility across 2 years and 2 treatments.

we know that a one percent point difference in IVOMD can effect stover fodder pricing and/or livestock productivity by 5% and more (Kristjianson and Zerbini, 1999; Blümmel and Rao, 2006). The relatively largest ranges in all stover quality traits among lines were observed for stover N, ranging from values that would present a severe N deficit for rumen digestion to meeting minimum N requirements for it. Sorghum stover N content was found to be positively correlated with voluntary feed intake and the ranges in stover N observed among lines (Table 1) will have substantial effects on sorghum stover intake (Ramkrishna Reddy et al., 2010). Similarly, ADF and NDS varied by around 10% units among lines in both background and water treatments and differences of such magnitude will affect stover intake and digestibility substantially (Ramkrishna Reddy et al., 2010; Van Soest, 1994). To summarize, livestock nutritionally significant variations were observed in all fodder traits among the sorghum lines. However, while lines differed highly significantly for all these traits, water treatment and years had always strong effects in background S-35 and in most cases in background R-16. Water management had generally the greatest statistical effect on fodder quality traits followed by year effects (Table 2). These findings agree with observations in a similar experiment conducted on pod and haulm traits in about 300 well watered and water stressed groundnut cultivars investigated over a period of two years (Blümmel et al., 2012). It also means that water stress interactions must be considered in the breeding for improved stover quality traits. However, water treatment had much greater effects on grain and stover yields, which increased between 25 and 48% under well watered condition, than on stover nutrient composition and overall quality (IVOMD) where effects of irrigation were less (<9%), see Table 1. In other words, water treatment affected overall biomass yield rather than biomass composition. These findings confirm the considerable degree of independency between agronomic yields and stover fodder quality traits that were also observed by Sharma et al. (2010), Blümmel et al. (2010) and Blümmel et al. (2012). On a very basic level, grain and stover yield were statistically unrelated, regardless of water treatment and background, see Fig. 1a and b. Wide cultivar-dependent variations in harvest indices – which results in loose, or even none-relation between grain and stover yield – were also observed by Blümmel et al. (2010) in a wide range of sorghum cultivars.

4.2. Relationships between grain and stover traits

For further analysis of possible trade-offs between grain and stover yields and stover fodder quality, two traits were selected:

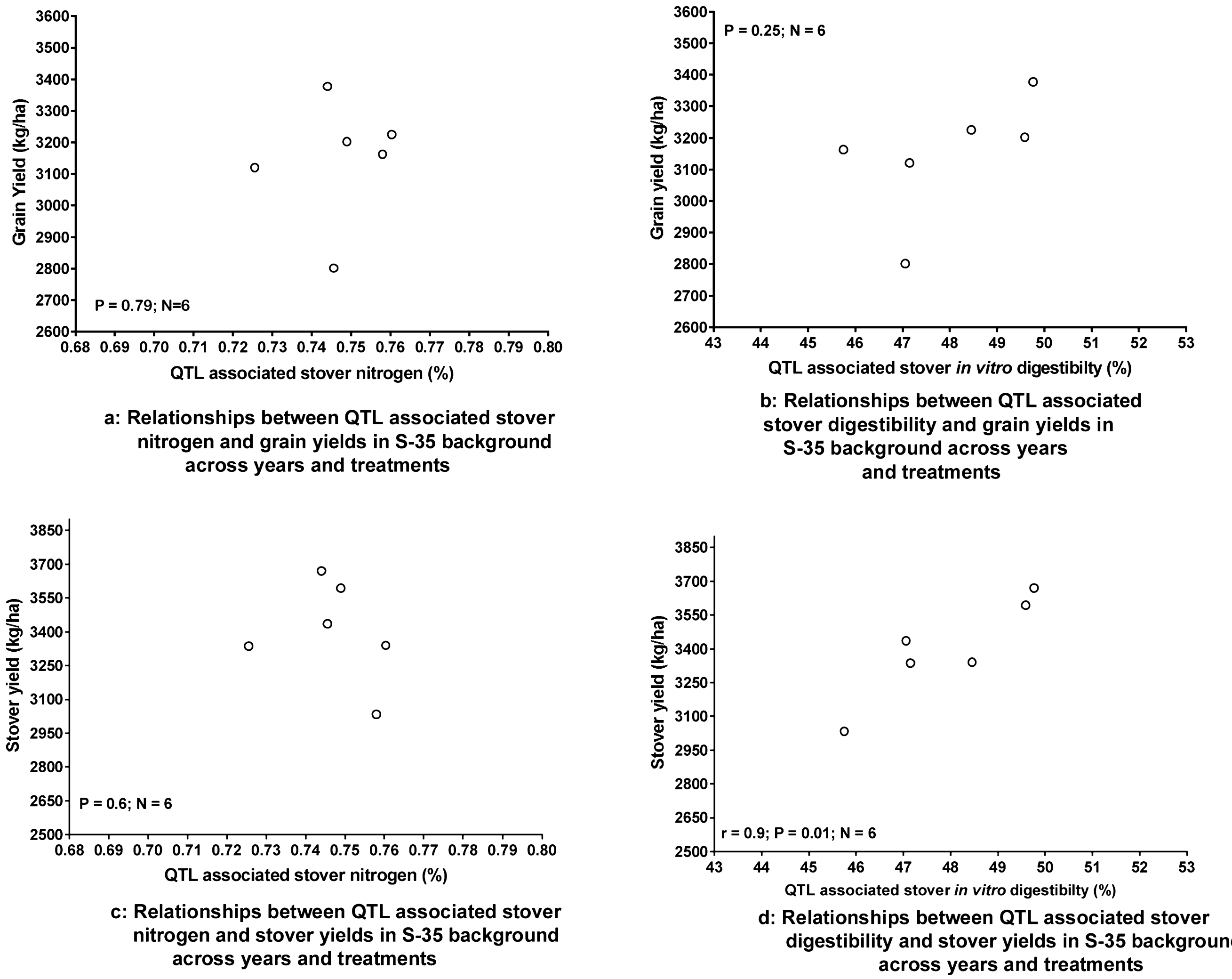

Fig. 6. (a) Relationships between QTL associated stover nitrogen and grain yields in S-35 background across years and treatments. (b) Relationships between QTL associated stover digestibility and grain yields in S-35 background across years and treatments. (c) Relationships between QTL associated stover nitrogen and stover yields in S-35 background across years and treatments. (d) Relationships between QTL associated stover digestibility and stover yields in S-35 background across years and treatments.

IVOMD as indicator for overall fodder quality and N as possible key limiting nutrient. Except for stover IVOMD under well watered condition in background R-16, stover N and IVOMD were statistically not related to grain yields (Figs. 2a, b and 3a, b). Relationship between stover quality and stover yield were different in that consistent significant inverse relationships were observed between stover N and stover yield while stover IVOMD was consistently significantly positively associated with stover yield (Figs. 2c, d and 3c, d), especially so in the S-35 background. Similar relationships were reported by Bidinger and Blümmel (2007) for pearl millet who reasoned that soil N is limited by fertilizer input which together with a dilution effect can result in inverse relationships between stover N and stover yield. Another interesting finding was the positive associations between stover yield and IVOMD, especially in S-35 background. This is particularly interesting from a sorghum stover value chain perspective, where the relative value of stover with respect to grain is increasing, reaching now 50% (Sharma et al., 2010), since any improvement of stover yield would also improve stover quality. It has been shown that stover quality enjoys a substantial price premium in the market (Blümmel and Rao, 2006). From a physiological point of view, it is unclear why a more productive stover would also be more digestible. This could be related to the fact that the staygreen expression in several of the staygreen introgression lines actually reflect a higher water availability during the grain filling period (Vadez et al., 2013b). Perhaps the stover of some of the staygreen lines has remained more photosynthetically active for a longer period, leaving some more soluble carbohydrates available and contributing to digestibility (Rama Reddy et al., 2014). This would have been even more important in S-35 background, *i.e.* a sweet-stemmed line. Indeed the NDS values in S-35 background were on average about 4% unit higher than in R-16 (Table 3).

4.3. Effect of QTL's on stover fodder quality traits

With the exception of stover N in S-35 background, highly significant differences were observed for stover fodder quality traits when grouped according to QTL (Table 3). These findings seem to confirm that QTL can affect important stover fodder quality traits (Rama Reddy et al., 2014). While water treatment and year had in most cases a strong impact on stover fodder quality traits, significant QTL interactions with treatment and year were largely absent (Table 3) suggesting that QTL effects on stover quality traits were expressed consistently across diverse environment and management practices. Staygreen QTL introgression had no significant effect on stover N in S-35 background (see Table 3 and Fig. 4a) but could significantly alter stover N in background R-16 (see Table 3 and Fig. 5a). The maximum differences in stover N caused by

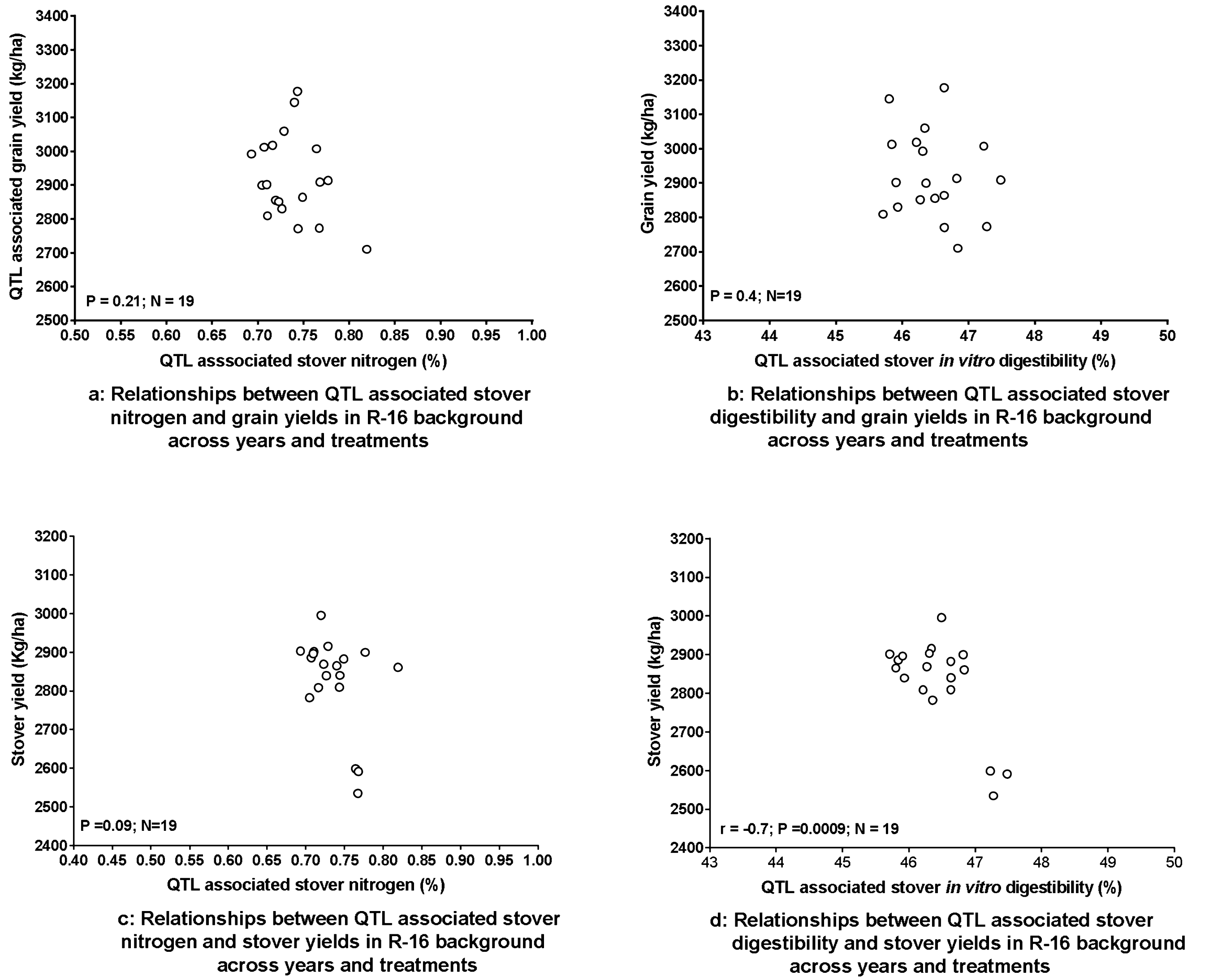

Fig. 7. (a) Relationships between QTL associated stover nitrogen and grain yields in R-16 background across years and treatments. (b) Relationships between QTL associated stover digestibility and grain yields in R-16 background across years and treatments. (c) Relationships between QTL associated stover nitrogen and stover yields in R-16 background across years and treatments. (d) Relationships between QTL associated stover digestibility and stover yields in R-16 background across years and treatments.

staygreen QTL introgression was 0.13% (0.69 *vs* 0.82%, Fig. 5a). In any case, the fact that staygreen QTL had different effects in different genetic background is worth mentioning, as it means the QTL effect is not solely dependent on the nature of any QTL and is dependent on the genetic background in which the QTL is introgressed. For instance Stg B had a major effect on the IVOMD of introgression lines in S-35 background (Fig. 4b), but not in introgression lines in R-16 background (Fig. 5b). Similar conclusions were drawn in a recent study with these same materials, where the introgression of Stg1 QTL increased water extraction in S-35 background but not in R-16 background, or where introgression of StgB QTL increased water use efficiency in R-16 background but not in S-35 background (Vadez et al., 2011). In the previous study, this was likely due to the fact that the water extraction capacity of S-35 was high, and so was the water use efficiency of R-16, so that no QTL could help improve these traits. The present work is somewhat different in that the stover N content and IVOMD of S-35 and R-16 are quite similar, and we have no explanation for the improvement of IVOMD in S-35 background and of stover N content in R-16 background. In any case, it indicates that the process of staygreen QTL introgression can bring substantial improvement in certain quality traits, but that these first need to be assessed to choose the best entries, and quality traits baselines need to be measured in potential recurrent parents.

4.4. QTL introgression for concomitant improvement of gain yield, stover yield and key stover quality traits

The attractive feature of stay green introgression resides with opportunities for multiple trait improvement such as water-use efficiency, agronomic yield and stover fodder quality traits (Rama Reddy et al., 2014). In the present work staygreen QTL introgression into background S-35 could indeed significantly increase IVOMD by about 2.5% units when StgB QTL was used and this QTL had also positive impact on grain and stover yield (Fig. 6b and d). This introgression generated a group of lines having highest grain and stover yield and highest IVOMD supporting the assumption that QTL introgression can indeed concomitantly improve these three important traits. An increase in IVOMD of 2.5 percent units might appear to be of low significance, however as pointed out by Blümmel and Rao (2006) a one percent difference in IVOMD in sorghum stover – trading was associated with price premiums of 5% and above. Anandan et al. (2010) fed two total mixed rations to dairy buffalo that differed only in the digestibility of the sorghum stover (47 and 52%

digestibility) that made up 50% of the ration. Milk potential on the two rations differed by about 5 kg/d (10 and 15 kg/d). Thus a difference of 2.5% unit in IVOMD does matter for livestock productivity and achieving this improvement by introgressions of staygreen traits will make sense economically. However depending on which QTL were introgressed, traits could also be reduced. For example, IVOMD was significantly reduced by 1.7% units when stg4 QTL was introgressed into background S-35 (Fig. 4b). It is also of concern that the success of staygreen introgression is so background-dependent that trial and error approaches might be required to best match QTL and background.

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